

**Studies of the increased gastrin release associated
with *Helicobacter pylori* infection in duodenal ulcer
disease**

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for the degree of Doctor of Medicine

by

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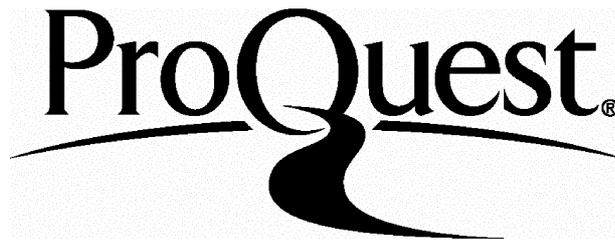
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Disease is very old, and nothing about it has changed. It is we who change as we learn to recognise what was formerly imperceptible

Jean Martin Charcot
French neurologist, 1825–93.
De l'expectation en médecine

To my parents, Renée and Gerry Moss

ABSTRACT

Duodenal ulcer disease is strongly associated with infection of the gastric antrum by *Helicobacter pylori* (*H pylori*), possibly through the exaggerated plasma gastrin response associated with this organism. The work described in this thesis investigates aspects of both the cause and the effect of this increase in gastrin secretion in duodenal ulcer patients.

Chapter 2 provides further evidence that *H pylori* is responsible for the exaggerated gastrin response since when duodenal ulcers are healed by sucralfate, which does not eradicate *H pylori*, the gastrin response is unchanged. However, unexpectedly, this treatment decreased basal gastric acid secretion.

The results of studies described in chapter 3 suggest that the exaggerated gastrin response occurs by a mechanism independent of luminal pH and with no apparent change in the meal-stimulated secretion of acid or pepsin. Despite the fall in meal-stimulated gastrin the peak acid output also remained unchanged a year after the eradication of *H pylori* (chapter 4) but the basal acid output appeared to decrease in this small study. The work described in chapter 5 confirms this fact; successful eradication of the organism decreased both basal plasma gastrin concentrations and basal acid secretion, without altering the sensitivity of the parietal cell to circulating gastrin. This may be how the eradication of *H pylori* prevents ulcer recurrences.

The cause of the hypergastrinaemia was addressed in the final chapters. Methods were developed to measure somatostatin mRNA from endoscopic biopsies as a surrogate marker of local somatostatin release. It was then established that the hypergastrinaemia seen in pernicious anaemia is associated with a deficiency of somatostatin mRNA (chapter 6). Finally, an increase in both somatostatin-secreting cells and somatostatin mRNA was found after the eradication of *H pylori*, implying that this bacterium increases gastrin release by the depletion of somatostatin.

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PUBLICATIONS ARISING FROM THIS WORK

ABSTRACTS

Moss S, Ayesu K, Li SK, Calam J. Gastrin, gastric acid and pepsin responses during intragastric titration in duodenal ulcer patients: effect of suppressing *Helicobacter pylori*. Gut 1991; 32: A 1206-7.

Moss S, Ayesu K, Calam J. Gastrin and gastric acid output 1 year after eradication of *Helicobacter pylori* in duodenal ulcer patients. Regul Pept 1991; 35: 251.

Moss SF, Bishop AE, Polak JM, Calam J. Increased antral somatostatin-immunoreactive cell density after eradication of *Helicobacter pylori*. Irish J Med Sci 1992; 161 (suppl 10): 30-31.

Moss SF, Legon S, Calam J. Gastrin and somatostatin mRNA in human endoscopic gastric biopsies. Gut 1992; 33 (suppl 1) : S74.

Moss SF, Legon S, Calam J. *Helicobacter pylori* infection decreases gastric somatostatin mRNA in duodenal ulcer patients. Gut 1992; 33 (suppl 2) : S27.

Moss SF, Legon S, Calam J. Somatostatin regulates antral gastrin synthesis in pernicious anaemia. Gut 1992; 33 (suppl 2) : S61.

Moss SF, Calam J. Changes in acid secretion and parietal cell sensitivity to gastrin in duodenal ulcer patients after eradication of *H pylori*. Gut 1992; 33 (suppl 2): S 28.

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Moss SF, Thomas DM, Ayesu K, Levi S, Calam J. Sucralfate diminishes basal acid output without affecting gastrin, *Helicobacter pylori*, or gastritis in duodenal ulcer patients. *Aliment Pharmacol Therap* 1992; 6: 251–8.

Moss SF, Legon S, Bishop AE, Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. *Lancet* 1992; 340: 930–2.

Moss S, Playford RJ, Ayesu K, Li SK, Calam J. pH dependent secretion of gastrin in duodenal ulcer disease: effect of suppressing *Helicobacter pylori*. *Digestion* 1992; 52: 173–8.

Moss SF, Calam J. Acid secretion and sensitivity to gastrin in duodenal ulcer patients: effect of eradication of *H pylori*. *Gut* (in press)

Moss SF, Legon S, Calam J. Reciprocal changes in antral gastrin and somatostatin mRNA in pernicious anaemia (submitted to *Dig Dis Sci*).

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ABBREVIATIONS

BAO	Basal Acid Output
CCK	Cholecystokinin
cDNA	complementary Deoxyribonucleic Acid
cpm	counts per minute
D-cell	somatostatin-secreting cell
DNA	Deoxyribonucleic Acid
DU	Duodenal Ulcer
EDTA	Ethylenediamine Tetra-acetic acid
G-17	Gastrin heptadecapeptide
G-34	Gastrin 34
G-cell	gastrin-secreting cell
GIP	Gastric inhibitory polypeptide
GRP	Gastrin releasing peptide
MOPS	3-[N-Morpholino] Propane-Sulphonic Acid
mRNA	messenger Ribonucleic Acid
PAO	Peak acid output
RNA	Ribonucleic Acid
rpm	revolutions per minute
rRNA	ribosomal Ribonucleic Acid
S-14	Somatostatin 14
S-28	Somatostatin 28
SDS	Sodium dodecyl sulphate
SSPE	Standard Saline Phosphate Ethylenediamine Tetra-acetic acid

CHAPTER 1 - INTRODUCTION

Introduction

The first pathological description of a duodenal ulcer (DU) is attributed to Hamberger in 1746 and this finding was rapidly followed by the first insight into a possible pathogenic mechanism when, in 1752, René de Réamur reported that meat was digested without putrefaction by his pet buzzard's vomit (Réamur, 1752). The current definition of a DU is a breach of the duodenal epithelium involving the full thickness of the mucosa (Morson *et al*, 1990).

Duodenal ulceration remains a common health problem worldwide, with a lifetime prevalence in the West of 5–10% (Langman, 1979). Despite the use of treatment with potent acid-suppressing drugs which heal ulcers effectively, the ulcers almost invariably recur once treatment has stopped. Therefore it would appear that some individuals have an underlying ulcerogenic tendency.

For much of this century physiologists and psychologists have attempted to unravel the factors responsible for the predisposition to a DU. They have attempted to establish whether the basic abnormality responsible is psychological or genetic, due to too much acid and pepsin or to a breakdown of natural defences. DU disease used to be perceived to be the common end result of a number of abnormalities of gastro-duodenal physiology such as excessive acid secretion, rapid gastric emptying and diminished duodenal prostaglandin synthesis. The recent epidemiological discovery that in the vast majority of cases the predisposition is related to the presence of an infectious agent, *Helicobacter pylori* (*H pylori*), and that the finding that this organism increases plasma concentrations of the acid-secreting hormone gastrin, has led once again to intense interest into the pathogenesis of DU disease. The key issues addressed in this thesis concern *H pylori* and gastrin in DU disease – namely establishing how *H pylori* increases gastrin release and assessing whether this increased gastrin release is accompanied by changes in the secretion of acid, likely to be important in the pathogenesis of a DU.

In this introductory chapter I shall cover three areas pertinent to understanding the regulation of gastrin and its role in *H pylori*-related DU disease. Firstly I shall review what is known about the normal physiology of gastrin, based upon animal and

human studies. Secondly, I shall evaluate the physiological abnormalities of secretion, including those of gastrin, which are present in patients with DU and which were first established prior to the discovery of *H pylori*. Finally, I shall examine the evidence for the rôle of *H pylori* in the causation of DU disease and consider the mechanisms by which the organism may do so.

GASTRIN – REGULATION IN HEALTH AND DISEASE

Molecular forms of gastrin and their biosynthesis

At the turn of the century JS Edkins showed that there was a substance in the pyloric mucosa which could stimulate gastric acid secretion when injected intravenously (Edkins, 1905; Edkins, 1906). However, because it was then discovered that histamine also stimulated acid secretion, it was not until 1964 that the separate existence of this antral hormone, gastrin, was finally proven (Gregory & Tracy, 1964).

The isolation and further analysis of this substance in porcine antrum which strongly stimulated gastric acid secretion, led to the discovery that the activity of gastrin might not be due to just one single peptide molecule. Initially two components of gastrin were identified, G-I and G-II, which could be separated by gel filtration or electrophoresis (Gregory & Tracy, 1964). Subsequent analysis revealed that these peptides were both 17 amino-acids in lengths with identical amino acid sequences but, unlike G-I, G-II had a sulphated tyrosine residue at position 12 (Gregory *et al*, 1964). Similarly, sulphated and non-sulphated forms of gastrin-17 were identified in other species, including man (Gregory, 1979; Kenner & Sheppard, 1968) with considerable sequence homology found between species.

Further purification of the gastrin-like bioactivity of antral extracts led to the discovery of a larger molecular form of gastrin in plasma (Yalow & Berson, 1971; Yalow & Berson, 1972, having a carboxyl terminal identical to G-17 but with an additional 17 amino-acid residues at the amino-terminal. This peptide is called 'big gastrin' or G-34 (figure 1.1) (Dockray *et al*, 1979; Kenner *et al*, 1981; Wunsch *et al*, 1981) and it too has been found in both sulphated and non-sulphated forms.

Smaller forms of gastrin have also been identified. G-14 ('minigastrin') was initially isolated in tissues (Gregory & Tracy, 1974), although it is not clear whether the presence of this small gastrin form in plasma is merely due to the enzymatic cleavage of G-17 (Blair *et al*, 1977). The carboxyl-terminal four (Rehfeld & Larsson, 1979) and six (Gregory *et al*, 1983) amino acid residues have also been found in the gut, although whether they play a physiological role is not known.

The relationships between these different forms of gastrin with similar carboxyl termini have now been established by the use of specific radioimmunoassays and the techniques of molecular cloning. The evidence indicates that they arise from differential cleavage and post-translational processing of a common precursor. The cDNA complementary to gastrin mRNA was first sequenced in the pig (Yoo *et al*, 1982) and subsequently in man (Boel *et al*, 1983; Kato *et al*, 1983b). It has been used both to determine the amino acid sequence of the gastrin precursor, progastrin, and also as a hybridisation probe for genomic gastrin (Ito *et al*, 1984; Kato *et al*, 1983a; Wiborg *et al*, 1984).

The human gastrin gene is now known to comprise 3 exons; exon 1 being untranslated and separated from exons 2 and 3 by over 3000 base pairs (Figure 1.1). Some of the factors which regulate the transcription of gastrin have recently been elucidated from studies of a transfected rat pituitary cell line. In this system epidermal growth factor switches on transcription through the interaction of a transcription factor with the gastrin promoter (Merchant *et al*, 1991) whilst somatostatin inhibits gastrin synthesis through the inhibitory G protein, G_i , which interacts with a regulatory element just upstream of the epidermal growth factor-responsive element (Bachwich *et al*, 1992). Investigating the regulation of gastrin expression from the stomach has been hampered by the lack of an antral gastrin cell line, but studies of canine cultured G-cells have now shown that epidermal growth factor increases the levels of gastrin mRNA in these cells too (Merchant *et al*, 1992). This raises the possibility that transforming growth factor alpha, which is homologous to epidermal growth factor and which is produced in the antrum (Beauchamp *et al*, 1989), may stimulate the expression of gastrin *in vivo*.

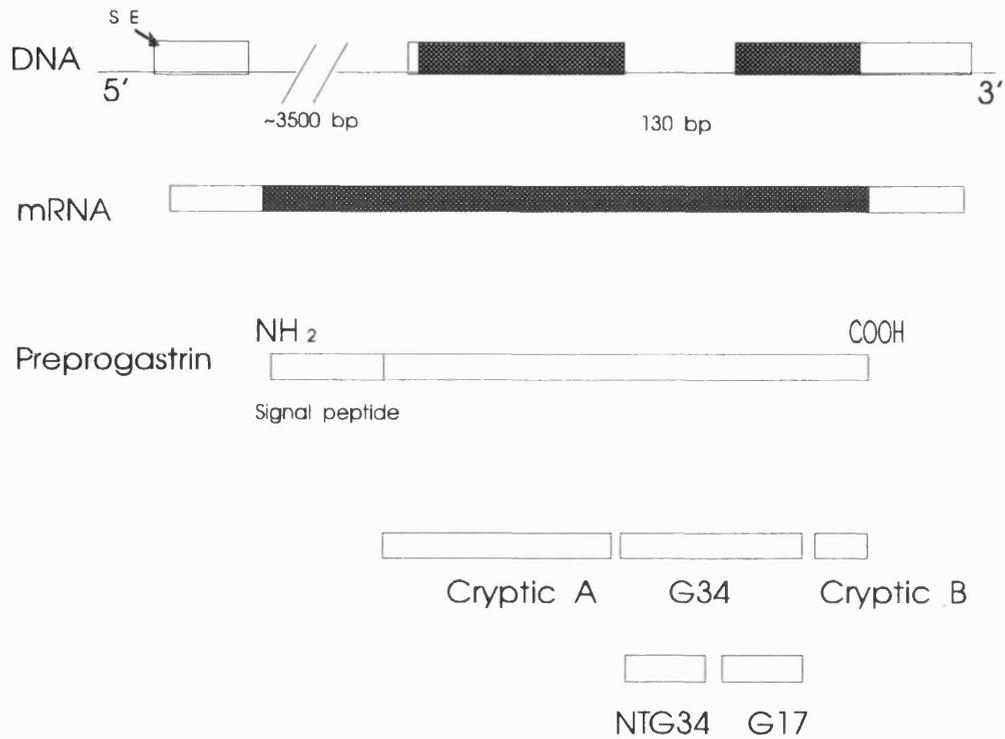


Figure 1.1 Structure of the gastrin gene, gastrin mRNA and preprogastrin with its cleavage products. In the gastrin gene the non-coding exons are shown as open boxes and the translated exons as shaded boxes. S and E represent the somatostatin and the epidermal growth factor-responsive elements of the gastrin promoter respectively. NTG34 is the N-terminal of G-34.

Progastrin consists of 101 amino-acid residues, with a 21 amino-acid signal sequence at the amino-terminal. Cleavage at pairs of basic residues by a trypsin-like enzyme yields G-17, G-34 and the flanking peptides termed cryptic A and cryptic B (Dockray & Gregory, 1989). Before G-17 and G-34 are secreted they undergo post-translational processing. The tyrosine residue is sulphated and a carboxyl terminal amide group, necessary for biological activity (Morley, 1968), is formed by the cleavage of a glycine residue.

Intact progastrin has been found in the human gastric antrum, comprising 5–10% of the total gastrin immunoreactivity (Pauwels *et al*, 1986) and the flanking peptides have been identified both in pig antrum (Desmond *et al*, 1985) and human gastrinoma tissue (Reeve *et al*, 1984). In the antrum most G-34 is cleaved to form G-17 and the biologically inactive amino-terminal of G-34 and the four products of progastrin are stored and secreted together. However, in the duodenum relatively more gastrin is secreted as intact G-34 (Dockray & Gregory, 1989).

Tissue distribution of gastrin

Gastrin is secreted by specialised neuroendocrine gastrin-producing (G)-cells, which can be identified by anti-gastrin antibodies. Because gastrin and cholecystokinin (CCK) share a common carboxyl-terminus pentapeptide sequence, the identification of G-cells in tissues such as the small intestine, which also contain CCK, requires the use of antibodies which are specific to regions other than the carboxyl terminus.

G-cells are most numerous in the gastric antrum, located approximately half way down the gastric glands where they are in close proximity to somatostatin-immunoreactive (D) cells. The apical (luminal) border of G cells possesses microvilli and the cells contain gastrin packaged in secretory granules. These secretory granules are about 200 nm in diameter and are concentrated at the basal region, in close proximity to nerves and blood vessels (Polak, 1989). The gastrin contained in these granules is almost all G-17, at a tissue concentration of around 10 nmol/g (Brand *et al*, 1984).

In contrast, in the duodenum the G-cells contain smaller granules and approximately half of their gastrin immunoreactivity is due to G-34 (Berson & Yalow, 1971; Calam *et al*, 1980; Malmstrøm *et al*, 1976). The gastrin concentrations in duodenal mucosa are 10 to 50% of those in the antrum (Malmstrøm *et al*, 1976; Nilsson *et al*, 1972b).

Gastrin is not normally found in the adult human pancreas, but it has been extracted from pancreatic gastrinoma tissue in the Zollinger–Ellison syndrome. Outside the gut, gastrin is also produced by the pituitary gland.

Normal gastrin physiology

Gastrin release

In the gut gastrin is a true endocrine mediator, exerting its effects after it is released into the systemic circulation. Exactly how much gastrin is released *in vivo* depends upon the balance between the factors which promote the release of gastrin and those which inhibit it. The release of gastrin is modulated in response to several different stimuli. The important luminal stimuli are the intragastric hydrogen ion concentration (pH) and the chemical composition of foods. The release of gastrin is also regulated by various neurotransmitters, contained within and released from nerve endings and by regulatory peptides which circulate in the bloodstream (Table 1.1).

PATHWAY	CHEMICAL MEDIATOR	ACTION
Nervous	Acetylcholine	Nicotinic: stimulation Muscarinic: stimulation and inhibition
	GRP	Stimulation
	Noradrenaline	β -adrenergic: stimulation
Hormonal	Secretin/GIP	Inhibition (? via somatostatin)
Paracrine	Somatostatin	Inhibition
Luminal	Amino-acids, amines, polypeptides	Stimulation (direct and indirect)
	Acid	Inhibition (via somatostatin)

Table 1.1. Mechanisms controlling the release of gastrin (from Dockray & Gregory, 1989). GIP = Gastric inhibiting polypeptide

i) Luminal stimuli & the rôle of somatostatin

Of the various luminal chemical stimuli, protein digests – particularly amino-acids – are far more potent gastrin releasers than are undigested proteins. In contrast, fats and carbohydrates are very poor gastrin-releasers (Debas *et al*, 1974; Richardson *et al*, 1976). The release of gastrin is stimulated by amino-acids both in their native form and after decarboxylation, probably by two distinct mechanisms (DeValle & Yamada, 1990). Whether the decarboxylated amines are important stimulants of gastrin release *in vivo* is currently unclear. Similarly, it remains unknown whether the effect of high concentrations of calcium on gastrin release are important *in vivo* (Reeder *et al* 1974). There is also some evidence that luminal stimuli may release

gastrin **indirectly**, via cholinergic nerves rather than by a direct effect on the G-cell (Saffouri *et al*, 1984b; Schiller *et al*, 1980).

It has been known for some years that luminal acid stimulates the release of somatostatin from the gastric antrum (Holst *et al*, 1983; Schusdziarra *et al*, 1978) and prolonged alkalinization of the stomach for more than 3 hours produces a rise in plasma gastrin (Peters *et al*, 1983). The rise in gastrin upon neutralisation is probably mediated by a decrease in somatostatin, since inhibition of gastrin release is generally achieved through the local release of somatostatin.

Somatostatin is a cyclic peptide with potent inhibitory actions. It was initially isolated from the hypothalamus by investigators studying the inhibition of growth hormone secretion from the pituitary (Brazeau *et al*, 1973; Burgus *et al*, 1973) and has subsequently been shown to be present in almost every tissue of the body (Yamada & Chiba, 1989). As for gastrin, the precursor molecule, preprosomatostatin, yields 2 main biological forms of somatostatin, S-14 and S-28, with similar carboxyl termini. The biosynthetic relationship between these two molecular forms is currently unclear but, again like gastrin, there is a tissue-specific ability to synthesise each of the forms. For example, the small intestine synthesises S-28 and S-14 approximately equally whereas in the rest of the gut and pancreas the total somatostatin-like immunoreactivity is almost all due to S-14 (Penman *et al*, 1983; Baldissera *et al*, 1985). Specialised somatostatin-secreting (D) cells are found throughout the gut and pancreas, usually communicating with the gut lumen (the "open type"), though in the gastric fundus they are of the "closed type" with no obvious luminal contacts (Yamada & Chiba, 1989) They typically possess long cytoplasmic processes which abut upon and thereby inhibit target cells through their local (paracrine) secretion of somatostatin (Larsson *et al*, 1979).

In the gastric antrum the target cells are the gastrin-producing G-cells. Because of the paracrine nature of somatostatin's inhibitory action, measuring the circulating concentrations of somatostatin by radioimmunoassay may not reflect the local inhibitory activity of this peptide. Measuring stored concentrations of somatostatin is equally unrepresentative: does a fall in mucosal somatostatin imply that less has been released or more? For these reasons studying the inhibition of gastrin by somatostatin is best performed by measuring somatostatin mRNA since this is

likely to reflect local peptide synthesis and to be closely linked to rates of release, as has been shown for the G-cell (Dockray *et al*, 1991). Studies of this type in the rat have shown that gastrin and somatostatin mRNA change in a reciprocal fashion. Thus the fall in circulating gastrin due to starvation is accompanied by a rise in somatostatin mRNA (Wu *et al*, 1991) and the elevation of intragastric pH achieved by the parietal cell proton pump inhibitor omeprazole, results not only in an increase in both circulating gastrin peptide and gastrin mRNA (Dockray *et al*, 1991) but also a fall in antral somatostatin mRNA (Brand & Stone, 1988). In experimental animals, immunoneutralisation of somatostatin with an anti-somatostatin monoclonal antibody increased gastrin mRNA, implying that under normal conditions, somatostatin tonically inhibits gastrin synthesis (Karnik *et al*, 1989). This may be achieved by both inhibiting gastrin gene transcription and increasing the turnover of the gastrin messenger RNA once it has been transcribed, thus decreasing the amount available for translation (Karnik & Wolfe, 1990).

It is likely that somatostatin not only mediates the inhibition of gastrin release in response to luminal stimuli but that it is also the final common pathway through which the peptides secretin, glucagon, gastric inhibitory polypeptide and vasoactive intestinal polypeptide (Chiba *et al*, 1980; Holst *et al*, 1983; McIntosh *et al*, 1984; Saffouri *et al*, 1984a) and prostaglandin E₂ (Saffouri *et al*, 1980) all inhibit antral gastrin release.

ii) Neurotransmitters

Gastrin release is also under the control of peptides and neurotransmitters released from nerve endings. During the cephalic phase of gastric secretion the vagus stimulates the release of gastrin, as demonstrated by sham feeding experiments (Dockray & Tracy, 1980; Nilsson *et al*, 1972a; Tepperman *et al*, 1972). The available evidence suggests that the vagus has antral nerve endings which stimulate and fundic endings which inhibit gastrin release and that there are non-cholinergic mechanisms involved too (Dockray & Gregory, 1989). Animal studies suggest that nervous reflexes also influence the gastrin response to **luminal** stimuli, during the gastric phase of gastric secretion. For instance, the anti-cholinergic drug atropine inhibits the gastrin

response to feeding in dogs (Hirschowitz *et al*, 1981), which would fit with the evidence that cholinergic nerves are involved in the gastrin response to luminal stimuli (Saffouri *et al*, 1984b; Schiller *et al*, 1982; Schubert *et al*, 1991). However, studies of this type may be complicated by the possibility that the change in gastrin release is secondary to some other action of pharmacological agents, for example on acid secretion and local somatostatin release. In man distension of different regions of the stomach may release gastrin by non-cholinergic neural pathways, independent of chemical stimulation (Schiller *et al*, 1980; Soares *et al*, 1977; Koop *et al*, 1990), possibly by a reflex involving beta-adrenergic receptors (Peters *et al*, 1982; Buchan, 1991).

The non-cholinergic neurotransmitter involved in many of these gastric reflexes is gastrin-releasing peptide (GRP). Nerve endings containing GRP have been identified in the human antral mucosa (Price *et al* 1984). In low doses in man GRP stimulates both gastrin and acid secretion (Walsh *et al*, 1981), whereas at higher doses it inhibits the secretion of acid (Varner *et al*, 1981; Hirschowitz & Molina, 1983). This dual effect of GRP may be explained by the finding that GRP also indirectly stimulates the secretion of somatostatin from the fundus (Schubert *et al*, 1991).

Circulating gastrin

To measure the very low circulating concentrations of gastrin in plasma (in the picomolar range) radioimmunoassay is required. The forms of gastrin which are detected by this method depend upon the specificity of the antibody which is used. These antibodies are most commonly directed against the carboxyl terminus and therefore bind to all forms of gastrin. However, they may also have cross-reactivity with CCK. More recently, antibodies have been raised with specificity against epitopes specific to a particular gastrin form, so that these different forms may be specifically and directly measured in plasma. An alternative method to assay the different plasma forms of gastrin is by first separating them by gel chromatography and then assaying the fractions independently. In practice both methods may be needed to identify the

various forms in plasma with precision, especially when the total circulating gastrin is low and concentration of the plasma is required (Dockray & Gregory, 1989).

As I shall discuss in further detail later, the circulating gastrin concentration is higher when there is gastric antral colonisation by *H pylori*. Information on plasma gastrin concentrations published before 1989, when this was first realised (Oderda *et al*, 1989; Levi *et al*, 1989a), should therefore be considered in the light of this confounding factor. Not controlling for *H pylori* infection may conceivably explain why women have been found to have higher plasma gastrin concentrations in some published studies (Feldman *et al*, 1983, Poulsen & Lovgreen, 1986; Calam *et al*, 1989) but not others (Lam & Ong, 1980; Goldschmiedt *et al*, 1991).

Feeding increases the plasma concentration of gastrin within a few minutes and this peaks at 20–30 minutes to reach 2–4 times the fasting gastrin concentration. This rise in gastrin on feeding is more exaggerated in the presence of *H pylori* infection (Levi *et al*, 1989a; McColl *et al*, 1989, Graham *et al*, 1990; Smith *et al*, 1990) as will be discussed in detail presently. In the fasting state most of the gastrin immunoreactivity is due to G-34 (Lamers *et al*, 1982), but after a meal G-34 and G-17 concentrations are approximately equal (Dockray & Taylor, 1976; Lamers *et al*, 1982).

Animal studies in which gastrin forms in the antral venous outflow are compared with those in the peripheral veins, have indicated that there is considerable post-secretory processing of gastrin shortly after release (Dockray & Gregory, 1989). This results in the generation of smaller gastrin forms but it is not known whether similar processing occurs in man.

The metabolism of gastrin

In man the plasma half life of unsulphated G-17 is about 3 minutes (Pauwels *et al*, 1985) but G-34 is much more persistent in the circulation, being cleared at a rate six to eight times slower than G-17 (Walsh *et al*, 1976). Animal studies have indicated that all organs remove about 25% of the gastrin flowing through them (Strunz *et al*, 1978b). The liver selectively removes over 90% of the small gastrin

forms, of 8 amino acids or less (Strunz *et al*, 1978a), thus preventing these small gastrin fragments released by the antrum from entering the systemic circulation. Degradation of gastrin by the liver involves removal of the carboxyl-terminal amide (Varro *et al*, 1973), which is essential for biological activity.

Actions of gastrin

Gastrin is the most potent gastric acid secretagogue (Makhlouf *et al*, 1965), with a D_{50} for acid secretion in man of 50–100 pmol/L. The acid secretory response to a meal of either steak, bread and butter (Blair *et al*, 1987) or peptone (Eysselein *et al*, 1992) is entirely explained by the rise in plasma gastrin which occurs on feeding and the importance of gastrin in this acid response has been confirmed by the effect on acid secretion of the immunoneutralisation of gastrin (Kovacs *et al*, 1989). Women may be less sensitive to circulating gastrin than men. In one study the intravenous dose of gastrin required to produce half-maximal acid secretion in women was twice that required in age-matched men (Feldman *et al*, 1983). Again, the possible confounding factor of *H pylori* may influence the interpretation of this study.

That gastrin has a trophic effect on the parietal (oxyntic) mucosa is demonstrated by the increased parietal cell mass of patients with very high circulating gastrin concentrations due to a gastrin-secreting tumour (Ellison & Wilson, 1967). Oxyntic gland hyperplasia can be produced experimentally by the administration of exogenous gastrin (Ryan *et al*, 1978) and, of more importance *in vivo*, when dogs are fed the gastrin response produces measurable increases in oxyntic gland DNA synthesis (Ryan *et al*, 1978). Whether in man physiological concentrations of gastrin have a trophic effect on the stomach is not known.

Animal experiments have shown that high concentrations of gastrin can have a host of other actions upon the gastrointestinal tract. These include the stimulation of water, electrolyte and enzyme secretion, actions on intestinal smooth muscle and hormone release and an effect upon visceral blood flow (Dockray & Gregory, 1989). However, it is unlikely whether, apart from gastrin's potent acid secretory action and its trophic effect, any of these other actions are important in man *in vivo*. Studies

which attempt to dissect the actions of gastrin by using isolated cells and specific gastrin receptor antagonists are necessary to fully resolve these issues.

Gastrin receptors

Gastrin exerts its effects after binding to specific receptors. Because some carboxyl-terminal antibodies bind to both gastrin and CCK, it was unclear initially whether CCK and gastrin had a common receptor. However, recent pharmacological and molecular studies have confirmed that gastrin exerts its effect after binding to a specific receptor which is exactly the same as the CCK_b receptor, the receptor responsible for CCK's action in the brain (Kopin *et al*, 1992). Gastrin receptors are found not only on parietal cells but also in an elutriation fraction of dispersed canine fundic mucosa which contains D-cells and mast cells (Soll *et al*, 1984). In addition gastrin-binding sites have been found in the duodenum, liver, spleen and kidney, though not in the antrum (Brown & Gallagher, 1978; Takeuchi *et al*, 1979). The use of gastrin/CCK_b receptor antagonists (Woodruff & Hughes, 1991) is likely to be of considerable use in unravelling further aspects of gastrin physiology.

Pathophysiology of hypergastrinaemia

Abnormally high plasma concentrations of gastrin are found in association with several clinical conditions. The importance of *H pylori*-related hypergastrinaemia in the aetiology of DU disease will be discussed shortly. Other clinical conditions associated with hypergastrinaemia include the rare but much described Zollinger-Ellison syndrome, characterised by excessive gastric acid secretion which results in ulceration of the duodenum and jejunum. This is caused by the autonomous and excessive production of gastrin by an endocrine tumour of the foregut, typically located in the pancreas or duodenum (McGuigan, 1989). Successful excision of the tumour results in a decline in gastric hypersecretion towards normal (Pisegna *et al*, 1992). A similar syndrome has also been described which is due to an as yet unidentified non-gastrin acid secretagogue (Chey *et al*, 1989).

In contrast to the Zollinger–Ellison syndrome where an excess of gastrin is the primary abnormality, in pernicious anaemia high plasma gastrin concentrations are secondary to a lack of secretion of gastric acid. Pernicious anaemia is a chronic autoimmune disease characterised by vitamin B₁₂ malabsorption due to autoantibodies directed against antigens expressed on the gastric mucosa (Morson *et al*, 1990), including the parietal cell's proton pump (Toh *et al*, 1990). A consequence of the resultant chronic inflammation is gastric mucosal atrophy in the corpus and fundus with impaired ability to secrete gastric acid (Weinstein, 1989). The highly elevated plasma concentrations of gastrin which are commonly seen in pernicious anaemia (McGuigan & Trudeau, 1970; Lanzon–Miller *et al*, 1987) are thought to be due to the antral mucosa, which is relatively spared from atrophy, responding to a lack of luminal acid by increasing the secretion of gastrin. Ultimately this leads to G–cell hyperplasia and hypoplasia of the adjacent somatostatin–immunoreactive (D) cells (Arnold *et al*, 1982). The molecular mechanisms responsible for the hypergastrinaemia of pernicious anaemia are the subject of chapter 6.

Decreased gastric acid secretion may also be due to drugs used in the treatment of acid–related disorders, particularly the histamine H₂–antagonists and the parietal cell proton pump inhibitors. As a result plasma gastrin concentrations are elevated to a moderate degree but this hypergastrinaemia appears to be of no clinical significance in man. Following vagotomy, an increased plasma gastrin concentration is commonly observed which may not be totally explained by the accompanying decline in acid secretion (Becker *et al*, 1973).

Hypergastrinaemia is also seen in patients with renal failure. This may be due to a deficiency of gastrin clearance by the renal capillaries, but it may also be explained by decreased gastric acid secretion in these patients (El Munshid *et al*, 1976; Taylor *et al*, 1980).

Finally, plasma gastrin concentrations are mildly elevated in colon cancer and in some cases may return to normal after the colonic tumour is resected (Borland, 1991). It is unclear at present whether the elevated gastrin is contributing to the growth of the colonic tumour, is due to gastrin secretion by the tumour or whether this is merely a chance association since the studies performed to date have been small and largely uncontrolled for *H pylori* and gastric acid secretion.

PHYSIOLOGICAL ABNORMALITIES IN DUODENAL ULCER DISEASE

Taken as a group there is evidence that patients with duodenal ulceration secrete more acid, pepsin and, in some cases, gastrin than do a cohort of their age-matched controls. The increased secretion of DU patients is often subtle and there is considerable overlap with controls, yet several studies have confirmed that differences exist between DU patients and controls, under a number of different conditions.

Sometimes familial tendencies to hypersecretion have been observed. Although this has generally been interpreted as evidence for a genetic predisposition to ulceration, a common environmental effect on affected family members cannot be excluded, especially since the discovery of *H pylori*.

Increased acid secretion

Acid secretion of hydrochloric acid into the lumen of the stomach has been measured under a variety of different conditions and has generally been performed by aspirating the gastric contents through a naso-gastric tube. By mathematical correction of the acid content, both for gastric acid lost through the pylorus and for collected juice of duodenal origin, the acid output under different conditions may be calculated (Baron, 1978).

In general, such studies have demonstrated that DU patients tend to have increased basal (unstimulated) acid output, BAO, (Kirkpatrick & Hirschowitz, 1980, Johnston & Jepson, 1967; Blair *et al*, 1987), nocturnal acid output (Feldman & Richardson, 1986), 24-hour acid secretion (Feldman & Richardson, 1986) and

increased peak acid output (PAO), (Blair *et al*, 1987; Baron, 1978), the latter in response to maximal stimulation with pentagastrin or histamine. In addition to a high BAO and PAO, DU patients as a group have a high BAO/PAO ratio, secreting proportionally more of their PAO under basal conditions (Kirkpatrick & Hirschowitz, 1980; Blair *et al*, 1987; Feldman *et al*, 1980). The tendency to a high PAO, a reflection of the parietal cell mass (Grossman & Elashoff, 1980), could reflect a genetic predisposition but it may also be an acquired consequence of sustained hypergastrinaemia inducing a trophic response in the fundic mucosa. Another explanation for the large parietal cell mass in these patients is that this may reflect a relative lack of fundic atrophy, a common accompaniment of aging (Weinstein, 1989).

In comparison with normal individuals, DU patients also tend to secrete excessive quantities of acid in response to a meal, their acid response to feeding is both excessive and prolonged (Blair *et al*, 1987; Malagelada *et al*, 1977).

There is some evidence to suggest that these excesses of acid secretion in DU patients may be due to a loss of the normal negative feedback of acid on gastrin secretion. Normally, acid in the lumen of the stomach inhibits gastrin release and the further secretion of acid. However, by keeping the gastric lumen at a constant pH by the technique of intragastric titration, Walsh and co-workers showed that in DU patients the normal inhibition of gastrin release and acid secretion at pH 2.5 was diminished (Walsh *et al*, 1975; Eysselein *et al*, 1992). Others (Malagelada *et al*, 1977; Cooper *et al*, 1985) were not able to confirm this observation, though this may be due to methodological differences and the selection of different patients and controls.

Might the excessive acid secretion of DU patients be due to the parietal cells being abnormally sensitive to circulating gastrin? Results supporting such a hypothesis have been obtained by some workers (Peterson & Myren, 1975; Isenberg *et al*, 1975; Lam *et al*, 1980; Lam & Koo, 1985) but such findings have not been universally confirmed (Hirschowitz, 1984; Hirschowitz *et al*, 1985). The interpretation of gastrin-acid dose-response studies is complicated by the use of more than one way to measure parietal cell sensitivity; this is further discussed in chapter 5.

Increased pepsin secretion

Compared to the many studies on acid secretion, relatively few workers have addressed the issue of pepsin secretion in DU disease, probably because pepsin secretion is more difficult to measure and there are several enzyme isoforms (Hersey, 1987). In general, acid and pepsin secretion increase in parallel (Baron, 1978). Since DU patients tend to secrete more acid than controls, they would also be expected to have a tendency to pepsin hypersecretion. This has been shown both by measuring the peptic activity of gastric aspirates (Baron, 1978) and by measuring serum levels of pepsinogens, the inactive zymogens, which diffuse back into the bloodstream (Samloff *et al*, 1975). Rotter *et al* (1979) showed that plasma concentrations of group I pepsinogens correlated strongly with peak acid output and that high levels ran in ulcer families, with more ulcers occurring in individuals with elevated levels. Although this was thought at the time to represent an autosomal dominant genetic trait, another interpretation of this study is that the families were infected with *H pylori* and that this organism was responsible for the familial clustering of cases.

Increased gastrin secretion

Despite evidence of a disturbance in the negative feedback of acid on gastrin (Walsh *et al*, 1975), prior to the discovery of *H pylori* there was only one report of elevated plasma gastrin concentrations in unselected DU patients. (Blair *et al*, 1987). A few case reports existed of individuals with primary hypersecretion of antral gastrin due to G-cell hyperplasia, so-called 'G-cell hyperfunction' (Lewin *et al*, 1984; Keuppens *et al*, 1980; Cooper *et al*, 1985) where increased gastrin release was particularly evident after meals. Two families were described with G-cell hyperfunction in association with hyperpepsinogenaemia (Taylor *et al*, 1981) but overall the evidence for there being increased meal-stimulated gastrin release in DU patients was slim. Interestingly, in a group of patients with G-cell hyperfunction, rather than a lack of negative feedback of acid on gastrin release, there was a

generalised exaggerated gastrin response to luminal amino-acids (Cooper *et al*, 1985). In retrospect it is rather surprising that the relationship between gastrin and DU disease was so weak, based upon what we now know about *H pylori*, gastrin and duodenal ulcer disease. However it is possible that the 'controls' for many of the negative studies were themselves infected with *H pylori*.

H PYLORI AND DUODENAL ULCER DISEASE

The discovery of *H pylori*

In 1983, Warren and Marshall, working in the same hospital in Australia, wrote separate letters which were published together in *The Lancet* (Warren 1983, Marshall 1983). They described small, curved, S-shaped bacilli in gastric biopsy specimens which were associated with gastric inflammation and they also noted, for the first time, that these bacteria could be grown in culture. It is informative to note that like many other breakthroughs in medical science the discovery was not only serendipitous, an unplanned prolonged culture had been performed owing to a long weekend, but also that the 'discoverers' were in fact extending observations which had been made many years before and long forgotten (Bizzozero, 1893; Salomon, 1896; Balfour, 1906; Lucet, 1910).

In the 10 years since this discovery, a wealth of information has been published on this organism and the bacterium has undergone two name changes, from *Campylobacter pyloridis* to *Campylobacter pylori* and finally to *Helicobacter pylori*, the last change reflecting the unique genus to which the organism belongs (Goodwin *et al* 1989; Anonymous, 1992).

Characteristics of *H pylori*

H pylori is a gram-negative, microaerophilic spiral-shaped bacterium which is motile by virtue of multiple flagellae (Hazell, 1991). It possesses a uniquely powerful urease enzyme capable of converting urea to ammonia (Mobley *et al*, 1988) and this is thought to be how it survives in a gastric environment which, by virtue of its extreme acidity, is hostile to other bacteria. Many of its other enzymes and proteins have been identified and characterised including adhesins, proteases, an oxidase, lipase and lipopolysaccharide (Hazell, 1991).

DNA fingerprinting and ribotyping have demonstrated that there is a remarkable genotypic heterogeneity within the species (Langenberg *et al*, 1986; Owen *et al*, 1992). Almost all the clinical isolates are different at the genomic level, with the exception of strains taken from the same individual or close family contacts. The biological significance of this extreme heterogeneity is unclear. *H pylori* lives within the mucus layer of the stomach, almost invariably in the antrum, but often also in the corpus. Colonisation of the first part of the duodenum may also occur, particularly in association with DU disease, but only when the duodenal mucosa has undergone gastric metaplasia (Goodwin, 1988; Moss & Calam, 1992).

Exactly how infection with *H pylori* is acquired is currently under investigation. Epidemiological studies have demonstrated that infection is increased by social deprivation (The gastrointestinal physiology working group, 1990) and that prevalence is increased by increasing age (Graham *et al*, 1988b; Perez-Perez *et al*, 1988). The effect of age might simply reflect more opportunities for infection to have occurred, or be the result of an epidemic in the past, a 'cohort effect'. However, in favour of the latter is the evidence that in China seroconversion occurs at a very young age, with a very low rate of new infection later in life (Mitchell *et al*, 1992) and that the seroprevalence of *H pylori* infection is determined more by social deprivation in childhood than as an adult (Mendall *et al*, 1992). Serological studies have also shown marked clustering in families (Drumm *et al*, 1990) and in an institution (Berkowicz & Lee, 1987). Therefore *H pylori* probably spreads person-to-person, largely in families and via a route that may depend on sub-optimal hygiene. *H pylori* has been cultured from dental plaque (Shames *et al*, 1989) and identified in

faeces by the polymerase chain reaction (Mapstone *et al*, 1992) and by direct culture (Thomas *et al*, 1992). Since in their preliminary report Thomas *et al* were able to culture *H pylori* from the faeces of over a third of a small group of randomly-selected children in West Africa, the faecal-oral route may be the most important way of transmitting the infection.

Diagnosis of *H pylori* infection

The diagnosis of *H pylori* infection can easily be made either at endoscopy, by antral mucosal biopsy, or by using non-invasive methods. The endoscopic tests commonly used are the urease test, bacterial culture and histology (Barthel & Everett, 1990; Deltenre *et al*, 1989). A patchy distribution of *H pylori* within the antrum occasionally leads to tests on biopsies being false-negative so that at least two, preferably three, biopsies are taken in clinical trials, with the definition of *H pylori* infection being made ideally by more than one method. Re-testing after treatment is performed at least four weeks after the end of the eradication regime, because of the tendency of *H pylori* to "lurk and revive" (Weil *et al*, 1988); *H pylori* may do this by assuming a dormant coccoid form under adverse conditions (Jones & Curry, 1992). The non-invasive tests for *H pylori* infection include the urea breath tests (Bell *et al*, 1987; Graham *et al*, 1987) and antibody testing. The clinical use of serology for follow-up is debatable since after the eradication of *H pylori* it can take six months for titres to fall sufficiently for diagnostic use. (Kosunen *et al*, 1992).

***H pylori* and the pathogenesis of duodenal ulcer disease**

The intense interest in *H pylori* has arisen because of its close association with chronic gastritis and DU disease and, to a lesser extent, with gastric ulcer and carcinoma (Dooley & Cohen, 1988; Moss & Calam, 1992; Correa, 1991).

In DU disease *H pylori* is found in the gastric antrum in over 90% of cases, compared with around 40% of age-matched controls (Dooley & Cohen, 1988). However, the strongest evidence for the role of *H pylori* in the pathogenesis of DU is the fact that DU patients treated with drug regimes in which *H pylori* is eradicated almost never get an ulcer recurrence, in contrast with the almost universal tendency of DUs to recur when *H pylori* is still present (Coghlan *et al*, 1987; Marshall *et al*, 1988; Borody *et al*, 1989; Rauws & Tytgat, 1990). In other words, *H pylori* is responsible for the predisposition to recurrent duodenal ulceration.

How does *H pylori* cause or promote duodenal ulceration and why **doesn't** *H pylori* cause ulcers in most individuals? The prevalence of *H pylori* in adults is close to their age in years so that by the age of 50 about half of the population have *H pylori* but only about 10% have DUs (Graham *et al*, 1988b). Because *H pylori* mainly resides in the gastric antrum whereas the ulcer is in the duodenum, possible explanations also need to account for this apparent paradox. Thus far there are two main and not necessarily exclusive explanations – firstly that *H pylori* has a direct effect on the host's duodenum, and secondly that antral *H pylori* infection has an indirect effect by disturbing gastric physiology in such a way as to promote the formation of a DU.

Direct effect–duodenal mechanisms

It was initially difficult to understand how *H pylori*, which only colonised gastric-type epithelium, might cause local damage within the duodenum. This is explained by the presence of patches of gastric metaplasia in the duodenum of patients with DU disease (Goodwin, 1988). It has been estimated that gastric metaplasia is present in about 90%, and is colonised with *H pylori* in about 50% of patients with DU disease (Marshall *et al*, 1988), but it is only present in 5 to 30% of non-ulcer *H*

pylori-colonised persons (Fitzgibbons *et al*, 1988; Wyatt *et al*, 1987). This raises the question of what causes gastric metaplasia? In man it is associated with acid hypersecretion (Wyatt *et al*, 1987) and may be diminished after prolonged suppression of acid (Wyatt and Rathbone, 1989a). In animals it has been induced experimentally by chronic gastric acid hypersecretion (Rhodes, 1964; Gaskin *et al*, 1975). However, gastric metaplasia is probably a non-specific response to injury. A similar phenomenon occurs in Crohn's disease, in association with local epidermal growth factor production (Wright *et al*, 1990) so an as-yet unidentified insult could be responsible for gastric metaplasia in DU disease. Patches of metaplasia may well reflect the sites of previous ulcers (Fullman *et al*, 1985) but what causes the first ulcer?

Although *H pylori* forms attachments with the gastric epithelial cells and can degrade mucus (Sidebotham & Baron, 1990), it has not generally been found to be locally invasive so that it does not cause ulcers by burrowing through the mucosa. Having colonised the duodenum *H pylori* might cause duodenal ulcers by producing injury itself, by stimulating immunocytes to cause damage, by allowing acid to injure the mucosa, or by all three. Some results support the idea that certain types of *H pylori* are more ulcerogenic than others. Preliminary observations suggest that strains specifically associated with DU disease may have a distinctive DNA finger print (Yoshimura *et al*, 1990). During culture *in vitro* some strains of *H pylori* produced a toxin which produces vacuoles in cultured cells; the 'vacuolating toxin' whereas other strains did not (Leunk *et al*, 1988). Moreover, antibodies to a protein thought to be the toxin were present in 100% of DU patients with *H pylori* compared with 61% of patients with *H pylori* but no ulcer (Cover *et al*, 1990). One such vacuolating toxin has recently been purified and characterised and found to have a molecular weight of 87 kDa (Cover & Blaser, 1992). However, studies of *H pylori*-derived toxins have produced different results in different laboratories; in one lab the toxin proved to be *H pylori*'s enzyme urease itself (Xu *et al*, 1990), whilst another contender for pathogenicity appears to be a cell surface protein of 120 kDa present on some but not all strains. This protein elicits both a systemic IgG response and the secretion of a specific mucosal IgA, the latter being specifically confined to those patients who develop peptic ulceration (Crabtree *et al*, 1991b).

H pylori provokes an intense immunological reaction, both humoral and cell-mediated, yet infection tends to persist. The organism is clearly recognised as foreign, with enhanced expression of MHC class II antigens on gastric epithelial cells (Engstrand *et al*, 1989) but once acquired it is rarely cleared spontaneously. There is some evidence that damage to the gastrointestinal epithelium may occur through an indirect mechanism, by the production of autoantibodies to antral mucosal cells (Negrini *et al*, 1991). *H pylori* can also secrete soluble proinflammatory factors including a potent peptide neutrophil chemotaxin (Mooney *et al*, 1991; Craig *et al*, 1992; Mai *et al*, 1992) and substances capable of activating peripheral blood monocytes *in vitro* (Mai *et al*, 1991) to add to the tissue damage. A number of cytokines have been identified from cultured *H pylori*-infected gastric mucosa, including interleukins 6 (Crabtree *et al*, 1991a) and 8 (Gupta *et al*, 1991; Crabtree *et al*, 1992; Atherton *et al*, 1992; Nouch *et al*, 1992), tissue necrosis factor alpha (Crabtree *et al*, 1991a) and platelet activating factor (Denizot *et al*, 1990). However, the nature of the inflammatory response in the duodenum has so far received little attention. Prostanoids and platelet activating factor may be involved here (Ackerman *et al*, 1990). The intensity of the immunological response and any consequent injury is likely to depend on both the antigens presented by the particular strain of *H pylori* and the vigour of the host's immune response.

Alterations of gastrin, acid and pepsin secretion

It is tempting to speculate that infection of the gastric antrum by *H pylori* may itself cause the known secretory abnormalities of DU disease, described in previous sections of this chapter. If this were so then it could explain how the eradication of *H pylori* causes long-term remission from ulceration – by reversing the hypersecretory tendency. The first evidence to support such a hypothesis was published in 1989.

In a study of DU patients (Levi *et al*, 1989a), it was found that the BAO, PAO and plasma gastrin levels were all greater in those with a positive urease test for *H pylori*, than in the few DU patients who had a negative urease test, indicating that colonisation with *H pylori* was light or absent. Suppression of *H pylori* also led to a

fall in the meal-stimulated gastrin response, though there was no change in basal gastrin concentration or acid secretion (Levi *et al*, 1989b). Several studies since then have further investigated the effect of *H pylori* on gastric function (reviewed by Rademaker & Hunt, 1991). Some caution is required in their interpretation, particularly in the studies of acid secretion. Firstly, the studies vary with respect to the individuals studied – some are asymptomatic controls, some non-ulcer dyspeptics, some DU patients and some are composed of mixed groups. In addition, some compare *H pylori*-infected individuals with uninfected individuals whereas other studies investigate the same patients before and after eradication (and sometimes suppression) of *H pylori*. In general, the latter type of study, in which a patient acts as their own control, is preferred – particularly when eradication of *H pylori*, rather than merely suppression (Weil *et al*, 1988), is achieved. The comparison of a group who are infected with *H pylori* with a group that is not is compounded by the fact that gastric fundic atrophy, which can decrease acid secretion, is a possible consequence of chronic infection with *H pylori* (Karnes *et al*, 1991b); the groups may therefore not be matched in terms of maximal acid secretory capacity and this may explain why infection with *H pylori* can be associated with lower acid secretion rates (Goldschmiedt *et al*, 1991). A second note of caution involves how the measurement of acid secretion is performed. There are many ways to measure acid secretion but not all of them test the gastrin-driven acid response and only some correct for pyloric losses and duodenogastric reflux (further discussed in chapter 5). Finally the measurement of intragastric acidity rather than acid output may depend more on intragastric buffering capacity than on acid secretion; for example, acidity goes down rather than up after a meal.

It is now well established that infection with *H pylori* increases the plasma concentration of gastrin; under basal conditions (McCull *et al*, 1991), in response to a meal (Levi *et al*, 1989a; Levi *et al*, 1989b; Graham *et al*, 1990; McCull *et al*, 1991; Karnes *et al*, 1991a), over a 24-hour period (Smith *et al*, 1990; Karnes *et al*, 1991a; Prewett *et al*, 1991) and even in response to a non-luminal stimulus such as gastrin-releasing peptide (Graham *et al*, 1991b; Beardshall *et al*, 1992). *H pylori*-associated hypergastrinaemia is not unique to DU disease – increased gastrin release is associated with *H pylori* infection in asymptomatic individuals too (Smith *et al*, 1990; Chittajallu

et al, 1992a). The form of gastrin responsible for the increased gastrin immunoreactivity associated with *H pylori* infection is gastrin-17 (Beardshall *et al*, 1992; Mulholland *et al*, 1992) implying that it is probably antral in origin. Exactly what causes the G-cell to be in a generally more excitatory state when *H pylori* is present is not known; possible explanations will be discussed shortly.

Despite the falls in plasma gastrin which accompany the eradication of *H pylori* (Levi *et al*, 1989b; McColl *et al*, 1991; Graham *et al*, 1990; Prewett *et al*, 1991), this has not generally been found to be accompanied by a fall in acid secretion (Rademaker & Hunt, 1991). In one report the eradication of *H pylori* resulted in a less acidic intragastric pH after feeding when measured one month after treatment (McColl *et al*, 1989), but this effect was lost six months later despite the gastrin remaining low (McColl *et al*, 1991). In attempting to explain how the eradication of *H pylori* decreases plasma gastrin without changing acid secretion it has been postulated that *H pylori* decreases the sensitivity of the parietal cells to circulating gastrin so that smaller gastrin concentrations are then required to produce equivalent acid secretion (Blaser, 1992). It is conceivable that this may occur through the production of an acid secretion-inhibiting toxin directed against parietal cells (Cave & Vargas 1989; King *et al*, 1992), since there are reports of acute infection with *H pylori* resulting in decreased acid secretion (Graham *et al*, 1988a). However, it is difficult to equate a loss of parietal cell sensitivity to gastrin with the evidence that, if anything, the parietal cells of DU patients have **increased** sensitivity to gastrin (Peterson & Myren, 1975; Isenberg *et al*, 1975; Lam *et al*, 1980; Lam & Koo, 1985). Finally, an interesting alternative explanation for the discordance between gastrin and acid secretion has been proposed by Graham *et al* (1992b). In a preliminary study, the excess immunoreactivity of gastrin in *H pylori* infection was attributed to biologically inactive progastrins.

There are relatively few studies concerning the effect of *H pylori* on pepsin secretion. Pepsinogens are normally secreted into the gastric lumen, where they are converted by the low intragastric pH to active proteolytic pepsins. Serum pepsinogen concentrations may be measured as a surrogate for intragastric peptic activity (Baron, 1978). However, serum levels represent only a small proportion of the pepsinogens which are secreted into the gastric lumen. They reach the bloodstream by back

diffusion (Hersey, 1987), so that they may be increased if there is an inflamed gastric mucosa leaking pepsin into the bloodstream as well as actually reflecting an increase in intragastric peptic activity. *In vitro*, *H pylori* does stimulate the secretion of pepsinogens (Cave & Cave, 1991; Young *et al*, 1992). However, in a study of gastric peptic activity there was, if anything, **lower** intragastric pepsin secretion in the individuals infected with *H pylori* although they had high serum pepsinogen levels (Mertz *et al*, 1992). Decreased mucosal gastric peptic activity in association with *H pylori* was also found in a study of gastric biopsy specimens from children; unlike gastric pepsinogen, the peptic activity increased following the eradication of *H pylori* (Yahav *et al*, 1992). In contrast to these reports of decreased gastric peptic activity in *H pylori* infection, several studies of serum pepsinogens have demonstrated that eradication of *H pylori* is associated with a fall in pepsinogens I and II (Oderda *et al* 1989; Chittajallu *et al*, 1992b; Fraser *et al*, 1992). The fall in serum pepsinogens associated with increased gastric peptic activity may represent the resolution of a leaky gastric mucosa after eradication therapy but this clearly needs to be resolved by further studies.

Mechanisms of *H pylori*-associated hypergastrinaemia

How does *H pylori* increase the release of gastrin? Several mechanisms have been suggested and these are summarised in figure 1.2 . It should be emphasised that the various mechanisms which have been proposed are not mutually exclusive.

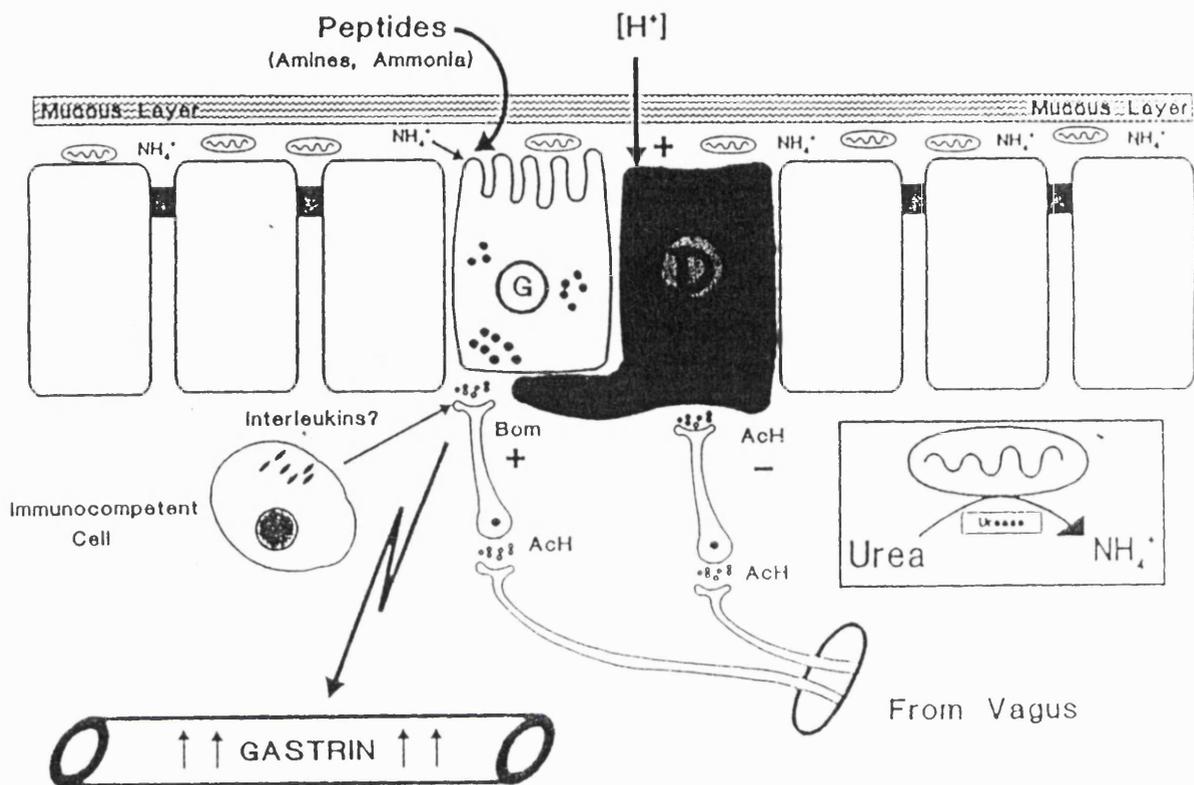


Figure 1.2 How does *H. pylori* increase the release of gastrin? The ways in which *H. pylori* might influence the release of gastrin from the G-cell are shown above (from Rangachari, 1991).

In the initial report of increased plasma gastrin concentrations associated with gastric infection by *H pylori*, it was suggested that the pH rise due to the production of ammonia in the gastric antrum might stimulate the release of gastrin (Levi *et al*, 1989a). It has now been confirmed that the pH of the antral mucus layer is indeed more alkaline in the presence of *H pylori* (Kelly *et al*, 1990; Beardshall *et al*, 1991). This may be due to the bacterium's urease generating ammonia, but it may also be due to other mechanisms, for instance a damaged epithelium may leak bicarbonate. The difference in pH between *H pylori* positive and negative patients is only 0.3 – 0.8; but we have no idea of the magnitude of change in the pH of the antral mucus layer that is required to affect gastrin release normally. How the intraluminal pH can normally influence the G-cell when the pH of the mucus layer overlying it is buffered close to neutral is unknown.

Against the hypothesis of urease-influenced pH change, it has been shown that neither the inhibition of *H pylori*'s urease (El Nujumi *et al*, 1991) nor the intragastric instillation of urea (Chittajallu *et al*, 1991b; Graham *et al*, 1991b) altered the release of gastrin in the short term. Whether these experiments are relevant to the chronic small juxtamucosal pH change which is produced by *H pylori* is not known.

An alternative idea to explain the elevated gastrin concentrations is that the increased gastrin release is a response to inflammation in the gastric antrum. This was suggested initially by Wyatt *et al* (1989b) who found increased basal gastrin levels in two patients with non-*H pylori* antral gastritis, possibly due to the inflammation produced by the pyloric reflux of bile (Calam & Tracy, 1980). In another study the decline in urease activity with eradication therapy occurred before the fall in gastrin and the decrease in inflammation, suggesting that the hypergastrinaemia was due neither to the pH change nor the inflammation (Chittajallu *et al*, 1991a).

How might antral inflammation influence the secretion of gastrin? As described before, mucosal infection by *H pylori* provokes a strong inflammatory response. Some pro-inflammatory cytokines have been demonstrated to release gastrin *in vitro* from enriched G-cell preparations, including interleukin 1 (Teichmann *et al*, 1990a; Weigert *et al*, 1992) and 2, (Teichmann *et al*, 1986), gamma interferon (Teichmann *et al*, 1986) and the leukotrienes C4 and D4 (Teichmann *et al*, 1990b). However, studies of this type using cell fractions are difficult to perform and may not reflect the situation

in vitro. The purity of such enriched fractions is also not absolute; the G-cell fraction contains some D-cells too (Schepp *et al*, 1990), so that the effect of a cytokine upon gastrin release may be through somatostatin or another intermediary.

The third possible explanation for the exaggerated gastrin release is that antral infection with *H pylori* decreases the normal inhibition of gastrin release and synthesis mediated by somatostatin (Yamada & Chiba, 1989). This possibility has been hitherto largely neglected, owing perhaps to the difficulty in the measurement of local somatostatin release. In chapters 6 and 7, I shall discuss the methods I have used to measure somatostatin mRNA from gastric biopsies, in order to investigate the effect of eradicating *H pylori* on local somatostatin release.

Treatment to eradicate *H pylori*

Because of the beneficial decrease in recurrence rates of DU disease once *H pylori* has been eradicated, there has been considerable interest in the development of drug regimes to eradicate the organism. Their only clinical indication at present is in the treatment of *H pylori*-associated, recurrent DUs which, in practice, is almost all DU disease.

In retrospect, the first step on the path of an *H pylori* eradication regime was the surprise finding by Martin *et al* (1981) that a DU stays healed for considerably longer after treatment with tripotassium dicitrato bismuthate (De-Nol) compared with H₂-antagonists. It is now known that DeNol has anti-*H pylori* activity both *in vivo* (McNulty *et al*, 1986; Rauws *et al*, 1988) and *in vitro* (McNulty *et al*, 1985) which presumably explains the longer disease-free interval. However, it was argued for some time that the long remission produced after De-Nol was due to bismuth's tendency to be stored in tissues after a course of treatment has finished (Gavey *et al*, 1989) or that bismuth produces non-*H pylori*-related benefits such as prostaglandin-mediated cytoprotection (Hall, 1988). Another idea was that H₂-antagonists might actually shorten remissions by producing rebound hypersecretion of acid-pepsin, but there is little evidence to support this theory (Chiverton & Hunt, 1989).

With the discovery of *H pylori*, bismuth-based therapies soon became central to eradication regimes. However, it was then discovered that bismuth alone usually suppressed, rather than eradicated *H pylori* (Weil *et al*, 1988) and that it was necessary to add antibiotics to bismuth to achieve the complete eradication of *H pylori*. The addition of antibiotics to De-Nol increases eradication rates from about 20% to about 80%, with the best eradication rates achieved with the use of two antibiotics, taken together with bismuth (Collins *et al*, 1992). The nitroimidazoles, particularly metronidazole, were particularly widely used in this respect. Because of the great variety of regimes that had been used in trials worldwide, an international working party met in 1990 and formulated a suggested regime for the eradication of *H pylori*. They recommended using De-Nol 120 mg qds (30 min before meals and at night), metronidazole 400 mg tds and tetracycline hydrochloride (or amoxycillin) 500 mg qds, all for 2 weeks (Tytgat *et al*, 1990; Anonymous, 1990). The success of such a complicated regime depends greatly on compliance (Graham *et al*, 1992a) and resistance to metronidazole (Glupczynski *et al*, 1990). Metronidazole resistance develops rapidly if the drug is given without bismuth (Glupczynski & Burette, 1990) and is present before treatment in about 15% of colonised females in the UK and about 80% of the population in parts of Africa (Glupczynski *et al*, 1990), related to past use of the drug for vaginal infection.

Most recently, in an attempt to simplify regimes and use agents with as little toxicity as possible, short therapies using two drugs have been used. These have combined the potent acid suppressing drug omeprazole together with just one antibiotic, with reported success rates in some studies comparable to "conventional" triple therapy (Unge *et al*, 1989; Unge *et al*, 1992; Logan *et al*, 1992). It remains to be seen whether these newer regimes maintain such good eradication rates in larger trials. Small, and often poorly-performed, clinical trials remain a major problem in the development of anti-*H pylori* therapy. The other large obstacle is the problem of extrapolating from *in vitro* to *in vivo* – laboratory sensitivity is only poorly predictive of the success or failure of the drug in the acidic environment of the stomach (Goodwin & McNulty, 1992).

In the studies described in this thesis the eradication regime I have employed is the triple therapy recommended by the International working party, with a further

two weeks of De-Nol following the initial fortnight. The purpose of the prolonged course of De-Nol is to give an ulcer-healing course of the drug (Collins *et al*, 1992), since I am studying patients with active ulcers. No additional drugs were administered unless otherwise stated in the individual chapters.

AIMS

In summary, the aims of the experiments described in this thesis are to address two questions concerning the hypergastrinaemia which is associated with *H pylori* infection:

- 1) Is the hypergastrinaemia important? Does it increase acid secretion and if not, why not?
- 2) What causes the increased gastrin release in *H pylori* infection? Does somatostatin change in a way that would explain this?

To answer these questions I have investigated various parameters of gastric function in DU patients and compared them before and after the eradication of *H pylori*. This approach has the advantage of allowing a homogeneous group of patients to act as their own controls. To further understand the mechanisms responsible for the regulation of gastrin release, I have also investigated the regulation of gastrin synthesis in some hypergastrinaemic patients with pernicious anaemia.

At the time that these studies were performed the methods of diagnosis of *H pylori* infection for clinical purposes and the definition of eradication were changing, both in our unit and elsewhere. This explains the minor changes in the methods of diagnosis of *H pylori* infection and its eradication between different studies in this thesis.

**CHAPTER 2 – THE EFFECT OF SUCRALFATE
ON *H PYLORI*, GASTRIN AND ACID
SECRETION IN DUODENAL ULCER PATIENTS**

INTRODUCTION

The eradication of *H pylori* from patients with active DUs is accompanied by a decrease in plasma gastrin (see introduction, page 39). The decline in gastrin may explain how treating DUs with drugs which eradicate *H pylori* results in long remission from recurrent DU disease.

However, the fall in gastrin following such a treatment may not necessarily be due to the eradication of *H pylori*. For instance, it is conceivable that it is due to the decline in antral inflammation which accompanies the eradication of *H pylori* or that the drugs themselves have an effect on the G-cell. Alternatively ulcer healing *per se* may lower the plasma gastrin concentration.

The purpose of the study described in this chapter was to help understand which of the above factors contributes to the fall in gastrin by using an alternative drug, sucralfate, to heal DUs. By acting as a control group to the original study of the effect of *H pylori* eradication on plasma gastrin (Levi *et al*, 1989a) by substituting sucralfate for De-Nol and metronidazole, we could test whether ulcer healing without the eradication of *H pylori* also resulted in a change in the gastrin response.

The reason for using sucralfate as the 'control' treatment is that this drug, which has a number of beneficial "cytoprotective" ulcer-healing effects upon the gastrointestinal epithelium (McCarthy, 1991), is thought not to affect *H pylori* infection (Barbara *et al*, 1990; Rauws *et al*, 1988). However, it is reported to improve or heal the antral inflammation which accompanies *H pylori* infection (Barbara *et al*, 1990; Hui *et al*, 1989).

The use of sucralfate to treat DU disease is also of interest because of the reports that although sucralfate heals duodenal ulcers at a similar rate to H₂-antagonists (Lam, 1990), the average duration of remission after sucralfate, as after De-Nol, is longer than following treatment with H₂-antagonists (Lam, 1989). This raises the possibility that sucralfate treatment may also decrease the plasma concentrations of gastrin, in contrast to the H₂-antagonists which cause a rebound hypergastrinaemia and possibly increased acid secretion after the end of treatment (Chiverton & Hunt, 1989, Kohn *et al*, 1991); there is no published literature concerning the effect of sucralfate on gastrin.

METHODS

Patients selection in general

In all the studies described in this thesis prior approval was obtained from the Hammersmith Hospital Research Ethics Committee. The patients were recruited from the gastroenterology outpatient clinic and informed consent was given. In the studies of DU patients, the entry criteria were ages 18–85 and good general health with an active, endoscopically–proven DU. We specifically excluded patients with any known cardiac, respiratory or renal disease. Other exclusion criteria were a DU not associated with *H pylori* infection, a DU complicated by bleeding or pyloric stenosis, a past history of gastric surgery, previous use of antibiotics or bismuth preparations in the preceding 6 months or histamine–H₂ receptor antagonists or omeprazole during the 14 days before endoscopy. These exclusion criteria were to ensure that the patients did not have any additional factors which could influence their gastrin response and to exclude patients who may have recently received anti–*H pylori* treatment.

Patients, biopsies and treatment

In this study 12 patients with a DU, 8 male and 4 female, aged 35–75 (mean 54) were recruited. At the time of endoscopy, 3 gastric antral biopsies were taken endoscopically with Olympus biopsy forceps, cup size 5 x 2 mm (Key–Med, Southend–on–Sea, Essex). One biopsy was used for the diagnosis of *H pylori* infection by the urease test (McNulty *et al*, 1989) and two were placed into formal saline (10% formaldehyde v/v, 0.9% saline w/v) for later histological examination. The patients were then treated with sucralfate 1g qds for four weeks and endoscopy with antral biopsies and acid and plasma gastrin estimations were then repeated within 3 days of the end of treatment. In three patients who received no additional treatment all the tests were repeated again six months after the end of the course of sucralfate.

Measurement of the acid and gastrin responses

All the gastric secretory studies described in this thesis were performed in accordance with the established protocols of the Hammersmith Hospital gastric clinic (Baron, 1978).

Patients attended the clinical investigation unit after fasting overnight. An indwelling 18G venous cannula (Venflon 2, Viggo-Spectramed, Helsingborg, Sweden) was placed in the left forearm for the collection of plasma samples for gastrin measurement and another placed in the right forearm for intravenous infusions. The cannulae were flushed with 5ml 0.9% w/v sterile saline after insertion and after each plasma collection. The first 10 ml blood sample, containing blood plus saline, was then discarded before drawing 8ml blood for each gastrin estimation. Patients were positioned semi-recumbent, with their abdomens at 45° to the horizontal. The nasal passagae and throat were sprayed with xylocaine spray (Astra Pharmaceuticals, King's Langley, Herts) and a modified size 16 French multifenestrated nasogastric sump tube (HW Anderson Inc, Oyster Bay, NY, USA) was passed and the tip positioned in the dependent part of the stomach by the water recovery test (Baron, 1978).

These nasogastric tubes have two lumina and were modified by the addition of an extra (third) lumen; radiopaque PVC tubing with I.D 1.0 mm, O.D. 2.0 mm (Portex, Hythe, Kent) being attached by tetrahydrofuran (Sigma, Poole, Dorset) to the original tube. These modified tubes then had three lumina, one major lumen for the aspiration of gastric juice, one minor one for the equalisation of intragastric and atmospheric pressure and a secondary minor lumen for the infusion of 0.15% phenol red, ending 15 cm from the tip of the major lumen.

After manually aspirating the resting juice, the gastric contents were constantly aspirated under 20 mm Hg negative pressure by a suction pump (Air Shields, Hatboro, PA, USA) and collected into beakers on ice. Patency of the major lumen tube was ensured by the manual insufflation of 20 ml air into the pressure equilibration lumen every 5 minutes and by the manual aspiration of the major lumen every 10 minutes. The gastric juice was collected under basal conditions for 60 minutes and during a 90-minute intravenous infusion of a maximal stimulus with pentagastrin 6 µg/kg/h, ('Peptavlon', ICI Pharmaceuticals, Wilmslow, Cheshire)

The acid content of the gastric aspirate was measured by back titration *in vitro* with 0.01 M sodium hydroxide and the acid output calculated for the basal 60-minute period and for the highest acid output obtained during pentagastrin stimulation, with correction being made for pyloric losses by phenol red recovery and for duodeno-gastric reflux by the sodium ion concentration (Baron, 1978).

Following extubation and a 20 minute rest period the patients ate a standard meal consisting of 2 eggs, 2 slices dry toast and 2 beef stock cubes in 120 ml warm water. Blood was collected at 10 minute intervals for one hour in order to measure the integrated plasma gastrin response to the meal by calculating the area under the time versus plasma gastrin curve, after subtraction of the basal values (Richardson *et al*, 1976).

Gastrin measurement

Blood was collected into chilled tubes containing EDTA (100 mmol/l disodium EDTA, 50 µl per ml of blood) and the plasma was separated promptly by centrifugation at 2,000 x g for 10 minutes and stored at -20°C. The plasma gastrin concentration was measured by an established radioimmunoassay using antiserum G179 kindly provided by Professor SR Bloom (Bryant & Adrian, 1982). This antiserum binds both gastrin-17 and gastrin-34 to an equal extent, and has very low cross-reactivity with CCK (Bryant & Adrian, 1982).

Antiserum G179 was used at a titre of 1:640,000 in 50 mmol/L sodium phosphate buffer containing 6 mmol/L potassium phosphate, 10 mmol/l disodium EDTA, 1.5 g/L bovine serum albumin and 8 mmol/l sodium azide, pH 7.4. The tracer used was 1,000 cpm per assay tube of synthetic non-sulphated gastrin-17 labelled with ¹²⁵I by the Chloramine-T method, which was also donated by Professor Bloom. Synthetic non-sulphated gastrin-17 in the range 0.05 - 50 pmol/L final concentration was used as standards.

When the concentration of gastrin in the plasma samples was to be measured, 100 µl plasma was included in each sample tube, and an equivalent volume of 'Haemaccel' degraded gelatine (Hoechst UK Ltd., Hounslow, Middlesex) was added to control (Ct) tubes and standard tubes to control for the non-specific binding of

plasma proteins to the antiserum. The total volume per assay tube was 1 ml. Tubes were incubated for 3 days at 4°C. Separation of free and bound tracer was achieved by the addition of 200 µl of 6% w/v charcoal ('Norit PN5', BDH Ltd.) with 0.3% dextran-4000 in assay buffer, followed by centrifugation at 2,000 x g at 4°C for 10 minutes. The radioactivity (counts per minute) in both the precipitate (free tracer) and supernatant (bound tracer) was measured using a gamma counter ('LKB 1260 Multigamma II', Pharmacia LKB, Turka Finland).

For each assay, control tubes were included (Ct) which contained buffer and tracer but no antiserum, in order to determine the level of non-specific binding of tracer. Results were calculated by the following method. The ratio of bound:free tracer (B:F) in standard and sample tubes was calculated using the formula:

$$B:F = \frac{B - (BC + FC)}{F}$$

where B is the total bound antigen, F is the total free antigen and C is calculated by the formula:

$$C = \frac{B^{ct}}{B^{ct} + F^{ct}}$$

where B^{ct} is the bound antigen in the control tube and F^{ct} is the free antigen in the control tube. Non-specific binding is therefore taken into account as a percentage of the total radioactivity. Results were calculated from a standard 'inhibition' curve of B:F against concentration of standard (log scale).

All the samples were assayed in duplicate and the result expressed as the mean of the duplicates. Inter- and intra-assay variations were determined using standard 'unknown' concentrations of gastrin and were 15±4% and 7±3% (mean ± standard deviation) respectively. The sensitivity of the assay, the smallest change in gastrin which can be measured with 95% confidence, is 1 pmol/L.

Histological grading

At each endoscopy two antral biopsies were fixed in formal saline and 2µm sections stained with haematoxylin and eosin. All biopsies were examined by a single histopathologist (Dr DM Thomas, Histopathology Dept, Hammersmith Hospital) who was unaware of the clinical details. The number of *H pylori* organisms, polymorphonuclear neutrophils and chronic inflammatory cells present (monocytes and lymphocytes) were then graded using a simple scoring system ranging from 0 (none) to 3 (numerous), in order to assess both the extent of *H pylori* infection and the degree of gastritis (Hui *et al*, 1989). The histological scores are expressed as the mean of the two biopsies.

Statistical analysis

Wilcoxon's matched pairs test was used to compare differences before and after treatment. The results are expressed as medians (ranges).

RESULTS

Ulcer healing

All 12 patients completed the study. DUs healed in eight of the patients after one month's treatment with sucralfate but in four of the eight 'healed' patients there was still some inflammation in the duodenum endoscopically.

Acid output

The basal acid output (BAO) fell in all the patients after treatment with sucralfate. BAO fell from a median of 4.8 (2.1–12.1) to 1.6 (0–4.8) mmol H⁺/h, $P < 0.01$, (Fig 2.1a). In contrast to the fall in BAO, there was no significant change in the peak acid output (PAO). This was 41 (21–59) mmol H⁺/h initially and 38 (24–55) mmol H⁺/h following treatment (figure 2.1b).

The volume of basal gastric juice secreted fell from 92 (56–180) to 72 (24–128) ml/h under basal conditions and from 240 (104–190) to 200 (152–156) ml/h during pentagastrin stimulation. The falls in the volumes of gastric juice secreted were not statistically significant ($P=0.08$ and $P=0.07$ respectively).

Plasma gastrin

Figure 2.2 demonstrates that the basal and meal-stimulated plasma gastrin response did not change significantly following sucralfate therapy. Before treatment the basal plasma gastrin concentration was 8 (2–17) pmol/L and the integrated meal response 732 (188–1045) pmol.min/L, these were 6 (2–17) and 600 (140–1302) respectively after therapy.

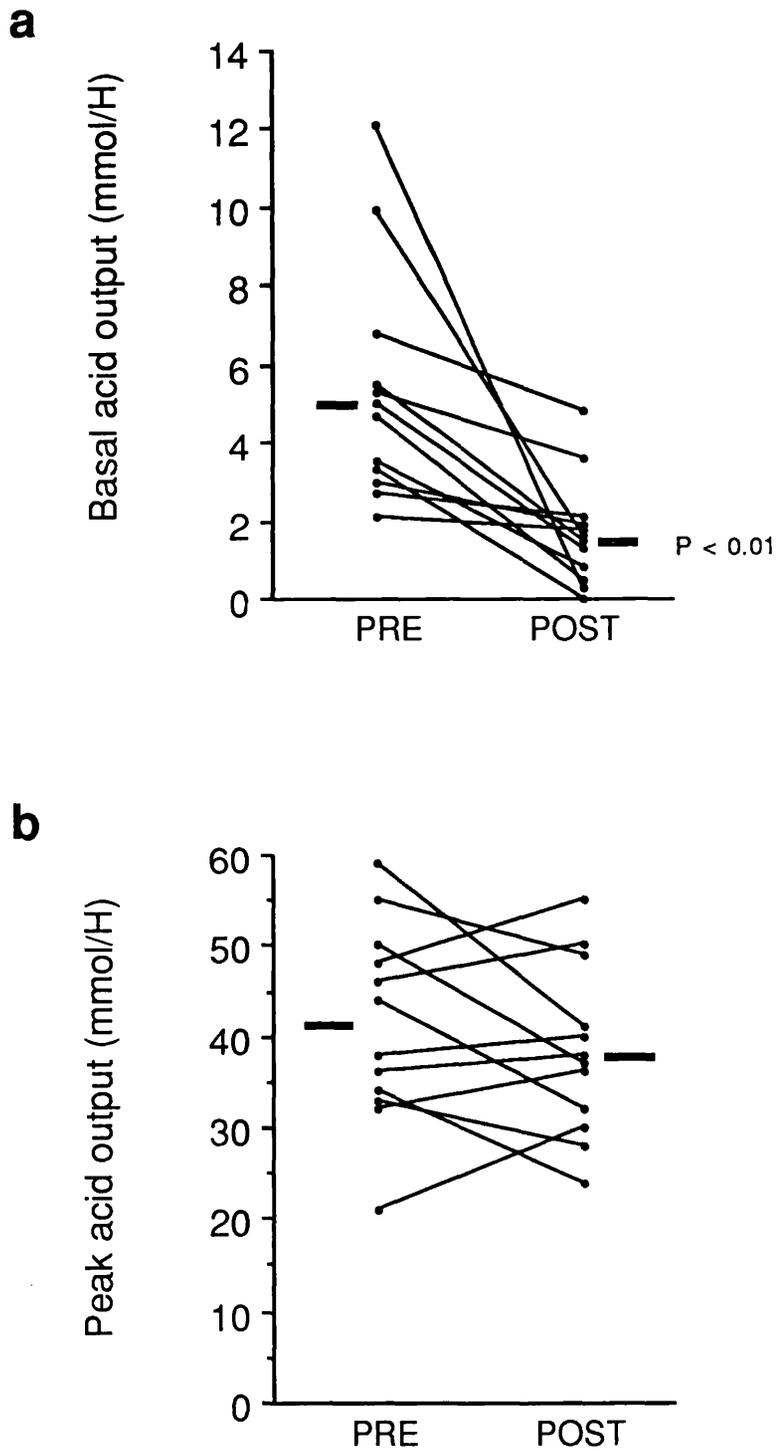


Figure 2.1 The effect of treatment with sucralfate on (a) basal and (b) peak acid secretion in 12 DU patients. The medians are indicated by bars.

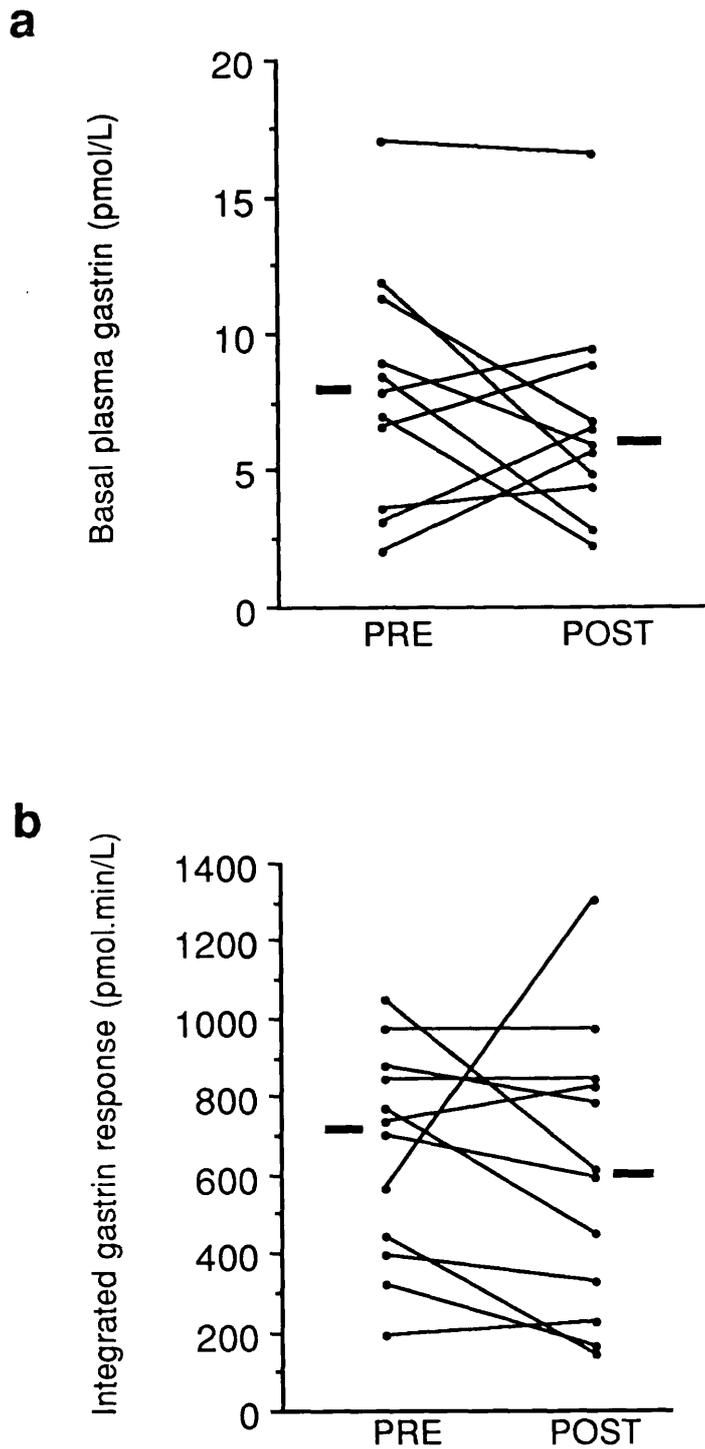


Figure 2.2 The effect of treatment with sucralfate on (a) basal plasma gastrin concentrations and (b) the integrated meal-stimulated gastrin response in 12 DU patients. The medians are indicated by bars.

***H pylori* infection**

The biopsy urease test remained positive in 11 of the patients. The single patient with a negative urease test at the end of treatment still had *H pylori* infection (grade 1) histologically, so that his negative urease post-treatment was a false negative. Sucralfate had no significant effect upon the degree of *H pylori* infection assessed by histology. The median score per biopsy was 1.0 (1-2) before and 1.0 (0-2) after therapy. The density of infecting organisms decreased in five patients, increased in two and was unchanged in five patients.

Histological gastritis

The median neutrophil infiltrate was 1.0 (1-3) before and 2.0 (0-3) after therapy, the scores for chronic inflammatory cells were 2.0 (1-3) and 2.0 (1-3) respectively. The median total gastritis scores were 3.0 (2-5) pre and 3.5 (0-5) post treatment and improved in only three patients, worsening in five. Thus the degree of antral gastritis was also unchanged by sucralfate therapy.

6 month follow-up

Of the 12 patients who were initially studied, nine were subsequently treated to eradicate *H pylori* since they remained symptomatic or rapidly relapsed soon after the end of their course of treatment. Three patients remained well whilst off therapy and we were able to measure their BAO six months later, at which time they had normal endoscopic findings. The results of these patients show that the BAO remained lower than the initial value before treatment:

Patient	Initial BAO	BAO immediately post-sucralfate	BAO 6 months post-sucralfate
PL	2.9	1.9	1.4
KN	6.8	4.8	1.8
PG	3.1	0.0	1.3

Table 2.1 6-month follow-up of basal acid secretion after sucralfate treatment for a DU. The basal acid secretion (in mmol H⁺/h) is shown before, immediately after and 6 months after treatment with sucralfate in 3 patients with duodenal ulcers.

DISCUSSION

The results of this study show no apparent effect of sucralfate on *H pylori* status, antral inflammatory infiltrate, or plasma gastrin after treatment of patients with DU disease with sucralfate. However, we did find a consistent and significant fall in BAO. The BAO was initially within the range found in untreated DU disease (Baron, 1978) and treatment reduced the rate of secretion to about one-third of initial values. The coefficient of variation of the measurement of BAO is around 50% (Baron, 1978) so that small changes in the measurement of secretion might be artefactual. However, our study has demonstrated a significant and considerable fall in the BAO in all 12 patients, implying that the change is a real effect of sucralfate.

On reviewing the literature, a diminished BAO after sucralfate was also noted incidentally in a study of parietal cell sensitivity by Marks *et al* (1989). In that study there was a fall in BAO of 50% after six weeks' treatment with sucralfate 2g b.d. when the BAO was measured 60–84 hours after the end of treatment, a similar time course to our study. Unlike our patients, the endoscopies of their 10 patients were all normal following treatment. The same group of workers have also measured the nocturnal acid output after sucralfate and found that this fell by a similar degree (Kummer *et al*, 1992).

Patients with DU disease tend to have increased basal acid secretion which has been implicated in ulcerogenesis (Kirkpatrick & Hirschowitz, 1980, Johnston & Jepson, 1967; Blair *et al*, 1987). The fall in BAO produced by sucralfate may therefore contribute to its therapeutic effect. It is noteworthy that Achord (1981) suggested that the healing of an ulcer may **itself** be associated with a fall in BAO, since a group of DU patients with healed ulcers had a lower BAO than a group in whom the ulcers were active. One could postulate that this may be because the ulcer could stimulate gastric acid secretion by a reflex involving nerves or the release of regulatory peptides. Alternatively, cyclical changes in acid secretion may occur, with the formation of an ulcer at a time when the basal acid secretion is at its maximum. In favour of the hypothesis that ulcer-healing is itself associated with a fall in acid secretion is our finding that the BAO remained low in three patients six months after the end of treatment. However, against this hypothesis, all our patients had decreased

BAO after treatment with sucralfate and the fall in BAO was of a similar degree whether or not the ulcer healed.

How sucralfate affects acid secretion remains open to speculation. It does not appear to occur through in gastrin since this did not change. However, the measured acid output inevitably reflects acid secreted minus gastric secretion of bicarbonate, and sucralfate is known to increase the latter (Shorrock *et al*, 1990; McCarthy 1991). Shorrock *et al* (1990) found that perfusion of the human stomach with sucralfate 8 mg/ml for one hour increased gastric bicarbonate secretion by about 0.5 mmol/h, from 1 to 1.5 mmol/h. In our study the BAO fell by two thirds but the basal gastric volume output was diminished by only around 20%. This suggests that the apparent fall in titratable acid output may have been chiefly due to increased bicarbonate secretion neutralising the gastric acid. If the fall in acid output were due to reduced acid secretion a corresponding fall in the volume of secretion would be expected. The mechanism of increased bicarbonate secretion by sucralfate is unclear. Sucralfate is a complex of aluminium hydroxide and sulphated sucrose. The evidence from animal models is that it is the aluminium moiety which is responsible for the increased secretion of bicarbonate (Crampton *et al*, 1988). Sucralfate does increase the synthesis of prostaglandin E₂ (Shorrock *et al*, 1990; Hollander & Tarnawski 1990), and prostaglandins of this type do inhibit gastric acid secretion (Wilson 1986) which may contribute to the fall in basal acid secretion. However, the stimulation of human gastric bicarbonate secretion by sucralfate is not inhibited by indomethacin and occurs before the rise in prostaglandin E₂ (Shorrock *et al*, 1990), suggesting that the rise in prostaglandins is not directly linked to the increased secretion of bicarbonate.

We did not observe a change in peak pentagastrin-stimulated acid output after treatment with sucralfate for four weeks, whereas Marks *et al* (1989) and Kummer *et al* (1992) recorded a small but significant fall in PAO after a six-week course of therapy. The 4-week rate of duodenal ulcer healing of 67% in the present study was as expected from larger studies (Lam 1989).

The lack of effect of sucralfate on *H pylori* that we observed is consistent with the findings of Barbara *et al* (1990) and Rauws *et al* (1988), but not with those of Hui *et al* (1989) who reported a significant fall in the number of organisms after sucralfate. Using a simple histological scoring system we found no effect of sucralfate on the

antral inflammatory cell infiltration. This was surprising since Hui *et al* (1989), who used a similar scoring system to us, and Barbara and coworkers (1990) who assessed gastritis by neutrophil infiltration only had reported an improvement in the gastritis. However, the results of Rauws *et al* (1988) who quantified gastritis by the degree of cellular infiltration, intraepithelial polymorphonuclear leukocytosis and superficial erosions were in agreement with our own – that sucralfate does not alter the degree of antral gastritis. It is not clear why the results of these different groups are not in agreement; there appears to be no methodological differences to account for this.

In view of the lack of change in *H pylori* status and inflammation, it is not surprising that sucralfate did not alter gastrin release. In this respect the study did not answer whether it is *H pylori* infection or antral inflammation which is responsible for the hypergastrinaemia. In fact, all studies of this type seem to show that a drug's effect or lack of it on improving antral gastritis is determined by its effect on the *H pylori* infection (Hui *et al*, 1991; Chittajallu 1991a) so that separating antral gastritis from *H pylori* infection in order to explain the hypergastrinaemia may ultimately prove to be impossible *in vivo*.

**CHAPTER 3 – pH DEPENDENT SECRETION OF
GASTRIN, ACID AND PEPSIN IN DUODENAL
ULCER DISEASE: EFFECT OF SUPPRESSING *H*
*PYLORI***

INTRODUCTION

One hypothesis to explain the increased gastrin release of *H pylori* infection is that *H pylori* alkalinises the gastric lumen or mucosa. If this were so, then *H pylori* might be expected to interfere with the normal negative feedback of acid on gastrin release which occurs at low intragastric pH and which has been reported to be abnormal in patients with DU disease (Walsh *et al*, 1975).

The purpose of the study described in this chapter is to test whether *H pylori* influences the gastrin response to intraluminal pH. For this purpose, we used a gastric perfusion method to measure the gastrin response to intragastric stimulation by 4% peptone solutions at pH 2.5 and at pH 5.5, both before and after the suppression of *H pylori*. The gastric pH was kept at a constant pH by titration with alkali and the meal was perfused through the stomach by a method which has been previously used in our department (Playford *et al*, 1991) to avoid the gastric distension which occurs in intragastric titration methods. We were keen to avoid even small degrees of distension since this increases the secretion of both gastrin (Koop *et al*, 1990) and acid (Feldman 1979).

We hoped that this method would also provide us with estimates of pH-dependent acid and pepsin secretion too, to see whether these were altered by the suppression of *H pylori*.

METHODS

Patients and treatment

We studied 11 patients with active duodenal ulceration and *H pylori* infection, 8 male and 3 female, aged 23–76 years (median 46). The criteria for entry into the study and methods used to diagnose *H pylori* infection were as described in chapter 2.

Following gastric perfusion, which was performed within 7 days of endoscopy, the patients were treated with tripotassium dicitratobismuthate (DeNol) 120 mg qds for four weeks together with metronidazole 400mg tds and amoxicillin 500mg tds for the first two weeks. Endoscopy and gastric perfusion were then repeated within one week of the end of treatment.

Gastric perfusion

After an overnight fast, two basal blood samples were taken for plasma gastrin measurement and a modified triple-lumen nasogastric tube was positioned in the dependent part of the stomach as described in chapter 2. Residual gastric juice was manually aspirated and discarded and then 100 ml of isotonic 4% peptone (Sigma Chemical Company, Poole, Dorset) at pH 2.5 was perfused over five minutes through the stomach from the proximal lumen and manually aspirated via the major lumen to achieve rapid equilibration of intragastric pH. Isotonic 4% peptone, pH 2.5, was then infused at a rate of 4 ml/min for 60 minutes and the gastric contents constantly aspirated from the antrum as described in chapter 2. The pH of the aspirate was monitored continuously by allowing it to flow over a pH electrode into a collecting vessel placed in an ice bucket and sufficient isotonic 0.16 M sodium bicarbonate was added to the infusion port to maintain the gastric contents at pH 2.5. At the end of the first hour the stomach was

washed with 100 ml isotonic 4% peptone at pH 5.5 and the titration repeated at an intragastric pH of 5.5.

Using the same gastric perfusion technique in 7 normal volunteers aged 21–45 years others in our department have shown that more than 87% of the gastric infusate was recovered in the aspirate, as estimated by polyethylene glycol dilution (Playford *et al*, 1991).

Gastrin measurement

Two venous blood samples were taken for basal gastrin measurement 10 minutes apart and further samples were taken at 10 minute intervals for the last 40 minutes of each hour of gastric perfusion, when a steady state had been established. The concentration of gastrin in plasma was measured as described in chapter 2.

Acid and pepsin outputs

The acid output for each period was taken to be equal to the amount of alkali necessary to keep the pH of the gastric aspirate constant at pH 2.5 for the first hour and at pH 5.5 for the second.

The pepsin output was assessed by measurement of the peptic activity and volume of the gastric juice. The peptic activity was measured by an autoanalytical method in routine use in the Hammersmith Hospital which is based upon the digestion of haemoglobin (Vatier *et al*, 1968). In brief, gastric juice was pre-diluted 1 in 20 with 0.01M hydrochloric acid and the peptic activity assessed by the breakdown of a 20g/L concentration of haemoglobin at pH 2.0. The liberated tyrosine residues are then dialysed and measured spectrophotometrically using alkaline sodium Ciocalteu's reagent (Sigma, Poole, Dorset) and the peptic activity compared to a standard preparation of pepsin A (BDH, Poole, Dorset).

To ensure that there had been sufficient mixing of the gastric contents at the times of sampling, calculations of both acid and pepsin secretion are based upon the results of the last 40 minutes of each hour, when a steady state had been reached.

Statistical analysis

As the data were not normally distributed, Wilcoxon's signed rank test for non-parametric data was used. The results are expressed as medians (ranges).

RESULTS

Clinical

There was complete healing of duodenal ulceration and *H pylori* became undetectable by histology and the biopsy urease test in 10 of the 11 patients. In one male aged 45 *H pylori* infection and duodenal ulceration persisted; for this reason he was excluded from further analysis.

Plasma Gastrin

After the eradication of *H pylori* there was a reduction in the plasma gastrin concentration under basal conditions, at pH 2.5 and at pH 5.5, with significant reductions at all but one time point (Fig 3.1).

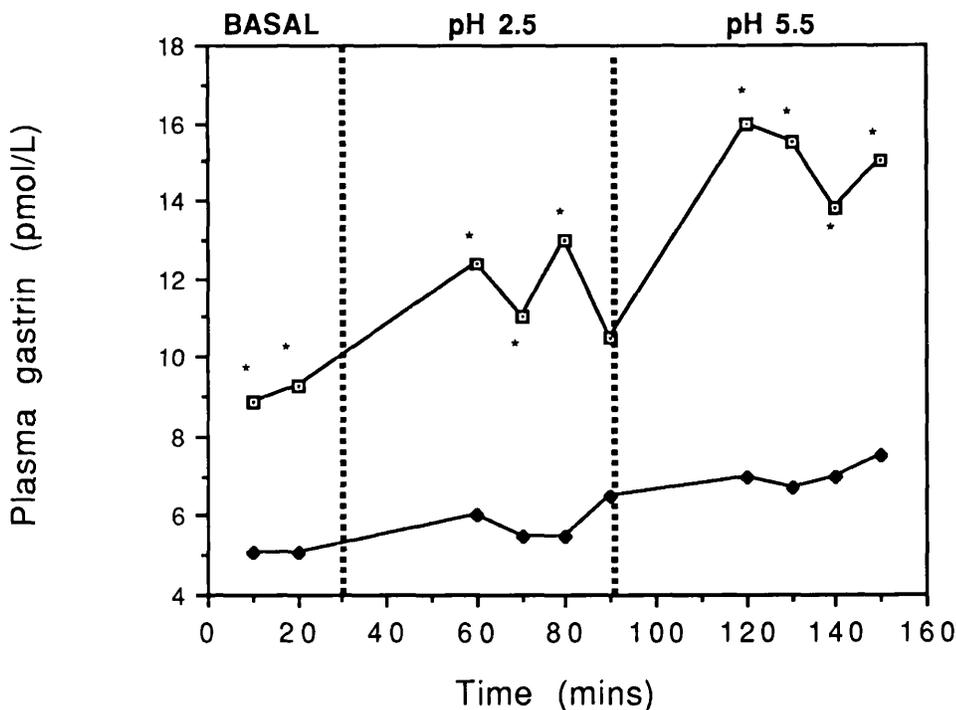


Figure 3.1 Median plasma gastrin concentrations during gastric perfusion under basal conditions, and at pH 2.5 and 5.5. The plasma gastrin concentrations are shown before (—□—) and after (—●—) the suppression of *H pylori* in 10 DU patients. * = $P < 0.05$, pre versus post treatment.

Overall, the mean basal plasma gastrin concentration decreased significantly after treatment, from a median of 9.2 (range 3.7–23) pmol/L to 5.1 (1.7–15) pmol/L, $P < 0.05$ (Table 3.1). After suppression of *H pylori* there was also a significant decrease in the gastrin response to peptone, at an intragastric pH of 2.5 and at pH 5.5. The mean plasma gastrin concentration at pH 2.5 fell from a median of 11.3 (3.8–29) pmol/L to 5.9 (5.7–6.1) pmol/L ($P < 0.05$) after suppression of *H pylori*; at pH 5.5 the fall was from 15.2 (3.9–32) to 7.2 (6.1–14) pmol/L, $P < 0.05$.

Before treatment 4% peptone at pH 2.5 produced a significant rise in mean plasma gastrin with a further significant increase during peptone infusion of pH 5.5, $P < 0.05$ in each case (Table 3.1). After the suppression of *H pylori*, the mean gastrin levels followed a similar pattern but they were only significantly greater than the basal levels during perfusion with peptone at pH 5.5 ($P < 0.05$).

The ratio of the mean plasma gastrin concentration at pH 2.5 compared with pH 5.5 was 0.8 (0.5–1.7) before treatment and 0.8 (0.5–1.1) afterwards. Therefore the suppression of *H pylori* was not accompanied by a change in the relationship between gastrin release at these two pHs.

	BEFORE	AFTER	P
Basal	9.2 (3.7–23)	5.1 (1.7–15)	<0.05
pH 2.5	11.3 (3.8–29)	5.9 (5.7–6.1) ^α	<0.05
pH 5.5	15.2 (3.9–32)*	7.2 (6.1–14)*	<0.05
pH 2.5/5.5	0.8 (0.5–1.7)	0.8 (0.5–1.1)	NSD

Table 3.1 Mean plasma gastrin concentrations in 10 DU patients before and after the suppression of *H pylori*. The data are expressed in pmol/L as medians (ranges). NSD = no significant difference. * $P < 0.05$, pH 5.5 vs basal.

^α $P < 0.05$, pH 2.5 vs basal.

Acid output

The rates of acid secretion during the last 40 minutes of each hour at each pH are shown in table 3.2. There was more acid secreted at pH 5.5 compared with pH 2.5, both before and after the suppression of *H pylori*. However, this difference was only significant **after** treatment. The suppression of *H pylori* did not significantly change the median acid secretion at either pH. There was, if anything, relatively **more** acid secretion at pH 2.5 compared to pH 5.5 after treatment, with a greater pH 2.5/5.5 ratio, again this was not statistically significant.

	BEFORE	AFTER	P
pH 2.5	7.9 (1.0–43)	6.8 (1.4–19)	NSD
pH 5.5	14 (5.5–27)*	13 (1.9–33) ^α	NSD
pH 2.5/5.5	0.5 (0.1–1.1)	0.7 (0.2–1.4)	NSD

Table 3.2 Acid secretion rates during intragastric perfusion in 10 DU patients before and after the suppression of *H pylori*. The data are expressed in mmol H⁺/h as medians (ranges). * = NSD, pH 5.5 vs 2.5. ^α = P < 0.02, pH 5.5 vs 2.5. NSD = no significant difference.

Pepsin secretion

The peptic activity in the gastric aspirate was measured for the last 40 minutes of each hour (Table 3.3). There were no significant differences in the rates of pepsin secretion at either pH 2.5 or pH 5.5 after the suppression of *H pylori*. Both before and after treatment the pepsin secretion was slightly greater at pH 5.5 than 2.5 but the ranges were large and the differences were not statistically significant.

	BEFORE	AFTER	P
pH 2.5	2.0 (1.2–8.6)	2.1 (1.1–3.9)	NSD
pH 5.5	2.3 (1.2–5.7)*	2.5 (1.2–4.1)*	NSD
pH 2.5/5.5	0.87 (0.4–4.4)	0.92 (0.6–1.2)	NSD

Table 3.3 The secretion of pepsin during intragastric perfusion in 10 DU patients before and after the suppression of *H pylori*. The data are expressed (in Anson units/h) as medians (ranges). * = NSD, pH 5.5 vs 2.5.
NSD = no significant difference.

DISCUSSION

The results of the present study confirm that suppression of *H pylori* diminishes plasma gastrin levels (Levi *et al*, 1989b; McColl *et al*, 1991; Graham *et al*, 1990; Smith *et al*, 1990; Prewett *et al*, 1991). Both basal and peptone-stimulated plasma gastrin concentrations were diminished. Moreover, the response to gastric perfusion with peptone was diminished to a similar extent, whether the intraluminal pH is low or high. However, in contrast to the fall in gastrin after the suppression of *H pylori*, we found that there was no accompanying change in the gastric secretion of acid or pepsin.

The fact that we found a fall in gastrin independent of luminal pH would tend to argue against the idea that *H pylori* stimulates gastrin release by blocking antral acid-sensors through ammonia generated by its urease (Levi *et al*, 1991a). Low pH inhibited peptone-stimulated gastrin release to a similar extent both before and after suppression of the organism, implying that *H pylori* does not interfere with the negative feedback of acid on gastrin release when the gastric lumen is very acidic. This finding is consistent with the evidence that the organism's urease is **not** responsible for the hypergastrinaemia; inhibition of *H pylori*'s urease with acetohydroxamic acid or by triple anti-*Helicobacter* therapy had no effect on plasma gastrin (Chittajallu *et al*, 1991a; El Nujumi *et al*, 1991), and neither did increasing intragastric ammonia by the intragastric instillation of urea (Chittajallu *et al*, 1991b). Thus the minor alkalization of the mucus layer produced by *H pylori* (Kelly *et al*, 1990; Beardshall *et al*, 1991) may not be responsible for the exaggerated gastrin release seen in *H pylori* infection.

Two other groups have also investigated whether *H pylori* stimulates gastrin release through a change in luminal pH. Chittajallu *et al* (1992c) found that in DU patients meal-stimulated gastrin release was increased to a similar degree whether the meal was eaten at an uncontrolled, physiological, pH or during an intragastric perfusion of a pH 7 buffer; in agreement with the present results. Similar results to our own were also obtained by Karnes *et al* (1991a) using intragastric titration rather than perfusion.

They studied groups of healthy volunteers and retrospectively assessed their *H pylori* status on stored sera. Like us, they found an exaggerated gastrin response at both pHs in the subjects who were *H pylori* positive but the ratio of the plasma gastrin concentration at pH2.5/5.5 was identical in the two groups. However, another study from the same unit (Tarnasky *et al*, 1991) did demonstrate diminished acid-inhibition of peptone-stimulated plasma gastrin release in subjects infected with *H pylori*, in accordance with this unit's earlier results in DU patients (Walsh *et al*, 1975). One difference between the study of Tarnasky *et al* and our own is that they compared non-ulcer patients with and without the bacterium, whereas we studied DU patients before and after the suppression of *H pylori*. Other methodological differences between our study and theirs should also be considered. It is possible that *H pylori* was not completely eradicated in some of our patients, which is a drawback of repeating the study soon after the end of a course of treatment to eradicate *H pylori*. We repeated the intragastric perfusion one week after the end of treatment because plasma gastrin concentrations return to normal at this time (Levi *et al*, 1989b). Although *H pylori* could not then be identified by our tests, the organism may have been lying dormant in some patients (Weil *et al*, 1988) but still be influencing gastric physiology.

Ideally the follow-up study should be performed at a time when *H pylori* can be completely excluded by negative tests, in practice at least one month after the end of treatment. Tarnasky *et al* used 1% and 8% peptone and an alkaline pH of 7.0. This contrasts with 4% peptone and an alkaline pH of 5.5, as used in our study and that of Karnes *et al* (1991a). In addition Tarnasky *et al* performed the intragastric titration for a total of two hours at each pH, which may have unmasked a change that was not evident after just one hour.

In contrast to Karnes *et al* (1991a) and Tarnasky *et al* (1991), we perfused the peptone through the stomach, with continuous aspiration of the antrum to avoid any effects of distension on gastrin release. Koop *et al* (1990) have demonstrated that fundic distension in man increases basal gastrin by 31% which may explain why our peptone-stimulated gastrin release was relatively small compared with previous studies of

intra-gastric titration (Walsh *et al*, 1975; Cooper *et al*, 1985). Although this approach has theoretical advantages over intra-gastric titration in assessing the gastrin response to a predominantly chemical stimulus, rather than to the chemical and distension stimuli which accompany intra-gastric titration, it has not been used previously to measure acid and pepsin secretion.

A secondary aim of this study had been to measure not only pH dependent gastrin secretion but also pH dependent acid and pepsin secretion. Our results suggest that there was more acid secreted at pH 5.5 than at pH 2.5, regardless of *H pylori* infection but this difference was only significant after suppression. However, neither the amount of acid nor the ratio of acid secreted at pH 2.5/5.5 was altered by the suppression of *H pylori*. Similar results were reported in the study of Karnes *et al* (1991a). Using intra-gastric titration, they found acid secretion rates approximately double those which we observed, with a much smaller range of acid secretion than we found, but their conclusions were similar – that infection with *H pylori* does not alter acid secretion, in absolute terms or in the ratio of acid secreted at pH 2.5/5.5, which was approximately 0.5 in all cases. In contrast, however, Tarnasky *et al* (1991) reported that infection with *H pylori* was associated with increased acid secretion at pH 2.5, when the stimulant was 8% peptone but not with 1% peptone. Interestingly other under conditions (pH 7.0, 8% peptone and pH 2.5 or pH 7.0, 1% peptone) the *H pylori* positive patients secreted, if anything, less acid. This report therefore implicates *H pylori* as the agent responsible for the excess acid secretion at a low intra-gastric pH, first reported by Walsh *et al* in 1975. It is important to emphasise that in Walsh's original study, the loss of negative feedback only became apparent after 90 minutes. In addition in the study of Tarnasky *et al* (1991) the increased in acid secretion was again only significant in the second hour. This may explain why we did not observe it during a one hour perfusion at each pH. Our negative result may therefore be subject to a type II error of failing to observe an effect when it was present. However, this may have occurred for other reasons apart from the length of the study. For instance, performing an intra-gastric perfusion rather than a titration may result in a variation in intra-gastric contents, dependent on the rates of aspiration and of infusion of bicarbonate.

In addition, we used 4% rather than 8% peptone as the meal stimulus and at pH 5.5 rather than 7.0, in accordance with the original study of Walsh *et al* (1975). Further studies will be necessary to finally establish whether some or all of these methodological differences are responsible for these different results.

We found that the peptic activity of gastric juice was unaffected by the suppression of *H pylori*. However, the measured peptic activity was the same at pH 2.5 and pH 5.5. Since we found that the secretion of acid was increased at pH 5.5 and it is known that acid secretion and pepsin secretion are closely linked (Baron, 1978), this raises some doubts over the measurement of pepsins in our study. Indeed, pepsins are irreversibly inactivated above pH 7 (Hersey, 1987) and it is possible that this pH was reached intragastrically close to the proximal lumen of the nasogastric tube from which the bicarbonate was infused in order to keep the aspirate, and by implication the gastric antrum, at pH 5.5. The measured peptic activity at this pH may therefore underestimate the true pepsin secretion rate. For this reason intragastric titration studies have not previously been used to measure pepsin outputs simultaneously.

In conclusion, this study has demonstrated that *H pylori* causes a pH independent increase in plasma gastrin concentration which does not support the hypothesis that *H pylori* alters the release of gastrin through changing luminal pH. The study did not show any effect of *H pylori* suppression on pH dependent acid or pepsin secretion but this may have been due to methodological flaws when studying gastric responses by an intragastric perfusion, rather than a titration technique.

**CHAPTER 4 – ONE YEAR FOLLOW-UP OF
ACID AND GASTRIN SECRETION FOLLOWING
ERADICATION THERAPY FOR *H PYLORI***

INTRODUCTION

It is now well-established that *H pylori* infection in DU patients is associated with increased gastrin release. Initial reports also suggested that *H pylori* was responsible for increased acid secretion (Levi *et al*, 1989a; McColl *et al*, 1989) but subsequent studies, including cross-sectional studies of different groups of patients did not support this view (Levi *et al*, 1989b; McColl *et al*, 1991; Rademaker & Hunt, 1991). One explanation which has been proposed for this apparent dissociation between gastrin and acid secretion in *H pylori* infection is that *H pylori* infection may decrease the sensitivity of the parietal cells to gastrin (Blaser, 1992); a study to address this question is the subject of chapter 5.

However, the occurrence of a decline in plasma gastrin concentration without a fall in acid secretion contrasts with the decrease in both basal and particularly peak acid secretion which occurs 3–6 months after the excision of a gastrin-secreting tumour and the subsequent fall from very high plasma gastrin concentrations to normal (Pisegna *et al*, 1992). Might the lack of change in acid secretion after the eradication of *H pylori* be due to re-testing too soon after anti-*H pylori* treatment? Two of the earliest follow-up studies were performed relatively soon after the end of anti-*H pylori* therapy (Levi *et al*, 1989b; Mountbriand *et al*, 1989) so that in some patients *H pylori* may have been suppressed rather than eradicated when the follow-up acid secretion tests were performed (Weil *et al*, 1988). Although the gastrin concentrations were reduced by the time of the follow-up studies, the trophic effect of previously high gastrin levels on the parietal cells may be more long-lasting – the gastric parietal cells turn over slowly, with a lifetime of 3 months in the rat (Lipkin, 1987), no data exist for man. In the one study with a longer follow-up period (McColl *et al*, 1991) there was again no change in either intragastric pH or nocturnal acid output; however, the peak acid secretory rate, an index of the parietal cell mass (Baron *et al*, 1978), was not tested. Although the basal acid output often remains elevated, the PAO returns to normal values 3–6 months after the curative resection of a gastrinoma (Pisegna *et al*, 1992) so that measurements of PAO are a good index of assessing the reversibility of any trophic effect of gastrin.

In the study described below, the gastrin and acid secretory responses were compared with pre-treatment values one year after the eradication of *H pylori* from DU patients, by which time the trophic effects of high gastrin levels would be expected to have diminished.

METHODS

A group of 24 patients who had initially all had a positive urease test for *H pylori* associated with an active ulcer were treated with tripotassium dicitratobismuthate (DeNol) 120 mg qds for one month and metronidazole 400 mg tds for the first two weeks. These patients had a median age of 35 (range 25–77), 17 were male. Those patients in whom the urease test had become negative two days after the end of treatment were then invited to reattend the endoscopy unit for a repeat endoscopy and estimation of their acid secretory and plasma gastrin responses 12 months after the beginning of their initial therapy. Some of these patients have been described elsewhere since they were part of our unit's original cohort of patients treated with an anti-*H pylori* regime (Levi *et al*, 1989b).

The inclusion and exclusion criteria for the study, the measurement of acid secretion, the measurement of the fasting and meal-stimulated gastrin response and the determination of *H pylori* status at 12-month follow-up (urease and histology tests) are all as described in chapter 2. If either of the tests for *H pylori* was positive the patient was regarded as infected.

RESULTS

Clinical

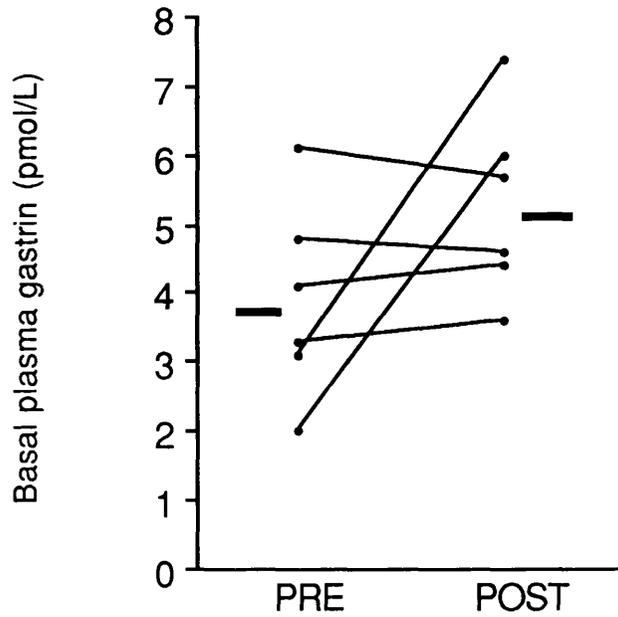
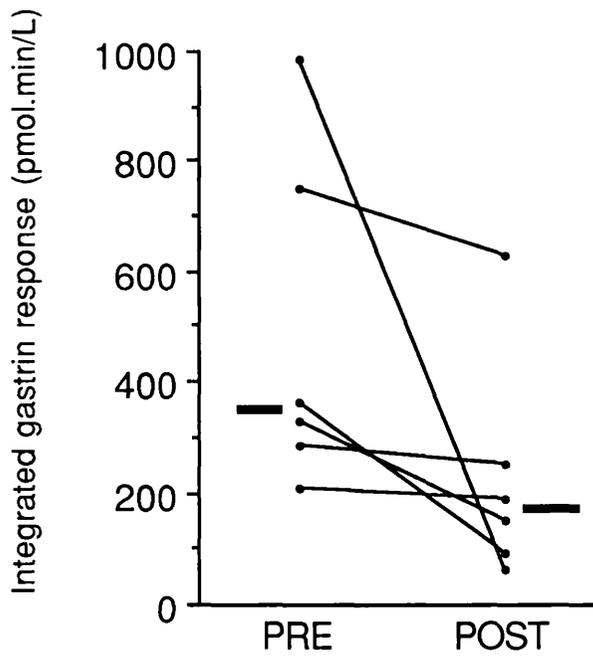
Of the 24 patients originally treated 4 were still infected with *H pylori* immediately following treatment, 2 had a symptomatic relapse with evidence of *H pylori* infection in the one-year period of follow-up, 3 developed medical conditions unrelated to DU disease and a further 6 patients declined further investigation or could not be contacted.

Endoscopies were then performed on the nine willing patients who remained symptom-free 12 months after the course of treatment to heal their ulcers and suppress *H pylori*. Eight of these patients had normal endoscopic appearances; one patient, who had a positive urease test, had duodenitis. Only six of the patients (all male, aged 33–62 years (median 51)) were still negative for *H pylori* and in these patients the acid secretory and gastrin responses were measured.

Acid and gastrin responses

Compared to initial (pre-treatment) values the median fasting plasma gastrin concentration was not changed significantly, from 3.7 pmol/L (2–6.1) to 5.7 (3.6–7.4) pmol/L at one year (Fig 4.1a). However, the one-hour meal-stimulated integrated gastrin responses were significantly lower than pre-treatment levels at one year; the median was 323 pmol.min/L, (range 207–985) before and 170 pmol.min/L (64–626) at one year, $P < 0.05$ (fig 4.1b).

Neither the median BAO nor the PAO changed significantly over this time. The median BAO was 6.3 (2.5–12) mmol H⁺/h initially and 3.8 (1–4.6) mmol H⁺/h at 12 months, falling in 5 of the 6 patients (fig 4.2a). This difference did not reach statistical significance, however. The respective peak acid outputs were 46 (36–55) and 48 (40–58) mmol H⁺/h (fig 4.2b).

a**b****Figure 4.1**

The fasting plasma gastrin concentration (a) and meal-stimulated integrated gastrin response (b) before and 11 months after treatment to eradicate *H pylori* from 6 patients with a DU. The median values are shown by the bars.

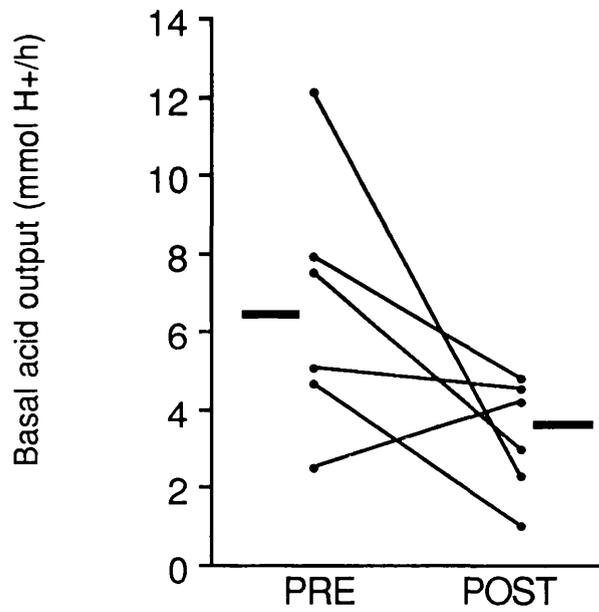
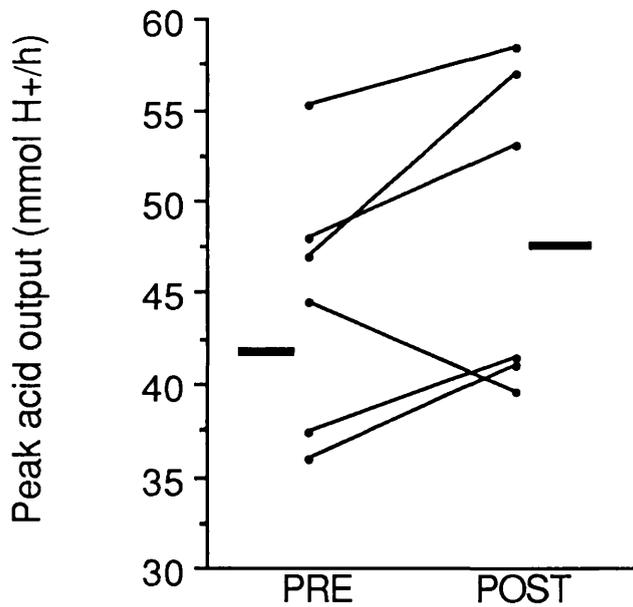
a**b**

Fig 4.2 The basal acid output (a) and peak acid output (b) before and 11 months after treatment to eradicate *H pylori* from 6 patients with DUs. The median values are shown by the bars.

DISCUSSION

The main finding of this study is that even one year after the eradication of *H pylori* from patients with active DU disease, the PAO does not change. This contrasts with the marked fall in PAO which occurs 3–6 months after the surgical removal of a gastrin-secreting tumour (Pisegna *et al*, 1992). Therefore the trend to increased PAO which is typical of patients with DU disease (Blair *et al*, 1987; Baron, 1978) is unlikely to be due to the trophic effect of the moderately increased plasma gastrin concentrations characteristic of infection with *H pylori*. Since this study was commenced others have confirmed this finding, reporting that the maximal acid output was unchanged one year after successful eradication of *H pylori* (Fullarton *et al*, 1991), although the plasma gastrin concentrations were not measured in that study.

In our study the integrated gastrin response to a meal remained lower than the pre-treatment value, as one would predict if the fall in gastrin release were due to the eradication of *H pylori*. An alternative explanation of the fall in the gastrin response, that this is due to the continuing effect of bismuth which may have accumulated in the body is unlikely. Three months after a six week course of tripotassium dicitratobismuthate the plasma bismuth levels return to normal although bismuth continues to be excreted in the urine for at least a further 9 weeks (Gavey *et al*, 1989). Since our study was performed 11 months after the end of treatment, we can exclude a prolonged effect of the treatment on both acid and gastrin secretion. Despite the fall in the meal-stimulated gastrin response the fasting gastrin concentration did not alter significantly. Although in some studies of DU patients the basal gastrin concentration falls when *H pylori* is eradicated (McCull *et al*, 1991; Chittajallu *et al*, 1992d), this has not always been found (Graham *et al*, 1991b; Levi *et al*, 1989b). It is not clear why a fall in basal gastrin is not always observed –perhaps because the degree of fall in basal gastrin in some of the studies is close to the intra-assay variation of the gastrin radioimmunoassay (Bryant & Adrian, 1982).

Since *H pylori* has an effect predominantly on **stimulated** gastrin release with only a small effect upon the basal gastrin concentration, this may explain why *H*

pylori is unlikely to be responsible for the increased PAO of DU patients. For most of a 24-hour period the circulating gastrin concentrations are only mildly elevated by *H pylori* (Smith *et al*, 1990; Prewett *et al*, 1991) whereas in the Zollinger–Ellison syndrome there are high gastrin concentrations throughout the day, including very high fasting levels. Indeed, the degree of gastrin hypersecretion in patients with the Zollinger–Ellison syndrome is much greater than that due to *H pylori*, with fasting gastrin concentrations increased typically 5–50 fold (McGuigan, 1989; Pisegna *et al*, 1992) and a corresponding increase in maximal acid output of 2 to 3 times normal (Baron, 1978; Pisegna *et al*, 1992).

An unexpected finding of this follow-up study is that the basal acid secretion fell in 5 of the 6 patients. Assessing whether or not this is a significant observation would require studying more patients in this way. We were able to study only a small number of patients after 12 months. This was because over a third of the patients were infected with *H pylori* following their initial treatment, with or without symptoms, and several other patients were not willing to have further invasive tests when they had been asymptomatic for a year after their initial treatment. Based upon my experience we would need to at least double the number of patients initially treated to establish whether the BAO is altered by the eradication of *H pylori*. However, using a more effective eradication regime would also be beneficial – treatment with triple therapy results in a higher *H pylori* eradication rate than the dual therapy which was in use in this hospital at the time that the study was commenced.

In another small follow-up study (Fullarton *et al*, 1991) there was also a fall in BAO one month after the end of treatment in patients with inactive DU disease but by one year the BAO had returned to the pre-treatment value, arguing an effect of *H pylori* on BAO. The initial fall in BAO could perhaps be attributed to bismuth (Gavey *et al*, 1989) or some other component of the treatment in that study. However, as was discussed in chapter 2, there is also some evidence that the basal acid secretion is lower in DU patients in whom the ulcer has healed than it was at the time of active ulceration (Achord *et al*, 1981) so that the presence of an ulcer crater may itself be associated with an increase in BAO, perhaps explaining why our one-year follow-up results differ from those of Fullarton *et al* (1991) who studied patients without active ulceration initially.

The small study described in this chapter does not invoke *H pylori* as the agent responsible for the higher than average PAO observed in DU patients. It is possible that a genetic or acquired predisposition to high PAO is the non-*H pylori* related co-factor necessary for duodenal ulceration, with the balance being tipped in favour of a DU when both factors are present. However, whether *H pylori* influences the BAO remains an open question, addressed further in the following chapter.

**CHAPTER 5 – ACID SECRETION AND
SENSITIVITY TO GASTRIN IN DUODENAL
ULCER PATIENTS : THE EFFECT OF
ERADICATING *H PYLORI***

INTRODUCTION

H pylori increases plasma gastrin concentrations by about 50–100% and the increased levels fall to those of non-infected individuals after the organism has been eradicated. However, despite the reduction in plasma gastrin, no consistent changes in rates of acid secretion have been documented after eradication therapy. One possible explanation for the apparent dissociation between a fall in gastrin but not acid secretion after eradication therapy is that *H pylori* infection might decrease the sensitivity of parietal cells to gastrin (Blaser, 1992). However, there is currently no evidence to support this view. For example, Chittajallu *et al*, (1992d) were unable to detect a change in the acid secretory response to stepped infusions of pentagastrin on eradication of *H pylori* from patients with DU disease, although circulating concentrations of pentagastrin were not measured in that study. Furthermore, earlier studies of patients with DU disease, who were presumably mostly infected, showed their parietal cells to be, if anything more sensitive to gastrin than those of controls (Isenberg *et al* 1975; Lam *et al* 1980), although it was not known whether the controls were infected with *H pylori* or not.

The study described in this chapter was performed to determine whether the sensitivity of the parietal cell is altered shortly after the eradication of *H pylori*. In this study intravenous G-17 was used as the stimulant of acid secretion since the elevation in plasma gastrin in *H pylori* infection is predominantly due to an increase in this form of gastrin (Beardshall *et al* 1992; Mulholland *et al* 1992).

METHODS

Patients and treatment

11 patients, 8 male, with a median age of 34 (21–76) years were studied; the inclusion and exclusion criteria for entry were as described in chapter 2. The patients were studied before and one month after anti-*H pylori* therapy.

Determination of *H pylori* infection

At each endoscopy three antral biopsies were taken for the diagnosis of *H pylori* infection: one for the biopsy urease test, a second for histological staining, as in chapter 2 and a third was placed into a transport medium consisting of brain heart infusion broth (Oxoid, Basingstoke, Hants) with 5% (v/v) foetal calf serum (Tissue Culture Services Ltd, Botolph Claydon, Bucks) and cultured within 2 hours. The biopsy for bacterial culture was streaked onto a blood agar plate containing Skirrow's supplement (vancomycin, trimethoprim and polymyxin B) (Skirrow, 1990) and left in a microaerophilic environment for up to 5 days. Infection with *H pylori* was confirmed by using Gram stain, oxidase, catalase and the Rapidec pylori kit (Bio Mérieux, Marcy-l'Etoile, France) which detects the presence of urease, alkaline phosphatase, gamma glutamyl transferase and nitrate. In all the patients at least two of the tests for urease, bacterial culture and histology were positive prior to treatment.

G-17 Dose-Response Studies

Acid secretory responses to gastrin were measured within 5 days of endoscopy, both before and after treatment. Patients fasted overnight and acid secretory rates were then determined by naso-gastric aspiration as described in chapter 2. G-17 (Gastrin I Human; Sigma, Poole, Dorset, UK) made up in 0.15M saline, containing 1%

albumin, was infused continuously through a venous cannula placed in the right forearm by an infusion pump (Vickers Medical, Basingstoke, Hants, UK) at doses of 0, 11, 33, 100, and 300 pmol/kg/h, each for a 30 minute period. The latter dose is known to produce maximal stimulation of acid secretion (Lam *et al* 1980; Hirschowitz *et al* 1985).

The BAO per hour was calculated by doubling the acid output during the 30 minute infusion of saline only. Other acid outputs were calculated by multiplying the acid output during the second 15 minutes of each infusion period by four. Blood samples were taken at the end of each infusion period from an indwelling venous cannula in the left arm, as described in chapter 2.

Statistical analysis

The data was analyzed with an Arcus 2 statistical package (Medical Computing, Aughton, W. Lancs). Wilcoxon's rank sum test was used to compare differences before and after treatment and the results are expressed as medians (ranges). $P < 0.05$ was considered to signify statistical significance.

RESULTS

Clinical

At repeat endoscopy ulcers had healed in 10 of the 11 patients. The one patient with a DU at the end of treatment (a male aged 31 years) had positive urease, histology and bacteriology for *H pylori* when retested and a female, aged 66 years, still had a positive urease test following treatment. These two patients were excluded from further analysis. In the remaining 9 patients *H pylori* was successfully eradicated, and the endoscopic findings were normal. Data from these 9 patients were then analyzed further.

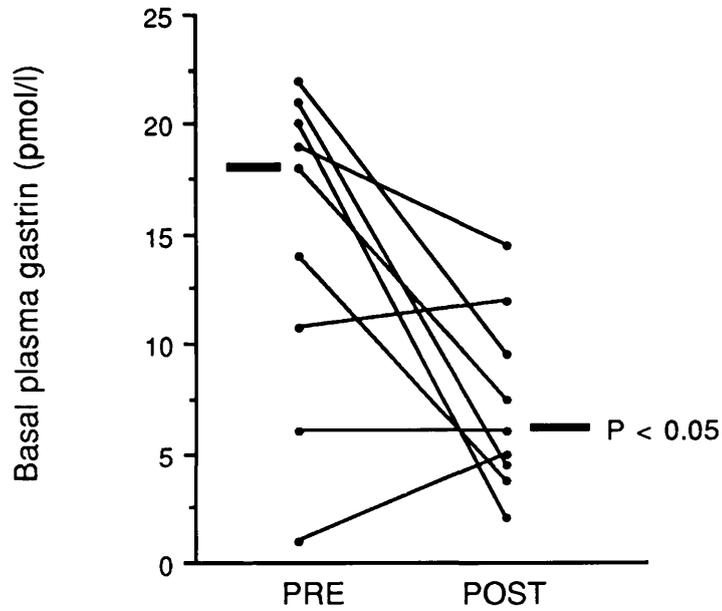
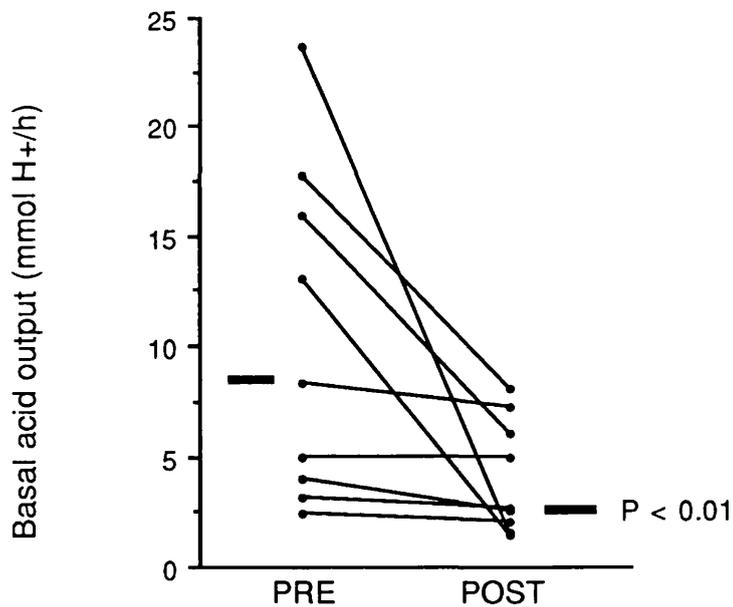
Basal plasma gastrin

The basal plasma gastrin concentrations fell significantly following the eradication of *H pylori* (Figure 5.1a). The median basal plasma gastrin concentration before treatment was 19 (range 1–22) pmol/L compared with 6 (2–15) pmol/L after, $P < 0.05$.

Basal and peak acid secretory output

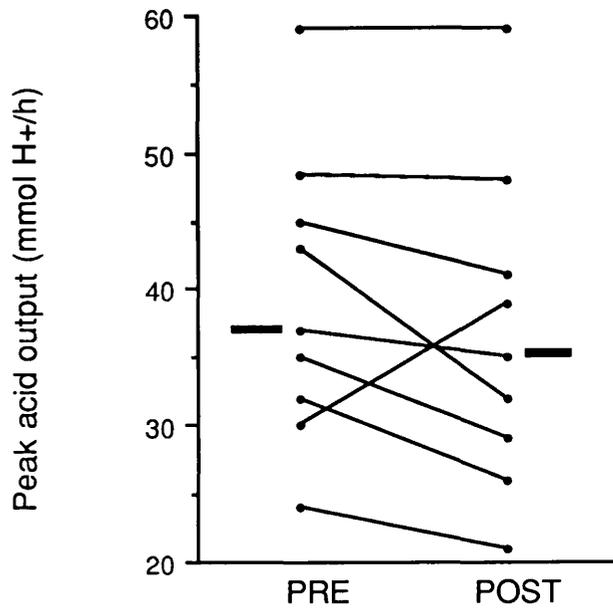
Figure 5.1b shows that after eradication of *H pylori* there was a reduction of BAO in eight patients and no change in one patient. The median BAO fell significantly from 8.3 (2.4–24) to 2.6 (1.4–8.1) mM H⁺/h, $P < 0.01$. The median volume of gastric juice secreted under basal conditions decreased from 220 (104–340) ml/h to 144 (76–252) ml/h following the eradication of *H pylori*, $P < 0.02$.

However, the peak acid output was unchanged, from 37 (16–59) to 37 (21–59) mM H⁺/h after treatment (Figure 5.2a). Before treatment the patients also secreted a greater proportion of their peak acid output during the basal state. The basal/peak acid output ratio fell from 0.28 (0.07–0.67) to 0.07 (0.03–0.20) following the eradication of *H pylori*, $P < 0.05$ (Figure 5.2b).

a**b****Figure 5.1**

Changes in basal plasma gastrin concentration (a) and basal acid secretion (b) in 9 DU patients following the eradication of *H. pylori*. Bars represent the median values.

a



b

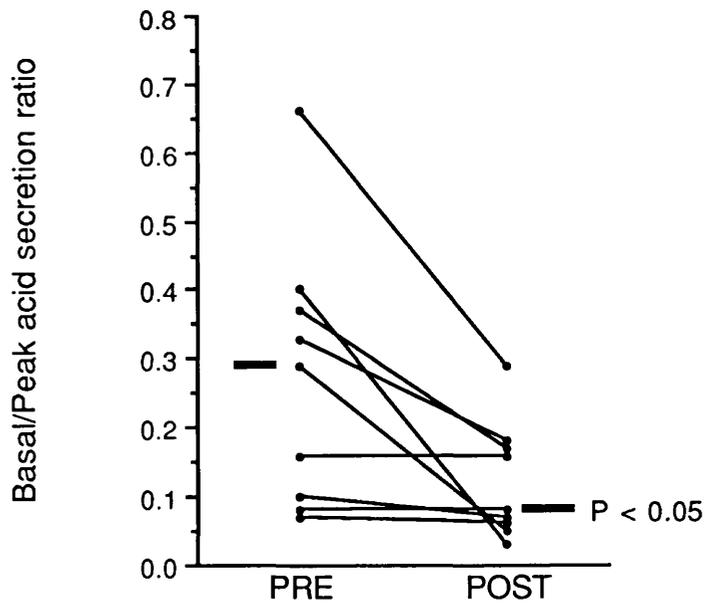


Figure 5.2

Peak acid secretion (a) and basal/peak acid secretion ratio (b) before and after the eradication of *H. pylori*. Bars represent the median values.

Parietal cell sensitivity to gastrin

Figure 5.3 shows that for the nine patients as a group, there was a linear relationship between the mean plasma gastrin during infusion of G-17 plotted on a log scale and the mean acid output at each of the G-17 doses. This relationship was linear both before and after treatment, with correlation coefficients of 0.99 ($P < 0.001$) and 0.98 ($P < 0.005$) respectively. The only difference between the two lines is that after treatment there was a lower mean plasma gastrin concentration and a lower mean acid secretion rate under basal conditions. Consequently, the mean concentration of gastrin necessary to produce half maximal acid secretion (EC_{50}) was identical, both before and after the eradication of *H pylori*.

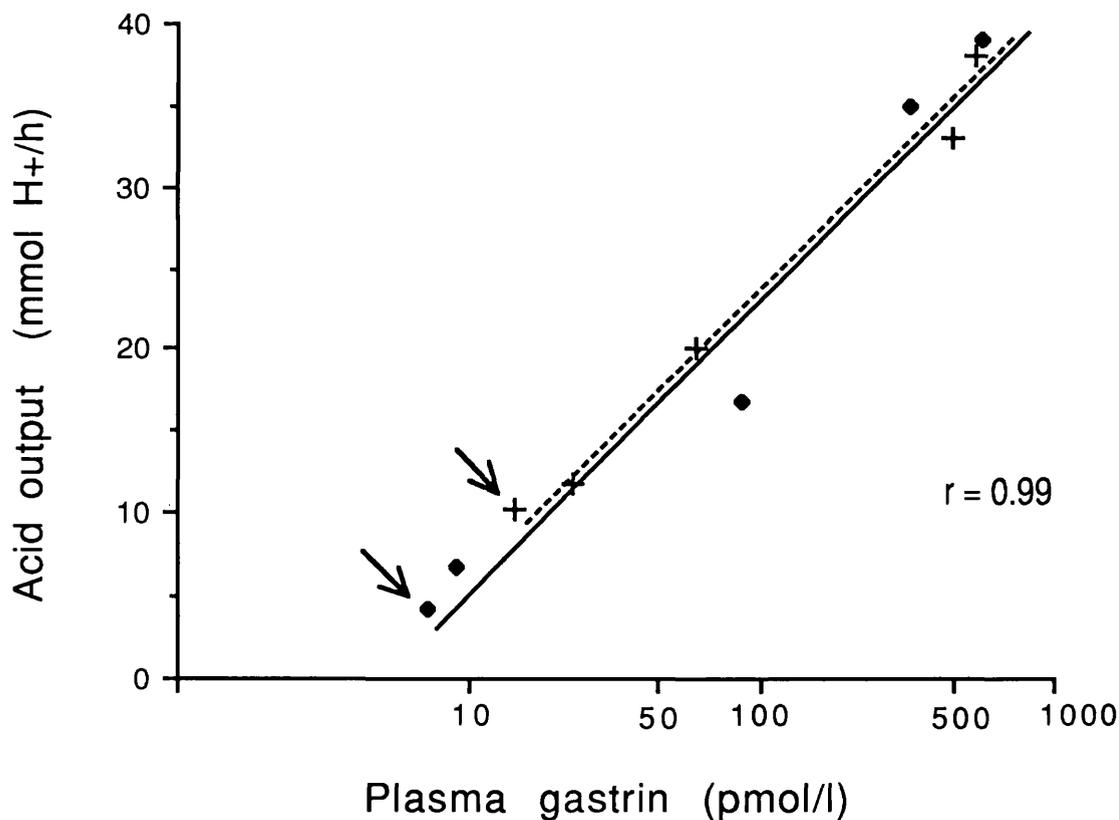


Figure 5.3 Gastrin versus acid dose-response plots before and after the eradication of *H pylori*. The relationship between mean plasma gastrin concentration, plotted logarithmically, and acid output during the last 15-minute period of each G-17 infusion is shown before (---+---) and after (—●—) the eradication of *H pylori*. The values under basal conditions are indicated by the arrows.

Individual dose–response curves for each patient were also plotted. From these the EC_{50} for each patient – rather than the group as a whole – was determined, both before and after eradication of *H pylori*. In the 9 patients in whom *H pylori* was eradicated, the median correlation coefficient of individual regression plot of log dose against response was 0.93 (0.85–0.96) before and 0.94 (0.89–0.99) after treatment. There was no significant difference in the EC_{50} after the eradication of *H pylori*. The median EC_{50} before was 41 (14.8–126) pmol/L and 33 (23–125) pmol/L after eradication.

The basal–subtracted EC_{50} , the concentration of gastrin necessary to increase acid secretion **from basal** to half maximal acid secretion, has been advocated to be a more appropriate index of parietal cell sensitivity (Hirschowitz, 1984). This is because this analysis excludes the effect of changes in basal acid secretion, which is thought to be non–gastrin dependent (Feldman *et al* 1980; Kirkpatrick & Hirschowitz, 1980). We therefore also plotted individual dose–response curves of log plasma gastrin against the **basal–subtracted** acid output. The correlation coefficients for these plots were 0.94 (0.85–0.96) and 0.94 (0.88–0.99) before and after treatment respectively. In the 9 patients in whom *H pylori* was successfully eradicated the median basal–subtracted EC_{50} fell significantly, from 104 (18–171) pmol/L to 48 (26–136) pmol/L, $P < 0.05$.

Metabolic clearance rates of G–17

By comparing the infused G–17 dose with the plasma gastrin response the metabolic clearance rate of G–17 can be calculated by two different methods, as described by Blair *et al* (1986). The clearance rate can be calculated by the reciprocal of the slope of the regression line between G–17 dose and plasma gastrin and by the plateau principle at an infusion rate of G–17 of 300 pmol/kg/h. The metabolic clearance rate of G–17 was 8 (6–93) before and 8 (6–76) ml/kg.min after treatment when calculated by the former method and 9 (6–74) and 8 (6–62) ml/kg.min respectively by the latter method. Therefore we found that the clearance of G–17 was not altered by the eradication of *H pylori*.

DISCUSSION

The results of this study confirm that treatment which both heals DUs and eradicates *H pylori* significantly decreases basal plasma gastrin concentrations. However, this study also shows that such treatment also decreases the basal gastric acid secretion significantly. Thus *H pylori* infection combined with active ulceration may cause the elevated basal acid secretion seen in DU disease.

Previous studies have demonstrated that eradication of *H pylori*, with or without ulcer healing, produces a fall in fasting plasma gastrin concentrations. This change in fasting gastrin is generally small and was only statistically significant in two published studies (McColl *et al*, 1991; Chittajallu *et al*, 1992d). However, despite a fall in the basal plasma gastrin concentration, it had not been shown previously that eradication of *H pylori* can significantly decrease the BAO. Two previous studies in which the BAO has been measured before and after treatment, two in DU patients (Levi *et al*, 1989a; Beardshall *et al*, 1992) and another in dyspeptics (Mountbriand *et al*, 1989), showed no significant change in BAO after anti-*H pylori* therapy. However in these three studies the patients were re-tested within a week of ending therapy so that in some cases infection may have persisted (Weil *et al*, 1988). In another study of parietal cell sensitivity, in this case to pentagastrin, Chittajallu *et al* (1992d) have found, in contrast to us, that the eradication of *H pylori* from DU patients resulted in a fall in fasting gastrin which was **not** accompanied by a fall in BAO. However, the design of their study differs in several ways from the study in this chapter, one of the ways being that they studied the effect of the eradication of *H pylori* from DU patients at a time when they did not have an active ulcer. As was discussed in chapter 2, there is evidence to support the idea that the presence of an ulcer in the duodenum may itself be associated with increased acid secretion, regardless of *H pylori* infection (Achord, 1981). Another difference between our studies and those of other units is that our acid secretion rates are calculated after correction for any pyloric losses or duodenogastric reflux (Baron, 1978). This enables us to obtain a more accurate assessment of acid secretory rates than is possible by merely measuring the uncorrected gastric acid secretion; particularly if, for example, *H pylori* were to have an effect on gastric emptying, as has been suggested recently (Tucci *et al*, 1992).

An alternative explanation for the fall in BAO is that this is due neither to the eradication of *H pylori*, nor the healing of an ulcer but a direct effect of the drugs themselves. Since bismuth may persist in the body after treatment has finished (Gavey *et al*, 1989) it is theoretically possible that it may lower acid secretion one month after the end of a course of treatment. However, this is very unlikely since when a group of DU patients were retested 24 hours after the end of a course of tripotassium dicitratobismuthate, when their ulcers had all healed, the BAO was unchanged from pre-treatment values (Baron *et al*, 1986). There is no published data concerning the long-term effects of the other components of the treatment, tetracycline and metronidazole, on gastric function.

Other evidence supports the idea that the fall in basal acid secretion is due to the eradication of *H pylori*. In the study described in chapter 4, when 6 DU patients in whom *H pylori* had been eradicated were re-tested after 12 months, the median BAO fell by about 40%, although the change was not significant. Fullarton *et al* (1991) observed a fall of about the same degree, 1 month after eradicating *H pylori* from 8 patients with inactive DU disease, but again the change was not statistically significant. In a previous study from our unit it was noted that the BAO was significantly greater in DU patients with *H pylori* than in DU patients without this infection (Levi *et al*, 1989a). However, it is difficult to deduce the 'physiological' effect of *H pylori* on basal secretion from this and other studies in which patients do not act as their own controls, because other differences between the groups might affect acid secretion, as discussed in the introductory chapter (page 40).

It is well established that patients with DU disease, tend to have a higher BAO than their age-matched controls although there is considerable overlap between the two groups (Johnston and Jepson, 1967; Baron, 1978; Achord, 1981; Blair *et al*, 1987). However, in retrospect it is very likely that the DU patients described in these 'pre-*H pylori*' studies were almost always infected with *H pylori* (Dooley & Cohen, 1988) whereas only some of the non-DU controls would have been, perhaps around half, given that they were mainly aged 40-60 years (Graham *et al*, 1988b; Perez-Perez *et al*, 1988). If the studies were repeated now, with all the controls being *H pylori*-negative, perhaps the difference in BAO between the two groups would be more obvious, since the implication from the study described in this chapter is that in

DU patients it is the presence of *H pylori* which may be responsible for the increased basal acid secretion.

Our present findings, in line with other studies which were reviewed in chapter 4, are consistent with the generally accepted view that peak acid output reflects the parietal cell sensitivity and is not altered by the eradication of *H pylori*.

The main purpose of the present study was to determine whether chronic infection with *H pylori* alters the sensitivity of the parietal cells to gastrin. This was suggested by the relative lack of change in rates of acid secretion and intragastric acidity on eradication of *H pylori* despite substantial falls in circulating gastrin concentrations, and by the evidence that *H pylori* may be capable of suppressing the secretion of acid, both *in vitro* (Cave & Vargas 1989; King *et al*, 1992) and, as occasionally reported, on first infection *in vivo* (Graham *et al*, 1988a; Hunt, 1992).

To obtain an index of the sensitivity of the parietal cell to gastrin, gastrin concentration versus acid response plots were constructed and the circulating gastrin concentration required to produce half-maximal acid secretion was determined. This method is superior to constructing gastrin dose-response curves, as performed by others (Chittajallu *et al*, 1992d) since measuring the plasma gastrin concentration reflects what the parietal cell 'sees'. It cannot be assumed that the infused gastrin dose is always proportional to the circulating gastrin concentration. For example, *H pylori* may alter the clearance of gastrin.

It has been debated whether the sensitivity of the parietal cell should be calculated with or without subtraction of the basal acid secretion. It is generally thought that the increased BAO in DU patients is determined by an increased vagal drive rather than an increase in fasting circulating gastrin. Kirkpatrick & Hirschowitz (1980) demonstrated that in DU patients who were selected on account of basal acid hypersecretion, reducing vagal cholinergic afferent input by atropine or surgery resulted in a marked reduction in BAO. In support of these findings, Feldman *et al* (1980) demonstrated that sham feeding, a strong vagal stimulus, did not provoke any extra acid secretion in 4 DU patients with already high BAO. The evidence from these studies, suggesting increased vagal drive in DU patients, is the rationale for determining the basal-subtracted EC_{50} (also referred to as the 'intrinsic' sensitivity) in some previous studies (Isenberg *et al*, 1975; Lam *et al*, 1980; Hirschowitz *et al*,

1985; Hirschowitz, 1984). We found that this parameter, the basal-subtracted EC_{50} , does fall significantly on eradication of *H pylori*, suggesting that the eradication of *H pylori* increased the sensitivity of the parietal cells to gastrin. However, the effect of eradication of *H pylori* on basal gastrin and acid secretion is more consistent with the idea that circulating gastrin is responsible for driving basal acid secretion in DU patients, because the fall in basal gastrin concentration which we observed on eradication was accompanied by a proportional fall in basal acid secretion. Therefore, the EC_{50} (sometimes called the 'apparent' sensitivity (Hirschowitz, 1984), rather than the derived basal-subtracted EC_{50} , is likely to be the most appropriate measure of parietal cell sensitivity and this EC_{50} did not change after healing with eradication of *H pylori*. On reflection it seems more likely that the change in basal-subtracted EC_{50} on eradication plus healing which was observed is probably a consequence of the fall in gastrin-driven basal acid secretion. This is best appreciated when the mean acid output is plotted against mean plasma gastrin concentration for each G-17 infusion (Figure 5.3); the two lines are almost identical when exogenous gastrin is infused. These graphs are similar to those obtained by Hirschowitz *et al* (1985) and by Blair *et al* (1986) who compared G-17 dose-response curves in ulcer and control subjects, after normalising for differences in peak acid secretion rates; the only difference between the two groups of patients was seen under basal conditions.

Our results suggest that in *H pylori*-associated DU disease the increased BAO is primarily driven by gastrin rather than the vagus nerve. They are therefore at variance with the conclusions of the earlier studies (Kirkpatrick & Hirschowitz, 1980; Feldman *et al* 1980). One might speculate that the increased vagal drive described by these workers is present in only a subset of patients with DU disease since the patients that were investigated in those studies were highly selected for extreme basal acid hypersecretion. However, our results suggest that for the majority of patients the BAO is gastrin-driven. It is worthwhile recalling that although it is now clear that *H pylori* increases the release of gastrin, most studies performed prior to this discovery did not find a link between gastrin secretion and DU disease. It may be necessary to control for *H pylori* infection when studying the effect of cholinergic stimulation, to resolve whether basal acid secretion is predominantly driven by the vagus or by gastrin.

Blair *et al* (1986) found that the metabolic clearance rate of G-17 was lower in DU patients than in controls. However, our results, in agreement with those of Eysselein *et al* (1984), do not demonstrate a difference in the clearance of G-17 on eradication of *H pylori*. The results that we obtained for the metabolic clearance rate of G-17 were similar to the results in the DU group in the study of Blair *et al* so that those results cannot be explained by the presence of *H pylori* in the DU patients.

The present results show that healing of duodenal ulcers using a regime that also eradicates *H pylori* does not alter the sensitivity of the parietal cells to circulating concentrations of G-17. However it does produce a fall in basal acid secretion which is associated with and may be due to the concomitant fall in basal plasma gastrin concentration.

**CHAPTER 6 – THE REGULATION OF GASTRIN
SYNTHESIS BY SOMATOSTATIN mRNA IN
PERNICIOUS ANAEMIA**

INTRODUCTION

In animal models, the release and synthesis of gastrin is under the control of the inhibitory peptide somatostatin – gastrin and somatostatin change in a reciprocal fashion. In investigating how *H pylori* increases plasma gastrin concentrations it is therefore important to consider the possibility that *H pylori*-related hypergastrinaemia is secondary to a lack of inhibition of gastrin synthesis and release by somatostatin.

However, as discussed in the introductory chapter, measuring the concentration of somatostatin in the plasma or tissues is inadequate when one is interested in measuring the local release of this paracrine peptide. Studying how somatostatin influences gastrin synthesis in animal models has necessitated the measurement of their respective mRNAs, but there has been no previous work in this area in man.

The work in this chapter describes how I have adapted the RNA extraction and Northern blotting methods used in laboratory animals and applied them to RNA extracted from human endoscopic gastric biopsies. In this way I could quantitate changes in **human** gastrin and somatostatin mRNA for the first time. This chapter describes in detail the methods which I employed to measure gastrin and somatostatin mRNA, initially in a group of patients with hypergastrinaemia due to pernicious anaemia in comparison with normal controls, prior to investigating the comparatively milder degree of hypergastrinaemia in *H pylori* infection which is the subject of chapter 7.

METHODS

Patients and biopsies

Six pernicious anaemia patients and twelve age- and sex-matched normal controls were studied. Pernicious anaemia had been diagnosed by the findings of megaloblastic anaemia, an abnormal part I Schilling test, the presence of gastric autoantibodies and atrophic gastritis with achlorhydria. All the patients with pernicious anaemia were being treated regularly with cyanocobalamin injections. They had a median age of 59 (range 47–78) and a median time from diagnosis of four (1–26) years, three were male and only one had a positive urease test for *H pylori*. The control group comprised patients who were attending the Hammersmith Hospital outpatient gastroenterology clinic for investigation of dyspeptic symptoms but who had normal endoscopic findings and an intragastric pH of 1–3; three of these patients had a positive urease test, 6 were female and they had a median age of 52 (range 27–70). Following investigation the final diagnosis for this group of patients was non-ulcer dyspepsia. None of them were taking any medication or had undergone previous gastric surgery.

Antral biopsies were taken 2 cm proximal to the pylorus with Olympus biopsy forceps, cup size 2 x 5 mm, (KeyMed, Southend-on-Sea, Essex) during videoendoscopy. In a pilot study it was established that five biopsies per patient were necessary to reproducibly yield at least 50µg total RNA. Therefore in this study five biopsies were taken and pooled for RNA extraction, and a further antral biopsy was taken for the diagnosis of *H pylori* infection by the biopsy urease test (McNulty *et al*, 1989).

Measurement of plasma gastrin concentration

The fasting plasma gastrin concentration was determined by radioimmunoassay as described in chapter 2.

Measurement of gastrin and somatostatin mRNA

Notes: All reagents were obtained from Sigma Chemical Company, Poole, Dorset unless otherwise stated. In order to minimise the degradation of RNA by RNAases, sterile gloves were worn throughout the following procedures and all laboratory apparatus was autoclaved prior to use wherever possible. The solutions were made in deionised, distilled water and then autoclaved before use.

Extraction of RNA

Five antral biopsies per patient were collected into 1.2 ml screw-top bio-freeze vials (Costar, Cambridge, MA, USA), snap frozen in liquid nitrogen and stored at -80°C . The frozen biopsy tissue was then transferred swiftly to Fisherbrand 1.5 ml microfuge tubes (Fisher Scientific, Springfield, NJ, USA) immediately prior to extracting RNA. The RNA was extracted using the method of Chomzynski and Sacchi (1987). The tissue was ground in liquid nitrogen and homogenised in 0.5 ml of 'solution D' (4M acid guanidinium isothiocyanate (Fluka Chemicals Ltd, Glossop, Derbys) containing 100mM mercaptoethanol, 0.5% sodium sarcosyl (Fluka) and 25 mM sodium citrate pH 7.0), by the use of a micro tissue grinder (Fisher Scientific, Springfield, NJ, USA). RNA was extracted by sequentially adding and mixing on ice 50 μl 2M sodium acetate pH 4.0, 100 μl chloroform / isoamyl alcohol mixture (49:1 v:v) and 0.5 ml water-saturated phenol; the latter solution was not neutralised. After 10 minutes centrifugation at 4°C at 12 000 rpm in a Micro Centaur centrifuge (MSE Scientific Ltd, Crawley, Sussex) the RNA-containing aqueous phase was collected and the RNA precipitated with 0.5 ml isopropanol at -20°C for 2 hours. Following centrifugation as above, the supernatant was removed by gentle suction through an extruded Pasteur pipette. The RNA pellet was then redissolved in 0.25 ml 4M acid guanidinium isothiocyanate with 100 mM mercaptoethanol and precipitated again, with 0.25 ml isopropanol. This was followed by resuspension and ethanol precipitation (Sambrook *et al*, 1989) and the RNA pellet was finally redissolved in deionised distilled water for quantitation of the total RNA content by the absorbance of ultraviolet light at a wavelength of 260 nm (Cecil CE 202 Spectrophotometer, Cecil Instruments Ltd, Cambridge).

Northern Blotting

10 μg total RNA per patient was denatured in 7.5% formaldehyde, 50% formamide and 0.02 M 3-[N-morpholino] propane-sulphonic acid (MOPS) with 0.1 M sodium acetate and 20 mM EDTA made up to pH 7.0 as a running buffer. The samples were electrophoresed on a 1% agarose, 7.5% formaldehyde gel in the MOPS running buffer over 4 hours. The RNA was transferred by capillarity overnight onto nylon membranes (Hybond N, Amersham, Bucks) and fixed by UV-irradiation for 45 seconds followed by baking for one hour at 80°C. Blots were prehybridized for four hours at 60°C in 0.25 M NaH_2PO_4 buffer pH 7.2, with 5% SDS, 2.5 mM EDTA, 25 μM aurin tricarboxylic acid and 0.5% dried milk powder (Asda Stores Ltd, Leeds). Hybridization with ^{32}P -labelled cDNA probes (about 5 μCi per hybridisation) was performed for 16 hours under the same conditions in a volume of 5 ml. The blots were then washed at 60°C for one hour in a buffer containing 0.25 M Na Phosphate pH 7.2 with 1 mM EDTA and 2% SDS followed by one hour in 0.2 x SSPE (30 mM NaCl, 1.8 mM NaH_2PO_4 , 0.2 mM EDTA) with 0.2% SDS.

Autoradiography was performed at -70°C in an X-ray cassette with an intensifying screen (Genetic Research Instrumentation Ltd, Bishops Stortford, Herts) and presensitised Kodak XAR-5 film, and bands were quantified in arbitrary units by scanning laser densitometry (Cromoscan 3, Joyce Loebel, Gateshead). The filter was originally probed for somatostatin mRNA and then stripped in 10 mM Tris.Cl (pH 7.4) with 1 mM EDTA and 0.2% SDS at 80°C for 10 minutes and reprobbed sequentially for gastrin mRNA and then for 18S ribosomal RNA, the latter to correct for small loading differences between the lanes. Hybridization with the rRNA oligonucleotide was also performed at 60°C in the same buffer as above but with the addition of 0.5 M NaCl and the omission of the second wash. The results are expressed as the ratio of specific gastrin or somatostatin mRNA to ribosomal RNA.

Probes

The gastrin probe was a 385–base Pst I–digested fragment of human gastrin cDNA in Bluescript plasmid (Ito *et al*, 1984), kindly provided by Prof K Agarwal, Chicago. The somatostatin probe was a 153–base PstI–fragment of clone pHS8–86 (Shen *et al*, 1982), from Prof W Rutter, San Francisco.

The probes were subcloned using standard laboratory methods (Sambrook *et al*, 1989). In brief, each cDNA was ligated into bluescript plasmid by T4 DNA ligase (Northumbria Biologicals Ltd, Cramlington, Northumberland) and transformed into a competent XLIB strain of *E. coli*. Successful transformation was confirmed by the growth of a white colony using the blue–white colour selection β –galactosidase assay and a transformed colony was then grown in the presence of tetracycline and ampicillin. The bacteria were then harvested and the DNA was extracted by protoplast lysis with SDS at high pH, followed by precipitation with potassium acetate, removal of RNA with RNAase and precipitation with polyethylene glycol. The bacterial DNA was then digested with the restriction endonuclease Pst I (Amersham International, Amersham, Bucks), separated by electrophoresis and the cDNA band cut from the gel. Following electroelution, the cDNA was extracted by phenol/chloroform, precipitated with ethanol and suspended and stored frozen at -20°C in distilled deionised water. For use as probes, the cDNA was labelled with ^{32}P dCTP (ICN Radiochemicals, Cleveland, OH, USA) by random primed synthesis (Feinberg & Vogelstein, 1983) to a specific activity of 10^9 cpm/ μg , purified by Sephadex G50 chromatography and denatured for hybridization by boiling for two minutes.

The ribosomal probe was a synthetic 30–nucleotide sequence complementary to the 3' terminus of 18S rRNA, labelled with terminal transferase (IBI Inc, New Haven, CT, USA) to give a mean of one addition of ^{32}P dCTP per molecule.

Statistical analysis

Differences between groups were compared by the Mann–Whitney U test and the degree of linear correlation was calculated using the least squares method. $P < 0.05$ was considered to signify statistical significance.

RESULTS

RNA Measurement

40–155 μg total RNA were obtained from the five pooled endoscopic biopsies per patient. Following electrophoresis the RNA samples were undegraded, with clearly visible ribosomal bands on ethidium–stained gels (Figure 6.1). Gastrin and somatostatin mRNA were identified by hybridization with their respective cDNA; each was seen to exist as a single species compatible with the size predicted by their published sequences of approximately 475 and 600 bases respectively (Figure 6.2).

Positive hybridization signals from the gastrin cDNA probe were visible after 16–64 hours whereas the somatostatin signal was seen only after a 2–3 week exposure, implying that the gastrin mRNA was much more abundant than somatostatin mRNA. Differences in mRNA abundance between the pernicious anaemia patients and the controls were seen for both gastrin mRNA and somatostatin mRNA (figures 6.3 & 6.4). The median gastrin mRNA/rRNA ratio was 10.4 (range 3.7–38.0) in the pernicious anaemia patients, compared with 1.7 (0.7–8.3) in the controls, $P < 0.02$. Somatostatin mRNA levels were altered in a reciprocal fashion; the median somatostatin mRNA/rRNA ratio was 0.84 (0.58–2.32) in the pernicious anaemia group and 2.04 (0.05–6.47) in the controls, $P < 0.05$.

Plasma gastrin concentrations

The median fasting plasma gastrin concentration in the five patients with pernicious anaemia was 640 (420–3500) pmol/L compared with 5 (2–58) pmol/L in the control group, $P < 0.001$. There was a strong positive correlation between the gastrin mRNA/rRNA ratio and the plasma gastrin concentration (figure 6.5), $r = 0.93$, $P < 0.0001$.

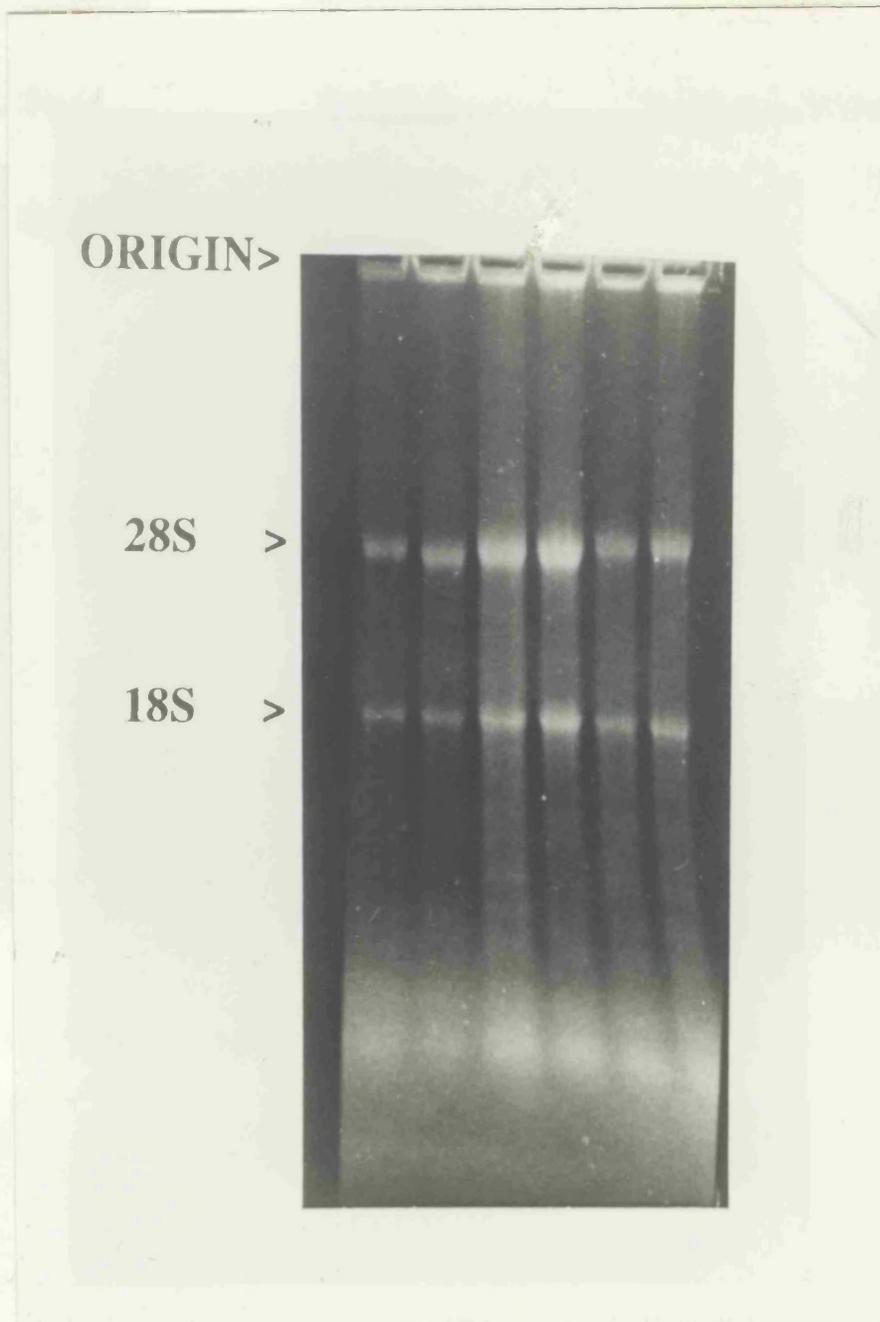


Figure 6.1

Photograph of an ethidium-stained formaldehyde-agarose gel of electrophoresed 10 μg samples of RNA, viewed under ultraviolet light. The 18S and 28S rRNA bands are clearly visible.

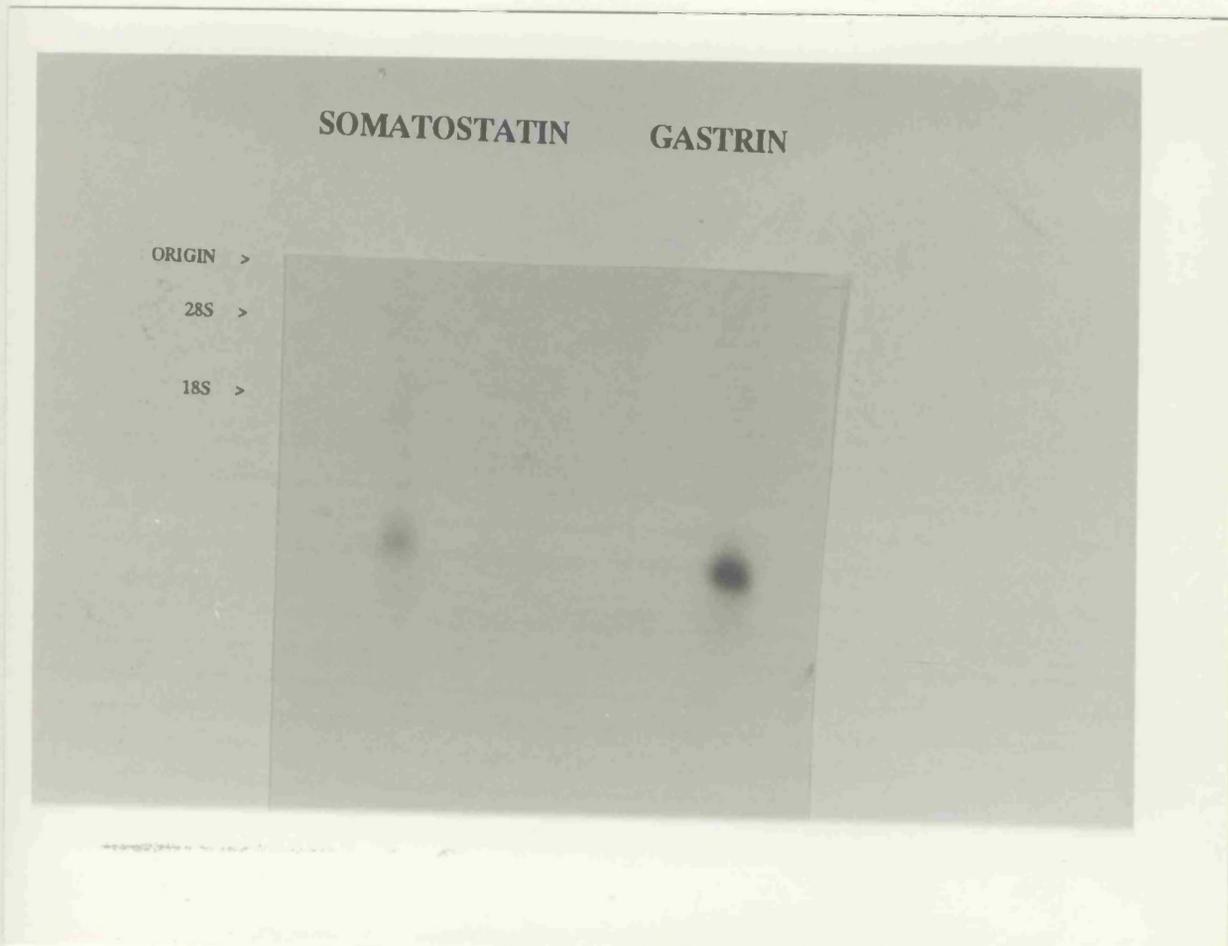


Figure 6.2

Figure 6.2

Representative autoradiogram of Northern hybridization of RNA.

Autoradiograph of a Northern blot obtained from two RNA samples, probed with (left) somatostatin and (right) gastrin cDNA probes. The positions of the origin and the ribosomal bands have been marked.

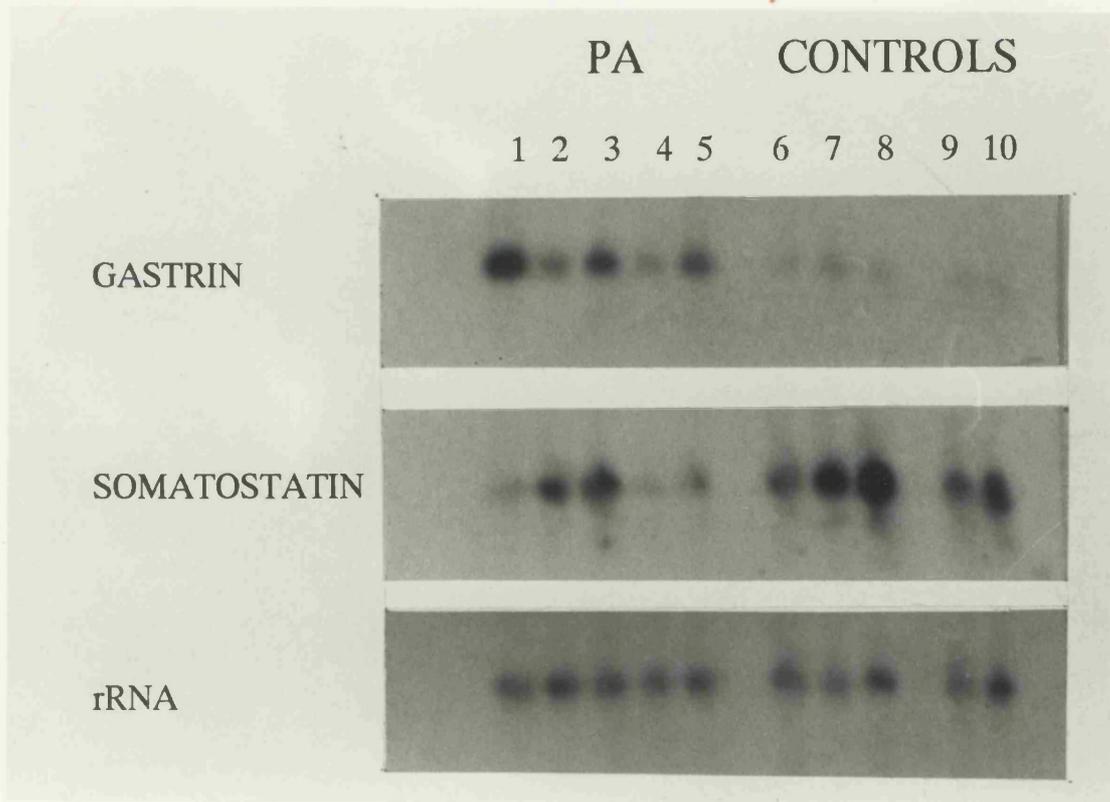


Figure 6.3

Representative autoradiogram of Northern hybridization of RNA samples from 5 patients with pernicious anaemia and 5 non-ulcer dyspepsia patients. The probes used were (top panel) gastrin cDNA, (middle) somatostatin cDNA and (bottom) an 18S rRNA oligonucleotide.

Figure 6.4

Quantifying the mRNA:rRNA ratio of gastrin (left) and somatostatin (right). The median value for each group of patients is shown as a horizontal line.

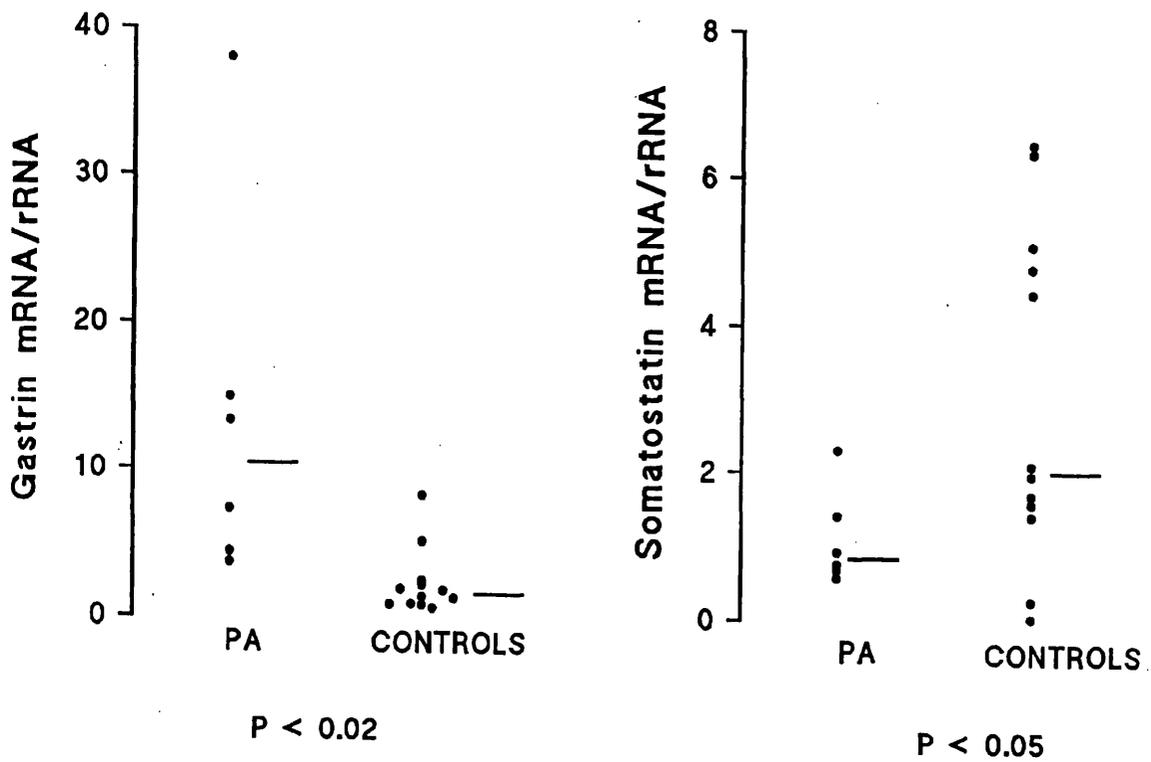
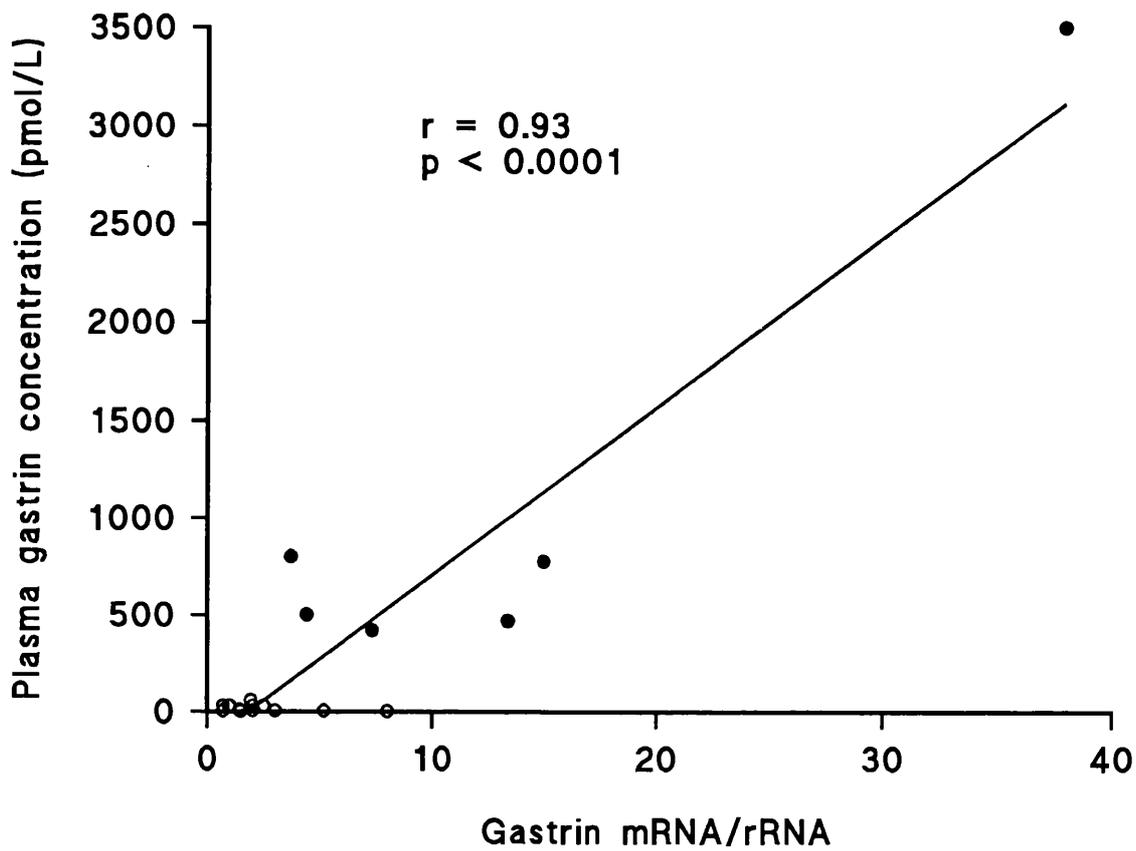


Figure 6.4

Scattergram demonstrating the mRNA/rRNA ratios of gastrin (left) and somatostatin (right). The median value for each group of patients is shown as a horizontal line.

**Figure 6.5**

Relationship between gastrin mRNA as determined by the gastrin mRNA/ rRNA ratio and the plasma gastrin concentration in 6 pernicious anaemia patients (closed circles) and 12 controls (open circles).

DISCUSSION

This is the first demonstration in man of the identification and quantitation of mRNAs encoding gastric regulatory peptides. The study has shown that patients with pernicious anaemia who secrete no acid have increased levels of antral gastrin mRNA, as well as increased circulating gastrin concentrations due to increased peptide release. This increase in G-cell function is accompanied by a decrease in somatostatin mRNA, suggesting that the local release of somatostatin peptide is also decreased in pernicious anaemia.

These findings are consistent with the idea that the increase in gastrin release associated with high luminal pH is due to decreased inhibition of the G-cells by somatostatin in man, as appears to be the case in laboratory animals. In animal isolated perfused organ models, luminal acid stimulates the release of somatostatin from specialised somatostatin-secreting cells (D-cells) within the gastric antrum and this leads to a fall in the release of gastrin (Yamada & Chiba, 1989; Schusdziara *et al*, 1978; Holst *et al*, 1983). However, somatostatin, a potent inhibitor of gastric secretion in general, inhibits adjacent G-cells over a short distance (paracrine inhibition) so that, as discussed in the introductory chapter, page 23–25, studying the inhibition of gastrin by somatostatin has been further investigated in animals by measuring somatostatin mRNA and the effect of blocking the actions of somatostatin with monoclonal antibodies. In the rat it is now clear that gastrin and somatostatin mRNA change in a reciprocal fashion in response to starving (Wu *et al*, 1991) and to the intragastric neutralisation produced by the parietal cell proton pump inhibitor omeprazole (Brand & Stone, 1988; Dockray *et al*, 1991). Somatostatin appears to inhibit the synthesis of gastrin by binding to a membrane receptor and then interacting through the inhibitory G protein, G_i, with a regulatory element just upstream of the epidermal growth factor-responsive gastrin promoter (Bachwich *et al*, 1992). Somatostatin also inhibits gastrin synthesis by increasing the turnover of gastrin mRNA once it has been transcribed, thus decreasing the amount available for translation (Karnik & Wolfe, 1990).

In this study a strong correlation between the gastrin mRNA/rRNA ratio and the plasma gastrin concentration was evident. However, the quantitative rise in circulating peptide (100-fold) was of a greater degree than the rise in the gastrin mRNA/rRNA ratio, which increased approximately eight times. It is interesting to note that a similar discrepancy was seen in omeprazole-treated rats after 12 hours fasting (Dimaline *et al*, 1991) but not in rats treated with omeprazole but with free access to food, who have elevations of gastrin mRNA and plasma gastrin of comparable degrees (Dockray *et al*, 1991). The results in our pernicious anaemia patients, who were necessarily fasting for endoscopy, are therefore similar to the rat model of omeprazole-induced achlorhydria and suggest that fasting decreases antral gastrin mRNA to a greater degree than the plasma gastrin concentration when the gastric contents are neutral. In this situation the gastrin mRNA is not so closely linked to the rate of release as it is in the fed state (Dockray *et al*, 1991). A change in mRNA half-life, in post-translational processing of the peptide or in clearance of the secreted peptide could all account for these differences.

Our results show that despite significant differences in somatostatin mRNA, gastrin mRNA and the plasma gastrin concentration, there was some overlap between the two groups of patients for both gastrin mRNA and somatostatin mRNA. A degree of overlap between the controls and pernicious anaemia patients was also observed in a morphological study of G-cell and D-cell numbers (Arnold *et al*, 1982) and may be due to different degrees of atrophy and intestinal metaplasia in individual patients; these histological changes are quite common in the age group we have studied (Morson *et al*, 1990). An additional confounding factor is the presence or absence of *H pylori*, which, as we shall see in the following chapter, decreases somatostatin mRNA (Moss *et al*, 1992). Three of our controls were positive for *H pylori* and all had a somatostatin mRNA/rRNA ratio below the median level for the control group. Of the pernicious anaemia patients, only one had a positive test for *H pylori*, in line with the low incidence of *H pylori* infection in these patients (Fong *et al*, 1991).

The elevated gastrin mRNA in our pernicious anaemia patients is accompanied by a relatively small decrease in somatostatin mRNA. It is possible that the decrease in somatostatin is sufficient to explain the rise in gastrin mRNA since second messenger systems may amplify the effects of a small change in the expression of this inhibitor of gastrin. However, there is some evidence to suggest that the hypergastrinaemia may be due to gastrin-releasing factors present in the juice of patients with pernicious anaemia, which increase

gastrin release **independent** of luminal pH (Deprez *et al*, 1991) and presumably independent of somatostatin. Therefore there may be more than one mechanism responsible for the increased gastrin synthesis which we have observed.

The present findings show that in pernicious anaemia there are reciprocal changes in gastrin and somatostatin mRNA. This supports the idea that a lack of local somatostatin release is at least in part responsible for the increased antral gastrin expression in this condition.

**CHAPTER 7 – *H PYLORI* AND ITS EFFECT ON
ANTRAL SOMATOSTATIN**

INTRODUCTION

H pylori increases the plasma gastrin concentration, but how it does so is not clear. Thus far, studies investigating whether *H pylori* influences gastrin release through an alteration of juxtamucosal pH or through the action of pro-inflammatory cytokines have been inconclusive. The work described in this chapter investigates whether *H pylori* increases the release of gastrin through changes in somatostatin.

As described in the introductory chapter, somatostatin is a strong inhibitor of gastrointestinal secretory function in general and gastrin release in particular, so that *H pylori* may conceivably increase gastrin through decreasing the inhibition of gastrin release and synthesis by somatostatin. This chapter describes how I investigated whether the eradication of *H pylori* is accompanied by a change in antral somatostatin activity in DU patients. Because of the problems inherent in the measurement of somatostatin in the plasma or mucosa by radioimmunoassay, two complementary biopsy-based methods were used to assess the antral somatostatin activity. These were quantitative immunocytochemistry of the somatostatin and gastrin secreting-cells, and quantitative Northern blotting for somatostatin and gastrin mRNA. The immunocytochemical staining identifies the cells which produce gastrin and somatostatin and the peptide mRNA levels are an index of local synthesis rates and provide a surrogate measure of local release.

D) THE EFFECT OF ERADICATING *H PYLORI* ON THE DENSITY OF G AND D-CELLS.

METHODS

Patients, biopsies and treatment

We recruited 21 patients with an active DU into this part of the study. The inclusion and exclusion criteria were as described in chapter 2 and the determination of *H pylori* infection was established as described in chapter 5. Fourteen of the patients were male and the age range was 24–68 years (median 44). When a DU was diagnosed at endoscopy, antral biopsies were taken 2 cm proximal to the pylorus using forceps with a cup size of 2 x 5 mm. Three biopsies were taken for the diagnosis of *H pylori* infection by histology, urease testing and bacterial culture and in addition, two antral biopsies were taken for quantitative immunocytochemistry. The patients were then treated with tripotassium dicitratobismuthate (DeNol) 120 mg qds for 1 month, with metronidazole 400 mg tds and tetracycline 500 mg qds taken concurrently for the first two weeks. Four weeks after the end of treatment the patients were endoscoped again and biopsies taken as before. Successful eradication of *H pylori* was presumed to have occurred if all the tests were negative at the follow-up endoscopy.

Quantitative immunocytochemistry

Antral biopsies were fixed in Bouin's solution and processed to wax. 5 µm sections were cut and stained with haematoxylin and eosin and well-orientated adjacent sections were then immunostained with polyclonal rabbit anti-human antibodies to gastrin (Polak *et al* 1973) and somatostatin (Polak *et al* 1978) by the peroxidase anti-peroxidase technique (Sternberger, 1979). Cells which stained positive for gastrin (G cells) and for somatostatin (D cells) were then counted in a minimum

of 3 high power fields per biopsy by Dr Anne Bishop (Department of Histochemistry, Royal Postgraduate Medical School) without reference to the clinical histories. The results are expressed as the number of positive cells per mm length of muscularis mucosae in accordance with the consensus paper on quantitative endocrine histochemistry (Solcia *et al*, 1988).

Statistical analysis

Statistical analysis was performed for the patients in whom *H pylori* was successfully eradicated. Wilcoxon's signed ranks (matched pairs) test was used to examine the effect of eradication of *H pylori*. The results are expressed as medians with ranges.

RESULTS

H pylori was successfully eradicated from 18 patients (11 males, median age 47 years, range 30–68) and this was accompanied by healing of the ulcers in all cases. Three patients (all male, median age 31 range 24–36) remained infected with *H pylori* and were excluded from further analysis. The typical appearance of an immunostained section from a patient prior to treatment is shown in figure 7.1. After the eradication of *H pylori* there was a significant increase in the somatostatin cell density, from a median of 9 (range 3–47) cells per mm muscularis mucosae to 19 (6–57), $P < 0.05$ (Figure 7.2a). In contrast, the density of G cells did not change significantly following treatment, from a median of 104 (18–175) before to 117 (20–159) after treatment, $P = 0.45$ (figure 7.2b). The G:D cell ratio decreased from 8.8 (2.8–57) to 4.0 (2.2–12) following the eradication of *H pylori*, $P < 0.001$.

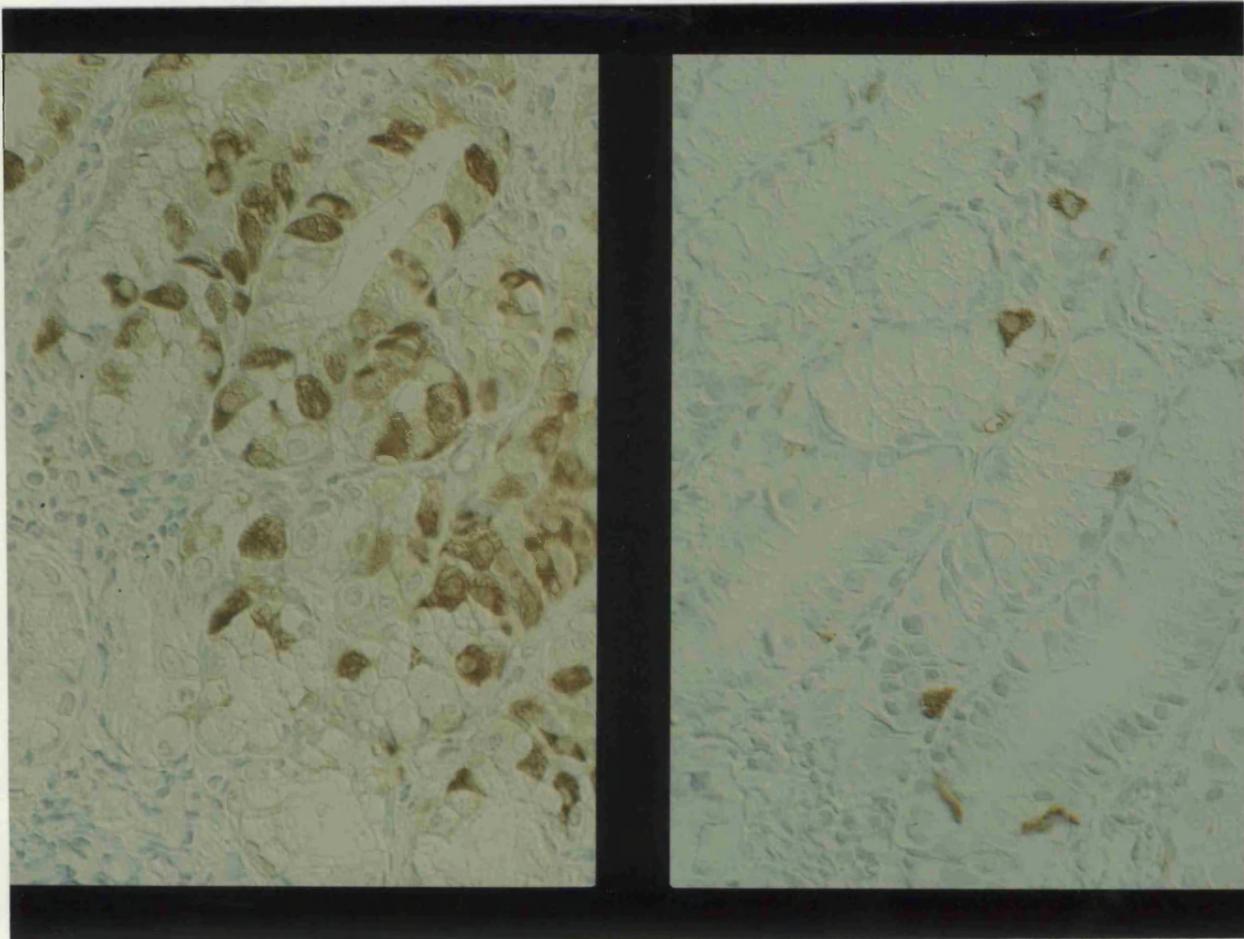
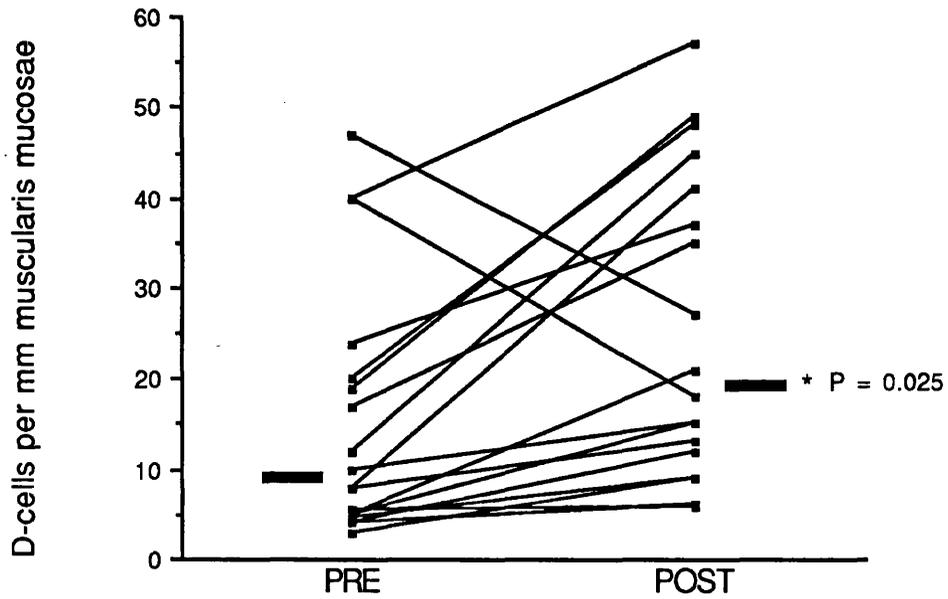


Figure 7.1 Sections of a gastric antral biopsy immunostained for (left) G-cells and (right) D-cells. The biopsy, which was taken from a DU patient before the eradication of *H pylori* shows numerous G-cells with relatively few D-cells. Original magnification x 200.

Figure 7.2 Scattergrams showing the densities of antral D-cells (a) and G-cells (b) before and after successful treatment (a) eradicate *H pylori*. The x-axis values are shown as bars.

a



b

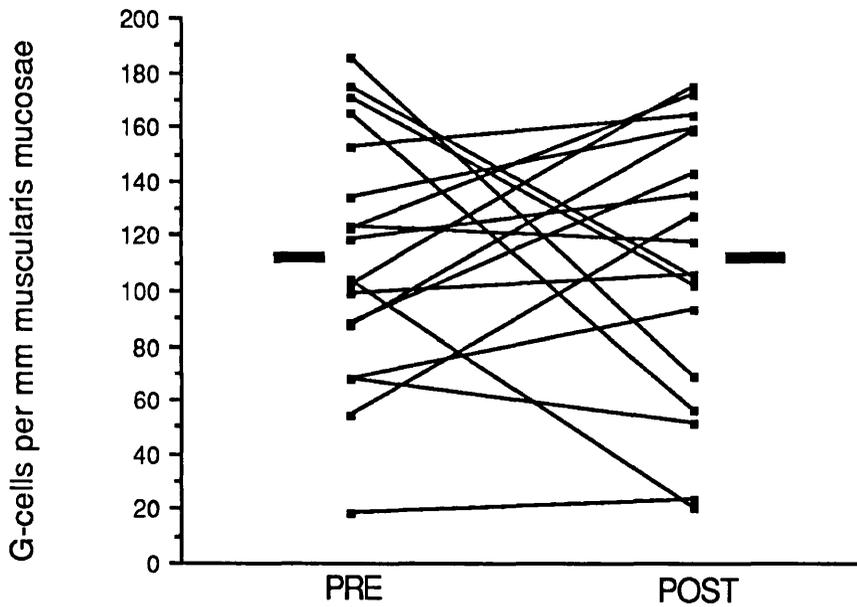


Figure 7.2 Scattergrams showing the densities of antral D-cells (a) and G-cells (b) before and after successful treatment to eradicate *H pylori*. The median values are shown as bars.

II) THE EFFECT OF ERADICATING *H PYLORI* ON GASTRIN AND SOMATOSTATIN mRNA.

METHODS

Fifteen patients with active DU disease were recruited into this arm of the study. The patients were aged 20–76, median 41, 12 were male. The inclusion and exclusion criteria for entry into the study, the methods used to diagnose *H pylori* infection, the treatment given and the timing of the endoscopies were identical to those in the immunocytochemistry study. At each endoscopy in addition to the three antral biopsies which were taken for the identification of *H pylori*, a further five biopsies were taken from sites 2 cm proximal to the pylorus and snap frozen in liquid nitrogen for the measurement of gastrin and somatostatin mRNA as described in chapter 6. To avoid the intra-assay variation inherent in the mRNA analysis, paired samples from each patient were run on adjacent gel lanes. Statistical analysis of the results are as for part I of this study.

RESULTS

Following treatment, *H pylori* was eradicated from 10 patients (8 males, median age 47, range 20–76). In these 10 cases the endoscopic appearances were also normal at follow-up. Five patients (4 males, median age 37, range 22–58) were still infected with *H pylori* following treatment.

The yield of total RNA from each set of 5 biopsies was at least 30 µg per patient. A representative autoradiograph of the effect of *H pylori* eradication in 4 patients is shown in Figure 7.3. Following the eradication of *H pylori* somatostatin mRNA increased significantly, from a median somatostatin mRNA/rRNA ratio of 50 (mean 61, range 25–160) to 95 (97, 40–180), $P = 0.01$, (Figure 7.4a). The somatostatin mRNA/rRNA ratio increased to a median of 164% (171, 81–245) of the pre-treatment level. Gastrin mRNA did not change significantly, from a median gastrin mRNA/rRNA ratio of 36 (58, 19–220) before to 41 (44, 7–118) following treatment, $P = 0.28$, (Figure 7.4b).

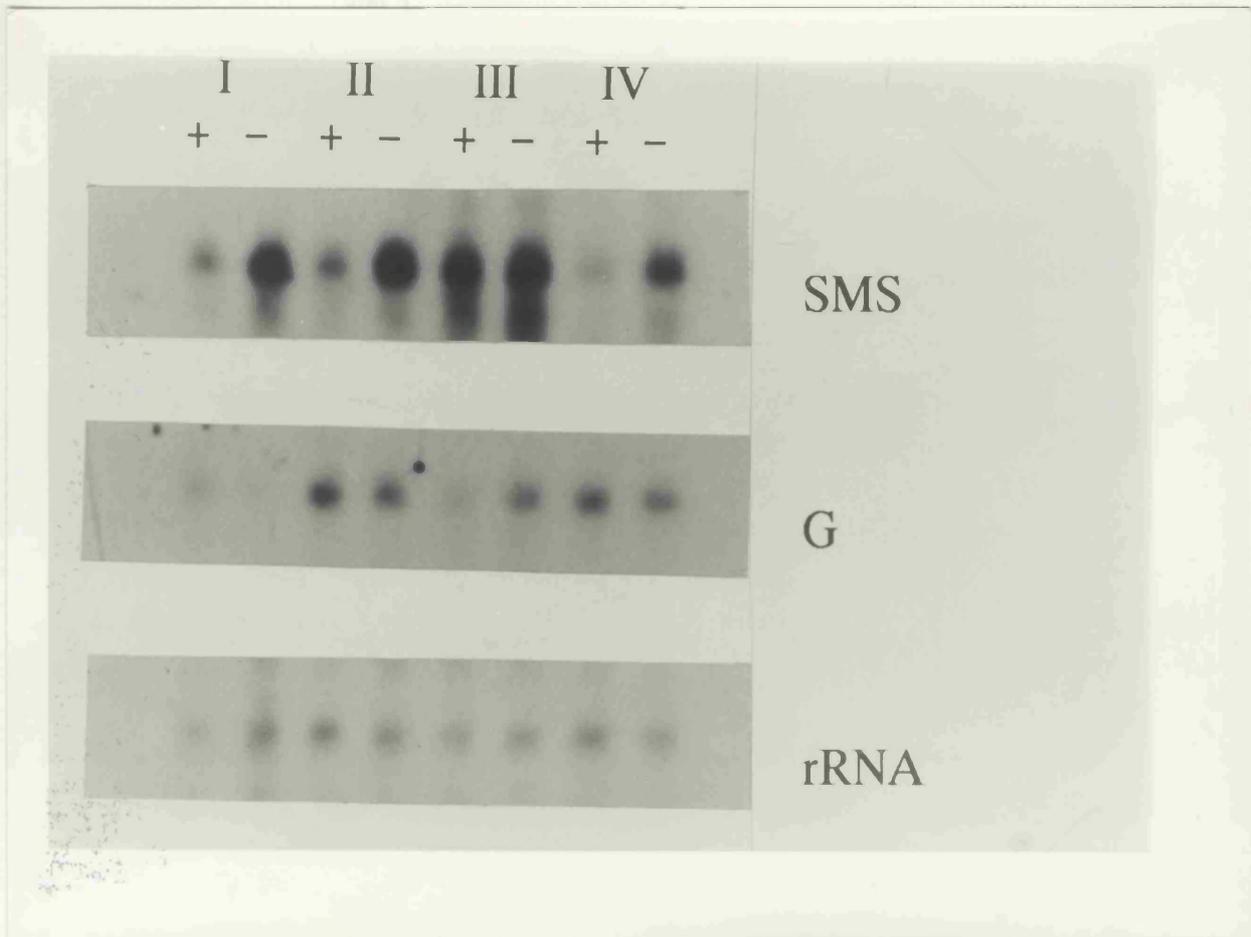


Figure 7.3

Representative autoradiograph from a Northern blot of RNA from 4 patients, represented by Roman numerals, before (+) and after (-) the eradication of *H. pylori*. The blots were probed for somatostatin mRNA (top panel), gastrin mRNA (middle panel) and 18S ribosomal RNA (bottom panel).

Figure 7.4

Scattergrams showing the somatostatin mRNA/rRNA ratio (left) and the gastrin mRNA/rRNA ratio (right) before and after successful treatment to eradicate *H. pylori*. The median values are shown as bars.

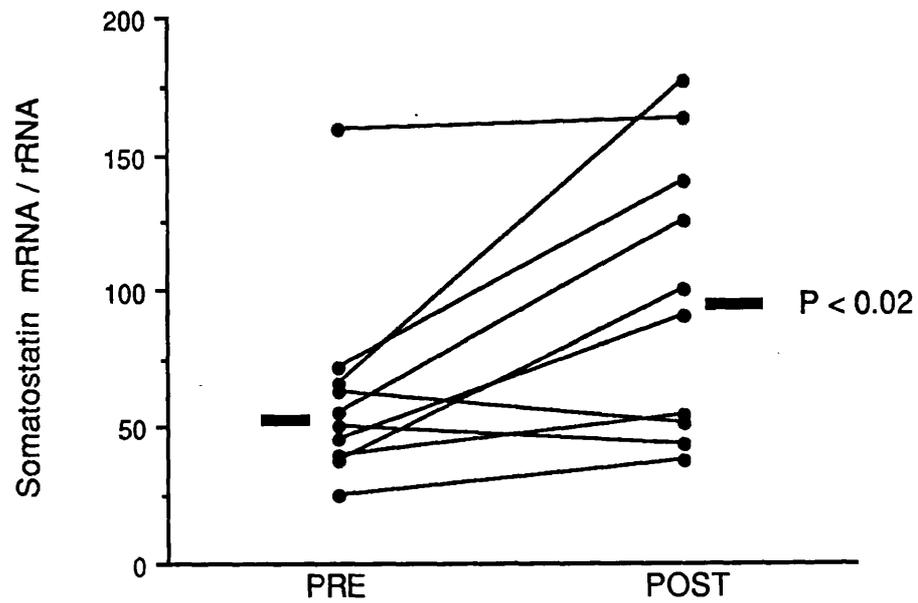
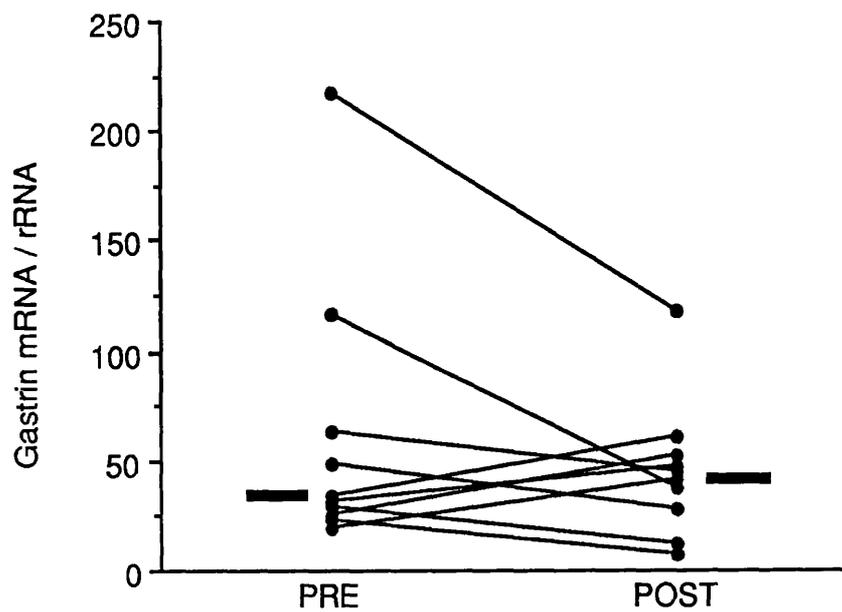
a**b**

Figure 7.4 Scattergrams showing the somatostatin mRNA/rRNA ratio (a) and the gastrin mRNA/rRNA ratio (right) before and after successful treatment to eradicate *H pylori*. The median values are shown as bars.

DISCUSSION

These results show that treating DU patients with drugs which eradicate *H pylori* is associated with an increased number of antral somatostatin-immunoreactive cells (D-cells) and a corresponding increase in the amount of antral somatostatin mRNA, and therefore by implication, synthesis and release of gastric somatostatin peptide.

The fall in antral somatostatin activity may explain the increased gastrin release which is characteristic of infection with *H pylori* infection. However, this study also demonstrated that the gastrin mRNA/rRNA ratio did not fall after eradication of *H pylori*. This may be because the biopsies were necessarily taken after overnight fasting, which inhibits gastrin synthesis. *H pylori* has most effect on plasma gastrin after meals; the effect on basal levels is small and not always significant (Levi *et al*, 1989a, Levi *et al*, 1989b, Graham *et al*, 1990). Under basal conditions most circulating gastrin is G-34 (Lamers *et al*, 1982), which probably derives from the duodenum (Berson & Yalow, 1971; Malmstrøm *et al*, 1976; Calam *et al*, 1980) so that the synthesis of gastrin by the antrum when fasting is likely to be relatively small. The relatively insensitive methods which we used may therefore miss a small change in gastrin mRNA following eradication.

The lack of change in gastrin cell numbers after eradication in this study has also been found by others (Graham *et al*, 1991a; Queiroz *et al*, 1992). However, Sankey *et al* (1990) found that in biopsies from patients infected with *H pylori* the G-cell immunostaining was more intense compared with *H pylori* negative tissue implying that *H pylori* infection was associated with an increase in the stored peptide. The decreased D-cell density in the presence of *H pylori* has also been recently described in a study of *H pylori*-positive gastritis patients (Murthy *et al*, 1992) and an increase in D-cell density following eradication therapy has also been demonstrated by Queiroz *et al* (1992). It is interesting to note that other studies, mainly from the pre-*H pylori* era, failed to demonstrate that DU had a deficiency of D-cells. (Polak *et al*, 1978; Arnold *et al*, 1982; Gutierrez *et al*, 1986; Hacker *et al*, 1990). The recent

finding that somatostatin immunoreactivity in gastric mucosal biopsies is decreased by *H pylori* (Haruma *et al*, 1992; Kaneko *et al*, 1992) is in accordance with our findings, although measuring extracted tissue levels of somatostatin immunoreactivity obviously reflects stored rather than secreted peptide.

We found an increase in somatostatin in most individuals. However, in 4 of the 28 DU patients the opposite trend was observed. It is possible that there may be genuine heterogeneity among DU patients and that these patients comprise a subgroup who have duodenal ulceration unrelated to *H pylori* or a deficiency of somatostatin. However, these patients, 2 male and 2 female, had a median age of 44 years (range 38–58) and were clinically indistinguishable from the other patients who were studied. Two of these 4 patients had very high pre-treatment somatostatin-immunoreactive cell densities, which fell considerably following treatment. This raises the possibility that the initial biopsies in these individuals may not have been truly representative of the situation throughout the antrum. We have attempted to minimise the effect of possible differences in somatostatin within the gastric antrum by pooling biopsies from 5 sites per patient when measuring RNA. However, for the measurement of somatostatin and gastrin cell densities, 3 fields per biopsy were examined but from only 2 sites per patient, so that sampling variation may have been responsible for these discordant results. Nevertheless, for the group as a whole, there was a significant increase in antral somatostatin after treatment, both in terms of D-cell density and somatostatin mRNA.

The most likely explanation for this result is that *H pylori* decreases gastric antral somatostatin activity. The somatostatin mRNA, the D-cell density and the D:G cell ratio all increased about two-fold after the eradication of *H pylori* suggesting that following treatment there was an increased number of normally functioning D-cells. This could be achieved initially through hyperfunction of the reduced D-cell population, followed by hyperplasia; time-course studies will be necessary to answer these issues. It is tempting to speculate that *H pylori* infection of the duodenum and gastric corpus and fundus may also decrease the D-cell populations in these sites, resulting in increased duodenal gastrin synthesis and a direct increase in the secretion of gastric acid respectively.

It is necessary to consider alternative explanations for the results of this study, unrelated to the eradication of *H pylori*. For example, the change in somatostatin may be an effect of the triple therapy unrelated to its anti-*H pylori* action. Similar hypotheses were evoked to explain the prolonged remission from duodenal ulceration and the fall in plasma gastrin after anti-*H pylori* treatment, although it is now clear that they are due to the eradication of the organism rather than to a component of the treatment. In the study by Queiroz *et al* (1992) *H pylori* was eradicated with a different drug regime; metronidazole, amoxicillin and furazolidone and yet the increase in somatostatin cell density which they found was comparable to our results. Perhaps the increase in antral somatostatin that we have observed following the eradication of *H pylori* is a consequence of ulcer healing *per se*, rather than the eradication of *H pylori*? Since we measured somatostatin in the gastric antrum, rather than in the duodenum where the ulcer is, the change in somatostatin is most likely to reflect the effect of eradication of antral *H pylori*. However, it is conceivable that the increase in somatostatin may be a consequence of a duodeno-antral reflex, mediated through nerve endings or regulatory peptide. Studying patients treated with various drug regimes which heal ulcers but do not eradicate *H pylori* will be necessary to fully determine which mechanism is responsible for the increase in somatostatin which we have observed.

The results of this study suggest that *H pylori* infection results in a decrease in somatostatin expression which may explain the exaggerated gastrin response. In fact, the decrease in somatostatin mRNA due to *H pylori* infection appears to be of an even greater magnitude than the increase in plasma gastrin. *H pylori* infection decreases somatostatin activity by approximately 50% which, as I have shown in chapter 6, might be expected to be accompanied by a rise in plasma gastrin of 10–100 fold. It is likely that other factors may be involved in limiting the gastrin response to the fall in somatostatin mRNA.

The fact that there is a decrease in somatostatin expression associated with *H pylori* infection does not demonstrate how the organism increases the release of gastrin. However, the findings are consistent with the hypothesis that *H pylori* has its initial effect upon the D-cell, either through a direct action, by altering the juxtamucosal pH or through the action of pro-inflammatory cytokines and that the G-cell response is secondary to this.

CHAPTER 8 – CONCLUSIONS

The hypothesis that an exaggerated gastrin response might be the missing link between *H pylori* infection and recurrent duodenal ulceration is an attractive one. However, when this idea was first proposed (Levi *et al*, 1989a), it was clear that this small study of DU patients raised more questions than answers. In this thesis I have investigated both ends of the 'gastrin link' – to see how *H pylori* might increase the gastrin response and to establish whether the increased gastrin response is of clinical importance – does it increase the secretion of gastric acid?

I first studied a group of patients with duodenal ulceration who were treated with sucralfate (chapter 2). The group was chosen as a control for the group of patients in whom the gastrin response was diminished after the suppression of *H pylori* (Levi *et al*, 1989b). I found that treatment with sucralfate had no effect on either *H pylori* infection or the gastrin response and, in addition, that the degree of antral inflammation was unchanged. Therefore this study did not shed light upon whether *H pylori* infection or inflammation *per se* was responsible for the exaggerated gastrin response. However, it did lead to the serendipitous discovery that when DU patients were treated with sucralfate there was a fall in basal acid secretion; this may be of importance in understanding how this commonly-prescribed drug works. Although the fall in BAO is likely to be related to the increase in bicarbonate secretion produced by sucralfate, it remains possible that it is merely an accompaniment of ulcer-healing, a subject to which I shall return. Further studies will be necessary to determine which of these mechanisms is responsible for the phenomenon, but if sucralfate treatment does lead to a long-term fall in BAO then this may explain the reports of its beneficial effect on ulcer recurrence.

In chapter 3, I showed that the *H pylori*-related increased gastrin response was independent of luminal pH, arguing against the role of the ammonia generated by *H pylori*-being responsible for the increased gastrin release through changing the juxtamucosal pH. The study was also designed to address the issue of whether the presence of *H pylori* was responsible for the increased meal-stimulated acid secretion reported in DU patients at low luminal pH (Walsh *et al*, 1975) but we found no significant change in pH-dependent acid secretion after the eradication of *H pylori*.

However, Tarnasky *et al* (1991), by stimulating acid secretion for longer did find a difference in acid secretion at low luminal pH between *H pylori* infected and non-infected healthy volunteers. Repeating a long intragastric titration in DU patients before and after the eradication of *H pylori* will be necessary to finally resolve whether the presence of *H pylori* increases acid secretion at low luminal pH.

The finding that the PAO remains high a year after successful treatment to eradicate *H pylori* (chapter 4) argues against the high PAO found in DU patients being secondary to the trophic effects of the mild elevation in plasma gastrin due to *H pylori*. However, this study suggested that the eradication of *H pylori* may be accompanied by a fall in BAO.

Further evidence that the 'gastrin link' is of clinical importance was found from the study described in chapters 5. Here I demonstrated, for the first time, that in DU patients the eradication of *H pylori* is accompanied by a fall in basal acid secretion. This finding may explain how the treatment to eradicate *H pylori* leads to a lower rate of ulcer recurrence. This discovery obviates the need to explain the apparent dissociation between gastrin and acid secretion; changes in the parietal cell sensitivity to circulating gastrin had been proposed but were not found in this study. The fall in BAO and basal plasma gastrin also suggests that in DU patients the BAO is mainly driven by gastrin, contrary to the evidence based upon a subgroup of patients with very high BAOs, that this was due to an excessive vagal cholinergic drive. However, the study could also be interpreted as demonstrating that ulcer healing itself lowers the BAO. Studying the effects of ulcer healing with treatments which do not influence *H pylori* or acid secretion and examining the effect of *H pylori* eradication from patients without ulcers will be needed to finally resolve these issues.

The final two chapters investigated whether somatostatin is involved in the regulation of gastrin in man, to see whether changes in gastric somatostatin could explain why patients with *H pylori* infection had an excessive gastrin response. Because of the difficulties involved in the measurement of the local release of this paracrine peptide, I adapted the Northern blotting methods, which had formerly been used in laboratory animals, to measure somatostatin and gastrin mRNA in man; extracting and measuring peptide mRNA had not previously been performed from human endoscopic biopsies. By adapting conventional RNA techniques for the small

amount of tissue which could be taken at biopsy, I was able to demonstrate that pernicious anaemia patients with high plasma gastrin concentrations had relatively high gastric antral mRNA and a relatively low somatostatin mRNA, suggesting that somatostatin is involved in the regulation of gastrin synthesis and release by luminal pH in man.

In chapter 7 I found that a similar depletion of somatostatin occurs in *H pylori* infection in DU patients – the eradication of *H pylori* led to a two-fold increase in both somatostatin mRNA and D-cell density, implying that the exaggerated gastrin release produced by *H pylori* may be secondary to the depletion of somatostatin from the antral mucosa. In understanding how *H pylori* alters gastric function we should therefore turn our attention away from the factors which increase gastrin release and towards those which inhibit the release of somatostatin. Might *H pylori* itself produce substances which alter the growth, differentiation or function of the D-cells? The depletion of somatostatin is consistent with the initial hypothesis that alkalization is responsible for the exaggerated gastrin release, but it still does not exclude the possibility that inflammatory mediators are responsible. In practical terms it has proven very difficult to disentangle the effect of inflammation from that of infection by *H pylori*. The *in vitro* evidence for gastrin release by inflammatory cytokines is still scanty and has not always been reproduced by different laboratories. We need to study antritis **not** associated with *H pylori*, but models which involve non-steroidal anti-inflammatory drugs or alcohol as the cause of antritis are unsuitable since these agents can also directly influence cytokine synthesis and acid secretion respectively.

Another important question is whether *H pylori* decreases somatostatin in the proximal stomach. Fundic somatostatin mediates the inhibition of acid secretion by GRP (Schubert *et al*, 1991) and a lack of fundic somatostatin may explain the greatly increased acid secretion which has recently been shown to occur in *H pylori*-infected DU patients in response to GRP (El-Omar *et al*, 1992).

Despite the increase in somatostatin I found that the number of G-cells and gastrin mRNA did not change significantly after the eradication of *H pylori*. This may be because under fasting conditions the changes were too small to be detected. Alternatively, changes in the expression of **duodenal** gastrin may be more important in the basal state – these questions will need to be addressed in the future.

The discovery of the relationship between gastrin and *H pylori* has stimulated many groups throughout the world to consider whether *H pylori* causes ulcer through alterations in gastric physiology. By exploring the mechanisms involved in detail, my studies have shown that the 'gastrin link' is plausible – *H pylori* infection in DU patients appears to deplete antral somatostatin and the resultant increased plasma gastrin is associated with increased basal acid secretion. It is tempting to speculate that this is how *H pylori* causes duodenal ulcers.

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APPENDIX

Reprints of published papers arising from this work

Moss SF, Thomas DM, Ayesu K, Levi S, Calam J. Sucralfate diminishes basal acid output without affecting gastrin, *Helicobacter pylori*, or gastritis in duodenal ulcer patients. *Aliment Pharmacol Therap* 1992; 6: 251-8.

Moss S, Playford RJ, Ayesu K, Li SK, Calam J. pH dependent secretion of gastrin in duodenal ulcer disease: effect of suppressing *Helicobacter pylori*. *Digestion* 1992; 52: 173-8.

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Sucralfate diminishes basal acid output without affecting gastrin, H. pylori or gastritis in duodenal ulcer patients

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SUMMARY

Twelve patients with active duodenal ulcer disease and *Helicobacter pylori* infection were treated with 1 g sucralfate q.d.s. for 1 month. Ulcers healed in 8 of the 12 patients without an alteration in the *H. pylori*-associated antral gastritis. Sucralfate produced a significant fall in basal acid output in all the patients, from a median of 4.8 (range 2.1–12.1) to 1.6 (0.4–8) mmol/h, $P < 0.01$, whereas peak acid output was unchanged from 41 (21–59) before to 38 (24–55) mmol/h after treatment. Basal plasma gastrin concentrations and the meal-stimulated integrated gastrin response were not altered significantly by sucralfate: 8 (2–17) pmol/L and 732 (188–1045) pmol. min/L pre-treatment and 6 (2–17) pmol/L and 600 (140–1302) pmol. min/L post-treatment, respectively. The fall in basal acid output observed may contribute to prolonged duodenal ulcer remission after treatment with sucralfate.

INTRODUCTION

Recurrence of duodenal ulcers is associated with *Helicobacter pylori* infection,¹ and

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low relapse rates after treatment with tripotassium dicitrato bismuthate² have been attributed to the anti-*H. pylori* effect of this drug.³⁻⁵ How *H. pylori* causes relapses is not known but its suppression decreases fasting and postprandial plasma gastrin concentrations⁶⁻⁸ and this might contribute to prolonged remission by tending to diminish gastric acid secretion.⁷ In contrast histamine H₂-receptor antagonists tend to elevate plasma gastrin concentrations, and it has been suggested that this contributes to earlier relapse after this treatment is stopped.⁹ Sucralfate heals duodenal ulcers at a similar rate to H₂-antagonists.¹⁰ However the average duration of remission after sucralfate, as after bismuth, is longer than following treatment with H₂-antagonists.¹¹ The reason for this is unclear.

Sucralfate is thought to heal ulcers through its cytoprotective effects, but there is also evidence that it decreases the antral inflammation^{12,13} which, according to Wyatt, may be involved in HP-associated gastrin release.¹⁴ We therefore examined the effect of sucralfate on *H. pylori*, antral inflammation, circulating gastrin and gastric acid secretion in patients with duodenal ulcer disease.

METHODS

Patients

The study was approved by the Hammersmith Hospital Research Ethics Committee. Twelve patients, 8 males and 4 females, aged 35–75 years (mean 54 years) were recruited from the gastroenterology outpatient clinic; informed consent was obtained. They all had active uncomplicated duodenal ulceration and *Helicobacter pylori* infection diagnosed by a biopsy urease test and histology. None had any complicating medical condition or previous history of gastric surgery, or had taken antibiotics or bismuth preparations in the previous 6 months. No patient took histamine-H₂ receptor antagonists during the 14 days before endoscopy and acid and gastrin responses were measured within 7 days of endoscopy. Patients were then treated with 1 g sucralfate q.d.s. for four weeks and endoscopy with antral biopsies, acid and plasma gastrin estimations were repeated within 3 days of the end of treatment.

Acid and gastrin responses

Patients attended the clinical investigation unit after fasting overnight. Blood was collected through an indwelling venous cannula for basal plasma gastrin measurement and then a nasogastric tube was passed and the tip positioned in the dependent part of the stomach. After aspirating the resting juice, acid output was measured under basal conditions and for one hour during maximal stimulation with pentagastrin, 6 µg/kg/h i.v., with correction being made for pyloric losses by phenol red recovery.¹⁵ Following extubation and a 20-min rest period the patients ate a standard meal consisting of 2 eggs, 2 slices dry toast and 2 beef stock cubes in 120 ml warm water. Blood was collected at 10-min intervals for 1 h in order to measure the integrated plasma gastrin response to the meal.

Gastrin measurement

Blood was collected into chilled tubes containing EDTA and the plasma separated promptly and frozen at -20°C . Plasma gastrin concentration was measured by radioimmunoassay using antibody G179 kindly provided by Professor Bloom.¹⁶ All samples were assayed in duplicate.

Histological grading

At each endoscopy 2 antral biopsies were taken with Olympus biopsy forceps, cup size 5×2 mm, and sections were stained with haematoxylin and eosin. All biopsies were examined by a single histopathologist (DMT) who was unaware of the clinical details. The number of *Helicobacter* organisms, polymorphonuclear leukocytes and chronic inflammatory cells present were then graded using a simple scoring system ranging from 0 (none) to 3 (numerous), in order to assess both the extent of *Helicobacter* infection and the degree of gastritis.¹³

Statistical analysis

Wilcoxon's matched-pairs test was used. The results are expressed as medians (ranges).

RESULTS

Ulcer healing

All 12 patients completed the study. Duodenal ulcers healed in 8 of the patients after one month of treatment with sucralfate.

Acid output

Basal acid output fell in all patients after treatment with sucralfate. Outputs fell from a median of 4.8 (2.1–12.1) to 1.6 (0–4.8) mmol/h, $P < 0.01$, (Figure 1). In contrast, there was no significant change in the peak acid output. This was 41 (21–59) mmol/h initially and 38 (24–55) mmol/h following treatment (Figure 2).

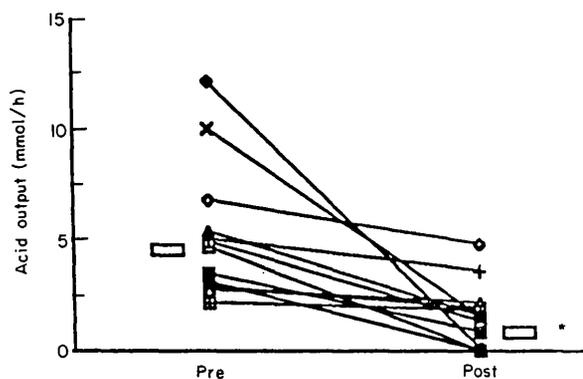


Figure 1. Basal acid output before and after 1 month of treatment with 1 g sucralfate g.d.s. in 12 duodenal ulcer patients. Bars represent medians. * $P < 0.01$.

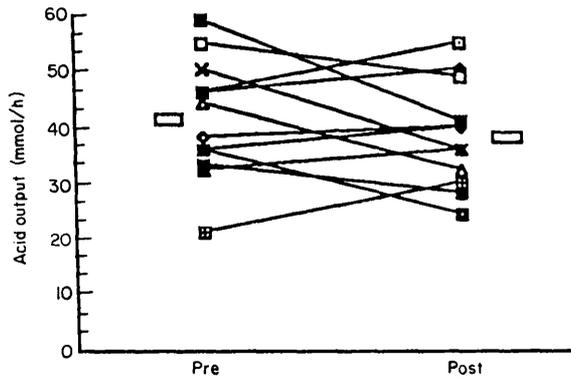


Figure 2. Peak acid output before and after 1 month of treatment with 1 g sucralfate q.d.s. Bars represent medians.

The basal gastric volume output fell from 92 (56–180) to 72 (24–128) ml/h under basal conditions and from 240 (104–190) to 200 (152–156) ml/h during pentagastrin stimulation. The falls in the volumes of gastric juice secreted were not statistically significant.

Plasma gastrin

Figures 3 and 4 demonstrate that basal and meal-stimulated plasma gastrin concentrations did not change significantly following sucralfate therapy. Before treatment the basal concentration was 8 (2–17) pmol/L and the 1-integrated meal response 732 (188–1045) pmol. min/L, these were 6 (2–17) and 600 (140–1302), respectively, after therapy.

Helicobacter infection

The biopsy urease rest remained positive in 11 of the patients. The single patient with a negative urease test at the end of treatment had *H. pylori* infection (grade 1)

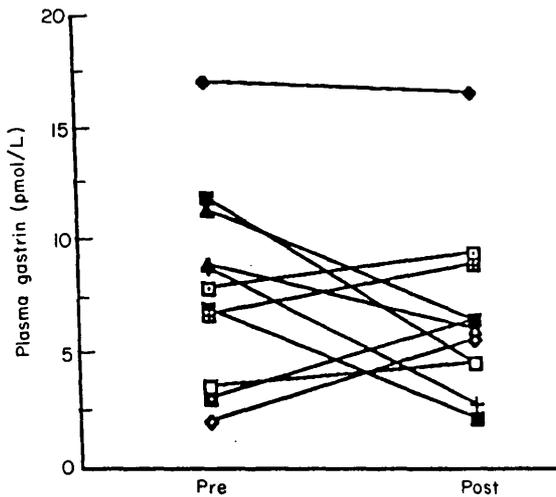


Figure 3. Basal plasma gastrin concentration: the effect of treatment with 1 g sucralfate q.d.s. for 1 month in 12 duodenal ulcer patients.

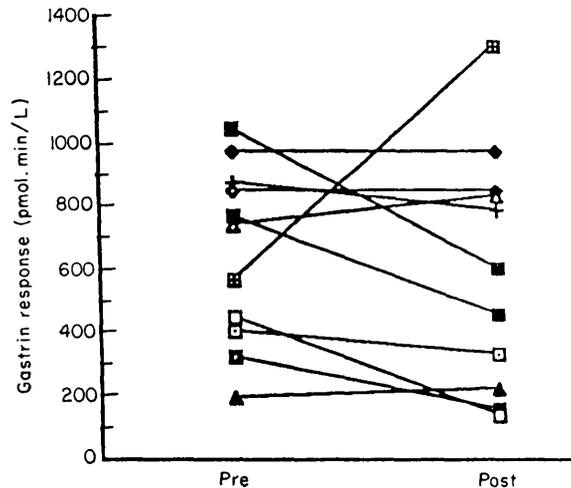


Figure 4. One-hour integrated plasma gastrin response to a standard meal before and after 1 month of sucralfate therapy in 12 duodenal ulcer patients.

histologically. Sucralfate had no significant effect on the extent of *Helicobacter* infection assessed by histology. The median score per biopsy was 1.0 (1–2) before and 1.0 (0–2) after therapy. The *H. pylori* density decreased in five patients and increased in two.

Histological gastritis

The median neutrophil infiltrate was 1.0 (1–3) before and 2.0 (0–3) after therapy, the scores for chronic inflammatory cells were 2.0 (1–3) and 2.0 (1–3), respectively. The median total gastritis scores were 3.0 (2–5) pre- and 3.5 (0–5) post-treatment and improved in only three patients, worsening in five. Thus antral gastritis did not decrease after sucralfate therapy.

DISCUSSION

The results of this study show no apparent effect of sucralfate on *H. pylori* status, antral inflammatory infiltrate, or plasma gastrin, but a consistent and significant fall in basal acid secretion after treatment of patients with duodenal ulcer disease with sucralfate. Basal acid secretion was initially within the range found in untreated duodenal ulcer disease¹⁵ and treatment reduced the rate of secretion to about one-third of initial values. The coefficient of variation of basal acid secretion is around 50%¹⁵ so that small changes in the measurement of secretion might be artefactual. However, our study has demonstrated a significant and considerable fall in basal acid output in all 12 patients, implying that the change is a real effect of sucralfate. Diminished basal acid output after sucralfate was also noted incidentally in a study of parietal cell sensitivity by Marks *et al.*¹⁷ Patients with duodenal ulcer disease tend to have increased basal acid secretion which has been implicated in ulcero-

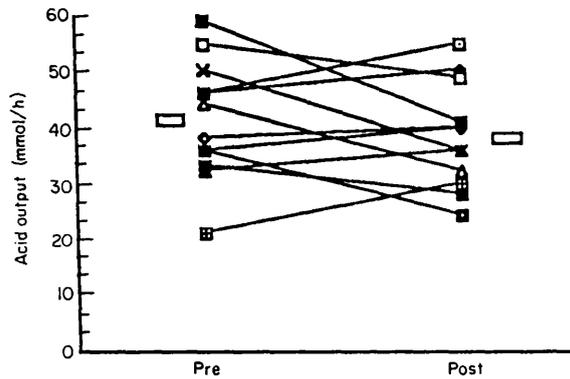


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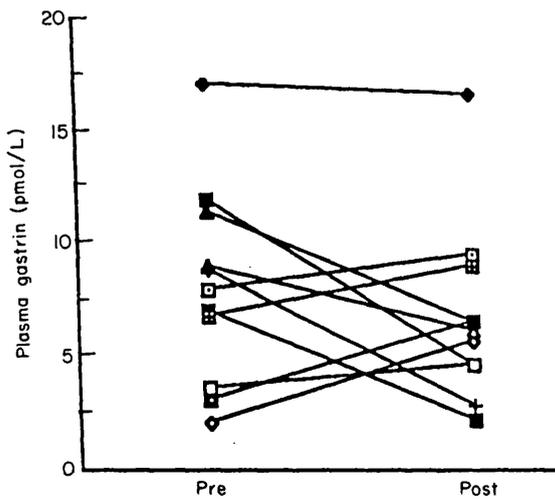


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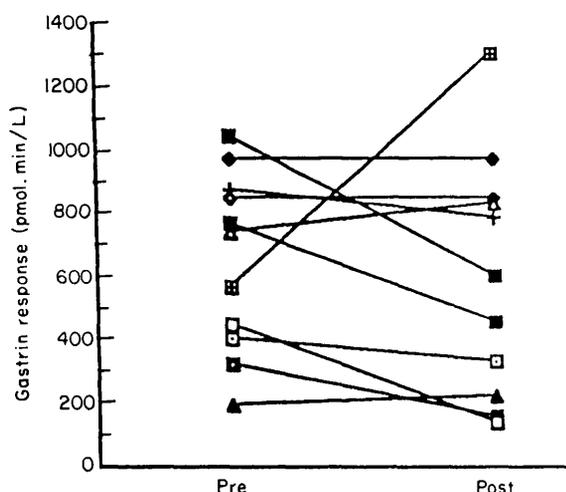


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genesis.¹⁸ Therefore the fall in basal acid secretion produced by sucralfate may contribute to its therapeutic effect.

How sucralfate affects acid secretion remains open to speculation, and gastrin concentrations did not change. However measured acid output inevitably reflects acid secreted minus gastric secretion of bicarbonate, and sucralfate is known to increase the latter.^{19,20} Shorrock *et al.* found that perfusion of the human stomach with sucralfate 8 mg/ml for 1 h increased gastric bicarbonate secretion by about 0.5 mmol/h, from 1 to 1.5 mmol/h.¹⁹ In our study the basal acid output fell by two thirds but the basal gastric volume output was diminished by only around 20%. This suggests that the apparent decrease in titratable acid output may have been chiefly due to increased bicarbonate secretion neutralizing the gastric acid. If the fall in acid output were due to reduced acid secretion, a corresponding fall in the volume of secretion would be expected. The mechanism of increased bicarbonate secretion by sucralfate is unclear. The stimulation of human gastric bicarbonate secretion by sucralfate was not inhibited by indomethacin suggesting that prostaglandins are not involved.¹⁹ However, sucralfate does increase the synthesis of prostaglandin E₂,^{19,21} and prostaglandins of this type do inhibit gastric acid secretion²² which may contribute to the fall in basal acid secretion. We did not observe a change in peak pentagastrin-stimulated acid output after treatment with sucralfate for 4 weeks, whereas Marks *et al.* recorded a small but significant fall in histamine-stimulated acid secretion¹⁷ after a 6-week course of therapy.

The 4-week rate of duodenal ulcer healing of 67% in the present study was as expected from larger studies.¹¹ The lack of effect of sucralfate on *H. pylori* that we observed is consistent with the findings of Barbara *et al.*¹² and Rauws *et al.*,²³ but not with those of Hui *et al.*¹³ who reported a significant fall in the number of organisms after sucralfate. Using a simple histological scoring system we found no effect of sucralfate on antral inflammatory cell infiltration, in agreement with Rauws *et al.*²³ who quantified gastritis by the degree of cellular infiltration, intraepithelial polymorphonuclear leukocytosis and superficial erosions. This finding is in contrast to the diminished gastritis observed by Hui *et al.*¹³ who used a similar scoring system to Rauws, and by Barbara and coworkers¹² who assessed gastritis by neutrophil infiltration only. We are unable to offer an explanation for these different results.

In view of the lack of change in *H. pylori* status and inflammation it was to be expected that sucralfate would not alter gastrin release. In contrast, antisecretory therapy with H₂-antagonists or proton pump inhibitors is accompanied by a rise in the plasma gastrin concentration. It has been suggested that this might contribute to earlier relapses after these forms of therapy by causing rebound hypersecretion,^{24,25} but the evidence for this is not conclusive.⁹

It seems likely that the fall in basal acid secretion that we have observed contributes to the therapeutic effect of sucralfate. It will be interesting to determine the mechanism and duration of this effect and whether it contributes to prolonged remissions after sucralfate therapy.

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pH-Dependent Secretion of Gastrin in Duodenal Ulcer Disease: Effect of Suppressing *Helicobacter pylori*

Key Words

Peptic ulcer
Gastrins

Abstract

Patients with duodenal ulcers and *Helicobacter pylori* infection have elevated plasma gastrin concentrations which fall after suppression of the organism. This may be due to *H. pylori* elevating the pH of the antral mucous layer, therefore preventing luminal acid from inhibiting gastrin release. To test this idea, we measured the plasma gastrin concentrations under basal conditions and in response to 4% peptone when the gastric lumen was maintained at pH 2.5 and at pH 5.5 by gastric perfusion. We studied 11 duodenal ulcer patients before and after suppression of *H. pylori*. Gastrin concentrations were significantly higher before suppression of *H. pylori* than after treatment in all three states; basal gastrin (pmol/l) fell from 9.2 (3.7-23, median and range) to 5.1 (1.7-15) after treatment; from 11.3 (3.8-29) to 5.9 (5.7-6.1) at pH 2.5 and from 15.2 (3.9-32) to 7.15 (6.1-14) at pH 5.5. The ratio of peptone-stimulated gastrin at pH 2.5/pH 5.5 was similar before (0.8; 0.5-1.7) and after (0.8; 0.5-1.1) suppression of *H. pylori*. These results indicate that infection with *H. pylori* increases basal and peptone-stimulated plasma gastrin concentrations, and that this response is independent of luminal pH.

Duodenal ulcer disease is strongly associated with infection of the gastric antrum by *Helicobacter pylori* [1], but it is not clear how this organism causes duodenal ulcers. It may

damage the duodenum directly after colonising patches of gastric metaplasia [2], or alternatively *H. pylori* might act in the stomach, altering its function in such a way as to pro-

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mote duodenal ulceration. In support of the latter, duodenal ulcer patients with *H. pylori* infection have elevated basal and meal-stimulated plasma gastrin levels and peak acid output [3]. In addition, *H. pylori* infection in asymptomatic young men results in an elevated 24-hour gastrin profile [4]. Plasma gastrin levels fall after eradication or suppression of *H. pylori* [5–9], confirming the effect of the organism on gastrin release.

It is not clear how *H. pylori* increases gastrin release. We proposed that the ammonia that it produces might increase the pH of the antral microenvironment, thus preventing luminal acid from inhibiting the release of gastrin. If this was the case, we would expect the effect of *H. pylori* on gastrin to be greater when the luminal pH is acid than when it is near neutral. To test this, we measured plasma gastrin concentrations during stimulation of the stomach with 4% peptone solutions at pH 2.5 and at pH 5.5 before and after suppression of *H. pylori*. The gastric pH was kept constant by titration with alkali, and the meal was perfused through the stomach by a method that we have previously used to avoid the effects of gastric distension [10–12].

Methods and Patients

Patients

The study was approved by the local ethics committee and 11 patients, 8 males and 3 females aged 23–76 years (median 46), were recruited from the outpatient gastroenterology clinic. Informed consent was obtained. All had active uncomplicated duodenal ulceration and *H. pylori* infection diagnosed by a biopsy urease test as described by McNulty et al. [13] and histological staining with haematoxylin and eosin at entry. None had any complicating medical condition or previous history of gastric surgery, and patients who had taken antibiotics or bismuth preparations in the previous 6 months or histamine H₂-antagonists in the previous 2 weeks were excluded.

Following gastric perfusion, which was performed within 7 days of endoscopy, patients were treated with tripotassium dicitrato bismuthate (DeNol) 120 mg q.d.s. for 4 weeks together with metronidazole 400 mg t.d.s. and amoxicillin 500 mg t.d.s. for the first 2 weeks. Endoscopy and gastric perfusion were then repeated within 1 week of the end of treatment.

Gastric Perfusion

After an overnight fast, two basal blood samples were taken for plasma gastrin measurement and a triple-lumen nasogastric tube was positioned so that its tip lay in the gastric antrum. The patients' abdomen remained at 45° to the horizontal throughout. Residual gastric juice was manually aspirated via a multi-fenestrated sump tube and discarded. 100 ml of isotonic 4% peptone (Sigma, Poole, UK) at pH 2.5 were then perfused over 5 min through the stomach from a lumen opening 20 cm proximally, in the proximal gastric body, and manually aspirated via the second lumen to achieve rapid equilibration of pH. Isotonic 4% peptone, pH 2.5, was then infused at a rate of 4 ml/min for 60 min, and the gastric contents constantly aspirated from the antrum under 20 mm Hg negative pressure. The nasogastric tube was aspirated manually every 10 min to ensure tube patency, the third lumen was for equilibration of intragastric and atmospheric pressures. The pH of the aspirate was monitored continuously and sufficient isotonic sodium bicarbonate was added to the infusion port to maintain the gastric contents at pH 2.5. The mean (\pm S.E.) volume of bicarbonate infused was 80 \pm 15 ml before and 49 \pm 10 after treatment. At the end of the 1st h, the stomach was washed with 100 ml isotonic 4% peptone at pH 5.5, and the titration repeated at an intragastric pH of 5.5. The volumes of bicarbonate added at this pH were 103 \pm 20 before and 83 \pm 15.6 ml after treatment, respectively.

In a previous study, we used the same gastric perfusion technique in 7 normal volunteers aged 21–45 years [10]. More than 87% of the gastric infusate was recovered in the aspirate, as estimated by polyethylene glycol dilution.

Gastrin Measurement

Two venous blood samples were taken for basal gastrin measurement 10 min apart and further samples were taken at 10-min intervals for the last 40 min of each hour of gastric perfusion, when a steady state had been established. Blood was collected through an indwelling venous catheter into chilled tubes containing

EDTA, centrifuged, and the plasma separated promptly and frozen at -20°C . Plasma gastrin was measured by radioimmunoassay using antibody G179 kindly provided by Prof. Bloom [14]. All samples were assayed in duplicate.

Statistical Analysis

As the data were not normally distributed, Wilcoxon's paired rank sum test for non-parametric data was used. The results are expressed as medians and ranges.

Results

There was complete healing of duodenal ulceration, and *H. pylori* became undetectable by histology and the biopsy urease test in 10 of the 11 patients. In 1 male aged 45, *H. pylori* infection and duodenal ulceration persisted, he was excluded from further analysis.

The basal plasma gastrin concentrations decreased significantly after treatment, from a median of 9.2 (range 3.7–23) to 5.1 (1.7–15) pmol/l, $p < 0.05$ (fig. 1). After suppression of *H. pylori*, there was also a significant decrease in the gastrin response to peptone, at an intragastric pH of 2.5 and at pH 5.5. The median plasma gastrin concentration at pH 2.5 fell from 11.3 (3.8–29) to 5.9 (5.7–6.1) pmol/l ($p < 0.05$) after suppression of *H. pylori*; at pH 5.5, the fall was from 15.2 (3.9–32) to 7.2 (6.1–14) pmol/l, $p < 0.05$.

Before treatment, 4% peptone at pH 2.5 produced a significant rise in plasma gastrin with a further significant increase during peptone infusion at pH 5.5, $p < 0.05$ in each case (fig. 1). After *H. pylori* suppression, median gastrin levels followed a similar pattern but plasma gastrin concentration were only significantly greater than the basal levels during perfusion with peptone at pH 5.5 ($p < 0.05$). The ratio of gastrin release at pH 2.5 compared with that released at pH 5.5 was 0.8 (0.5–1.7) before treatment and 0.8 (0.5–1.1) afterwards. Therefore, *H. pylori* suppression

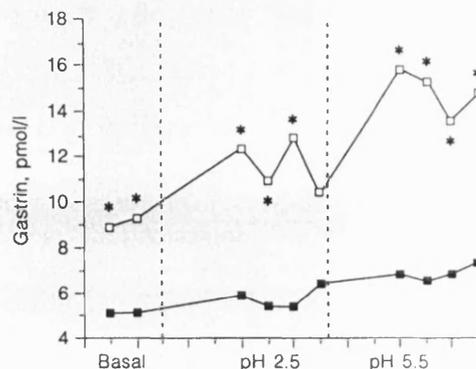


Fig. 1. Median plasma gastrin concentrations during gastric perfusion before and after suppression of *H. pylori* in 10 duodenal ulcer patients. The open squares represent gastrin before, and the dark squares after suppression of *H. pylori*. The horizontal axis is marked at 10 min intervals. * $p < 0.05$, before vs. after treatment.

was not accompanied by a change in the relationship between gastrin release at these two pHs.

Discussion

H. pylori infection in duodenal ulcer disease is associated with increased plasma gastrin concentrations [3]. The results of the present study confirm that suppression of *H. pylori* diminishes plasma gastrin levels [5–9]. Both basal and peptone-stimulated plasma gastrin concentrations were diminished. Moreover, the response to gastric perfusion with peptone is diminished to a similar extent, whether the intraluminal pH is low or high.

It is not clear how *H. pylori* affects gastrin release. One idea is that *H. pylori* might make the parietal cells respond less to gastrin so that more gastrin is then required to achieve a par-

ticular rate of acid secretion. Our results do not support this suggestion because more gastrin was released in the presence of *H. pylori*, even when the intragastric pH was kept constant. Furthermore, Chittajallu et al. [15] found that *H. pylori* has no effect on rates of acid secretion in response to different doses of pentagastrin [15]. We suggested that a local alkalinisation of the antrum by the urease of *H. pylori* might impair the inhibition of gastrin release by low luminal pH [3]. This could explain the diminished inhibition of gastrin release by low luminal pH that has been observed in duodenal ulcer patients, who were more likely to be infected with *H. pylori* than controls [16]. It has now been shown that *H. pylori* does indeed increase the pH of the antral microenvironment, but the effect is small [17, 18]. The present results argue against the idea that *H. pylori* blocks antral acid sensors; a low pH inhibited peptone-stimulated gastrin release to a similar extent before and after suppression of the organism. This finding is consistent with evidence from others that the urease of the organism is not responsible for the hypergastrinaemia; inhibition of urease of *H. pylori* with acetohydroxamic acid or by triple anti-*Helicobacter* therapy had no effect on plasma gastrin [19, 20], and neither did increasing intragastric ammonia by the intragastric instillation of urea [21]. An alternative explanation for the effect of *H. pylori* on gastrin was proposed by Wyatt et al. [22], who found elevated basal gastrin concentration in patients with non-*H. pylori* gastritis and suggested that inflammation itself might release gastrin. Several inflammatory mediators have indeed been shown to have this effect in vitro [23–25].

The present results differ from those of Tarnasky et al. [26] who found diminished acid inhibition of peptone-stimulated plasma gastrin release in subjects infected with *H. pylori*. One difference between their study and

ours is that they compared non-ulcer patients with and without the bacterium, whereas we studied duodenal ulcer patients before and after suppression of *H. pylori*. This raises the question as to whether the healing of ulcers, or the treatment itself, might affect gastrin release. However, in a previous study, we showed that healing of duodenal ulcers with sucralfate, which had no effect on *H. pylori*, did not affect basal or meal-stimulated plasma gastrin concentrations [27]. Methodological differences might also have been responsible for the different results. It is possible that *H. pylori* was not completely eradicated in some of our patients. We repeated the intragastric perfusion 1 week after the end of treatment because plasma gastrin concentrations return to normal at this time [5]. Although *H. pylori* could not then be identified by our tests, the organism may have been lying dormant in some patients [28] and still influencing gastric physiology. Tarnasky et al. [26] used 8% peptone and an alkaline pH of 7.0, compared with 4% peptone and an alkaline pH of 5.5 in our study. In addition, we perfused the peptone through the stomach, with continuous aspiration of the antrum to avoid any effects of distension on gastrin release. Koop et al. [12] have demonstrated that fundic distension in man increases basal gastrin by 31%, which may explain why our peptone-stimulated gastrin release was relatively small compared with previous studies of intragastric titration [17, 29]. In addition to distending the stomach, the method of intragastric titration may also chemically stimulate gastrin cells to a greater extent since the peptone is not perfused through the stomach as it was in our study, but is relatively static.

Further work is required to elucidate abnormalities in the control of gastrin release in disease states. In particular, we need to know more about how *H. pylori* affects gastric func-

tion, especially in view of the physiological abnormalities present in duodenal ulcer disease and the strong association between *H. pylori* eradication and long-term remission from duodenal ulceration [1, 30, 31].

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Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease

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JULIA M. POLAK JOHN CALAM

Infection of the gastric antrum by *Helicobacter pylori* is associated with recurrent duodenal ulcer disease but the mechanism of ulcerogenesis is unclear. Since pathways inhibiting gastric secretion are defective in patients with duodenal ulcers, we investigated whether *H pylori* interferes with the normal gastric inhibition that is mediated by somatostatin.

We studied 28 patients with active duodenal ulcers in whom *H pylori* was eradicated successfully. In 18 patients, we measured the density of antral somatostatin-immunoreactive cells and in a further 10 subjects, the amount of somatostatin mRNA before and after eradication of *H pylori* was determined. After eradication, the median density of somatostatin-immunoreactive cells increased significantly from 9 (range 3–47) to 19 (6–57) cells per mm muscularis mucosa ($p=0.025$). The median somatostatin mRNA/rRNA ratio increased from 50 (25–160) to 95 (40–180) ($p=0.01$). The number of gastrin cells and quantity of gastrin mRNA did not change significantly.

Our results suggest that in duodenal ulcer disease, gastric secretory function is disinhibited through the suppression of mucosal somatostatin.

Lancet 1992; 340: 930–32.

Introduction

Although studies of gastric function commonly focus on factors that stimulate secretion, the abnormalities of gastric physiology which are found in duodenal ulcer disease suggest that it is a normal inhibitory mechanism that is lacking. For instance, in patients with duodenal ulcer disease, acid secretion is suppressed less during fasting,¹ secretion of gastrin and acid are inhibited less by luminal acid,² and acid secretion is not only excessive³ but is also slow to return to baseline⁴ after eating. Recent work indicates that *H pylori* infection might be the cause of this failure of gastric inhibition. Eradication of *H pylori* from patients with active duodenal ulcer disease leads to falls in basal plasma gastrin^{5,6} and basal acid secretion.⁷ In addition, *H pylori* interferes with the normal negative feedback of gastric acid on gastrin release, resulting in more acid secretion when the lumen is acid in patients infected with *H pylori*.⁸

Somatostatin, released locally from D cells in the gastric mucosa, is an inhibitor of gastric secretory function. Laboratory animal studies have shown that somatostatin mediates the inhibitory effects of luminal acid and fasting on gastrin release;^{9,10} these processes are defective in duodenal ulcer disease and *H pylori* infection. Thus, a reduced production or release of somatostatin may account for the

secretory abnormalities in duodenal ulcer disease. Clinical studies of gastric somatostatin have been hampered by an inability to measure its local release in vivo, but the amount of mucosal peptide mRNA is likely to reflect the rate of peptide synthesis and release; such an association has been shown for gastrin.¹¹ We have measured somatostatin and gastrin mRNA, together with the numbers of D and G cells that release these peptides, in gastric mucosal biopsy specimens. By comparing the same patients before and after treatment, we investigated the effect of eradicating *H pylori* on mucosal somatostatin synthesis in those with active duodenal ulcer disease.

Patients and methods

36 patients from an outpatient endoscopy clinic who had an active duodenal ulcer were invited to take part in this study. Ethics committee approval and informed consent were obtained and the two parts of the study were done consecutively. We excluded patients with other illnesses and those who had undergone previous gastric surgery. We also excluded patients taking any medication other than antacids, notably H₂-antagonists within 2 weeks, omeprazole within 4 weeks, or bismuth or antibiotics within 3 months. When a duodenal ulcer was diagnosed at endoscopy, antral biopsy specimens were taken from the lesser curve, 2 cm proximal to the pylorus, with forceps (cup size 2 × 5 mm). 3 tissue samples were taken for *H pylori* diagnosis by the urease test, bacterial culture, and histology. At least two of these tests were positive in all patients. In 21 of the 36 patients, 2 antral biopsy specimens per patient were obtained for quantitative immunocytochemistry and, in a further 15 patients, 5 specimens were taken for mRNA measurement. Patients were treated with tripotassium dicitratobismuthate (DeNol) 120 mg four times daily for 1 month, with metronidazole 400 mg thrice daily and tetracycline 500 mg four times daily taken concurrently for the first 2 weeks. 4 weeks after the end of treatment, patients were re-endoscoped and biopsy specimens taken as before. 3 patients in the histochemistry part of the study (all male, median age 31, range 24–36) and 5 in the mRNA arm (4 males, median age 37, range 22–58) were still infected with *H pylori* after treatment; they were excluded from statistical analysis.

The 5 biopsy specimens per patient were pooled, snap frozen in liquid nitrogen, and the RNA was then extracted.¹² Total RNA was quantified by ultraviolet absorbance at 260 nm, and 10 µg paired samples, before and after eradication, were electrophoresed on a 1% agarose formaldehyde gel, transferred to nylon membranes (Hybond N, Amersham, Bucks), and fixed by UV irradiation and baking. To avoid the intra-assay variation inherent in the method, paired samples from each patient were run on adjacent gel lanes. Hybridisation and washing were done as described elsewhere.¹³ Filters were probed sequentially for somatostatin mRNA, gastrin mRNA, and 18S ribosomal RNA to correct for any slight loading differences between lanes. The gastrin probe was a 385-base PstI-fragment of the cDNA clone in Bluescript plasmid¹⁴

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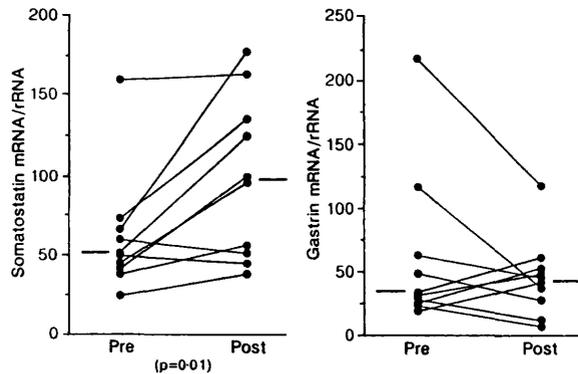


Fig 1—Somatostatin mRNA (left) and gastrin mRNA (right) after eradication of *H pylori* in 10 patients with duodenal ulceration.

These data are expressed as specific mRNA/ribosomal RNA ratios to correct for small differences in sample loading. Median values are indicated by the bold bars.

(provided by Prof K. Agarwal, Chicago, USA). The somatostatin probe was a 153-base *Pst*I-fragment of clone pHS8-86¹⁵ (Prof W. Rutter, San Francisco, USA). They were labelled with ³²P dCTP by random primed synthesis to a specific activity of 10⁹ cpm/μg. The ribosomal probe was a synthetic 30-nucleotide sequence complementary to the 3' terminus of 18S rRNA, labelled with terminal transferase to give a mean of one addition of ³²P dCTP per molecule. Autoradiography was completed at -70°C with pre-sensitised Kodak XAR-5 film; bands were quantified in arbitrary units by scanning laser densitometry (Cromoscan 3, Joyce Loeb, Gateshead, UK). Results are expressed as ratios of gastrin or somatostatin mRNA to ribosomal RNA.

Antral biopsy specimens were fixed in Bouin's solution and processed in wax. 5 μm sections were cut and stained with haematoxylin and eosin, and well-oriented adjacent sections were then immunostained with polyclonal antibodies to gastrin and somatostatin by the peroxidase/anti-peroxidase technique. Cells that stained positive for gastrin (G cells) and for somatostatin (D cells) were then counted in a minimum of three high-power fields per specimen, without reference to the clinical histories. The results are expressed as the number of positive cells per mm length of muscularis mucosae.¹⁶

Statistical analysis was done for patients in whom *H pylori* was successfully eradicated. Wilcoxon's signed-ranks (matched pairs) test was used to examine the effect of eradication of *H pylori*. The results are expressed as medians (ranges).

Results

Paired biopsy specimens before and after successful eradication of *H pylori* were obtained for histochemical study from 18 patients (11 males, median age 47 years, range 30-68) and for mRNA measurements in 10 patients (8 males, median age 47, range 20-76).

We obtained a yield of 40 μg to 155 μg total RNA from 5 endoscopic biopsies. The samples were undegraded, with clearly visible ribosomal bands when separated on ethidium-stained gels. Gastrin and somatostatin mRNA were identified by hybridisation with their respective cDNA; each existed as a single species. After eradication of *H pylori*, somatostatin mRNA increased significantly from a median somatostatin mRNA/ribosomal RNA ratio of 50 (mean 61, range 25-160) to 95 (97, 40-180) ($p=0.01$, fig 1, left). The somatostatin mRNA/rRNA ratio increased to a median of 164% (171, 81-245) of the pretreatment level. Gastrin mRNA did not change significantly: before eradication, the median gastrin mRNA/ribosomal RNA ratio was 36 (58, 19-220); after eradication, 41 (44, 7-118) ($p=0.28$, fig 1, right).

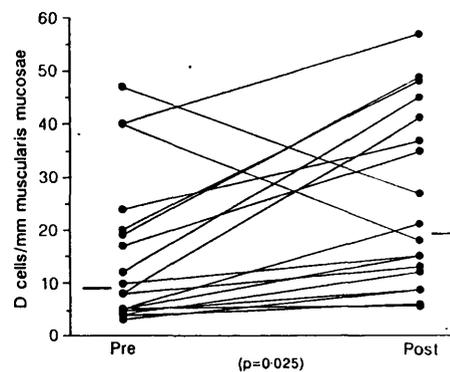


Fig 2—Somatostatin-immunoreactive cell density after eradication of *H pylori*.

Points represent individual patients, with medians indicated by the bold bars.

After eradication of *H pylori*, there was a significant increase in somatostatin cell density from a median of 9 (range 3-47) cells per mm muscularis mucosae to 19 (6-57) ($p=0.025$, fig 2). By contrast, the density of G cells did not change significantly following treatment: median, 104 (18-175) before and 117 (20-159) after treatment ($p=0.45$).

Discussion

We have found that eradication of *H pylori* in patients with active duodenal ulcer disease is associated with an increased number of antral somatostatin-immunoreactive cells (D cells), an increased amount of somatostatin mRNA, and, therefore, by implication, an increased synthesis and release of gastric somatostatin.

Somatostatin is a potent inhibitor of many gastrointestinal secretory functions. D-cells often act in inhibitory pathways as the penultimate effector cell, summing various inputs and then inhibiting adjacent secretory cells through the paracrine release of somatostatin. Since somatostatin in peripheral blood is derived from several organs, plasma concentrations do not reflect local gastric release. Our inability to measure gastric somatostatin release in human beings has caused considerable research difficulty. Mucosal somatostatin concentrations are not easy to interpret because most peptide is found in the intracellular pool, which may increase or decrease according to the rate of new synthesis. Thus, does a fall in mucosal somatostatin imply that less has been released or more? Studies that measure extracted tissue concentrations of somatostatin immunoreactivity¹⁷ do not address this biologically important issue. However, the measurement of somatostatin mRNA provides a solution; this reflects peptide synthesis, and is likely to be closely linked to rates of release.¹¹

Our findings explain several abnormalities of gastric secretion found in duodenal ulcer disease. For instance, data from laboratory animal experiments show that somatostatin inhibits gastrin release, especially in the fasting state or when the gastric lumen is acid.^{9,10} Suppression of somatostatin might also explain the increased plasma gastrin concentrations,^{5,6} and the lack of suppression of gastrin release and acid secretion by luminal acid, which were first observed in patients with duodenal ulcer disease^{2,18} and recently shown to be due to *H pylori*.⁸ Suppression of somatostatin may also explain the delayed return of acid secretion to baseline after eating⁴ and the increased basal

acid secretion,³ both of which are found in patients with duodenal ulcer disease. This explanation may account for the typical attacks of pain at night.

We found that the gastrin mRNA/rRNA ratio did not fall after eradication of *H pylori*. This result may be because the biopsy specimens were taken after overnight fasting, which inhibits gastrin synthesis.¹⁰ *H pylori* has most effect on plasma gastrin after meals; the effect on basal concentrations is small and not always significant.^{5,6} The constancy in gastrin cell numbers after eradication has been reported elsewhere.¹⁹

Although we found an increase in somatostatin in most individuals, in 4 of 28 patients with duodenal ulceration the opposite trend was observed. There may be genuine heterogeneity among these patients and they may comprise a subgroup who have duodenal ulceration unrelated to *H pylori* or a deficiency of somatostatin. However, these patients, 2 male and 2 female, had a median age of 44 years (range 38–58) and were clinically indistinguishable from the others who were studied. 2 of these patients had high pretreatment somatostatin-immunoreactive cell densities, which fell considerably after treatment. This finding raises the possibility that the initial biopsy specimens in these individuals may not have been truly representative of the entire antrum. We have attempted to limit the effect of possible differences in somatostatin within the gastric antrum by pooling biopsies from five sites per patient when measuring RNA. However, for the measurement of somatostatin and gastrin cell densities, three fields per biopsy specimen were examined but from only two sites per patient, so that sampling variation may have been responsible for these discordant results. However, for the group as a whole, there was a significant increase in antral somatostatin after treatment and we believe that there are three possible explanations for this finding. First, the change in somatostatin may be an effect of the triple therapy unrelated to its anti-*H pylori* action. Similar hypotheses were suggested to explain the prolonged remission from duodenal ulceration and the fall in plasma gastrin after anti-*H pylori* treatment, although it is now clear that this observation was due to the eradication of the organism.²⁰ Second, the increase in antral somatostatin, which we have observed after *H pylori* eradication, may be a consequence of ulcer healing rather than the eradication of *H pylori*. Since we measured somatostatin in the gastric antrum, rather than in the duodenum where the ulcer is, we believe that the change in somatostatin is more likely to reflect the effect of eradication of antral *H pylori*. However, the increase in somatostatin may be a response to changes in the duodenum. Third, the increase in somatostatin may be due to the eradication of *H pylori*; if this were so, then antral somatostatin would not increase after treatments that do not eradicate the organism.

When we reported that *H pylori* infection increased gastrin release, we suggested that alkali generated locally by *H pylori* may account for the lack of response of local cells to acid.²¹ This idea remains controversial, but since alkalinisation increases gastrin release through suppression of somatostatin, our present results support this idea. However, *H pylori* releases several factors that can have specific effects on host cells.²⁰ For instance, one that is toxic to gastric parietal cells increases cell membrane permeability to potassium and hydrogen ions.²² Thus, the antral pH receptor, located on the luminal membrane of antral D cells might be disturbed. In addition, the inflammatory cells attracted and activated by *H pylori* release cytokines capable

of affecting endocrine cell function.²³ Our work will now examine how *H pylori* inhibits the expression of somatostatin and how this mediates the increased gastric secretion found in patients with duodenal ulcer.

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