STUDIES OF PLASMA GASTRIN AND HELICOBACTER PYLORI INFECTION IN DUODENAL ULCER DISEASE

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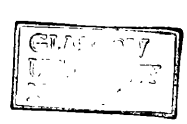

PREFACE

Peptic ulceration and it's complications result in a great deal of morbidity and mortality. The discovery of Helicobacter pylori has given us an opportunity to alter the natural history of peptic ulceration. This exciting possibility got me interested in Helicobacter pylori.

All the studies included in this thesis have been published. Advice and assistance of various colleagues was sought and these have been acknowledged. The studies in this thesis as well as writing of them was entirely my own work.

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TABLE 11 : Response (in mmol/h) to increasing doses of pentagastrin in 8 patients before and after eradication of H.pylori infection.

ABSTRACT

Chronic Helicobacter pylori infection of the gastric antrum is seen in over 95% of duodenal ulcer patients. Eradication of this infection results in a lowering of circulating gastrin concentration. A considerable proportion of the population are infected with H.pylori but only some of them develop duodenal ulceration. In this thesis I looked at the possibility that the development of duodenal ulceration in subjects with H.pylori infection is determined by the degree of hypergastrinaemia induced. In addition, I attempted to determine the mechanism by which this hypergastrinaemia may be brought about.

Consistent with earlier studies eradication of H.pylori infection in duodenal ulcer patients resulted in a fall in both basal and meal stimulated plasma gastrin concentrations. These values were similar in H.pylori positive duodenal ulcer patients and H.pylori positive asymptomatic volunteers. This suggests that factors in addition to elevated plasma gastrin are responsible for predisposition to duodenal ulceration in these patients.

The mechanism by which H.pylori infection induces hypergastrinaemia is unclear. It has been postulated that by virtue of its high urease activity the organism produces ammonia locally at the gastric antrum and this may directly stimulate gastrin release by the G cells or may act indirectly by elevating antral surface pH. There was, however, no change in plasma gastrin concentrations with either increased bacterial ammonia production by intragastric urea infusion or suppression of bacterial urease activity by triple therapy (amoxycillin, metronidazole, tripotassium dicitrato bismuthate). In addition, examining the effect of gastric alkalinisation on basal and meal stimulated plasma gastrin concentrations before and after eradication of H.pylori did not support this hypothesis. It was also noted that reversal of the acute inflammatory component of the antral gastritis produced by the infection is not associated with a change in plasma gastrin concentrations.

The possibility that the hypergastrinaemia is a physiological response to inhibition of parietal cell function by H.pylori was examined. There was no change in parietal cell sensitivity to pentagastrin after eradication of H.pylori. The mechanism by which chronic H.pylori infection of the gastric antrum induces hypergastrinaemia remains unclear. Further research is needed to elucidate this.

SUMMARY

Helicobacter pylori infection of the gastric antrum is seen in over 95% of patients with duodenal ulceration. Eradication of the infection reduces ulcer relapse rate. The mechanism by which this infection predisposes to duodenal ulceration is not known.

Eradication of H.pylori infection in duodenal ulcer patients resulted in a fall in basal and meal stimulated plasma gastrin concentrations, which is consistent with earlier studies.

H.pylori infection is present in a large number of healthy individuals. Only a proportion of them, however, go on to develop duodenal ulceration. This may be related to a variable elevation in plasma gastrin concentration produced by H.pylori in different individuals. The basal and meal stimulated plasma gastrin concentrations in H.pylori positive and negative asymptomatic volunteers were compared with corresponding values in H.pylori positive duodenal ulcer patients. The H.pylori positive asymptomatic volunteers and the H.pylori positive duodenal ulcer patients showed no difference in their basal or meal stimulated plasma gastrin concentrations and these values were higher than the corresponding values in H.pylori negative asymptomatic volunteers. This suggested that other factors in addition to elevated

plasma gastrin may be responsible for the predisposition to duodenal ulceration in patients with H.pylori positive duodenal ulcers.

The mechanism by which chronic H.pylori infection of the gastric antrum elevates plasma gastrin concentrations is not clear. It may be due to elevation of antral surface pH or direct stimulation of gastrin release by the ammonium produced as a result of the bacterium's urease activity. The antral gastritis induced by the infection may increase gastrin release by the G cells or the hypergastrinaemia may be a physiological response to inhibition of parietal cell function by H.pylori .

The effect of increasing H.pylori ammonium production by urea infusion on plasma gastrin concentrations in duodenal ulcer patients was studied. Despite a greater than three fold increase in bacterial ammonium production there was no change in plasma gastrin concentrations. This did not support the hypergastrinaemia being due to local ammonium production. It, however, did not exclude this possibility as the amount of ammonia produced by H.pylori under normal conditions may be sufficient to produce maximum gastrin response by this mechanism.

To further elucidate this, Triple therapy (amoxycillin, metronidazole and tripotassium dicitrato

bismuthate) was used to suppress H.pylori urease activity in duodenal ulcer subjects to that seen in H.pylori negative individuals. Despite this profound suppression in bacterial urease activity there was no change in basal or meal stimulated plasma gastrin concentrations. This confirms that the hypergastrinaemia in H.pylori infection is not a direct result of ammonia production by bacterial urease activity or via elevation of elevation of antral surface pH. It was also noted that the acute inflammatory cell component of the antral gastritis resolved rapidly after commencement of H.pylori eradication therapy and was not associated with a fall in plasma gastrin concentration.

If H.pylori induced hypergastrinaemia were due to elevation of antral surface pH the difference in plasma gastrin concentration between H.pylori positive and eradicated patients would largely or completely disappear at neutral pH. In addition, antral alkalinisation which results in an increase in meal stimulated gastrin response, would produce less of an increase in the integrated gastrin response to a meal in H.pylori positive than in H.pylori eradicated patients. There was, however, a persistent difference in plasma gastrin concentrations between H.pylori positive and eradicated patients even at neutral pH.

The integrated gastrin response to a meal rose to a similar degree in the presence and absence of H.pylori infection. Both these findings exclude H.pylori induced hypergastrinaemia being due to elevation of antral surface pH.

H.pylori induced hypergastrinaemia may be a physiological response to inhibition of parietal cell function by the organism. The parietal cell sensitivity to pentagastrin before and after eradication of H.pylori was examined in duodenal ulcer subjects by determining the acid output to graduated doses of pentagastrin. There was no difference in parietal cell sensitivity to pentagastrin following eradication of H.pylori. This finding does not support the hypergastrinaemia being due to diminished parietal cell sensitivity in the presence of H.pylori infection.

These studies have demonstrated that chronic H.pylori infection induces hypergastrinaemia. Some of the possible mechanisms by which this can be brought about have been examined and excluded. Other possible mechanisms are via the chronic gastritis induced by the infection. It is also possible that the elevated plasma gastrin concentrations consist of forms of gastrin that are immunoreactive but not functionally active. Further research is needed to elucidate this.

SECTION I

INTRODUCTION AND BACKGROUND

CHAPTER 1

HELICOBACTER PYLORI

1.1 INTRODUCTION

The discovery of Helicobacter pylori (H.pylori) has revolutionised thinking about duodenal ulcer disease. Although its presence in gastric biopsies had been noted for many years the significance of this infection was not realised until it was first cultured in 1983 (WARREN et al 1983). Then it was called Campylobacter pyloridis and has since undergone two changes in its name. Initially to Campylobacter pylori and in 1989 to its present name, Helicobacter pylori (GOODWIN et al 1989).

The organism is mainly confined to the gastric epithelium of the antrum and body of the stomach. In addition, areas of gastric metaplasia anywhere along the gastrointestinal tract can be infected (PAULL et al 1988, WYATT et al 1987, CASELLI et al 1988). It, however, does not infect areas of intestinal metaplasia in the stomach (MEYRICK-THOMAS 1984). It is an extracellular organism and is found in the gastric pits and on the luminal surface of the epithelial cells.

1.2 STRUCTURE

H.pylori is a sinusoidal Gram negative bacillus (Fig 1). It is 2-6.5 microns long by 0.5-0.6 microns wide. Its surface is smooth and has 4-6 unipolar sheathed flagellae which have a terminal bulb. There is

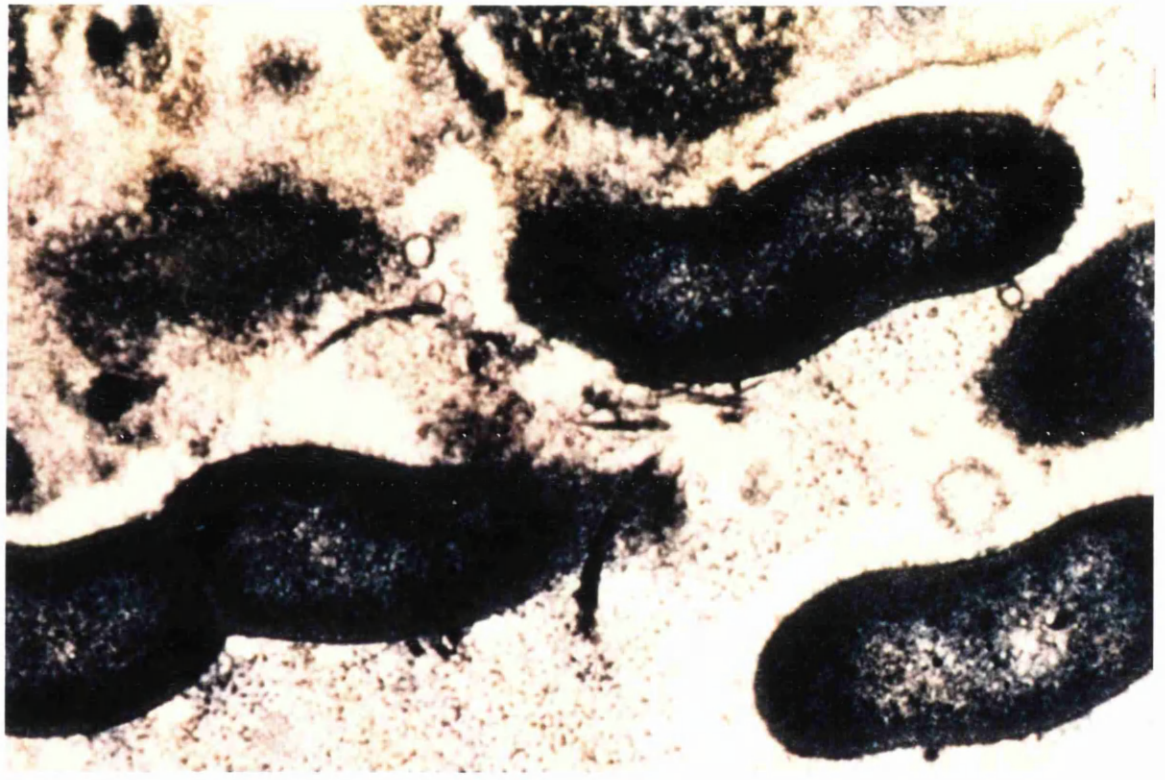

Figure 1 Photomicrograph of H.pylori

also a coccoid form which may be the organism's attempt at adapting to a hostile environment (BODE et al 1988). Only the sinusoidal form, however, is found in vivo.

1.3 ENZYMES

H.pylori is rich in a number of enzymes. The urease enzyme, however, is the one that has attracted the most attention. The activity of this enzyme in H.pylori is greater than that seen in any other bacteria (MOBLEY et al 1988). The enzyme has a molecular weight of 625,000 +/- 15,000 Daltons, pHi of 5.9, Km of 0.8 mmol of urea, an optimal temperature of 45 degrees C and optimal pH of 8.2 (MOBLEY et al 1988). It has a narrow substrate specificity and hydrolyses urea to produce ammonium and bicarbonate (BLAKELEY et al 1969, GORIN 1959). Elevated concentrations of ammonia, however, do not seem to have a feed back inhibitory effect on urease synthesis (FERRERO et al 1988). In addition to urease, H.pylori also possess superoxide dismutase, catalase and lipase.

1.4 EPIDEMIOLOGY

The prevalence of H.pylori infection differs worldwide. There seems to be higher prevalence in the underdeveloped countries than in the developed ones, with a 15-25% prevalence in the general population of

the USA, Western Europe, Australia and New Zealand and 60-70% prevalence amongst Chinese and Tongans (GRAHAM et al 1988, MORRIS et al 1986, LANGENBERG et al 1984, BARTHEL et al 1988, BERKOWICZ et al 1987). The prevalence of the infection increases with age. Few children harbour the infection, whereas in the 60-84 age group over 82% of people are infected (GRAHAM et al 1988). This high prevalence of infection throws doubt on what is now accepted as gastric physiology, as many of the subjects in these studies were probably infected with the organism.

1.5 NATURAL HISTORY

Ingestion of H.pylori results in acute onset of dyspeptic symptoms associated with acute gastritis (MARSHALL et al 1985, MORRIS A. et al 1987). In some this is followed by resolution of the infection and gastritis (MARSHALL et al 1985) but in others the organism persists with development of chronic gastritis (MORRIS et al 1987). Once established, the infection does not clear spontaneously. Amongst individuals in whom the infection persists, most remain asymptomatic but some go on to develop signs and symptoms of duodenal ulceration. The reason for only some of the individuals with the infection developing ulceration is not clear.

1.6 TRANSMISSION OF INFECTION

The mechanism of spread of this organism is not known. Evidence from various studies suggests that there is a person to person spread. An Australian study done in an institution where infections with person to person spread have higher incidence found that, in younger people, H.pylori infection was higher in the residents of the institution than in a control group and in each age group a higher proportion showed presence of the infection (BERKOWICZ et al 1987). It has also been shown that there is increased prevalence of the infection in family members of cases of H.pylori than in the general population (MITCHELL et al 1987) . Studies done on abattoir workers and veterinarians show an increased prevalence of H.pylori in these occupational groups suggesting that animals may be an important reservoir of H.pylori (MORRIS et al 1986, VAIRA et al 1988). The infection is probably acquired by ingestion of the organisms as was shown in volunteers who swallowed a culture of H.pylori and in subjects undergoing repeated gastric secretory studies (RAMSEY et al 1979, MORRIS et al 1987, GRAHAM et al 1988) as well as endoscopy staff (MORRIS et al 1986, RAWLES et al 1987).

1.7 ROLE IN GASTRITIS

ACUTE GASTRITIS

In acute infection with H.pylori , as occurred following ingestion of cultures of H.pylori , there is an acute gastritis. The antral mucosa shows infiltration by large numbers of polymorphonuclear leucocytes and the antral mucosal surface becomes irregular. The organism can be seen in the gastric mucus close to the epithelial cells lining the mucosal surface and gastric pits (MARSHALL et al 1985, MORRIS et al 1987, GRAHAM et al 1988).

CHRONIC GASTRITIS

Chronic gastritis can be classified into

	Pathogenesis
- Type A	autoimmune
- Type B	bacteria associated
- Type C	chemical injury such as enterogastric reflux, Nonsteroidal anti-inflammatory drugs, Alcohol
- Lymphocytic	? hypersensitivity
- Eosinophilic	allergy
- Granulomatous	? hypersensitivity

There is good evidence showing that chronic H.pylori infection is the major cause of type B gastritis. This evidence comes from studies looking at the effect of ingestion of H.pylori, correlation between type B gastritis and H.pylori positivity and the effect of eradication of H.pylori on gastritis.

Ingestion of H.pylori results in an acute gastritis with a spontaneous resolution of the inflammation (MARSHALL et al 1985). In some, however, this is followed by chronic gastritis (MORRIS et al 1987).

H.pylori infection is seen in 86-100% of subjects with chronic type B gastritis (MARSHALL et al 1985, VAIRA et al 1988, RATHBONE et al 1986, ANDERSEN et al 1987, TYTGAT et al 1985, RAUWS et al 1988) and is uncommon in subjects with normal gastric mucosa (TYTGAT et al 1985, RAUWS et al 1988). Eradication of the infection results in resolution of the gastritis (RAUWS et al 1988, MARSHALL et al 1987, McNULTY et al 1986). In those in whom the infection persists the severity of the gastritis, however, remains unchanged (RAUWS et al 1988). Patients in whom there is clearance but not eradication of the infection, the gastritis shows improvement and with relapse of the infection the gastritis increases (RAUWS et al 1988). In chronic

infection of the gastric antrum, there is an intraepithelial and interstitial neutrophil polymorph infiltrate in addition to lymphocytes and plasma cells. There may be loss of continuity in the epithelial cell lining resulting in erosions. The inflammation is predominantly in the antrum but in some, the body is involved as well. ANDERSEN et al (1987) showed a spatial relation between the polymorph infiltrate and the position of the H.pylori . This, however, was not confirmed by THOMSEN et al (1990) who were also unable to demonstrate any correlation between the polymorph infiltrate and density of infection. The epithelial cells show changes with infection. There is loss of microvilli, depletion of mucin content and cellular oedema (THOMSEN et al 1990, MARSHALL et al 1985, CHEN et al 1986).

In chronic H.pylori infection the bacteria are seen in the mucus layer in the crypts and on the luminal surface of gastric epithelial cells. They show preference for the upper portion of the gastric pits. It was initially thought that the organism had some predilection for the epithelial junctions which were believed to be a source of nutrients (CHEN et al 1986) but this has not been confirmed (THOMSEN et al 1990).

1.8 LOCAL AND SYSTEMIC IMMUNE RESPONSE

The immune response to the infection is directed against the surface of the organism including the body and flagella. The serum antibodies are mainly of the IgG and IgA class but rarely IgM (BOOTH et al 1986, RATHBONE et al 1986). The lack of a significant elevation of the IgM titre may be because the H.pylori colonisation is long standing. This antibody response, however, is unable to eliminate the infection or prevent recrudescence after temporary clearance. The gastric juice, and tissue culture supernatants of duodenal and gastric biopsies from patients with H.pylori infection have specific anti-H.pylori antibodies, but unlike the circulating antibodies they are of the IgA class though some IgM is also present (RATHBONE et al 1986). This antibody response is appropriate for elimination of bacteria from mucosal surfaces. There is also a cellular response with an increased number of T cells of both CD4 and CD8 positive subsets. So there is an adequate humoral as well as cellular response but despite this the H.pylori infection persists. The reason for this is not clear.

1.9 ROLE IN GASTRODUODENAL PATHOLOGY

1.9.1 ACUTE INFECTION

Experience on the effects of acute ingestion of H.pylori is from the human ingestion studies and from cases of spontaneous hypo/achlorhydria seen in subjects participating in gastric secretory studies (MARSHALL et al 1985, GRAHAM et al 1988). The symptoms developed by these patients were varied and included epigastric fullness, halitosis, nausea, vomiting, early morning hunger, irritability and headaches. This was associated with an acute increase in gastric acid, pepsin and mucus output followed by a fall in both acid and pepsin output for varying lengths of time (GRAHAM et al 1988, RAMSEY et al 1979). Following this there is, in most people, a spontaneous recovery.

1.9.2 CHRONIC INFECTION

In most cases this is asymptomatic. In some patients it leads to duodenal ulceration with its associated symptoms and complications. Its role in the causation of gastric ulcer, non ulcer dyspepsia and gastric cancer is less clear.

H.pylori AND DUODENAL ULCER

The association between this infection and

duodenal ulceration is strong. It is seen in over 95% of those with duodenal ulcer (O'CONNOR et al 1986, RAUWS et al 1988). In races which have a low incidence of duodenal ulcer, such as the Aborigines from Northern Australia, the infection is seen in less than 1%. In the Aborigines who live in urban and semi-urban conditions, however, duodenal ulceration is seen more commonly and is associated with an infection rate of 50% (BATESON 1976, DWYER et al 1988). Eradication of the infection results in a fall in the ulcer relapse rate to 20-27% whereas in patients, not eradicated of H.pylori , the ulcer relapse rate is 74-88% (COGHLAN et al 1987, SMITH et al 1988, LAMBERT et al 1987, BORODY et al 1988, GOODWIN et al 1988, MARSHALL 1988).

The mechanism by which H.pylori predisposes to duodenal ulceration is not clear but may be via increase in gastrin release from the G cells. Gastrin is trophic to parietal cells and is a strong stimulus to gastric acid secretion. So elevated plasma gastrin may increase gastric acid output. An increased acid output in patients with H.pylori infection would be consistent with earlier studies which showed a higher mean rate of gastric acid secretion in duodenal ulcer disease than in normal individuals (ISENBERG et al 1975, PETERSEN et al 1975). This increased gastric

acid output could induce gastric metaplasia in the duodenum. This metaplasia is thought to represent a response to injury to the duodenal mucosa due to an increased acid load. This is supported by studies in man which have shown that gastric metaplasia in the duodenum only occurs when the fasting intragastric pH is low and it correlates with maximal acid output (JAMES 1964, PATRICK et al 1974). It is extensive in patients with the Zollinger Ellison syndrome (PARRISH et al 1965). In low acid output states such as atrophic gastritis, gastric metaplasia is not seen and its prevalence is lower in patients who have undergone highly selective vagotomy (WYATT et al 1987).

H.pylori only infect gastric epithelium, therefore areas of gastric metaplasia in the duodenum may be infected by spread from the gastric antrum. Once the infection establishes in these areas of metaplasia it may have a local cytopathic effect resulting in reduced mucosal resistance. The organism also elaborates a lipase and protease which may weaken the mucus barrier. Incubation of gastric mucus with a filtrate of H.pylori has been shown to result in a 35% reduction in its viscosity (SAROSIEK et al 1988). These changes may reduce the mucosal resistance to gastric acid and pepsin resulting in erosions and ulceration.

If the infection produced ulceration by weakening the mucus barrier, one would expect these ulcers to be more common in the antrum where the infection is seen more often and the pH is lower. This, however, is not the case. To explain this discrepancy it has been postulated that in the gastric antrum the organism, which is motile, moves along the epithelial surface allowing repair of the damaged mucus layer to occur. So there is a continuous cycle of damage and repair. In the areas of gastric metaplasia in the duodenum, however, the mobility may be restricted as H.pylori cannot infect normal duodenal epithelium. So there is no opportunity for repair of the damaged mucus layer resulting in progression to erosions and ulceration.

H.pylori AND GASTRIC ULCER

Chronic H.pylori infection of the gastric antrum is seen in about 70% of patients with gastric ulcer (MARSHALL et al 1984, O'CONNOR et al 1987). The association of the infection with gastric ulcer, therefore, is less strong than with duodenal ulceration. However, it is possible that this is due to inclusion of nonsteroidal anti-inflammatory drug induced ulcers in these studies.

H.pylori AND GASTRIC CANCER

The prevalence of H.pylori in gastric cancer patients varies between 20-70% (FENG et al 1988, JIANG et al 1987, RATHBONE et al 1988, LAMBERT et al 1985, GILMAN et al 1987, CHENG et al 1987). The organism, however, does not infect gastric cancer cells (JIANG et al 1987).

Chronic gastritis is commonly seen with gastric carcinoma (MORSON 1955) and its presence may be used as an indicator of risk of gastric cancer (CORREA 1983, SIURALA et al 1966). H.pylori is by far the most common cause of chronic gastritis and it is possible that this infection predisposes to the development of gastric cancer. The prevalence of H.pylori infection is greater in patients with gastric cancer than in healthy controls but patients with colorectal cancer have a similar prevalence (TALLEY et al 1990).

The role, if any, of H.pylori in development of gastric cancer is not clear and further work needs to be done before any pathogenic role can be attributed to H.pylori .

H.pylori AND NON ULCER DYSPEPSIA

Nonulcer dyspepsia is defined as upper abdominal or retrosternal pain, discomfort, heartburn, nausea, vomiting or other symptoms considered to be referable

to the proximal alimentary tract and lasting for more than 4 weeks, unrelated to exercise and for which no focal lesion or systemic disease can be found responsible. H.pylori infection is seen in 50% of patients with nonulcer dyspepsia (MARSHALL et al 1984, BLOMBERG et al 1988). The association between H.pylori and non ulcer dyspepsia, however, is not clear because of the high frequency of colonisation with H.pylori in individuals without peptic ulceration. In addition, studies done so far have used different definitions for this condition.

In order to show that there is a link between nonulcer dyspepsia and H.pylori one would have to prove that there is an increased prevalence of H.pylori in this condition than controls and secondly treatment should produce a significantly greater improvement in symptoms in those eradicated of H.pylori than in those in whom the infection persists.

Many studies have looked at the prevalence of H.pylori in nonulcer dyspepsia. In 292 blood donors, never diagnosed to have peptic ulcer,the prevalence of H.pylori in subjects with dyspepsia was twice that of the rest (MARSHALL 1988). RAUWS (1988) showed a significantly higher prevalence of H.pylori in nonulcer dyspepsia subjects than controls. The controls in this study were, however, 18 years younger.

In a study on 247 blood donors, however, the occurrence of previous investigation for dyspepsia was associated with H.pylori infection only in those aged over 40 years (WYATT et al 1988). In 64% of those with positive serology there was no history of clinically significant dyspepsia. So there is at present no good evidence for a higher prevalence of H.pylori infection in subjects with nonulcer dyspepsia than in controls.

If the infection caused symptoms of nonulcer dyspepsia, eradication of it should improve these symptoms. In a group of patients with nonulcer dyspepsia, improvement in symptoms occurred in those eradicated of H.pylori , however, a similar improvement was seen in the noneradicated group (McNULTY et al 1986). There was no association between resolution of gastritis following treatment and resolution of symptoms. Others have done studies addressing the same problem but none have convincingly demonstrated an improvement in symptoms following eradication of the infection (BORODY et al 1987, HOLCOMBE et al 1991). H.pylori does not appear to be major factor in the pathogenesis of nonulcer dyspepsia. There is therefore, at present, no indication for eradication of H.pylori in nonulcer dyspepsia except within the confines of a clinical trial.

1.10 DIAGNOSIS

Infection with H.pylori can be diagnosed by various methods.

(1) Microscopy

(2) Rapid Urease tests

(3) 13/14C-urea breath test

(4) Urea concentration in gastric juice

(5) Serology

(6) Culture

(1) Microscopy

Gastric antral biopsies collected during endoscopy can be used to determine H.pylori status of an individual. The biopsies are usually stained with Giemsa or haematoxylin and eosin stain. With chronic infection, there is a chronic active gastritis i.e. there is a neutrophil polymorph infiltrate in the epithelium as well as the lamina propria in addition to the chronic inflammatory infiltrate (Fig 3). The inflammation is maximal in the antrum but the body may be involved to a variable extent. Eradication of the infection results in an improvement in this gastritis.

RAUWS (1988) developed a scoring system for the activity of the gastritis. In this system, the density of the chronic inflammatory infiltrate in the lamina propria is given a score of 0-2, polymorphonuclear

leucocytes in the lamina propria 0-3, intraepithelial polymorphonuclear leucocytes 0-3 and superficial erosions 0-2. For each parameter, 0 is none, 1 is mild, 2 is moderate and 3 is severe.

(2) Rapid urease tests

These tests utilise the organism's high urease activity to detect its presence. They only detect presence of preformed enzyme as further production is inhibited by addition of a bacteriostatic agent. In one test a modified urea broth is used and in the other, which is commonly used clinically, a commercially prepared slide (CLO test) is used. This CLO test slide has a dimple which contains a gel pellet. This pellet contains urea, phenol red and a bacteriostatic agent. The gel is buffered to an acid pH at which it has a bright yellow colour. An antral biopsy taken during endoscopy is put in the gel and the slide incubated at body temperature. In the presence of H.pylori the urea is hydrolysed to ammonium and bicarbonate resulting in elevation of the pH of the gel (Fig 2).

$$\underset{\text{urea}}{NH_2.CO.NH_2} + 2H_2O + H^+ \xrightarrow{\text{urease}} \underset{\text{ammonium}}{2NH_4} + \underset{\text{bicarbonate}}{HCO_3}$$

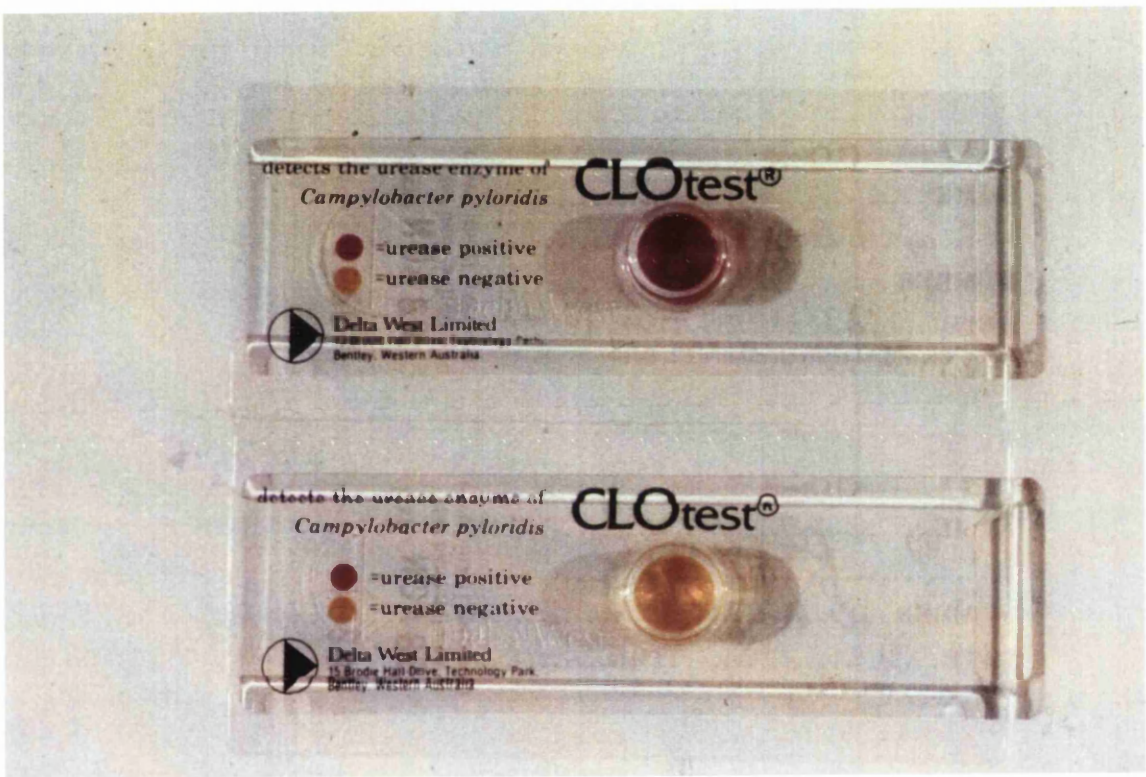

Figure 2 CLO test. Top is a positive test and bottom is a negative test.

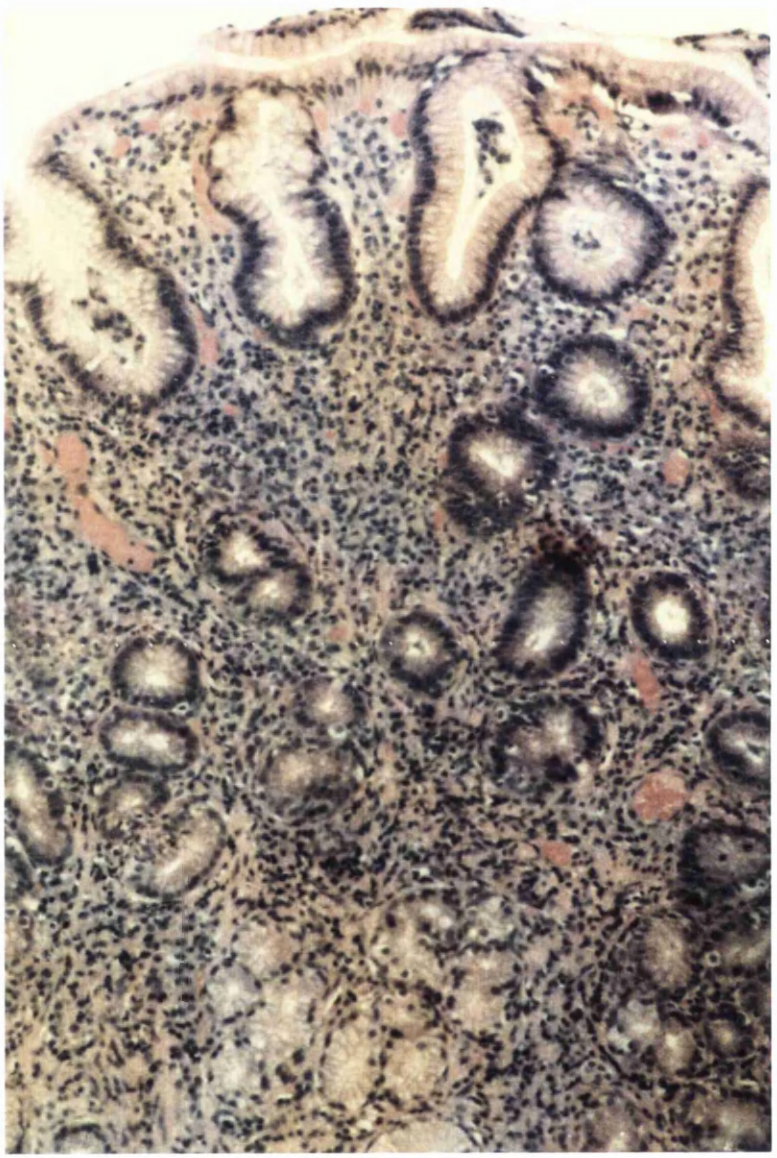

Figure 3 H.pylori induced chronic active gastritis.

This produces a change in the colour of the phenol red from the bright yellow to red. A positive test is the development of a red colour within 24 hours.

Urease tests are simple and have a specificity of near 100% and sensitivity of at least 98% (MARSHALL et al 1987). The sensitivity is however lower when it is used in specimens obtained from patients who have received treatment with antibiotics or bismuth.

(3) 13/14C-urea breath tests

This diagnostic test also relies on detecting the organism's high urease activity. It is a noninvasive, inexpensive and reproducible test to determine H.pylori status.

The patients present themselves after an overnight fast when they are given 250mls of Ensure Plus followed immediately by 0.4Mbq of 14C urea (Amersham International) in 20 mls of water. The Ensure Plus, which is a fatty meal, is given to delay gastric emptying of the 14C urea. Breath samples are collected before and at 10 minute intervals after ingestion of the 14C urea, for 2 hours. In the presence of H.pylori the 14C urea is hydrolysed to release 14C carbon dioxide which is exhaled. By measuring the 14C activity in the exhaled carbon dioxide using a scintillation beta counter one can determine the

presence or absence of H.pylori. The results are expressed in % 14C dose per mmol CO2 X 100 X Kg body weight. The 20 minute value has been shown to be most discriminating with a value of >40 confirming presence of H.pylori (BELL et al 1987). Treatment with H2 antagonists does not effect this test but antibiotic therapy is likely to influence it (WEIL et al 1989).

The radiation from this dose of 14C urea is less than that from a chest X-ray. It is better avoided in children and pregnant women. In these groups as well as in studies in which repeated assessments of H.pylori status are required a 13C urea breath test is better suited. The protocol for this test is similar to that for the 14C urea breath test except that the non radioactive isotope, 13C urea, is used instead of the radioactive 14C urea. The disadvantage, however, is that measurement of the 13C isotope requires a mass spectrometer which is not available at most hospitals.

(4) Urea concentration in gastric juice

The high urease activity of the organism hydrolyses the urea in gastric juice resulting in a fall in the urea and a rise in ammonium concentration in gastric juice. A urea concentration of less than 1 mmol/l in the gastric juice is indicative of infection with H.pylori . The ammonium concentration, however,

is not as useful in determining H.pylori status. Determination of urea: ammonium ratio in aspirated gastric juice has been shown to be superior to measurement of urea or ammonium concentration alone in H.pylori infection (NEITHERCUT et al 1990). This test may be used in patients who vomit, have nasogastric tubes or in whom gastroscopy and /or gastric biopsy is contraindicated.

(5) Serology

Anti-H.pylori antibodies can be detected by various serological assays such as complement fixation, haemagglutination, bacterial agglutination and immunofluorescence but the enzyme linked immunosorbent assay (ELISA) is the test of choice. Serum IgA and G antibodies are elevated in infected individuals but the IgM antibody is not significantly different (RATHBONE et al 1986, STEER et al 1987). Following eradication of the infection the titres fall although they may take a while to do so. So, elevated H.pylori IgG antibody titres are not a good indicator of active infection with this organism.

(6) Culture

A variety of media, such as blood agar, chocolate agar and brain heart infusion agar, have been used to

culture H.pylori from biopsies. For primary isolation both selective and nonselective media should be used. They are incubated in a microaerophilic atmosphere with an oxygen concentration of 5-7%. Growth is detected after 3-6 days of incubation. The advantage of culturing the organism is that its antibiotic sensitivity can be determined and it provides definitive proof of the presence of H.pylori .

1.11 TREATMENT

In determining efficacy of therapy, suppression of the organism has to be differentiated from eradication of the infection. Confirmation of absence of the organism 4 weeks after completion of therapy is true eradication. Tests done earlier can be used to show persistence of the infection but not eradication.

Monotherapy using antibiotics or bismuth compounds have been shown to be relatively ineffective in eradicating the infection. Antibiotics though effective in vitro are disappointing when used alone, in vivo. Trials using antibiotics such as nitrofurantoin and amoxycillin achieve an eradication rate of 20% (RAUWS et al 1988, GILMAN et al 1987). Various bismuth compounds have been used on their own to eradicate the organism. Bismuth subcitrate and subsalicylate have an eradication rate of 20-30%

(LAMBERT et al 1987, BORSCH et al 1988). Combining the bismuth preparation with an antibiotic such as amoxycillin improves this to 40% (RAUWS et al 1988, BAYERDORFFER et al 1987) but in a study using tinidazole with bismuth subcitrate H.pylori was eradicated in 75% of cases (GOODWIN et al 1987).

Combining a bismuth compound with metronidazole and amoxycillin/tetracycline (triple therapy) improves the eradication rate to more than 90% (BORODY et al 1988, BORSCH et al 1988). This is in contrast to the poor eradication rates achieved when these drugs are used individually.

The duration of therapy has been different in various studies. The long courses used initially are now felt to be unnecessary, with one and two week courses of triple therapy achieving as high an eradication rate as a 4 week course. The dosage of Tripotassium Dicitrato Bismuthate (DeNol) effects the therapeutic efficacy of this drug, with a dose of 120mg QID being more effective than 240mg BID.

The drugs used in the triple therapy are individually quite safe. Tripotassium dicitrato bismuthate causes darkening of the tongue and stool. Metronidazole can cause nausea, vomiting and a disulfiram like reaction with alcohol. Amoxycillin can cause loose stools and anaphylactic reaction in

penicillin allergic individuals, in whom tetracycline may be used instead. The combination of three drugs carries a risk of drug hypersensitivity and antibiotic associated colitis.

The other problem with eradication of H.pylori is the emergence of metronidazole resistance. Different studies have shown the percentage of organisms resistant to metronidazole to vary between 6 to 27% (WEIL et al 1990, GLUPCZYNSKI et al 1990, BECX et al 1990). This resistance may be due to treatment with this drug in the past, for other indications, (WEIL et al 1990, BECX et al 1990) or may occur during the course of treatment, especially when nitroimidazoles are used as mono-therapy (GOODWIN et al 1988, GLUPCZYNSKI et al 1987). In view of this, metronidazole should not be used as mono-therapy to eradicate H.pylori and unrestricted use of this drug for H.pylori infection should be discouraged.

The side effect profile and occurrence of metronidazole resistance in H.pylori suggest that eradication therapy should be prescribed only in the context of a research study and not for routine clinical use.

CHAPTER 2

SERUM GASTRIN AND HELICOBACTER PYLORI

2.1 INTRODUCTION

Gastrin was discovered by Edkins in 1905. The hormone is found in three main molecular forms - G-34 or 'big gastrin',G-17 or 'little gastrin' and G-14 or 'mini-gastrin'. The amino acid composition of G-14 is identical to the carboxyl terminal tetradecapeptide of G-34 and G-17 and they share the same biological actions. They all occur in sulfated and non-sulfated forms.

There are, in addition, the carboxyl terminal glycine extended peptides corresponding to each molecular form of gastrin and the NH2 terminal tetradecapeptide fragment of gastrin-17, called (1-13)G17. These have different biological effects to the other gastrins and inhibit gastric acid secretion (PETERSEN et al 1983). This, however, has not been confirmed in later studies (HILSTED et al 1988, PAUWELS et al 1985). Higher concentrations of glycine extended gastrin and (1-13)G17 have been reported in active duodenal ulcer disease but not when the ulcer is inactive.

2.2 SOURCE OF GASTRIN

Gastrin is secreted by the G cells which are mainly located in the antropyloric mucosa. Some G

cells are also found in the proximal small intestine but are not present in the oxyntic mucosa, ileum or colon (LARSSON et al 1977). In the stomach, they are found in the pyloric glands of the antrum and are most abundant in the mid portion of these glands. G cells have a flask shape with a broad base and a narrow neck. The microvilli on the mucosal surface of the G cell may contain receptors for stimulation and inhibition of by intragastric contents.

Somatostatin secreting D cells are found in very close relationship to the G cells. They have long cytoplasmic processes which come in contact with different glandular epithelial cells. Many of these processes end on G cells in the antropyloric mucosa and on parietal cells in the oxyntic mucosa (LARSSON et al 1979). Somatostatin inhibits gastrin release under basal conditions as well as following a meal (RAPTIS et al 1978). The ratio of these two cells is controlled by antral pH; the G:D ratio increases in reduced acid secretory states and decreases in hypersecretory states (ARNOLD 1982).

2.3 POTENCY OF GASTRIN

The carboxyl terminal tetrapeptide of the gastrin molecules has all the biological actions of the whole molecule. The G-17 and G-34 gastrins have equal

potency for stimulation of acid secretion. Sulfated and non-sulfated forms are equipotent (EYSSELEIN et al 1984).

2.4 COMPOSITION OF PLASMA GASTRIN

In the fasting state, the molar ratio of G-34 to G-17 is about 2:1. After a meal the concentration of G-34 doubles but that of G-17 increases four times so that the ratio becomes 1:1 (LAMERS et al 1979).

2.5 CATABOLISM

The half lives of G-17 and G-34 in the circulation are about 8 and 44 minutes (WALSH et al 1976,EYSSELEIN et al 1984). The half life of G-14 is similar to that of G-17 (DEBAS et al 1974). In pernicious anaemia patients, the disappearance half time of plasma gastrin after antral acidification is 7 minutes (YALOW et al 1970).

The mechanism by which gastrin is rapidly cleared from the circulation is not clear. The liver plays a minor role in the catabolism of G-17 and G-34 (TRONBERG et al 1983, STRUNZ et al 1978) although it actively secretes pentagastrin into the bile (WYLLIE et al 1974). The kidney was thought to be the most important site of catabolism of gastrin as elevated plasma gastrin concentrations are seen in patients with renal

failure (DAVIDSON et al 1973). The elevation of gastrin concentrations is proportional to the glomerular filtration rate. Serum gastrin levels remain elevated in hemodialysed patients and are lower after renal transplantation (HANSKY et al 1975, HALLGREN et al 1978, DOHERTY et al 1978, WESDORP et al 1981). The concentration of gastrin in the renal artery and vein during the basal state and during suppression of endogenous gastrin release by antral acidification are, however, not significantly different (BOOTH et al 1973). Following endogenous release of gastrin the concentration of gastrin in the renal vein is 35% less than in the renal artery. This, however, was not associated with significant excretion of gastrin in urine (BOOTH et al 1973). This fall in gastrin concentration across the renal circulation, however, does not differ significantly from the arterio-venous difference seen in the intestine, limb and head circulations (STRUNZ et al 1978). The removal of circulatory gastrin, therefore, appears to occur at multiple sites and the kidney has no special role in its catabolism.

2.6 STIMULATION OF GASTRIN RELEASE

Gastrin release is stimulated by

- vagal excitation

- gastric distention
- food stimulation
- blood borne chemical stimulation

VAGAL EXCITATION

In normal man, vagal excitation induced by modified sham feeding stimulates acid secretion (MAYER et al 1974, RICHARDSON et al 1977, FELDMAN et al 1979). The acid secretory response to sham feeding at an intragastric pH of 5, as measured by intragastric titration revealed a small rise in serum gastrin (RICHARDSON et al 1977, FELDMAN et al 1979) which, however, was not demonstrated in other studies which used direct gastric aspiration to measure gastric acid output (MAYER et al 1974). This rise in gastrin concentration can explain only some of the acid response to sham feeding (FELDMAN et al 1980). Vagal stimulation of acid secretion is, therefore, probably brought about by gastrin release and direct cholinergic stimulation of parietal cells. It appears that gastrin release produced by vagal stimulation is mediated by gastrin releasing peptide (DOCKRAY et al 1979). SCHUBERT et al (1985) have demonstrated this by abolishing vagally mediated gastrin release using specific bombesin antisera.

GASTRIC DISTENTION

In dogs and pigs, distention of isolated antrum (DEBAS et al 1974, STADAAS et al 1974) and separated innervated fundus (DEBAS 1974) stimulates gastrin release. This effect is probably mediated by local cholinergic reflexes as it can be blocked by atropine. A similar response to gastric distention could not, however, be demonstrated in humans (SCHRUMPF et al 1974). SOARES et al (1977), however, demonstrated a significant rise in gastrin at an intragastric pressure of 15cm H20.

FOOD STIMULATION

Proteins, peptones and amino acids stimulate gastrin release. Of the amino acids, phenylalanine and tryptophan are the most potent stimuli of gastrin release and acid secretion (BYRNE 1977). In contrast, fat and glucose are weak stimulants of acid secretion and gastrin release (RICHARDSON et al 1976, SAINT HILAIRE 1960). Gastrin release is inhibited by restricting entry of amino acids into the gastrin cell, where as, it is stimulated by increase in intracellular amine levels (LICHTENBERGER et al 1982, LICHTENBERGER et al 1986).

The predominant increase in serum gastrin after meals is in the G-17 form. Using a G-17 antiserum,

TAYLOR et al (1979) demonstrated that 50% of the postprandial gastrin was due to G-17.

CHEMICAL STIMULATION

Calcium stimulates gastrin release when given orally (LEVANT et al 1973) or intravenously (REEDER et al 1974). Intravenous epinephrine produces a rise in plasma gastrin concentration (STADIL et al 1973) but intense exercise, which releases endogenous epinephrine does not produce the same effect (HOSTEIN et al 1978) suggesting that it does not have a role in gastrin release under physiological conditions. Bombesin, a peptide isolated from frog skin, is a potent stimulus of gastrin release.

2.7 INHIBITION OF GASTRIN RELEASE

The following have been shown to inhibit gastrin release

VAGAL EXCITATION

The vagus in addition to stimulating gastrin release also inhibits it. Atropine increases serum gastrin concentrations following insulin hypoglycaemia (FAROOQ et al 1975) and eaten meals (KORMAN et al 1971, WALSH et al 1971, BECKER et al 1974) and all forms of vagotomy result in a rise in basal gastrin

concentrations (KORMAN et al 1972, McGUIGAN et al 1972, KRONBORG et al 1973, STERN et al 1973, JAFFE et al 1974). These studies suggest that the vagus has cholinergic fibres which inhibit gastrin release and when they are blocked by atropine, result in a rise in gastrin concentrations seen basally and following stimulation of the vagus. The rise in gastrin over preoperative levels is higher following selective proximal vagotomy than after truncal vagotomy (LAM et al 1978). This suggests that the vagal inhibitory pathways are located in the proximal stomach. This rise in gastrin is abolished by antrectomy implying that it arises from the antrum (LAM et al 1978). Other investigators have, however, been unable to demonstrate any change in basal gastrin with atropine (HANSKY et al 1973, WALSH et al 1971, SCHRUMPF et al 1974).

GASTRIC ACIDITY

Gastrin release by the gastric antrum is inhibited by antral acidification (WOODWARD et al 1960). There is a negative feedback control of gastrin release whereby gastrin stimulates acid secretion by the parietal cells and when the antral pH falls to about 1.5 there is inhibition of further gastrin release. Chronic achlorhydric states, such as pernicious

anaemia, are associated with elevated levels of gastrin (PETERS et al 1983). Intragastric instillation of hydrochloric acid in these patients results in a fall in gastrin in 5-15 minutes (YALOW et al 1970). In addition, the gastrin response to a meal is inhibited by acidification (WALSH 1975). The suppression of meal stimulated gastrin release by acid may be due to protonation of dietary amines, preventing their diffusion into the G cell and thereby their stimulation of gastrin release (LICHTENBERGER et al 1986).

The effect of antral alkalinisation, during fasting, on gastrin release is unclear. Some studies have shown no change (LEVANT et al 1973, HIGGS et al 1974, KLINE et al 1975, FELDMAN et al 1978) while others have shown a rise after 5 hours of antral alkalinisation (PETERS et al 1983).

CHEMICAL INHIBITION

A variety of gastrointestinal hormones inhibit gastrin release and its action on parietal cells. These include secretin (THOMPSON et al 1972), vasoactive intestinal polypeptide (RAYFORD et al 1974), Gastrin inhibitory polypeptide (RAYFORD et al 1974), glucagon (BECKER et al 1975), somatostatin (RAPTIS et al 1978) and calcitonin (BECKER et al 1974). Whether

these effects have a physiological role is not clear. Somatostatin inhibits gastrin by paracrine action. Histological studies have demonstrated that cytoplasmic processes from mammalian somatostatin cells in the antrum make direct contact with gastrin cells (LARSSON et al 1979, KUSUMOTO et al 1979). Intravenous somatostatin inhibits release of gastrin from gastrin cells as well as the action of gastrin on parietal cells (SCHRUMPF et al 1978). Using antibodies directed against somatostatin an increase in basal and stimulated gastrin release has been demonstrated in an isolated perfused stomach (SHORT et al 1985).

2.8 ACTIONS OF GASTRIN

Gastrin has two major physiological effects on the stomach. Firstly, it stimulates the parietal cells to secrete acid and is the major stimulant of acid secretion after food (FELDMAN et al 1978, RICHARDSON et al 1976).

It does this

- directly, by stimulating gastrin receptors on parietal cells. Activation of the gastrin receptor also potentiates histamine stimulated acid secretion.

- indirectly, by stimulating release of histamine from mast cells which then stimulates acid secretion.

Secondly, it stimulates division of stem cells

within gastric oxyntic glands (JOHNSON et al 1976). These cells in turn can differentiate into parietal cells, surface epithelial cells and enterochromaffin like cells.

Studies done to correlate gastrin with acid secretion between individuals resulted in controversial findings which may be due to variable parietal cell sensitivity to endogenous gastrin (TRUDEAU et al 1971, GEDDE-DAHL 1975, PETERSEN et al 1975, WESDORP 1974). However, within individuals, there is correlation between serum gastrin concentrations and acid secretion (LAM et al 1979). In addition, the maximal acid output is inversely related to post prandial integrated gastrin response (LAM et al 1976) suggesting there is an inverse relationship between G cell mass and parietal cell mass.

Gastrin also stimulates pepsin secretion and increases gastric mucosal blood flow. Contraction of the lower oesophageal sphincter appears to occur only at supra-physiological concentrations of gastrin.

Postprandial levels of gastrin have been shown to stimulate gastric mucosal DNA synthesis (JOHNSON 1977). Antrectomy results in mucosal atrophy in most of the gastrointestinal tract which can be prevented by exogenous gastrin administration (SEIDEL et al 1985). This trophic effect of gastrin on gastric mucosa is

antagonised by secretin and vasoactive intestinal polypeptide (STANLEY et al 1972). A similar effect is seen with proglumide, an antagonist of gastrin that acts by competitively binding to gastrin receptors (JOHNSON et al 1984). Conditions associated with prolonged elevations of gastrin, as Zollinger Ellison syndrome, are associated with gastric mucosal hypertrophy. The trophic effect of gastrin on colonic mucosa, however, is not clear.

2.9 GASTRIN AND DUODENAL ULCERATION

There is evidence that gastrin may play a role in the pathogenesis of duodenal ulceration. Basal serum gastrin has been demonstrated to be elevated in duodenal ulcer patients when compared with controls (GEDDE-DAHL 1975). Other studies, however, have been unable to show any difference (GANGULI et al 1972, McGUIGAN et al 1973, STADIL et al 1973). The post-prandial gastrin response is also unclear, with some studies demonstrating a higher response in ulcer patients than controls (McGUIGAN et al 1973, TAYLOR et al 1979, NYREN et al 1986) and others unable to demonstrate any difference (HUGHES et al 1976, LAM et al 1980, HIRSCHOWITZ et al 1985, BLAIR et al 1987). WALSH et al (1975) reported that the normal inhibition of gastrin release by a fall in intragastric pH is

impaired in ulcer patients suggesting a defect in autoregulation. Maximal acid output, which reflects parietal cell mass, (CARD et al 1960, CHENG et al 1977) may be normal or elevated in duodenal ulcer patients (BARON et al 1973). The acid secretory response to a meal in duodenal ulcer patients, though not higher, is more prolonged than in controls (BLAIR et al 1987). Ulcer patients are more sensitive than normals to the stimulation of acid secretion by pentagastrin (ISENBERG et al 1975) as well as endogenous gastrin (LAM et al 1980).

There have been conflicting reports concerning the concentration of gastrin in antral mucosa of duodenal ulcer patients compared with controls with studies showing higher, lower and no difference in concentration of gastrin in antral mucosa of ulcer patients (MALMSTROM et al 1975, CREUTZFELDT et al 1976, MALMSTROM 1976). The G cell mass in ulcer patients was higher than controls but showed a wide variation (ROYSTON et al 1978).

It, therefore, appears that duodenal ulcer patients are a heterogeneous group with a wide range of parietal and gastrin cell masses.

2.10 OTHER CAUSES OF HYPERGASTRINAEMIA

Conditions in which elevated levels of plasma

gastrin have a pathogenic role are Zollinger Ellison syndrome and isolated retained antrum syndrome. In addition, elevated levels of gastrin are seen in antral G cell hyperplasia (POLAK et al 1972), pyloric stenosis (TANI et al 1977), chronic renal failure (HANSKY et al 1975, HALLGREN et al 1978) intestinal resection (STRAUS et al 1974), vagotomy (WALSH et al 1975, LAM et al 1978) and in atrophic gastritis with or without pernicious anaemia (TRUDEAU et al 1971, GANGULI et al 1971, STRICKLAND et al 1971).

2.11 PATHOGENESIS OF HYPERGASTRINAEMIA IN CHRONIC HELICOBACTER PYLORI INFECTION

H.pylori induced gastritis is predominantly in the antrum. Eradication of the infection results in a fall in both basal and meal stimulated plasma gastrin concentrations (McCOLL et al 1991,ODERDA et al 1989). The mechanism by which H.pylori raises plasma gastrin concentration is unclear. This may be brought about by

(1) Elevation of antral surface pH

H.pylori has a high urease activity by which it hydrolyses urea to produce ammonium and bicarbonate. The ammonium may raise antral surface pH resulting in loss of the physiological inhibition of gastrin release by luminal acid. This would result in increased release

of gastrin from the G cells.

(2) Direct stimulation of gastrin release by bacterial ammonium

The ammonium produced by the organism may be a direct stimulus to gastrin release by the G cells. In rats,dietary ammonia produces an elevation in serum gastrin and this elevation is greater than that seen in controls (LICHTENBERGER et al 1981).

(3) Inducing antral gastritis

Antral gastritis induced by the infection may increase gastrin release from the G cells. In a study on dyspeptic patients, the fasting serum gastrin in the group with H.pylori positive or negative gastritis was greater than that seen in the group with normal mucosa (WYATT et al 1989). These findings suggest that gastritis, independent of H.pylori status, results in raised serum gastrin. Immunohistochemical studies of gastric mucosal biopsy specimens with H.pylori-associated gastritis demonstrate the presence of an increased number of T cells (ENGSTRAND et al 1989). TEICHMANN et al (1989) have shown that the T-cell products interleukin-2 and gamma-interferon induce gastrin release in isolated perfused canine antrum.

(4) a physiological response to inhibition of parietal cell function by the infection

H.pylori may produce a factor which inhibits the parietal cells resulting in reduced gastric acid output and the hypergastrinaemia may be a physiological response to this.

BORODY et al (1989) studied the effect of eradication of H.pylori with triple therapy in 14 patients with H.pylori positive hypochlorhydria. On reassessment 8 weeks after commencement of therapy, the gastric pH showed a significant fall from a pretreatment value of 6.9 to 1.5 post-treatment. In the 6, in whom the organism was not eradicated, the corresponding values were unchanged at 6.8 and 5.3 .

DEFIZE et al (1988), in a study on isolated guinea pig parietal cells showed a 50% inhibition of histamine stimulated acid production by H.pylori.

CAVE et al (1989) measured the effect of H.pylori on 14C-aminopyrine uptake by rabbit gastric epithelial cells. There was a significant inhibition of acid secretion equivalent to that produced by 0.0001 mol/l of cimetidine. This occurred with whole organisms and sonicates and was not due to a toxic effect of the inhibitor on the epithelial cells.

All these studies suggest that the infection produces a factor that inhibits acid production by the

parietal cells.

H.pylori may raise serum gastrin by one or a combination of these mechanisms or by other still undiscovered means. The aim of this thesis is to determine which of these mechanisms is responsible for the hypergastrinaemia seen with chronic Helicobacter pylori infection.

SECTION II

EXPERIMENTAL WORK

CHAPTER 3

THE DEGREE OF HYPERGASTRINAEMIA INDUCED BY HELICOBACTER PYLORI IS THE SAME IN DUODENAL ULCER PATIENTS AND ASYMPTOMATIC VOLUNTEERS

3.1 Introduction

There is considerable evidence that Helicobacter pylori infection of the gastric antrum predisposes to duodenal ulceration. The mechanism by which the H.pylori predisposes to duodenal ulceration is unclear but may be related to the excessive release of gastrin by the inflamed antral mucosa. Eradication of H.pylori results in a 27-33% fall in basal plasma gastrin and a 30-58% fall in the integrated gastrin response to a meal.

H.pylori infection is also found in a considerable proportion of the healthy non-ulcer population and its prevalence increases with age. In asymptomatic Caucasian individuals the organism is present in approximately 10% of those under 20 years of age compared with 70% of those more than 80 years old (GRAHAM et al 1988). There has been no direct comparison of the effect of H.pylori infection on gastrin in duodenal ulcer patients versus asymptomatic volunteers. The relative effect of H.pylori on serum gastrin in these groups should indicate the importance of the hypergastrinaemia in the pathogenesis of the ulcer disease.

I have examined basal and meal stimulated gastrin concentrations in H.pylori positive and negative healthy volunteers and compared these with values in

duodenal ulcer patients.

3.2 Patients

Eleven duodenal ulcer patients (mean age 41 years, one woman) in whom active duodenal ulceration within the previous year had been confirmed endoscopically, were studied. H.pylori infection had been confirmed in each patient by 14C-urea breath test, rapid urease test (CLO test, Delta West Ltd, Bentley, Western Australia) and antral histology performed within the previous month. Acid inhibitory agents were discontinued at least 2 weeks before entry into the study. None had received any bismuth preparations.

Eleven asymptomatic volunteers with H.pylori infection (mean age 40 years, all men) and 11 asymptomatic volunteers without H.pylori infection (mean aged 37 years, one woman) were also examined. Their H.pylori status had been confirmed by either the 14C- (n=10) or 13C- (n=12) urea breath test.

The 14C-urea breath test was performed as previously described and the 20-min value used as this has been shown to be the most discriminating (BELL et al 1987). The mean 20-min value (%14C dose per mmol CO2 X 100 X body weight(kg)) of the four volunteers to be positive with this test was 160 (range 108-227) and the values for those negative was 5 (range 4-7). For

the 13C-urea breath test, the volunteers were given 250ml Ensure Plus (Abbot laboratories, Maidenhead, UK) to delay gastric emptying, immediately followed by 0.1g of 13C-urea in 50ml of water. Breath samples were collected before and at 40 and 60 min after administration of the isotope for 13CO2 analysis. A rise in the breath 13CO2 over the baseline, of more than five parts per 1000 was taken as a positive result. The mean rise in breath 13CO2 (parts per 1000) in the five volunteers considered negative for H.pylori was 1.35 (range 0.31-3.66) and that in those positive was 15.85 (range 10.08-28.00). The 13C-urea breath test has been shown to be an accurate means of determining H.pylori status (DILL et al 1990).

3.3 Materials and Methods

Basal plasma gastrin and the integrated gastrin response to a standardised meal was measured in all the patients and volunteers. They presented one morning after an overnight fast. Blood samples were collected at 15-min intervals for 30 minutes for determination of basal plasma gastrin. They then drank, over 5 minutes, a standardised peptone meal consisting of two OXO beef cubes (OXO Ltd., Croydon, UK) in 200ml of water at 50 degrees C. A blood sample was collected 5 minutes after completing the drink and further samples at

10-minute intervals for 90 minutes, for gastrin determination.

Blood samples for plasma gastrin estimation were collected and analysed as described before. Samples from duodenal ulcer patients and healthy volunteers were assayed in the same batch. The basal gastrin value was taken as the mean of the three samples taken prior to the meal.

Statistical significance was assessed by the Mann Whitney U test. The study was approved by the Western Infirmary Ethical Committee and each patient gave written informed consent.

3.4 Results

The median basal plasma gastrin in the H.pylori positive volunteers was 47ng/l (range 27-95) and was not different from that in the duodenal ulcer patients with H.pylori infection (53ng/l, 13-103)($p=0.4$). The median basal gastrin in the H.pylori negative volunteers was 20ng/l (range 7-45) which was lower than the H.pylori positive volunteers ($p<0.001$) and duodenal ulcer patients ($p<0.002$) (Fig 4, Table 1).

The median integrated gastrin response to the meal was also similar in the H.pylori positive volunteers and duodenal ulcer patients with respective values of 2650 ng/l.min (range 900-8500) and 2225 ng/l.min (range

H.pylori positive duodenal ulcer patients		H.pylori positive asymptomatic volunteers		H.pylori negative asymptomatic volunteers	
Basal gastrin	Integrated plasma gastrin	Basal gastrin	Integrated plasma gastrin	Basal gastrin	Integrated plasma gastrin
83	2100	40	2150	17	900
58	8725	27	1500	30	225
58	5500	95	8250	40	300
42	1100	37	3150	20	450
13	550	48	1800	15	750
103	2350	85	900	35	550
23	7225	35	1050	10	525
53	800	43	5150	7	225
38	2860	47	6700	20	1125
53	1900	50	8500	22	600
87	2225	47	2650	45	2925

Table 1 Basal plasma gastrin (ng/L) and integrated plasma gastrin response (ng/l.min) to a standardised meal in H.pylori positive duodenal ulcer patients and H.pylori positive and negative asymptomatic volunteers.

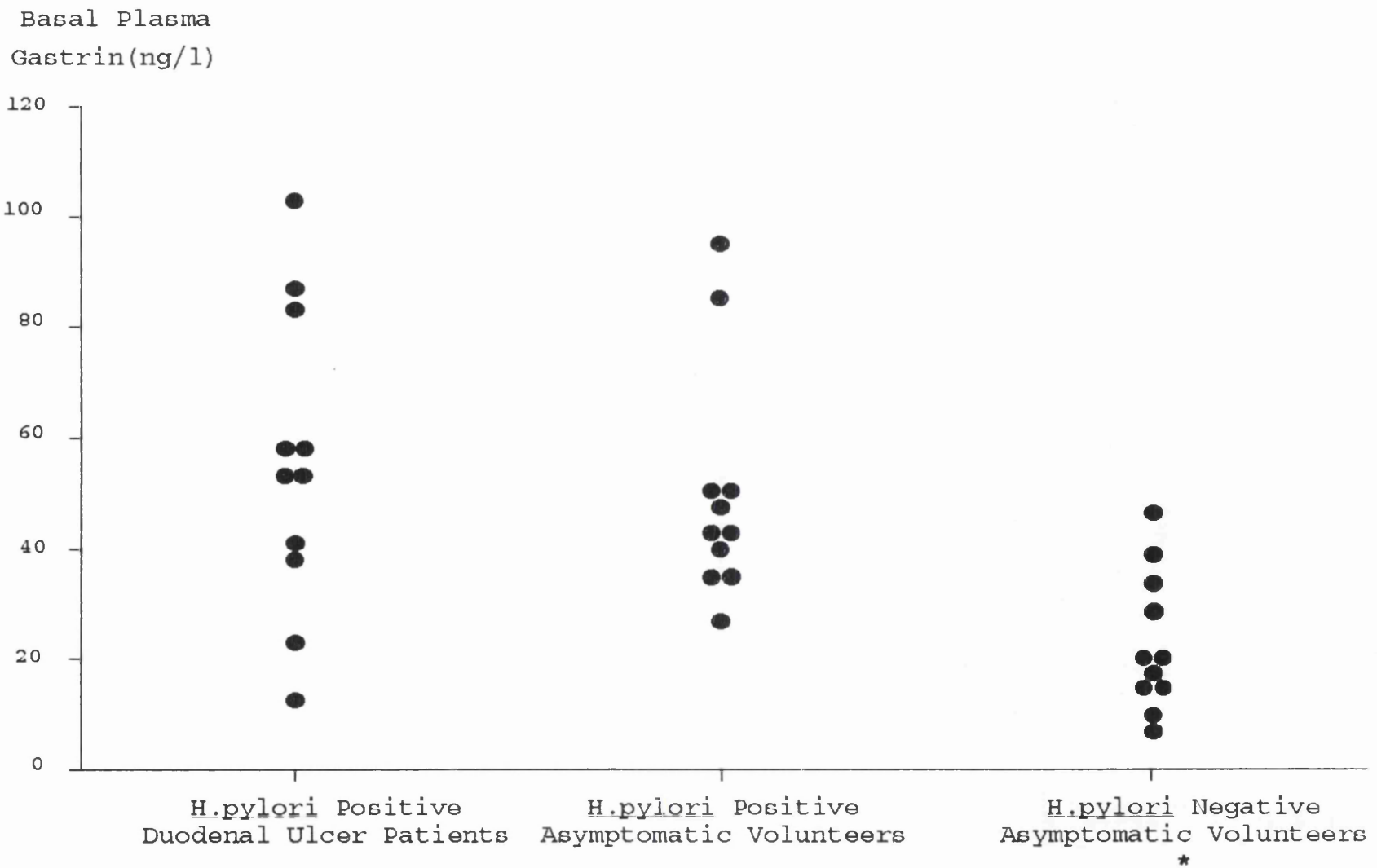

Figure 4 Basal plasma gastrin concentrations (ng/l) in H.pylori positive duodenal ulcer patients and H.pylori positive and negative asymptomatic volunteers .
* Indicates less than H.pylori asymptomatic volunteers at $p<0.001$ and less than H.pylori positive duodenal ulcer patients at $p<0.002$.

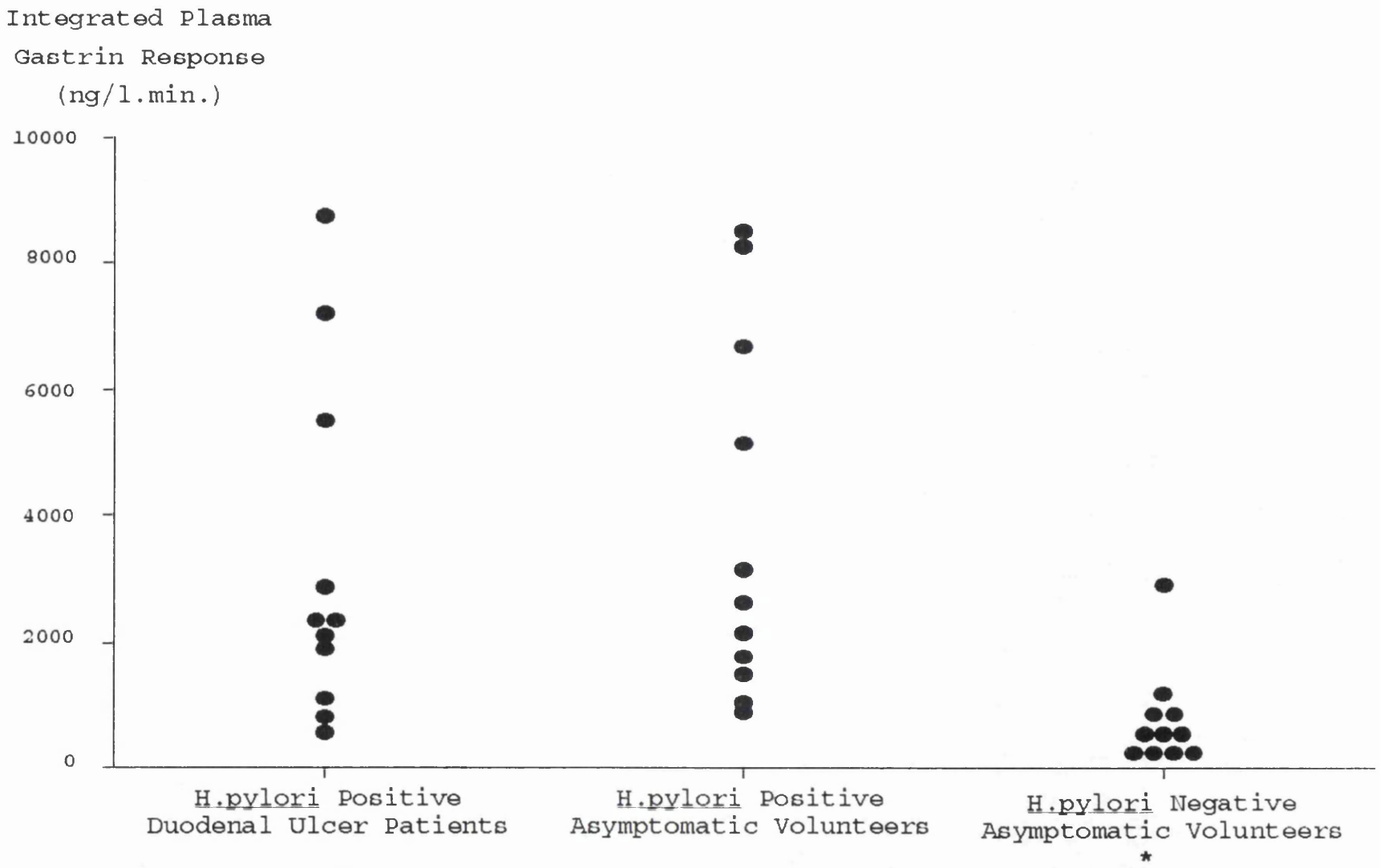

Figure 5 Integrated plasma gastrin response (ng/l.min) in H.pylori positive duodenal ulcer patients and H.pylori positive and negative asymptomatic volunteers.
* Indicates less than H.pylori positive asymptomatic volunteers at $p<0.001$ and less than H.pylori positive duodenal ulcer patients at $p<0.002$.

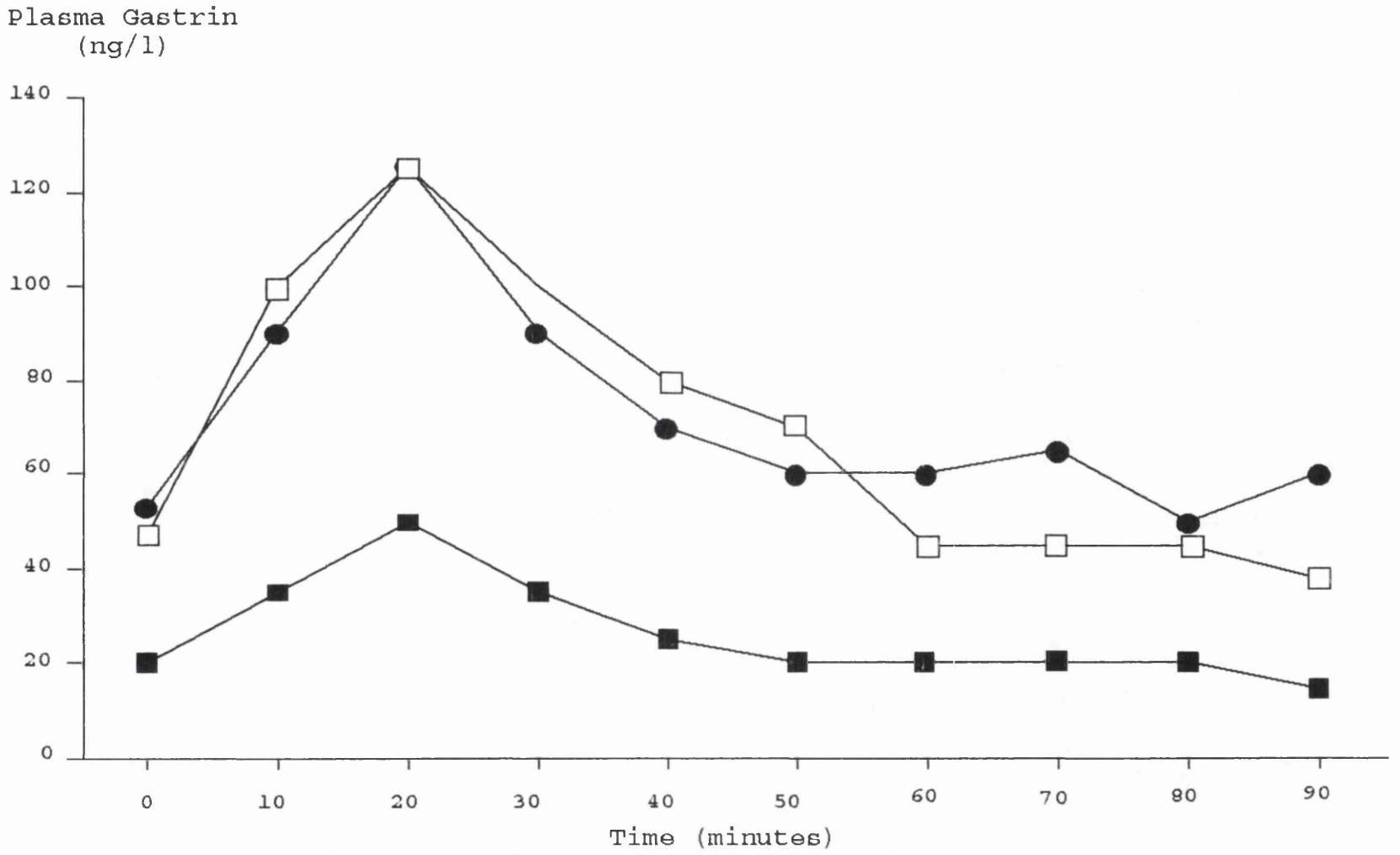

Figure 6 Median plasma gastrin response (ng/l) to the standardised meal in the H.pylori positive duodenal ulcer patients and H.pylori positive and negative asymptomatic volunteers (n=11 for each group).

■, H.pylori positive duodenal ulcer patients □, H.pylori positive asymptomatic volunteers ● , H.pylori negative asymptomatic volunteers

550-8725)(p=0.7). The integrated gastrin response in the H.pylori negative volunteers was 550 ng/l.min (range 225-2925) which was less than that in the H.pylori positive volunteers (p<0.001) and duodenal ulcer patients (p<0.002) (Fig 5, Table 1).

More detailed analysis was performed to investigate any possible difference between the gastrin response in the H.pylori positive duodenal ulcer patients and H.pylori positive volunteers. This showed that there was no difference between these groups with respect to the gastrin values at any of the 12 time points studied. In addition there was no difference in the time for gastrin to return to its basal value following the meal in the two groups (Fig 6).

3.5 Discussion

There is a strong association between H.pylori infection and duodenal ulceration (O'CONNOR et al 1986, RAUWS et al 1988). However, the infection is found in approximately 50% of adults in the Western world (GRAHAM et al 1991) whereas duodenal ulceration affects only about 10% of this population (LANGMAN 1979). The reason for less than one in five people with H.pylori infection developing duodenal ulceration is not clear. The present study investigated the possibility that the likelihood of developing duodenal

ulceration may be related to the degree of hypergastrinaemia induced by the chronic infection.

The results demonstrate that H.pylori infection raises plasma gastrin concentrations in healthy volunteers as well as duodenal ulcer patients. My study also demonstrates that basal and meal stimulated gastrin concentrations in the H.pylori positive volunteers were not different from those in H.pylori positive duodenal ulcer patients. This finding indicates that the explanation for only the minority of the population with H.pylori infection developing duodenal ulceration is not the degree of hypergastrinaemia induced by the infection.

In conclusion, the finding that H.pylori raises plasma gastrin to a similar level in duodenal ulcer patients and asymptomatic volunteers indicates that factors other than hypergastrinaemia must play a role in the pathogenesis of ulcer disease.

CHAPTER 4

EFFECT OF INCREASING HELICOBACTER PYLORI AMMONIA PRODUCTION BY UREA INFUSION ON PLASMA GASTRIN CONCENTRATIONS

4.1 INTRODUCTION

Patients with Helicobacter pylori infection of the gastric antrum have raised plasm gastrin concentrations (LEVI et al 1989) which fall when the infection has been eradicated (CHAPTER 5, ODERDA et al 1989). Gastrin may be the link between chronic H.pylori infection and duodenal ulceration (LEVI et al 1989). The mechanism by which H.pylori infection increases the plasma gastrin concentration is unknown but could be related to its high urease activity (MARSHALL et al 1986). The ammonia produced by the bacterial urease could directly stimulate gastrin release by G cells or indirectly by raising antral surface pH.

To determine whether the raised plasma gastrin concentrations is the result of ammonia production by H.pylori I studied the effect of altering the rate of ammonia formation on plasma gastrin concentration by infusing urea intragastrically.

4.2 PATIENTS

Eight patients (five men) with a history of endoscopically confirmed duodenal ulceration within the previous year were studied. Their ages ranged from 26 to 62 years. Each of the patients had H.pylori

infection of the gastric antrum confirmed by histology of antral biopsy specimens, rapid urease test (CLO test)(MARSHALL et al 1987) and 14C urea breath test (BELL et al 1987). None had taken any acid inhibitory agents or bismuth preparations in the month before the study.

4.3 METHODS

The plasma gastrin response to increasing H.pylori ammonia production was investigated by infusing a urea solution into the gastric antrum. Each patient acted as his or her own control by having the urea infusion repeated one month after a 3 week course of tripotassium dicitrato bismuthate (De-Noltab) 120mg QID and metronidazole 400mg TID designed to eradicate H.pylori. Confirmation of eradication of H.pylori was achieved by repeating the endoscopic antral biopsies and 14C urea breath test one month after the completion of treatment.

After a 16 hour fast a dual lumen size 16 F gastric tube (Andersen Inc, New York) was passed nasogastrically and positioned radiographically so that its tip lay in the distal part of the stomach. An intravenous cannula was inserted in the antecubital vein. At the end of a 30 minute basal period the stomach was emptied, and over the following hour

dextrose solution (328 mmol/l) that did not contain urea was infused into the stomach at a rate of 2 ml per minute. After this control period, a dextrose solution containing urea (50 mmol/l) was infused at a rate of 2 ml per minute for four hours. The concentration of dextrose in the urea solution was reduced by 50 mmol/l so that the osmolalities of the two infusions were the same. In addition, the pH of the solutions was reduced to 1.8 by adding 1 ml concentrated HCl per 500 ml dextrose to prevent them raising intragastric pH.

Venous blood samples were obtained at 30 minute intervals throughout the experiment for plasma gastrin determination and were collected in lithium heparin tubes containing 4000 KIU aprotonin (Trasylol). The blood was centrifuged at 3000g for 10 minutes at 4 degrees C. and the plasma was stored at -20 degrees C. During the intragastric infusions, 10 ml samples of gastric juice were collected every 15 minutes for measurement of the ammonium and urea concentration and determination of pH. At the end of each hour all the gastric contents were aspirated to prevent the accumulation of infusate.

The plasma gastrin concentration was measured by radioimmunoassay using antibody R98 which has a lower limit of detection of 5-10 ng/l (ARDILL 1973).

Gastric aspirate urea concentrations were measured by a urease method (SMAC I Technicon, Basingstoke, UK) and ammonium concentrations by an enzymatic method (SIGMA, Dorset, UK). The pH of the gastric aspirate was measured with a glass electrode (Radiometer ETS 822).

Statistical significance was assessed by Wilcoxon signed rank sum test. The study was approved by the Western Infirmary Ethical committee and each patient gave informed written consent.

4.4 RESULTS

In seven patients the H.pylori infection was successfully eradicated as confirmed by the absence of organisms and resolution of gastritis seen on antral biopsy specimens, negative CLO test and negative 14C urea breath test four weeks after completing the antibacterial treatment. Their median 20 minute breath test value was 135% of administered dose per mmol CO2 X kg body weight X 100 (range 104-251) before treatment and fell to 4.5 (range 0.6-7) one month afterwards. The one patient in whom the organism was not cleared was excluded from further analysis.

BASAL VALUES

The median concentration of ammonium ions in the

basal gastric aspirate before eradication of H.pylori was 4.4 mmol/l (range 1.8-14.7) and this fell after eradication to 0.7 mmol/l (range 0.3-1.4) ($p<0.02$). The median concentration of urea in the basal gastric aspirate was 1.1 mmol/l (range 0.3- 1.6) and rose to 2.5 mmol/l (range 1.0-3.4) after eradication ($p<0.02$). The median plasma concentration of gastrin was 30 ng/l (range 15-60) and this fell to 20ng/l (range 15-25) ($p<0.05$) after eradication. The median pH of the basal gastric aspirates did not change significantly - it was pH 1.7 (range 1.2-1.9) before and 1.6 (range 1.2-1.7) after eradication of H.pylori (Fig 7, Tables 2,3,4,5).

EFFECT OF INTRAGASTRIC INFUSIONS

During the 60 minute control infusion of dextrose solution, both before and after eradication, there was a progressive fall in urea and ammonium concentrations in the gastric aspirate due to dilution by the infusate. Plasma gastrin concentration did not change over this period.

Before eradication of H.pylori, the urea infusion resulted in a rise in the gastric aspirate urea concentrations which reached a plateau after 60 minutes at a median value of 15.5 mmol/l (range 7.9-21.3). The median gastric juice ammonium concentration

		Basal	Dextrose		Urea + Dextrose							
	Time (hours)	0	0:30	1:00	1:30	2:00	2:30	3:00	3:30	4:00	4:30	5:00
Patient 1	Pre-treatment	0.3	0.0	0.3	11.9	21.8	19.0	11.8	17.4	18.7	23.2	21.8
	Post-treatment	2.4	0.9	1.3	11.2	17.8	13.2	14.7	16.4	14.6	19.2	10.1
Patient 2	Pre-treatment	1.1	0.9	0.7	16.7	14.3	14.3	17.6	12.5	25.4	19.6	17.1
	Post-treatment	2.8	1.9	2.1	19.5	19.5	12.5	18.4	12.5	13.6	19.8	14.9
Patient 3	Pre-treatment	1.0	0.5	0.5	15.8	18.8	20.1	19.8	11.8	10.5	17.9	15.9
	Post-treatment	2.0	0.9	1.9	23.6	29.4	24.0	21.7	33.6	28.6	22.5	20.0
Patient 4	Pre-treatment	0.9	0.5	0.4	11.9	14.6	7.3	13.6	13.9	14.8	12.4	15.0
	Post-treatment	3.1	1.1	1.2	7.7	17.0	15.3	18.3	16.4	17.1	13.0	12.8
Patient 5	Pre-treatment	0.9	0.6	0.5	14.7	20.5	22.3	17.6	17.0	16.3	18.4	12.9
	Post-treatment	1.5	0.6	0.5	16.6	16.1	11.6	15.2	14.8	15.0	15.5	13.0
Patient 6	Pre-treatment	1.2	0.0	0.6	8.1	12.0	17.1	11.8	14.1	13.5	15.4	15.5
	Post-treatment	2.1	1.1	1.3	14.6	10.5	16.3	15.8	19.9	12.9	18.8	19.5
Patient 7	Pre-treatment	0.5	0.2	0.1	11.3	8.8	2.7	7.7	10.3	6.9	17.0	12.2
	Post-treatment	3.4	1.3	1.2	12.7	23.6	21.3	19.0	19.8	17.4	13.9	17.5

Table 2 Effect of intragastric infusion of dextrose and urea on gastric juice concentrations of urea (mmol/l)

		Basal	Dextrose		Urea + Dextrose							
	Time (hours)	0	0:30	1:00	1:30	2:00	2:30	3:00	3:30	4:00	4:30	5:00
Patient 1	Pre-treatment	5.2	2.4	2.3	2.0	5.4	6.6	5.8	5.1	7.4	7.2	7.4
	Post-treatment	0.3	0.5	0.8	0.6	0.7	0.8	0.6	0.8	0.6	0.5	0.5
Patient 2	Pre-treatment	3.8	3.2	1.8	3.7	3.4	3.7	4.9	7.3	3.8	5.9	5.1
	Post-treatment	0.8	0.5	0.4	0.3	0.2	0.2	0.5	0.4	0.2	0.6	0.3
Patient 3	Pre-treatment	4.4	4.4	3.6	8.4	9.8	12.1	13.8	9.0	9.3	10.4	9.0
	Post-treatment	1.4	0.4	0.9	0.8	0.1	1.2	0.7	0.7	0.5	0.5	1.2
Patient 4	Pre-treatment	2.8	2.4	2.0	3.0	4.2	4.1	6.5	5.1	6.3	4.7	6.2
	Post-treatment	0.6	0.2	0.3	0.4	0.4	0.5	0.4	0.6	0.6	0.4	0.3
Patient 5	Pre-treatment	1.8	1.4	1.3	1.6	2.4	1.2	2.6	2.8	2.6	3.2	1.8
	Post-treatment	0.4	0.2	0.2	0.3	0.4	0.3	0.1	0.3	0.2	0.1	0.4
Patient 6	Pre-treatment	8.0	5.1	5.1	5.0	8.1	7.0	6.8	7.3	7.4	10.0	11.6
	Post-treatment	0.9	0.5	0.1	0.7	0.1	0.3	0.3	0.5	0.4	0.8	0.4
Patient 7	Pre-treatment	14.7	5.7	5.9	16.8	9.4	17.4	9.1	14.5	9.8	8.4	8.9
	Post-treatment	0.9	0.3	0.8	0.8	0.8	0.6	0.3	0.6	0.3	0.5	0.5

Table 3 Effect of intragastric infusion of dextrose and urea on gastric juice concentrations of ammonium (mmol/l)

		Basal	Dextrose		Urea + Dextrose							
	Time (hours)	0	0:30	1:00	1:30	2:00	2:30	3:00	3:30	4:00	4:30	5:00
Patient 1	Pre-treatment	1.2	1.5	1.3	1.2	1.6	1.6	1.3	1.3	1.3	1.5	1.6
	Post-treatment	1.3	1.4	1.5	1.3	1.5	1.6	1.4	1.6	1.4	1.7	1.5
Patient 2	Pre-treatment	2.0	1.3	1.6	1.5	1.5	1.4	1.5	1.5	1.5	1.4	1.2
	Post-treatment	1.5	1.5	1.3	1.5	1.9	1.3	1.5	1.4	1.3	1.5	1.2
Patient 3	Pre-treatment	1.9	1.6	1.6	1.6	1.7	1.7	1.7	1.8	1.7	1.4	1.8
	Post-treatment	1.2	1.7	1.4	1.4	1.5	1.8	1.7	1.8	1.5	1.6	1.8
Patient 4	Pre-treatment	1.4	2.1	1.7	1.2	1.6	1.8	1.6	1.9	1.5	1.1	1.6
	Post-treatment	2.0	1.8	2.1	1.8	1.9	1.8	2.3	2.7	2.1	1.9	1.4
Patient 5	Pre-treatment	1.1	1.4	1.4	1.1	1.3	1.1	1.4	1.2	1.2	0.9	1.6
	Post-treatment	1.4	1.4	1.3	1.6	1.3	1.3	1.6	1.3	1.7	1.5	1.4
Patient 6	Pre-treatment	1.4	1.3	0.9	1.3	1.2	1.2	1.3	1.3	1.3	1.4	1.1
	Post-treatment	1.5	1.6	1.5	1.4	1.1	1.4	1.4	1.3	1.3	1.5	1.3
Patient 7	Pre-treatment	1.9	1.8	1.9	2.5	3.0	2.3	2.9	2.8	2.6	1.8	3.8
	Post-treatment	1.6	1.6	1.5	1.6	1.5	1.4	1.4	1.8	1.5	1.5	1.6

Table 4 Effect of intragastric infusion of dextrose and urea on gastric juice pH.

		Basal	Dextrose		Urea + Dextrose							
	Time (hours)	0	0:30	1:00	1:30	2:00	2:30	3:00	3:30	4:00	4:30	5:00
Patient 1	Pre-treatment	30	30	35	25	30	30	35	30	30	35	45
	Post-treatment	15	15	15	15	10	10	15	15	15	15	20
Patient 2	Pre-treatment	20	35	20	30	50	20	25	30	30	15	30
	Post-treatment	15	40	45	30	20	15	20	15	15	15	20
Patient 3	Pre-treatment	60	15	15	15	15	15	15	15	15	15	15
	Post-treatment	25	32	31	37	20	35	27	20	28	46	41
Patient 4	Pre-treatment	40	35	35	30	25	35	40	35	50	50	55
	Post-treatment	15	15	15	15	15	20	15	5	10	15	15
Patient 5	Pre-treatment	40	35	40	20	50	30	45	45	30	40	30
	Post-treatment	20	25	30	35	25	20	20	20	15	30	20
Patient 6	Pre-treatment	25	25	30	25	25	15	10	25	15	10	15
	Post-treatment	20	30	30	30	20	20	35	25	15	15	25
Patient 7	Pre-treatment	15	25	30	20	15	15	20	15	20	15	15
	Post-treatment	20	15	15	15	10	15	10	15	15	15	18

Table 5 Effect of intragastric infusion of dextrose and urea on plasma gastrin concentration (ng/l)

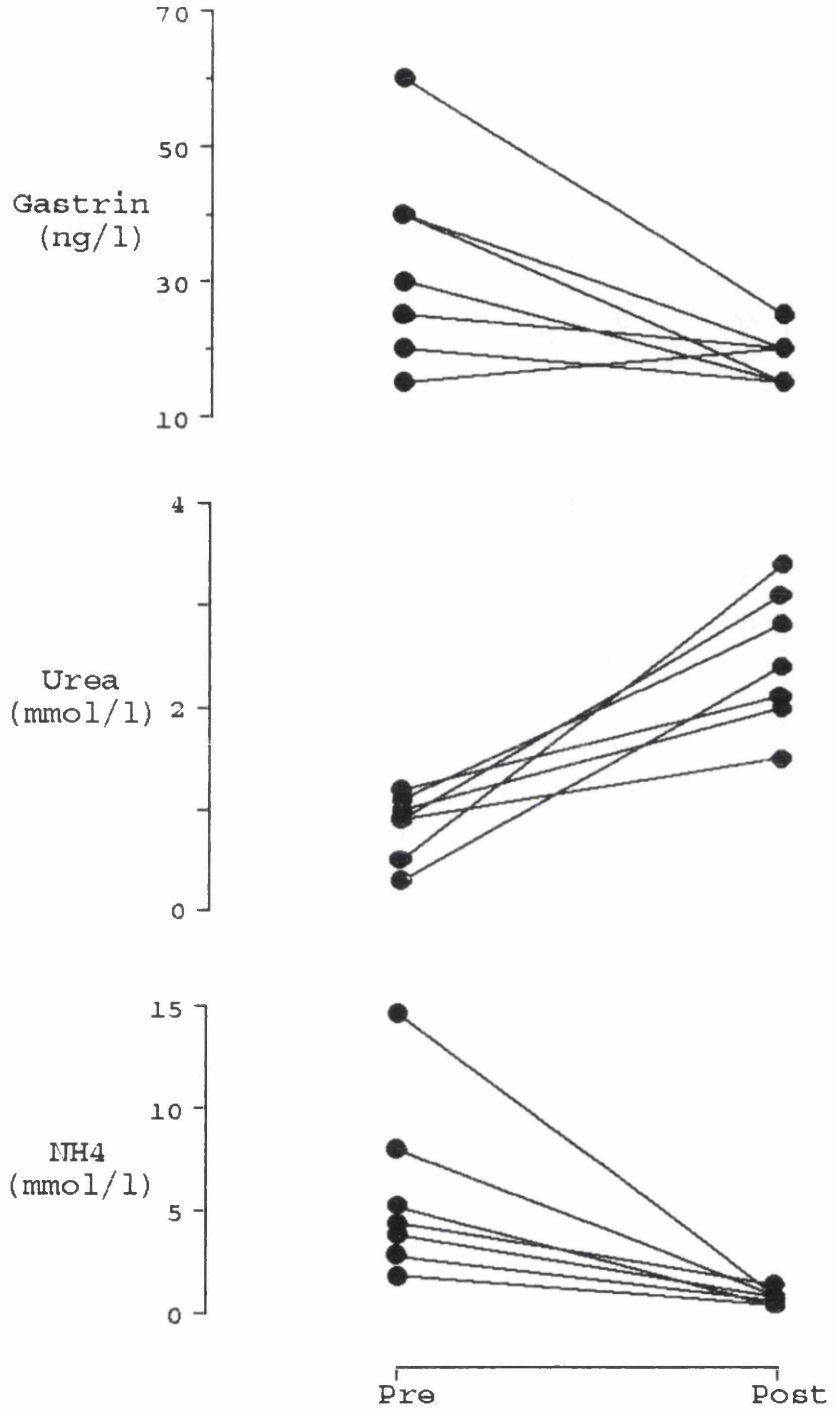

Figure 7 Gastric juice concentrations of urea (mmol/l) and ammonium (mmol/l) and plasma gastrin concentrations (ng/l) before and after eradication of H.pylori infection in 7 duodenal ulcer subjects.

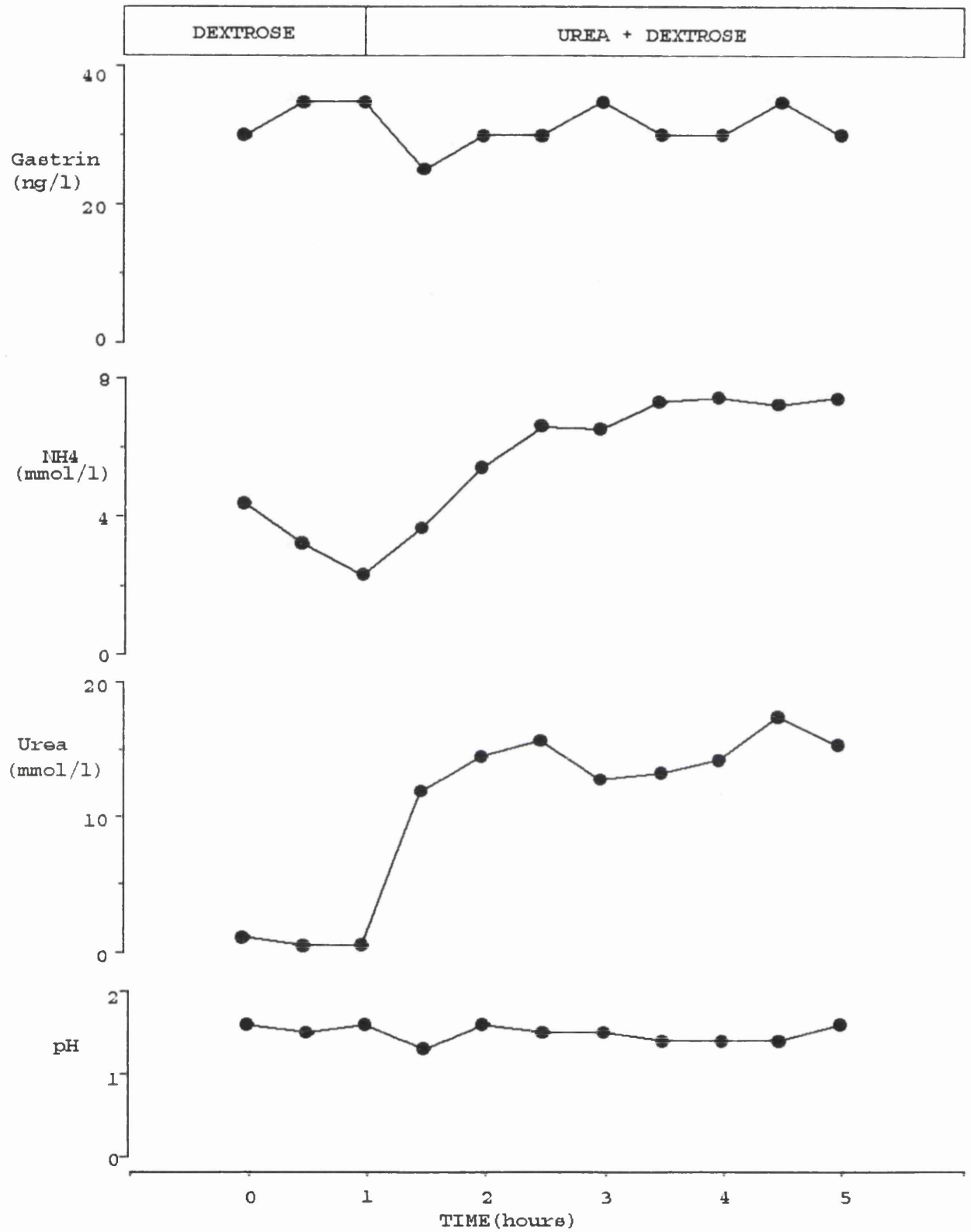

Figure 8 Effect of intragastric infusion of urea on gastric juice concentrations of urea(mmol/l) and ammonium(mmol/l), intragastric pH and plasma gastrin concentration(ng/l) in 7 patients with H.pylori infection of the gastric antrum. Values are medians.

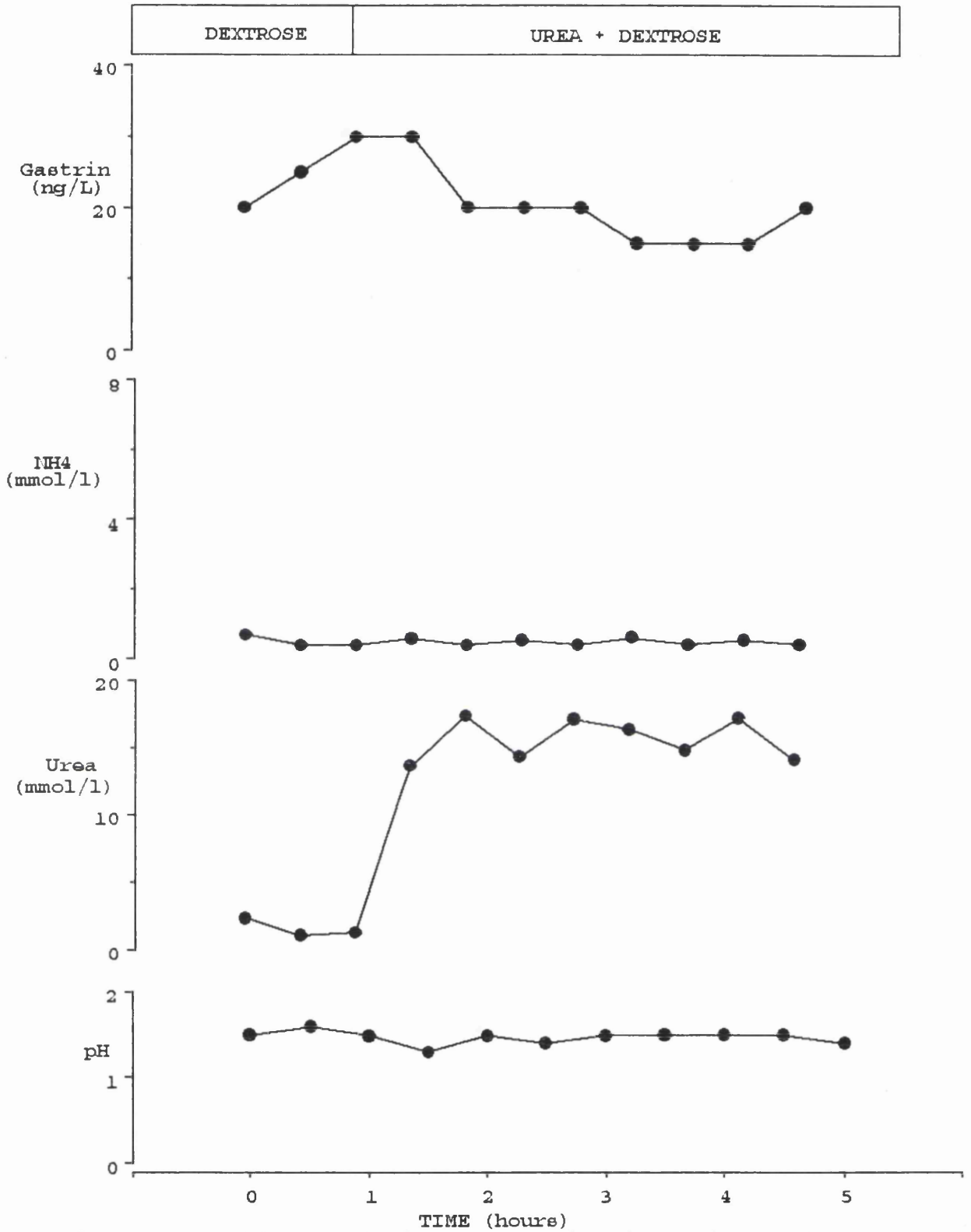

Figure 9 Effect of intragastric infusion of urea on gastric juice concentrations of urea (mmol/l) and ammonium (mmol/l), intragastric pH and plasma gastrin concentration (ng/l) in 7 patients following eradication of H.pylori infection of the gastric antrum. Values are medians.

immediately before beginning the urea infusion was 2.3 mmol/l (range 1.3-5.9) and rose over 90 minutes to reach a median plateau value of 6.1 mmol/l (range 4.2-11.9). This rise in ammonium production was not accompanied by any change in plasma gastrin concentration (Fig 8, Tables 2,3,4,5).

After eradication of H.pylori, the rise in gastric juice urea concentration during urea infusion was similar to that before eradication. On this occasion, however, there was no rise in the ammonium concentration. The median value immediately before beginning the infusion was 0.4 mmol/l (range 0.1-0.9) and it was 0.4 mmol/l (range 0.3-1.2) at the end of the infusion. The median gastrin concentration, which was lower after eradication of H.pylori, was unaffected by the urea infusion (Fig 9, Tables 2,3,4,5).

The pH of gastric aspirates remained between 1.5 and 2.0 throughout the studies (Fig 8,9).

4.5 DISCUSSION

In agreement with work done by McCOLL et al (1989), this study demonstrated a considerable lowering of plasma gastrin concentrations after eradication of H.pylori infection in duodenal ulcer subjects. The finding that increasing H.pylori ammonia production

failed to change the plasma gastrin concentration does not lend support to the hypothesis that the hypergastrinaemia is caused by the ammonia. It seems unlikely that the degree of increase in ammonia production was inadequate as the rise in the ammonium ion concentration at the antral epithelial surface, where the bacteria are found close to the gastrin secreting G cells, would have been greater than the three fold rise noted in the gastric aspirate. It should be stressed, however, that the failure to cause a further increase in gastrin by augmenting ammonia production does not exclude the possibility that the raised gastrin value was caused by the organism's ammonia production. The amount of ammonia produced by the bacterium under normal conditions may be sufficient to produce the maximum gastrin response via this mechanism.

It has been proposed by LEVI et al (1989) that the increased plasma gastrin concentrations are caused by the organism's ammonia production raising antral surface pH. This would interfere with the physiological suppression of gastrin release by luminal acid. Our finding that augmenting H.pylori ammonia production fails to stimulate gastrin does not refute this hypothesis. The local alkalinising effect of H.pylori under basal conditions may already be

sufficient to prevent suppression of gastrin release by gastric acid. In addition, the duration of the urea infusion in our study was four hours and earlier studies have shown that gastric alkalinisation was required for five hours before there was any rise in serum gastrin (PETERS et al 1983). The effect of antral alkalinisation on serum gastrin in humans is controversial, however, as a more recent study could not detect any increase after raising gastric pH for 10 hours (PETERSON et al 1986). Unfortunately, there are no studies of the effect of gastric alkalinisation on serum gastrin in patients of known H.pylori status.

In conclusion, though lowering intragastric ammonia concentration by eradicating H.pylori is accompanied by a lowering of plasma gastrin concentration, augmenting the bacterium's ammonia production is not accompanied by a further increase in plasma gastrin.

CHAPTER 5

EFFECT OF COMPLETE SUPPRESSION OF H.PYLORI UREASE ACTIVITY ON SERUM GASTRIN

5.1 INTRODUCTION

The mechanism by which the chronic antral H.pylori infection predisposes to duodenal ulceration is unclear but stimulation of excessive gastrin release may be important (ODERDA et al 1989, LEVI et al 1989, GRAHAM et al 1990). Eradication of H.pylori infection results in a fall in the basal gastrin concentration of 27-33% and a fall of 30-58% in the integrated gastrin response to a meal. The mechanism by which H.pylori infection raises plasma gastrin concentration is unclear and may be related to the organism's high urease activity or the antral gastritis induced by the infection.

In an attempt to differentiate between the effects of the H.pylori urease activity and the antral gastritis I decided to examine serum gastrin concentrations soon after commencing H.pylori eradication therapy. At this early time I hoped to achieve complete suppression of bacterial urease activity without significant resolution of the antral gastritis.

5.2 PATIENTS

Ten patients (7 male,age range 31-65) with a history of endoscopically confirmed duodenal ulcer were studied. In each of the patients H.pylori infection

had been demonstrated by histology of antral biopsy and rapid urease test (CLO test) (MARSHALL et al 1987). All the patients discontinued any therapy with acid inhibitory agents for at least two weeks before entering the study. During this period they were allowed antacids except for the 24 hours preceding the first study day. None had taken bismuth preparations previously.

Four volunteers who were shown to be negative for H.pylori by a 14C-urea breath test served as a control group.

5.3 MATERIALS AND METHODS

In 8 of the patients the following studies were conducted. Following a 14C-urea breath test to determine H.pylori urease activity, upper gastrointestinal endoscopy was performed and two antral biopsies taken for assessment of severity of antral gastritis. Within 3 weeks following this the patients reported fasted one morning and two samples of venous blood were collected 15 minutes apart for estimation of basal serum gastrin concentration. They then drank over 5 minutes, 250mls of Ensure Plus (Abott laboratories, England) which contains 12.5g fat, 15.6g protein and 50g carbohydrate. The gastrin response to this was measured by taking serum samples at 5 minutes

after completing the drink and then at 10 minute intervals for 90 minutes.

On the morning after completing the above tests they commenced tripotassium dicitrato bismuthate (De-Nol) 120mg qid, amoxycillin 500mg tid and metronidazole 400mg tid (Triple therapy). They took one complete day's treatment and reported fasted the following morning. Their morning dose of therapy was administered at 08:00h and one hour later two samples of blood were obtained 15 minutes apart for estimation of basal serum gastrin. Immediately following this a 14C-urea breath test was performed. The breath test involves drinking 250mls of Ensure Plus to delay gastric emptying of the 14C urea. It was therefore possible to simultaneously reassess the gastrin response to this. Studies have shown that a fall in meal stimulated gastrin response following eradication of H.pylori occurs with various meals (GRAHAM et al 1990, LEVI et al 1989) and is not specific to the OXO meal. At 13:00 hours an upper gastrointestinal endoscopy was repeated and further antral biopsies obtained for a CLO test and to reassess the severity of the gastritis. The four hour delay between the breath test and endoscopy was to allow gastric emptying of the Ensure Plus. The patients then went on to complete the 3 week course of triple therapy. One month after

completion of therapy their H.pylori status was reassessed by 14C-urea breath test, antral histology and CLO test.

The other two patients were studied in a similar fashion except that their gastrin response to a standardised OXO meal (OXO Ltd.,Croydon, England) was assessed immediately prior to performing the 14C-urea breath test. This prolonged the second study day and meant that upper gastrointestinal endoscopy could not be performed. The response to the OXO meal was undertaken as the original observation of a marked fall in integrated gastrin response following eradication of H.pylori used this meal. The standardised OXO meal was prepared by dissolving 2 OXO cubes in 200mls of water at 50 degrees Centigrade. They drank this over 5 minutes. The first sample of serum for gastrin estimation was collected 5 minutes after completion of the meal and further samples at 10 minute intervals for 90 minutes.

The 4 healthy volunteers without H.pylori infection served as the control group. They had their gastrin response to a standardised OXO meal measured before and 24 hours after commencement on the same triple therapy regimen as the patient group.

5.4 ANALYSES

The blood samples for estimation of plasma gastrin were collected and assayed as previously described. All samples for each patient were measured in the same assay.

The antral biopsies were stained with haematoxylin and eosin stain and examined double blind by a single pathologist and given an aggregate gastritis score of between 1-10 as described by RAUWS (1988). Chronic inflammatory infiltrate in the lamina propria was scored as 0-2, lamina propria polymorph infiltrate as 0-3, intra-epithelial polymorph infiltrate as 0-3 and mucosal erosions as 0-2.

Statistical significance was assessed by Wilcoxon signed rank sum test. The study was approved by the Western Infirmary Ethical Committee and each patient gave informed written consent.

5.5 RESULTS

The 24 hours of triple therapy produced profound suppression of H.pylori urease activity. The breath test 20 minute reading (%14C dose per mmol CO2 X 100 X Kg body weight) fell from a median pretreatment value of 176 (range 116-504) to 5 (range 2-15)($p<0.005$)(Fig 10, Table 6). The CLO test which was positive at 5 hours in all patients pretreatment, was

negative at >24 hours when repeated 28 hours after commencement on triple therapy. There was also evidence of some resolution of the gastritis within this period. The median pretreatment aggregate antral gastritis score in the 8 patients studied was 6 (range 4-6) compared with 3 (range 2-5) after 28 hours therapy ($P<0.02$). The change was mainly in the polymorph infiltrate in the epithelium and lamina propria with little change in the chronic inflammatory infiltrate in the lamina propria. The sum of the scores of the polymorph infiltrate in the epithelium and the lamina propria fell from a median pretreatment value of 4 (range 3-4) to 1 (range 1-3) after 28 hours therapy ($p<0.02$). The score of the chronic inflammatory infiltrate in the lamina propria remained unchanged at 2 (range 1-2)(Fig 13, Table 7).

Despite profound suppression of urease activity and significant reduction in the severity of the polymorphonuclear infiltrate in the antral mucosa neither the basal nor the meal stimulated serum gastrin concentrations were altered. The median pretreatment basal gastrin concentration of the 10 patients was 7 ng/l (range 45-77) compared with 59ng/l (range 45-80) after 24 hours of triple therapy (Fig 11, Table 6). The median pretreatment integrated gastrin response to the Ensure Plus meal in the 8 patients was 4265

	Pre-Treatment			24 hrs Triple Therapy		
	Breath Test 20 min value	Basal Gastrin	Integrated Plasma Gastrin	Breath Test 20 min value	Basal Gastrin	Integrated Plasma Gastrin
Patient 1	238	65	4690	2	71	4345
Patient 2	123	51	8350	3	58	6495
Patient 3	504	62	1975	2	60	2075
Patient 4	137	63	3840	3	69	2515
Patient 5	116	55	3230	8	53	5250
Patient 6	191	56	7775	13	56	2230
Patient 7	256	77	5785	5	80	4770
Patient 8	162	58	3245	9	47	4200

Table 6 Breath test 20 minute value (%14C dose per mmol CO2 X 100 X Kg body weight), basal plasma gastrin (ng/l) and integrated plasma gastrin response (ng/l.min) to Ensure Plus before and 24 hours after commencement on triple therapy

	Pre-Treatment			24 hrs Triple Therapy		
	Aggregate antral gastritis score	PIELP	CIILP	Aggregate antral gastritis score	PIELP	CIILP
Patient 1	6	4	1	3	1	2
Patient 2	6	4	2	4	3	1
Patient 3	6	4	2	2	1	1
Patient 4	5	3	2	3	1	2
Patient 5	6	4	2	5	3	2
Patient 6	5	3	2	5	3	2
Patient 7	4	3	1	3	2	1
Patient 8	6	4	2	3	1	2

Table 7 Aggregate antral gastritis score, polymorphonuclear infiltrate in epithelium and lamina propria (PIELP) and chronic inflammatory infiltrate in lamina propria (CIILP) before and 28 hours after commencement on triple therapy

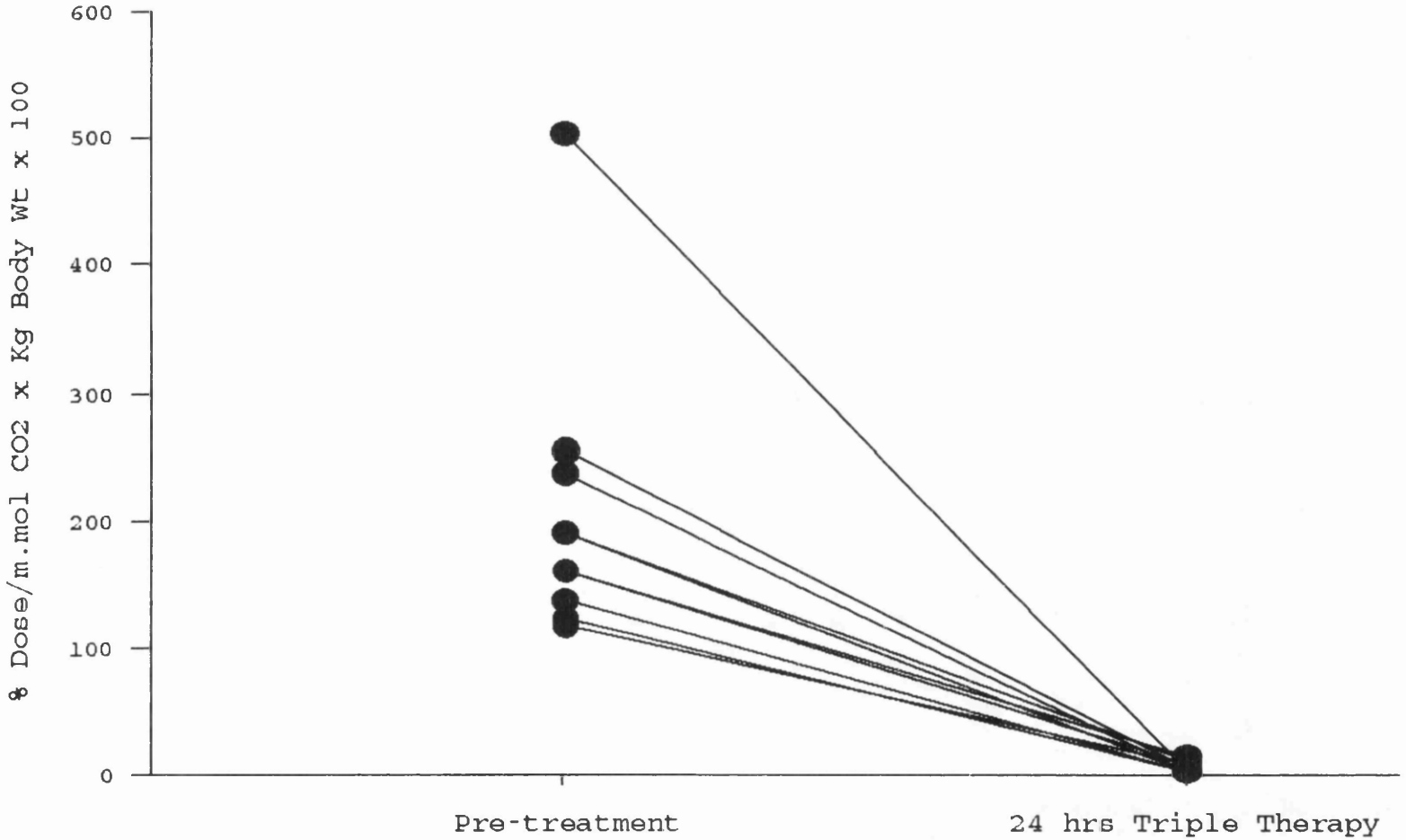

Figure 10 Breath test 20 minute value (% dose/mmol CO2 X Kg body weight X 100) before and 24 hours after commencement on triple therapy in 10 patients.

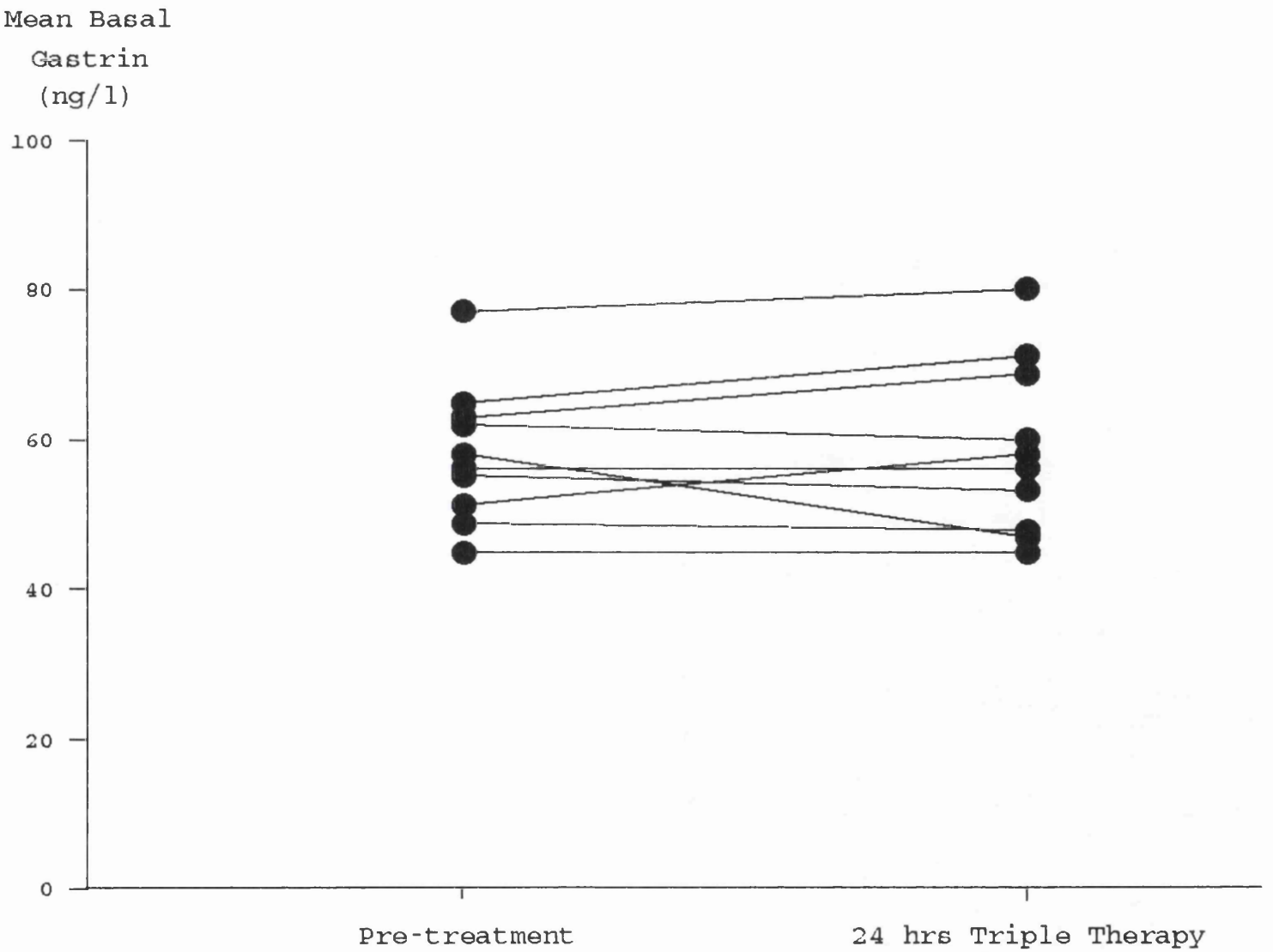

Figure 11 Basal plasma gastrin (ng/l) before and 24 hours after commencement on triple therapy in 10 patients.

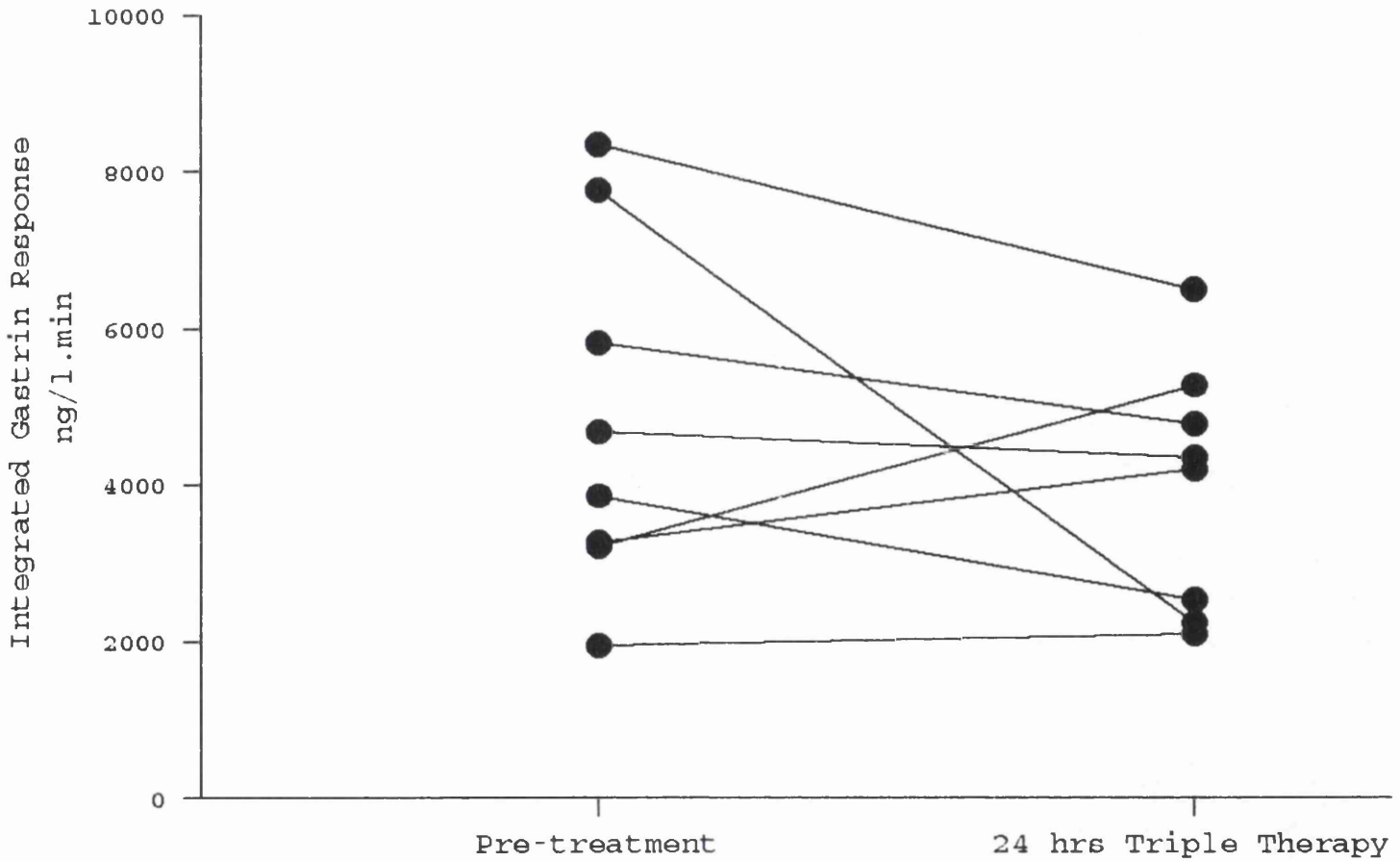

Figure 12 Integrated gastrin response (ng/l.min) to Ensure Plus before and 24 hours after commencement on triple therapy in 8 patients

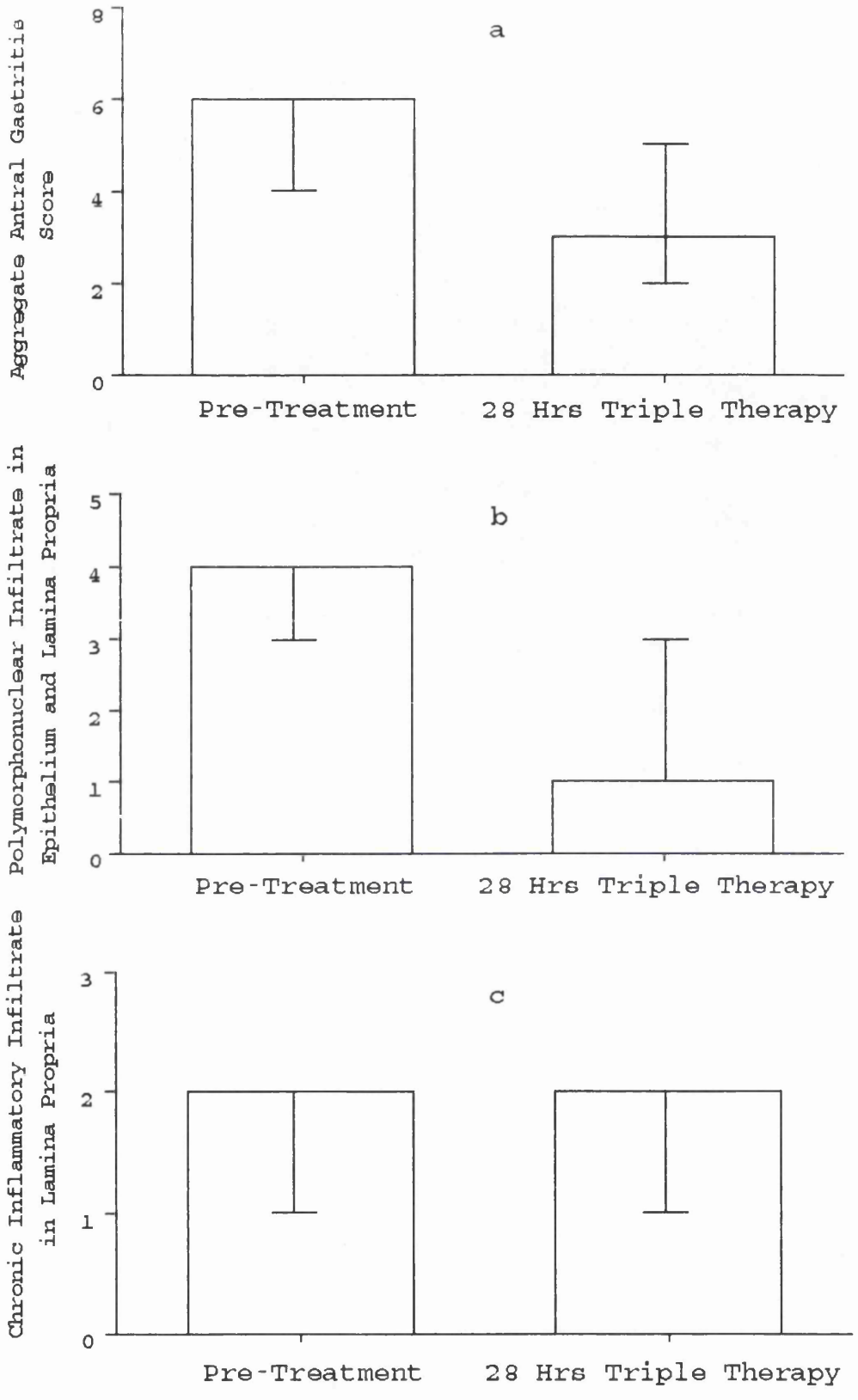

Figure 13

Change in antral gastritis 28 hours after commencement on triple therapy. The columns represent the median value of 8 patients and the error bars, the range

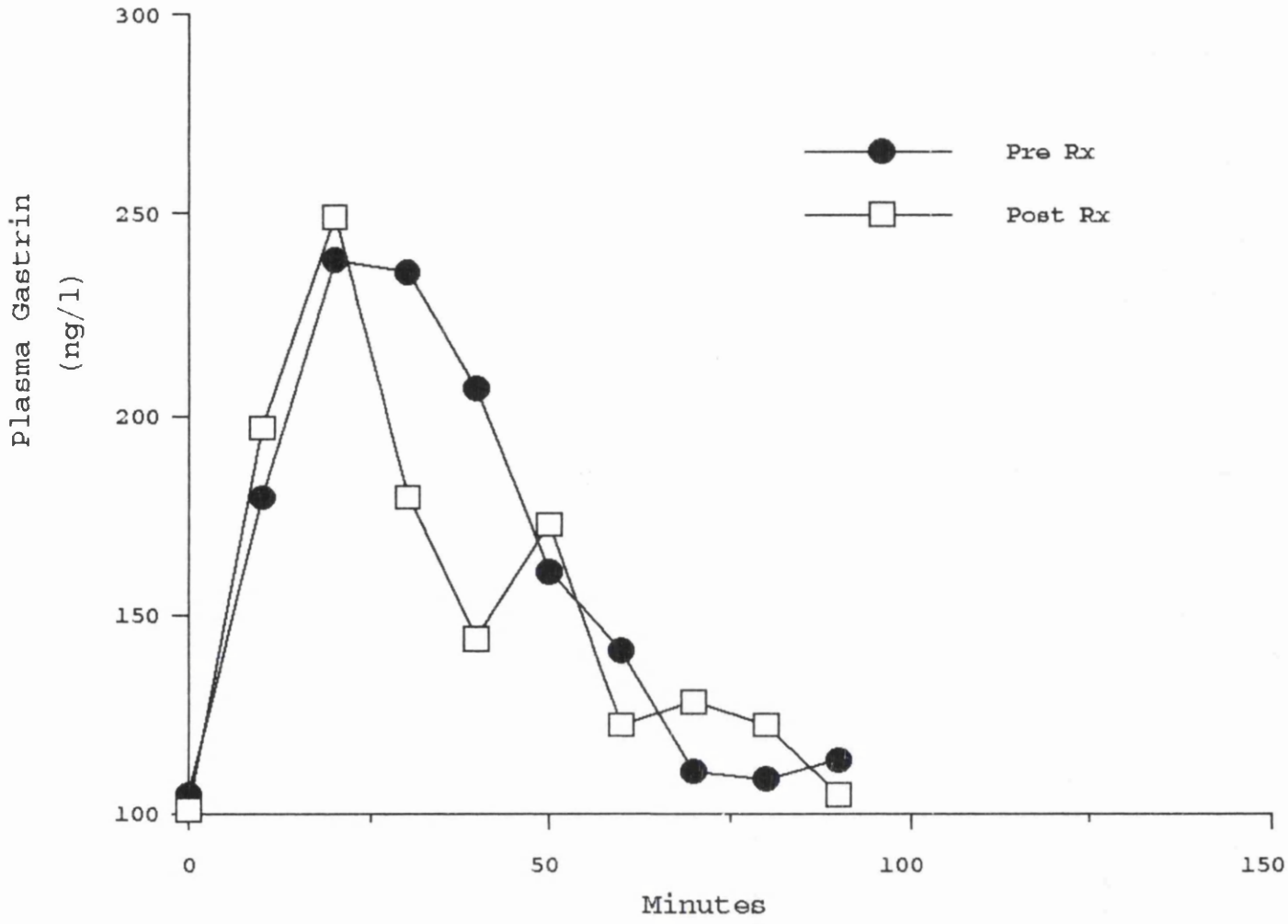

Figure 14 Representative gastrin response (ng/l) to OXO meal before and 24 hours after commencement on triple therapy. The meal was commenced at time 0.

ng/l.min (range 1975-8350) and after 24 hours of triple therapy was 4272 ng/l.min (range 2075-6495) (Fig 12, Table 6). The integrated gastrin response to the OXO meal was also unchanged in the 2 patients studied, their pretreatment values being 5455 and 1760 ng/l.min and after 24 hours triple therapy were 5070 and 1595 ng/l.min, respectively (Fig 14).

Of the 10 patients, 6 were eradicated of H.pylori when reassessed one month following completion of triple therapy.

In the 4 healthy volunteers without H.pylori infection there was no change in the integrated gastrin response to an OXO meal after 24 hours of triple therapy. Their mean pretreatment value was 4500 ng/l.min (range 2000-6000) and after 24 hours therapy was 3760ng/l.min (range 2000-6500).

5.6 DISCUSSION

It has been postulated that the hypergastrinaemia associated with chronic H.pylori infection is secondary to the production of ammonia by the organism close to the antral epithelial cell surface (LEVI et al 1989). In this study the triple therapy produced complete suppression of the bacterial urease activity. This is not surprising as bismuth has been shown to be a powerful inhibitor of H.pylori urease activity as well

as being bactericidal (SAROSIEK et al 1990). Our observation that this degree of suppression of H.pylori urease activity did not result in a fall in basal serum gastrin or meal stimulated gastrin response does not support the hypergastrinaemia being directly related to the bacterial urease activity. If the hypergastrinaemia were due to elevation of antral surface pH by bacterial ammonia, the hormone level should fall rapidly following suppression of ammonia production and thereby fall in antral surface pH. In patients with grossly elevated gastrin concentrations due to achlorhydria, intragastric instillation of hydrochloric acid causes the plasma gastrin to fall within 5-15 minutes (YALLOW et al 1970).

It is now necessary to consider alternative explanations for the hypergastrinaemia. It is possible that it is related to the effects on the antral G cells of the chronic inflammation that H.pylori infection induces in the antral mucosa. At 28 hours after commencing anti-H.pylori therapy there was already early resolution of the antral gastritis but no change in the gastrin concentration. The change in the gastritis score, however, only affected the degree of infiltration of the epithelium and lamina propria with polymorphonuclear cells and there was no change in the chronic inflammatory infiltrate. It has been shown in

isolated perfused canine antrum that the T lymphocyte products, Interleukin-2 and Gamma Interferon, stimulate gastrin release (TEICHMANN et al 1986). It is possible that resolution of the chronic inflammatory infiltrate is required before a change in the plasma gastrin concentration can be observed.

In conclusion, this study indicates that the hypergastrinaemia in patients with H.pylori infection is not directly linked to the bacterial urease activity and ammonia production. It also highlights the rapid rate of resolution of the acute inflammatory cell component of the antral gastritis following commencement of anti-H.pylori therapy.

CHAPTER 6

HELICOBACTER PYLORI RELATED HYPERGASTRINAEMIA IS NOT DUE TO ELEVATION OF ANTRAL SURFACE pH BY THE ORGANISM'S UREASE ACTIVITY

6.1 INTRODUCTION

It is well established that acidification of the gastric antrum suppresses the release of gastrin both basally and in response to eating (BEFRITS et al 1984, WALSH et al 1975, FELDMAN et al 1980). However, the effect of acute alkalinisation of the antrum on gastrin release is less clear, with some studies showing increased release of the hormone (PETERS et al 1983, HANSKY et al 1971) but others no change (SVENSSON et al 1979, PETERSEN et al 1986, LEVANT et al 1973, HIGGS et al 1974, KLINE et al 1975, FELDMAN et al 1978). The reason for these conflicting findings is unknown.

Eradication of H.pylori results in a lowering of both the basal and meal stimulated serum gastrin concentration (ODERDA et al 1989, LEVI et al 1989, GRAHAM et al 1990). It has been proposed that the ammonia produced by the bacterium's urease raises antral surface pH and thus stimulates gastrin release (LEVI et al 1989). If H.pylori infection does effect gastrin release via an effect on antral pH, the conflicting reports concerning the gastrin response to acute alkalinisation might be explained by differing H.pylori status of the groups studied.

I have investigated the influence of H.pylori

infection on the gastrin response to antral alkalinisation under both basal and meal stimulated conditions in duodenal ulcer patients.

6.2 PATIENTS

Eight patients (7 male, age range 32-60 years) with endoscopically confirmed duodenal ulceration within the previous year were entered into the study. Infection with H.pylori was confirmed by a rapid urease slide test (CLO test) histology of antral biopsy and 14C-urea breath test. All patients were off acid inhibitory agents for at least two weeks before entry into the study.

6.3 MATERIALS AND METHODS

In each patient, the gastrin response to a standardised meal at uncontrolled pH and at pH>6 was compared before and after eradication of H.pylori. In six of them the gastrin response to 5 hours of antral alkalinisation was also studied before and after eradication of H.pylori.

On the first morning, the gastrin response to a standardised peptone meal was measured. The meal was prepared by dissolving 2 OXO cubes (OXO Ltd., Croydon, England) in 200 mls of water at 50 degrees Centigrade and was drunk over 5 minutes. Three venous blood samples were obtained at 15 minute intervals

prior to the meal. A further venous blood sample was collected 5 minutes after completion of the meal and at 10 minute intervals after that for 90 minutes.

On a second morning, within a week of the above test, the effect of gastric alkalinisation on the gastrin response to the standardised meal was assessed. A 16F dual lumen tube was passed orogastrically (Andersen Inc.,New York), the stomach emptied and a sample of gastric juice stored for measurement of pH. A sample of venous blood was collected for determination of basal plasma gastrin. Further samples of gastric juice and venous blood were collected at 15 minute intervals over the next 30 minutes. At the end of this period the stomach was emptied and an intragastric infusion of pH 7 citrate buffer (530mmol/l) was commenced at a rate of 6 mls/min. One hour after commencement of the infusion the peptone meal was taken over 5 minutes. The infusion was continued for a further 90 minutes and 20ml samples of gastric juice collected every 20 minutes for pH determination with a glass electrode (Radiometer ETS 822). Venous blood samples were collected as described above for gastrin determination.

In 7 of the 8 patients the plasma gastrin response to gastric alkalinisation alone was assessed. An orogastric tube was passed and basal

gastric juice and venous blood samples collected as described above. After this an intragastric infusion of pH 7 citrate buffer (530mmol/l) was commenced at a rate of 4mls/min. and continued for 5 hours. Throughout this time samples of blood were collected every hour and gastric juice every 30 minutes for measurement of plasma gastrin and pH. The stomach was emptied at the end of each hour to prevent distention.

Following the above studies, the patients were commenced on a three week course of H.pylori eradication therapy consisting of Tripotassium Dicitrato Bismuthate 120mg QID, Metronidazole 400mg TID and Amoxycillin 500mg TID. One month after completion of therapy their H.pylori status was reassessed by CLO test, antral histology and 14C-urea breath test. After this the plasma gastrin response to the standardised peptone meal with and without gastric alkalinisation and to gastric alkalinisation alone was reassessed.

Blood samples were collected for determination of plasma gastrin concentration were collected and assayed as previously described. All the samples from a patient were assayed in the same batch.

Statistical significance was assessed by the Wilcoxon signed rank sum test. The study was approved by the Western Infirmary Ethical Committee and each patient gave informed written consent.

6.4 RESULTS

All patients were eradicated of H.pylori when reassessed one month after completion of the triple therapy. The 14C-urea breath test 20 minute value fell from a median pretreatment value of 126 (range 55-26) to 4 (range 2-8) one month after completion of therapy. The CLO test which was positive at <5 hours pretreatment was still negative at 24 hours when repeated at one month post-treatment.

The basal plasma gastrin assessed on the day the peptone meal was given without alkalinisation, fell from a median pretreatment value of 57ng/l (range 20-103) to 42ng/l (range 18-80)($p<0.05$) following eradication of H.pylori (Fig 16(a)). The median integrated gastrin response to the peptone meal without alkalinisation was 2525ng/l.min. (range 550-8725) pretreatment and fell to 725ng/l.min.(range 250-2925) following eradication ($p<0.01$)(Fig 16(a), Table 9). On the day the meal was given with alkalinisation the infusion of citrate buffer raised the intragastric pH to >6 within 30 minutes and this was maintained for the duration of the study. The median integrated gastrin response to the peptone meal with gastric alkalinisation was 3700ng/l.min.(range 1900- 14100) pretreatment and fell to 1400ng/l.min.(range 400-3400) one month after completion of therapy ($p<0.01$) (Fig

16(b), Table 9).

Alkalinisation of the stomach resulted in a rise in the integrated gastrin response to the peptone meal in 6 of the 8 patients both before and after eradication of H.pylori. The median % change in the 8 patients was +73% (range -63 to +500) pretreatment which was similar to that seen after eradication of the infection, median +48% (range -19 to +386%)(NS, p=0.9)(Table 9).

After eradication of H.pylori, the median fall in the integrated gastrin response to the peptone meal alone was 69% (range 36-89) and was similar to that seen with the peptone meal plus gastric alkalinisation, 61% (range 0-97) (Fig 16).

On the day that the effect of gastric alkalinisation without a meal was studied there was a rise in the plasma gastrin concentration in 6 of the 7 patients before and in the same number after eradication of H.pylori. The others did not show any change in the plasma gastrin with alkalinisation. The median plasma gastrin concentration after 5 hours of alkalinisation was greater before, (65ng/l, range 48-185), than after, (40ng/l, range 25-82), eradication of H.pylori ($p<0.02$). The percentage change in gastrin with alkalinisation alone was similar before (median = 13%, range -4 to 225) and after (median=25%,

	H.pylori-positive			H.pylori-eradicated		
	Basal gastrin before alkalinisation (ng/l)	Basal gastrin after 5h pH>6 (ng/l)	Percentage change	Basal gastrin before alkalinisation (ng/l)	Basal gastrin after 5h pH>6 (ng/l)	Percentage change
Patient 1	83	185	+122	62	63	+2
Patient 2	65	112	+72	73	82	+12
Patient 3	65	70	+8	52	67	+29
Patient 4	52	50	-4	18	25	+38
Patient 5	20	65	+225	18	35	+94
Patient 6	55	62	+13	40	40	0
Patient 7	45	48	+7	28	35	+25

Table 8 Effect of raising intragastric pH to >6 for 5 hours on basal plasma gastrin (ng/l) in 7 duodenal ulcer patients before and after eradication of H.pylori

	H.pylori-positive			H.pylori-eradicated		
	IGR without alkalinisation (ng/l.min)	IGR at pH>6 (ng/l.min)	% change with alkalinisation	IGR without alkalinisation (ng/l.min)	IGR at pH>6 (ng/l.min)	% change with alkalinisation
Patient 1	2350	14100	+500	250	400	+60
Patient 2	2150	7700	+258	450	500	+11
Patient 3	5175	1900	-63	1850	1775	-4
Patient 4	2700	4025	+49	750	1025	+37
Patient 5	550	2150	+291	350	1025	+193
Patient 6	5500	10875	+98	700	3400	+386
Patient 7	8725	3375	-61	2925	2375	-19
Patient 8	2025	2300	+14	950	1400	+147

Table 9 Effect of maintaining intragastric pH at >6 on the integrated gastrin response (IGR) (ng/l.min) to a peptone meal in 8 duodenal ulcer patients before and after eradication of H.pylori

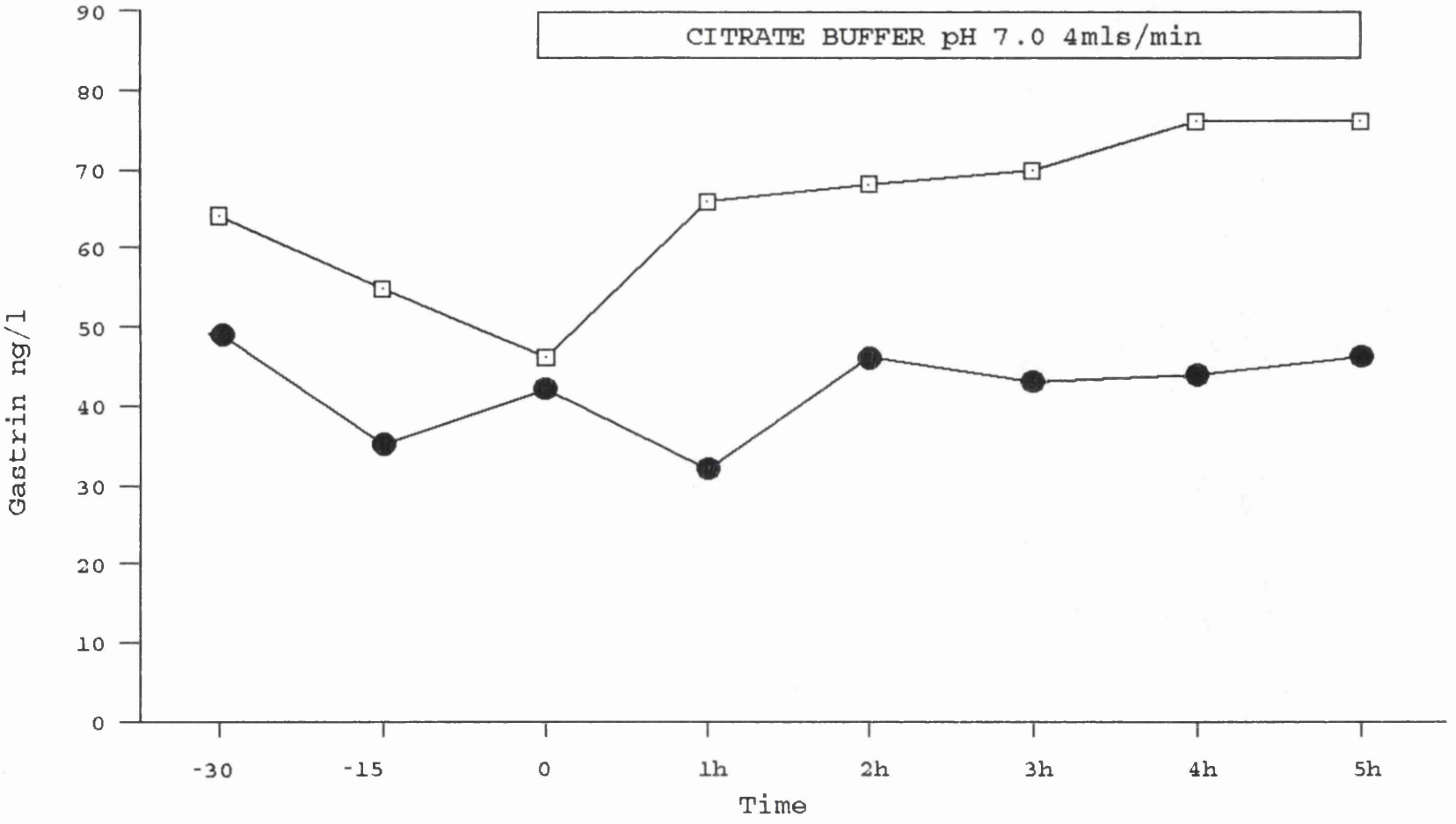

Figure 15 Plasma gastrin response to gastric alkalinisation before (—●—) and 1 month after (—⊡—) eradication of H.pylori in seven patients Values are means.

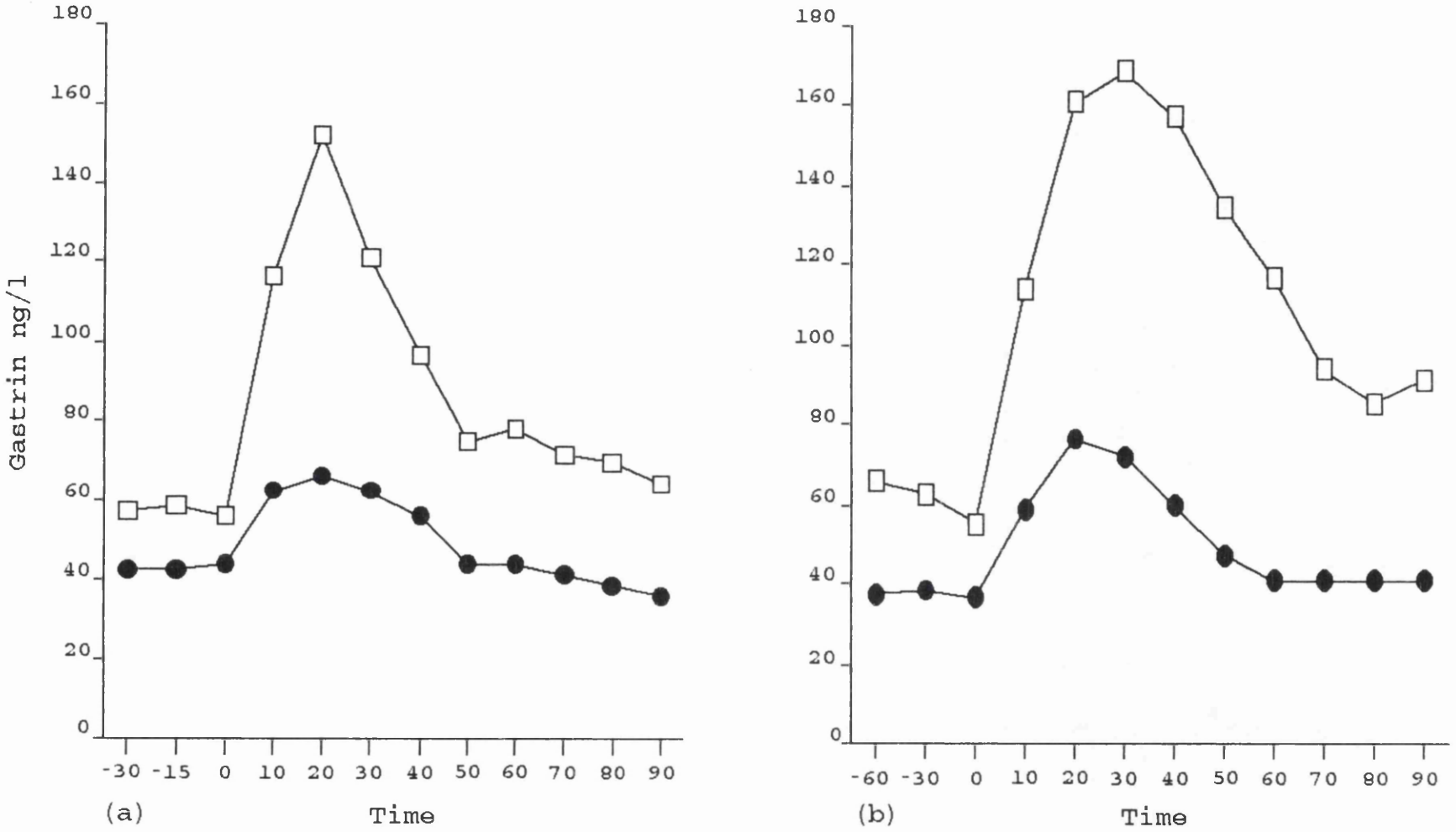

Figure 16 Plasma gastrin response to peptone meal (ng/l.min) at uncontrolled pH (a) and neutral pH (b), before (—□—) and 1 month (—●—) after eradication of H.pylori in eight patients. Values are means.

range 0-94) eradication of H.pylori (NS,p=0.2) (Table 8).

6.5 DISCUSSION

There are conflicting reports concerning the effect of alkalinisation of the antrum, during fasting, on gastrin release in man. Several studies have reported that acute elevation of antral pH does not alter the serum gastrin concentration (LEVANT et al 1973, HIGGS et al 1974, KLINE et al 1975, FELDMAN et al 1978). However, PETERS et al (1983) noted that maintaining antral pH above 6 for 5 hours produced an increase in serum gastrin. HANSKY et al (1971) also reported an increase in serum gastrin in both duodenal ulcer subjects and healthy volunteers which was apparent within 30 minutes of alkalinisation. These conflicting studies were all conducted before H.pylori infection was recognised and its effect on gastrin release appreciated.

In this study we have shown that the fall in gastrin is equally pronounced when the intragastric pH is maintained above 6. When the peptone meal is taken without alkalinisation the buffering effect of the meal raises the intragastric pH above 2 for less than 15 minutes (McCOLL et al 1991) It has been postulated that H.pylori raises plasma gastrin by its ammonia

production blocking the inhibitory effect of gastric acid on gastrin release (LEVI et al 1989). If this were true, the difference in plasma gastrin concentrations between H.pylori positive and eradicated patients would largely or completely disappear when examined at neutral pH. Our finding that the difference remained the same at neutral pH indicates that H.pylori induced hypergastrinaemia is not due to elevation of antral surface pH.

Alkalinisation of the antrum has an immediate effect on the meal stimulated gastrin response (WALSH et al 1975) and this is consistent with the finding that the gastrin response to the meal was increased by alkalinisation. The finding that alkalinisation of the antrum increased the meal stimulated gastrin response to a similar degree in the presence and absence of H.pylori again excludes H.pylori induced hypergastrinaemia being due to elevation of antral surface pH. The reason for two of the subjects not showing increased gastrin response to alkalinisation either before or after eradication of H.pylori is unclear.

Our studies of the effect of alkalinisation without a meal also showed a similar response before and after eradication of H.pylori. If basal gastrin concentration were raised due to elevation of antral

surface pH by H.pylori, raising intragastric pH should produce less of a rise in plasma gastrin concentration before than after eradication of H.pylori and the plasma gastrin level achieved after 5 hours of alkalinisation should be similar on both occasions. However, we, found that the % change in the plasma gastrin with alkalinisation was similar in the presence or absence of H.pylori and the gastrin levels achieved after 5 hours remained higher in the H.pylori positive group.

The findings of the present study confirm that H.pylori induced hypergastrinaemia is not due to elevated antral surface pH.

CHAPTER 7

EFFECT OF HELICOBACTER PYLORI ON PARIETAL CELL SENSITIVITY TO PENTAGASTRIN IN DUODENAL ULCER SUBJECTS

7.1 INTRODUCTION

Numerous studies have demonstrated that chronic H.pylori infection results in raised basal and meal stimulated plasma gastrin concentrations which fall after eradication of the organism (McCOLL et al 1991, ODERDA et al 1989, LEVI et al 1989,GRAHAM et al 1990). The mechanism by which the infection raises these gastrin concentrations is unknown but does not appear to be related to the organism's high urease activity (El NUJUMI et al 1991, CHAPTER 8,9).

The role of the hypergastrinaemia in the pathogenesis of the ulcer disease is also unclear as lowering the gastrin level by eradicating H.pylori does not reduce basal or maximal acid output (LEVI et al 1989). Meal stimulated acid output is also unchanged after eradication of H.pylori (FULLARTON et al 1991) even though gastrin is the main determinant of meal stimulated secretion (KOVACS et al 1989). Inappropriately high gastrin values relative to intragastric acidity also occurs in healthy volunteers with H.pylori infection. SMITH et al (1990) noted that the integrated 24h gastrin concentrations in H.pylori positive volunteers was twice that in H.pylori negative volunteers but there was no difference in integrated 24 hour intragastric acidity between the two

groups. The same group noted that eradication of H.pylori in healthy volunteers lowered the gastrin concentration but did not alter intragastric acidity (PREWETT et al 1991). PETERSON et al (1991) noted that healthy volunteers with H.pylori had increased basal and meal stimulated gastrin concentrations compared with uninfected volunteers yet there was no difference between the two groups with regard to basal, maximal or meal stimulated acid outputs. The reason for the inappropriately high gastrin concentrations for the degree of acid secretion in the presence of H.pylori infection is unknown.

When H.pylori is first contracted it may produce a period of hypochlorhydria which persists for several months (GRAHAM et al 1988). In addition in vitro studies have demonstrated that the organism produces a substance that specifically inhibits parietal cell function (DEFIZE et al 1988, CAVE et al 1989). It is possible that some inhibition of parietal cell function persists during chronic H.pylori infection and that the hypergastrinaemia represents a compensatory response to re-establish normal acid secretion.

To investigate this I examined the effect of eradicating H.pylori on the parietal cell sensitivity to pentagastrin in duodenal ulcer patients.

7.2 PATIENTS

Eight patients (6 male) with a history of endoscopically confirmed duodenal ulceration within the previous year were entered into the study. Their median age was 45 years (range 33-63) and median weight 71kg (range 58-80). None of them had active ulceration at the time of the study. H.pylori infection was confirmed in each patient by each of the following three tests - microscopy of the antral biopsy specimen, the rapid urease test (CLO test) and the 14C-urea breath test. Acid inhibitory agents were withdrawn for at least 2 weeks before the first study day. None had previously been treated with bismuth preparations.

7.3 MATERIALS AND METHODS

Gastric acid output in response to increasing doses of intravenous pentagastrin was determined before and after eradication of H.pylori . Basal plasma gastrin concentrations were also measured.

The patients presented at 0900h after an overnight fast. A 16F dual lumen tube (Andersen Inc., New York) was passed orogastrically, the stomach emptied and the positioning of the tube in the dependent portion of the stomach confirmed by the water recovery test. Two 15 minute specimens of basal gastric juice were collected. An intravenous infusion of pentagastrin was then

commenced at a rate of 0.031ug/kg/hour using an infusion pump (Braun Medical Ltd., UK). The rate of the pentagastrin infusion was progressively increased at 45 minute intervals to 0.062, 0.124 and 0.6ug/kg/hour. Three 15 minute collections of gastric juice were obtained at each infusion rate.

A venous blood sample for gastrin determination was collected at the commencement of the gastric aspiration and at 15 minute intervals until commencement of the pentagastrin infusion.

After the above tests the patients received a 3 week course of H.pylori eradication therapy consisting of tripotassium dicitratobismuthate (DeNol), 120mg four times daily; metronidazole, 400mg three times daily; and amoxycillin, 500mg three times daily. One month after completing this treatment, their H.pylori status was reassessed by microscopy of the antral biopsy specimen, the CLO test and the 14C-urea breath test and their secretory and gastrin studies were repeated.

7.4 ANALYSES

The volume and pH of each collection of gastric juice was recorded and the hydrogen ion concentration determined by titration with 0.1N sodium hydroxide to pH7 using an autotitrator (Radiometer ETS 822). The

basal acid output (mmol/h) was calculated by multiplying by 2, the sum of the hydrogen ion output in the two basal 15 minute collections of gastric juice. The acid output of each pentagastrin dose (mmol/h) was the sum of the results of the second and third 15 minute collection at each infusion rate multiplied by two.

Gastrin determination was performed by radioimmunoassay using antibody R98, as previously described (ARDILL 1973). Basal gastrin was taken as the mean value of the 3 samples obtained prior to commencing the pentagastrin infusion.

Power calculations were performed assuming alpha=0.05, beta=0.2 and the tests were two-sided. Under such conditions it would be expected that with n=8 we could detect a difference equivalent to one standard deviation using a parametric test.

The study was approved by the Western Infirmary Ethical Committee and each patient gave informed written consent.

7.5 STATISTICAL ANALYSIS

The dose-response data for each patient before and after eradication were analysed using an exponential model $V=V_{max}\ (1-exp^{c-bD})$ where V= output and D= dose of pentagastrin (this model has previously

been used by HIRSCHOWITZ et al (1984). From this analysis, estimates of V_{max}, K_m=(1n 2/b) and D_{50}=(K_m-c/b) were obtained, D_{50} is the dose of pentagastrin required to raise the output from basal to 50% of the estimated maximum and K_m is the amount of pentagastrin needed to raise the output to 50% of maximum if basal output was zero. D_{50} and K_m are measures of apparent and intrinsic sensitivity respectively. A schematic representation of these ideas is shown in Figure 17.

The data were fitted using BMDP (PAR)(1985). Estimates of V_{max}, K_m and D_{50} from these fits were only used in further analysis if the square of R (co-efficient of determination) was greater than 75%. Statistical testing was performed using Wilcoxon signed-ranks matched pairs and correlations by using Spearman Rank test with $p<0.05$ being taken as significant.

7.6 RESULTS

All the patients were cleared of H.pylori when they were reassessed one month after completion of therapy.

The median basal gastrin concentration was 48ng/l (range 22-77) before treatment and fell to 33ng/l (range 8-37) ($p=0.03$) after eradication of

Eradication of the infection did not produce any change in either the basal acid output or the acid output in response to the highest dose of pentagastrin (Table 11). The median basal acid output was 5.0 mmol/h (range 1.0-10.8 mmol/h) before treatment and 4.6 mmol/h (range 1.3- 9.0mmol/h) after eradication of H.pylori . The median output to the highest dose of pentagastrin was 37.0 mmol/h (range 15.5- 63.8 mmol/h) before and 31.5 mmol/h (range 18.0-66.0 mmol/h) after eradication of H.pylori (Fig 18(a)). The acid output in response to the submaximal doses of pentagastrin was also similar before and after eradication of H.pylori (Table 11). Analysis of dose-response showed that there was no significant change in either the maximum output of acid or the sensitivity to pentagastrin. The median estimated maximum acid output (V_{max}) was 39.2 (range 15.2-63.3) before treatment and 32.3 (range 18.2-67.2) after treatment (Fig 18(b)). The 95% confidence intervals for the median fall was -3.1 to 9.2 i.e. no significant change. Neither the intrinsic (K_m) or the apparent (D_{50}) sensitivity showed a significant change. The median fall (and 95% confidence interval) in intrinsic sensitivity was 0.004 (-0.019,0.019)(Figure 19(a)) and in apparent sensitivity was 0.0007 (-0.015,0.016)(Figure 19(b)). All the fits had coefficients of determination greater

	Before	After	Percentage Change
Patient 1	22	8	-64
Patient 2	55	32	-42
Patient 3	47	58	+23
Patient 4	48	33	-31
Patient 5	42	30	-29
Patient 6	37	37	0
Patient 7	62	33	-47
Patient 8	77	35	-54

Table 10 Basal plasma gastrin concentrations (ng/l) for the 8 patients before and after eradication of H.pylori infection.

	Before eradicating H.pylori Pentagastrin µg/kg/h					After eradicating H.pylori Pentagastrin µg/kg/h				
	Basal	0.031	0.062	0.124	0.600	Basal	0.031	0.062	0.124	0.600
Patient 1	10.80	26.0	37.7	42.1	63.8	9.0	16.6	26.4	36.5	55.7
Patient 2	4.60	6.0	18.0	22.0	21.0	4.8	3.1	12.7	14.0	19.0
Patient 3	9.00	18.0	44.0	44.0	38.0	6.5	16.0	42.0	32.0	35.0
Patient 4	1.04	2.9	11.7	10.2	15.5	1.6	2.1	15.4	14.6	18.0
Patient 5	4.10	14.0	20.0	21.0	36.0	1.3	2.0	14.3	16.2	22.2
Patient 6	5.40	8.6	33.0	38.6	42.0	7.4	10.5	34.2	39.0	40.0
Patient 7	1.60	1.2	19.0	23.0	33.0	2.7	8.0	19.0	24.0	28.0
Patient 8	9.00	6.5	37.0	48.0	57.0	4.3	6.2	40.0	51.0	66.0

Table 11 Response (in mmol/h) to increasing doses of pentagastrin in 8 patients before and after eradication of H.pylori infection.

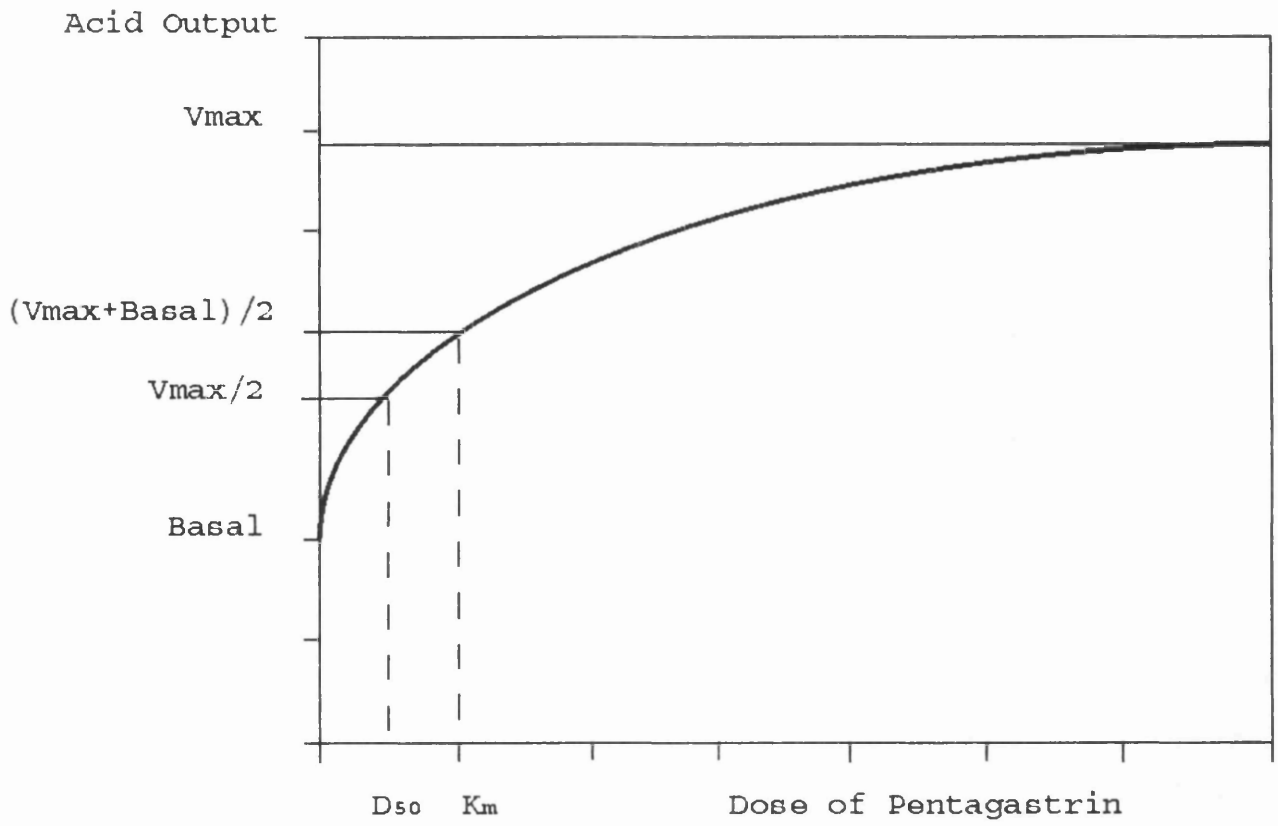

FIGURE 17 Schematic diagram of the definitions of the parameters obtained from fitting the model $V=Vmax\ (1-exp^{-bD})$

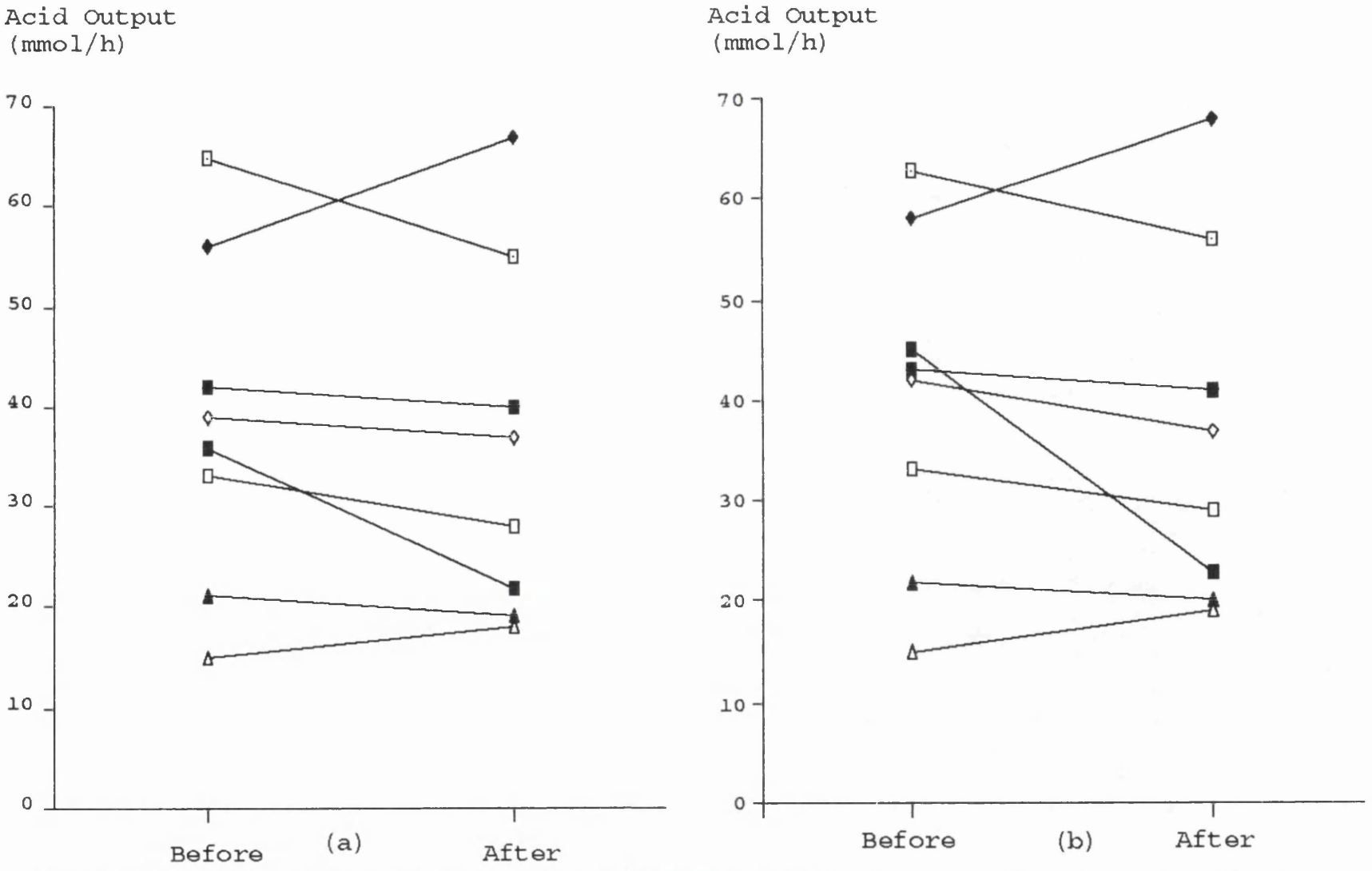

Figure 18 'Maximum' acid output (mmol/h) before and after eradication of Helicobacter pylori. 18a. 'Maximum' is the acid output obtained at the maximum dose of pentagastrin administered (0.6mcg/kg/h). 18b. 'Maximum' is the estimate of Vmax obtained from fitting the exponential model.

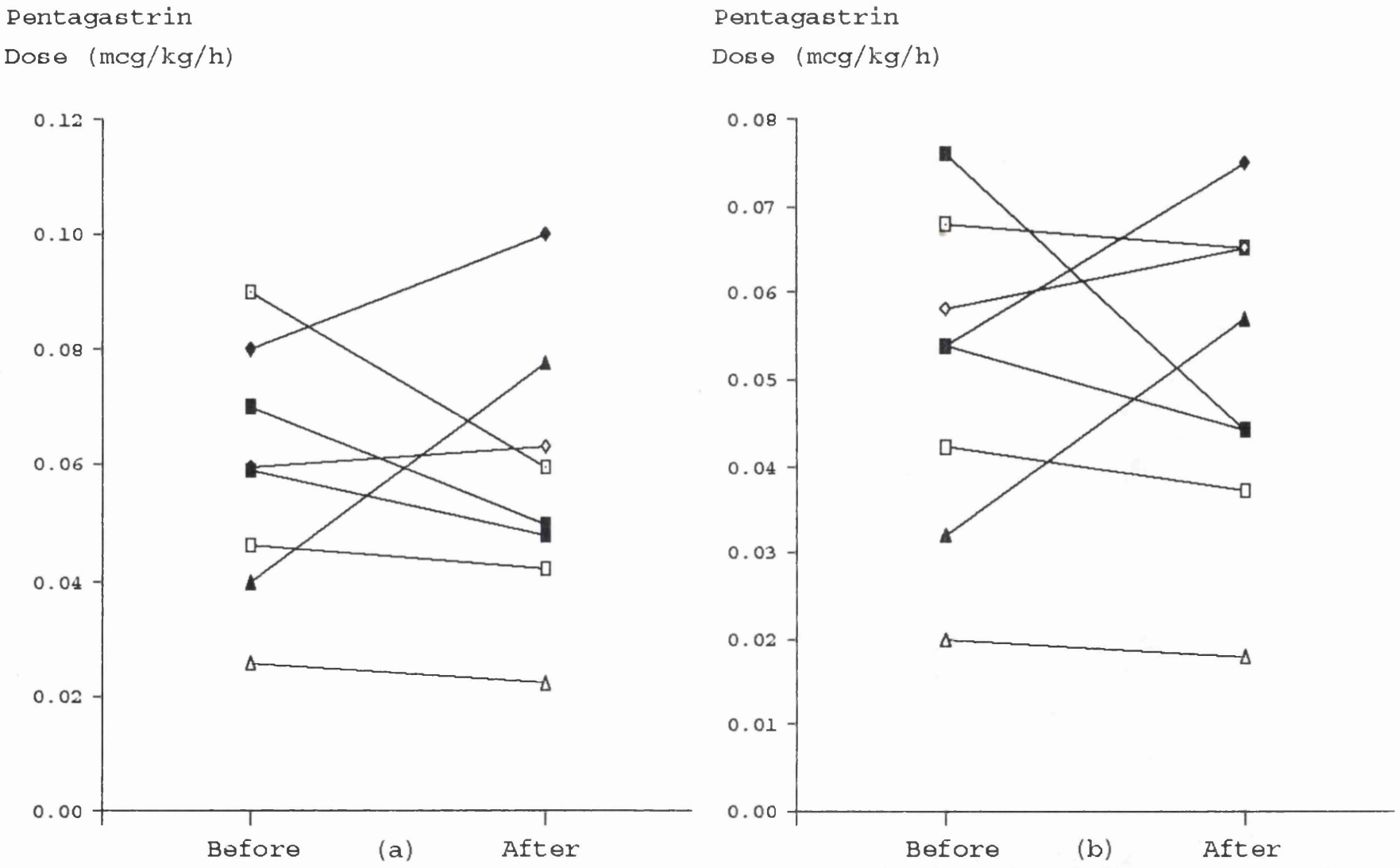

Figure 19 Sensitivity to pentagastrin before and after eradication of Helicobacter pylori. 19a. Intrinsic sensitivity estimated by Km obtained from fitting the exponential model. 19b. Apparent sensitivity estimated by D50 obtained from fitting the exponential model.

All the fits had coefficients of determination greater than 75%.

There was no correlation of basal plasma gastrin and basal acid output either before (rs=-0.34,N.S.) or after (rs=0.16, N.S.) eradication of H.pylori . In addition there was no correlation between basal serum gastrin and the value for $[K_m-D_{50}]$ either before (rs=-0.52,N.S.) or after (rs=-0.18, N.S.) eradication of H.pylori .

7.7 DISCUSSION

The mechanism of the increased gastrin concentration in chronic H.pylori infection is unclear. In this study we investigated the possibility that chronic H.pylori infection reduces the sensitivity of parietal cells to gastrin and consequently higher concentrations of the hormone are required to maintain the same level of acid secretion in the presence of the infection. The sensitivity of parietal cells to gastrin was assessed by measuring gastric acid secretion to graduated doses of pentagastrin and using the data to construct dose-response curves. This demonstrated that there was no difference in parietal cell sensitivity to pentagastrin following eradication of H.pylori.

SECTION III

OVERALL DISCUSSION AND CONCLUSIONS

CHAPTER 8

DISCUSSION AND CONCLUSIONS

Helicobacter pylori infection of the gastric antrum is seen in over 90% of patients with duodenal ulceration (O'CONNOR et al 1986, RAUWS et al 1988). Eradication of the infection results in a marked fall in ulcer relapse rate (MARSHALL 1988, BORODY et al 1988, SMITH et al 1988). The mechanism by which this infection predisposes to duodenal ulceration is not clear.

H.pylori only infects gastric epithelium and areas of gastric metaplasia. The predominant site of infection is the gastric antral epithelium which is the location of the gastrin producing G cells. Gastrin has an important role in gastric acid secretion and any alteration in its plasma concentration by H.pylori could result in a predisposition to duodenal ulceration. McCOLL et al (1991) examined the effect of eradication of H.pylori on basal and meal stimulated plasma gastrin concentrations in 10 duodenal ulcer patients. Eradication of the infection resulted in a 33% fall in basal plasma gastrin and a 50% fall in the integrated gastrin response to an OXO meal. A similar fall in basal gastrin concentration was observed in children with various upper gastrointestinal disorders of H.pylori (ODERDA et al 1989). In duodenal ulcer

subjects LEVI et al (1989) noted a similar fall in meal stimulated gastrin concentrations, 2 days after completion of H.pylori eradication therapy. These observations were confirmed in my studies (Chapters 5, 7 and 8). It is, therefore, possible that the H.pylori infection by raising plasma gastrin concentration has a trophic effect on parietal cells and increases gastric acid output, thus predisposing to duodenal ulceration.

A large proportion of the general population is infected with H.pylori. The prevalence of the infection increases with age (GRAHAM et al 1988) and varies in different parts of the world (GRAHAM et al 1991) with approximately 50% of adults in the western world being infected. Duodenal ulceration, however, affects only about 10% of the adults in the western world (LANGMAN 1979). The reason why less than one in five people with H.pylori infection develops duodenal ulceration is not clear. I investigated the hypothesis that the development of duodenal ulceration is related to the degree of hypergastrinaemia induced by the chronic infection.

The results demonstrated that H.pylori infection raises plasma gastrin concentration in healthy volunteers as well as duodenal ulcer patients. This finding is consistent with that of SMITH et al (1990)

who noted higher median integrated 24-h gastrin concentrations in eight volunteers,who had immunoglobulin (Ig) G antibodies to H.pylori, than in 87 who were sero- negative. My, study also demonstrates that basal and meal stimulated gastrin concentrations in the H.pylori positive volunteers were not different from those in H.pylori positive duodenal ulcer patients. The development of duodenal ulceration in a minority of the population with H.pylori infection is therefore not a reflection of the degree of hypergastrinaemia induced by the infection. Other factors must, therefore, play a major role in the pathogenesis of ulcer disease.

The mechanism by which H.pylori infection raises plasma gastrin concentration is not known. It has been proposed by LEVI et al (1989) that the ammonia produced by hydrolyses of urea by the organism's urease results in elevation of antral surface pH. The physiological inhibition of gastrin release by luminal acid is therefore lost resulting in elevation of the plasma gastrin concentration. In addition, ammonia may be a direct stimulus to gastrin release by the G cells. The hypergastrinaemia may be due to the chronic inflammation induced by H.pylori infection or physiological response to inhibition of parietal cell function by the infection.

To determine whether the raised plasma gastrin concentrations seen with H.pylori infection is the result of ammonia production by H.pylori, I studied the effect of altering the rate of ammonia formation on plasma gastrin by infusing urea intragastrically. Increasing H.pylori ammonia production failed to change the plasma gastrin concentration. This does not lend support to the hypothesis that the hypergastrinaemia is caused by the ammonia. It seems unlikely that the degree of increase in ammonia production was inadequate as the rise in ammonium ion concentration at the antral epithelial surface, where the bacteria are found close to the gastrin secreting G cells, would have been greater than the three fold rise noted in the gastric aspirate. It should be stressed, however, that the failure to cause a further increase in gastrin by augmenting ammonia production does not exclude the possibility that the raised gastrin value was caused by the organism's ammonia production. The amount of ammonia produced by the bacterium under normal conditions may be sufficient to produce the maximum gastrin response via this mechanism.

My finding that augmenting H.pylori ammonia production failed to stimulate gastrin does not refute the hypothesis that the organism stimulates gastrin release by raising antral surface pH. The local

alkalinising effect of H.pylori under basal conditions may already be sufficient to prevent suppression of gastrin release by gastric acid. In addition, the duration of the urea infusion in my study was four hours and earlier studies have shown that gastric alkalinisation was required for five hours before there was any rise in serum gastrin (PETERS et al 1983). The effect of antral alkalinisation on serum gastrin in humans is controversial, however, as a more recent study could not detect any increase after raising gastric pH for 10 hours (PETERSON et al 1986).

This study also provides information on the regulation of H.pylori ammonia production in vivo. The almost undetectable intragastric concentrations of urea found before eradication of the organism and the rapid rise in the gastric ammonia concentration after the infusion of urea indicate that the gastric juice concentration of urea and its rate of diffusion across the gastric mucosa are factors that limit the generation of ammonia by H.pylori. It has been proposed that the organism's ammonia production is a means by which it protects itself from intragastric acid by producing a local alkaline micro-environment (GOODWIN et al 1986). As gastric pH varies constantly and rapidly, one would expect such a protective mechanism to be under pH dependent control rather than

under the control of substrate availability, which cannot be altered.

In an attempt to further elucidate the role of the urease enzyme in the increased plasma gastrin concentration seen in chronic H.pylori infection I performed the next study. The aim of this study was to examine plasma gastrin concentration soon after commencing H.pylori eradication therapy. At this early time I hoped to achieve complete suppression of bacterial urease activity without significant resolution of the antral gastritis. The latter has been postulated as another possible mechanism by which the infection raises plasma gastrin.

The triple therapy, (tripotassium dicitrato bismuthate, amoxycillin and metronidazole) used in this study, produced complete suppression of the bacterial urease activity. This is not surprising as bismuth has been shown to be a powerful inhibitor of H.pylori urease activity as well being bactericidal (SAROSIEK et al 1990). My observation that this degree of suppression of H.pylori urease activity did not result in a fall in basal plasma gastrin or meal stimulated gastrin response does not support the hypergastrinaemia being directly related to the bacterial urease activity. If the hypergastrinaemia were due to elevation of antral surface pH by bacterial

ammonia, the hormone level should fall rapidly following suppression of ammonia production and thereby fall in antral surface pH. In patients with grossly elevated gastrin concentrations due to achlorhydria, intragastric instillation of hydrochloric acid causes the plasma gastrin to fall within 5-15 minutes (YALOW et al 1970).

The possibility must be considered that the antibacterial therapy could have independently stimulated gastrin release and thus obscured a fall in gastrin due to their suppression of urease activity. However, the absence of any rise in gastrin following 24h triple therapy in the H.pylori negative controls excludes this.

The findings in this study are consistent with those of El NUJUMI et al (1991) who observed that inhibiting H.pylori urease activity with acetohydroxamic acid did not lower the serum gastrin concentration in duodenal ulcer patients. In this study with acetohydroxamic acid only partial suppression of urease activity was achieved [median 20 minute value of the 14C-urea breath test 22 (range 14-95)] and there was the possibility that the suppression of ammonia production may have been inadequate to produce a fall in serum gastrin. In the present study, with 24 hours triple therapy, the urease

activity, as assessed by the 14C-urea breath test, was suppressed to a median value of 5 (range 2- 15) which was similar to the value 2 (range 0-5) previously observed in patients one month post eradication. The lack of association between H.pylori urease activity and serum gastrin in these studies is also consistent with the previous observation that augmenting H.pylori ammonia production by administering urea did not raise gastrin in duodenal ulcer subjects.

It is now necessary to consider alternative explanations for the hypergastrinaemia. It is possible that it is related to the effects on the antral G cells of the chronic inflammation that H.pylori infection induces in the antral mucosa. At 28 hours after commencing anti-H.pylori therapy there was already early resolution of the antral gastritis but no change in the gastrin concentration. The change in the gastritis score, however, only affected the degree of infiltration of the epithelium and lamina propria with polymorphonuclear cells and there was no change in the chronic inflammatory infiltrate. It has been shown in isolated perfused canine antrum that the T lymphocyte products, Interleukin-2 and Gamma interferon, stimulate gastrin release (TEICHMANN et al 1986). It is possible that resolution of the chronic inflammatory infiltrate is required before a change in the plasma

gastrin concentration can be observed.

Urease has been proposed as being essential for the survival of H.pylori in the low pH environment of the stomach (GOODWIN et al 1986). By making ammonia the organism is thought to make the pH of its environment more favorable to its survival. Despite total suppression of urease activity in all the patients with triple therapy, eradication was achieved in only 4 of them. This indicates that urease activity is not essential for the organism's survival once the infection is established within the gastric mucosa layer. It does not, however, exclude the possibility that the organism's ammonia production protects it from gastric acid when the infection is first being contracted.

This study indicates that the hypergastrinaemia in patients with H.pylori infection is not directly linked to the bacterial urease activity and ammonia production. It also highlights the rapid rate of resolution of the acute inflammatory cell component of the antral gastritis following commencement of anti-H.pylori therapy.

The hypothesis proposing that ammonia produced by the bacterium's urease enzyme raises antral surface pH and thus stimulates gastrin release (LEVI et al 1989) was further tested in my next study. Here, I

investigated the influence of H.pylori infection on the gastrin response to antral alkalinisation under both basal and meal stimulated conditions in duodenal ulcer subjects.

There are conflicting reports concerning the effect of alkalinisation of the antrum,during fasting, on gastrin release in man. Several studies have reported that acute elevation of antral pH does not alter the serum gastrin concentration (LEVANT et al 1973, HIGGS et al 1974, KLINE et al 1975, FELDMAN et al 1978). SVENSSON et al (1979) failed to observe any rise in gastrin after maintaining antral alkalinisation for 3 hours and likewise PETERSEN et al (1986) found no change even after 10 hours in duodenal ulcer subjects. However, PETERS et al (1983) noted that maintaining antral pH above 6 for 5 hours produced an increase in serum gastrin; in those with duodenal ulceration it increased from 53 pg/ml to 71 pg/ml representing a rise of 35% and in the healthy controls from 25 pg/ml to 37.5 pg/ml representing an increase of 50%. HANSKY et al (1971) also reported an increase in serum gastrin in both duodenal ulcer subjects and healthy volunteers which was apparent within 30 minutes of alkalinisation. These conflicting studies were all conducted before H.pylori infection was recognised and its effect on gastrin release appreciated.

In this study I have demonstrated that the fall in gastrin is equally pronounced when the intragastric pH is maintained above 6. When the peptone meal is taken without alkalinisation the buffering effect of the meal raises the intragastric pH above 2 for less than 15 minutes (McCOLL et al 1991). If H.pylori raised plasma gastrin by elevating antral surface pH, the difference in plasma gastrin concentrations between H.pylori positive and eradicated patients would largely or completely disappear when examined at neutral pH. The finding that the difference remained the same at neutral pH indicates that H.pylori induced hypergastrinaemia is not due to elevation of antral surface pH.

Alkalinisation of the antrum has an immediate effect on the meal stimulated gastrin response (WALSH et al 1975) and this is consistent with the finding that the gastrin response to the meal was increased by alkalinisation. The finding that alkalinisation of the antrum increased the meal stimulated gastrin response to a similar degree in the presence and absence of H.pylori again excludes H.pylori induced hypergastrinaemia being due to elevation of antral surface pH. The reason for two of the subjects not showing increased gastrin response to alkalinisation either before or after eradication of H.pylori is

unclear.

The studies of the effect of alkalinisation without a meal also showed a similar response before and after eradication of H.pylori. If basal gastrin concentration were raised due to elevation of antral surface pH by H.pylori, raising intragastric pH should produce less of a rise in plasma gastrin concentration before than after eradication of H.pylori and the plasma gastrin level achieved after 5 hours of alkalinisation should be similar on both occasions. However, I, found that the % change in the plasma gastrin with alkalinisation was similar in the presence or absence of H.pylori and the gastrin levels achieved after 5 hours remained higher in the H.pylori positive group.

The finding that the percentage rise in gastrin induced by H.pylori is independent of intragastric pH is consistent with recent studies in patients receiving therapy with the H+/K= ATPase inhibitor pantoprazole (McCOLL et al 1992). It was noted that the serum gastrin concentration during this acid inhibitory therapy was higher in the H.pylori positive subjects than in the H.pylori eradicated ones and the difference between them was similar before and during acid inhibitory therapy.

The findings of the present study,that H.pylori

induced hypergastrinaemia is not due to elevated antral surface pH is consistent with the previous studies in which I found no association between the organism's urease activity and gastrin concentration. Neither increasing the organism's ammonia production by intragastric urea infusion, nor inhibiting it with acetohydroxamic acid (El NUJUMI et al 1991) nor completely suppressing it with 24 hours triple therapy alters the plasma gastrin concentration.

Acute H.pylori infection produces hypochlorhydria which persists for several months (GRAHAM et al 1988). In vitro studies have demonstrated that the organism produces a substance that inhibits parietal cell function. It is therefore possible that the hypergastrinaemia induced by chronic H.pylori infection is a physiological response to reestablish normal acid secretion. To investigate this, I have examined the effect of eradicating H.pylori on the parietal cell sensitivity to pentagastrin in duodenal ulcer patients. In this study the sensitivity of parietal cells to gastrin was assessed by measuring gastric acid secretion to graduated doses of pentagastrin and using the data to construct dose-response curves. This showed that there was no difference in parietal cell sensitivity to pentagastrin after eradication of H.pylori.

One practical difficulty in calculating parietal cell sensitivity from the acid response to graduated doses of pentagastrin concerns basal acid output. It is difficult to know whether the basal acid output should be plotted against the zero dose of pentagastrin or should be subtracted from the outputs at each pentagastrin dose and itself excluded from the sensitivity calculations (PETERSEN et al 1975). This consideration is important when the basal acid output values are different in the two groups being compared- for example, duodenal ulcer patients versus healthy volunteers- but is less important in the present study, in which the basal acid output was similar before and after eradication of H.pylori. To allow for this potential problem, we adopted the method of HIRSCHOWITZ (1984). This calculates both the apparent sensitivity (D50)- that is, the dose of pentagastrin required to raise the output from basal to 50% of estimated maximum- and the intrinsic sensitivity (Km)- that is, the dose needed to raise the output to 50% of maximum if basal output was zero. The estimated sensitivity to pentagastrin was unchanged after eradication of H.pylori irrespective of whether it was calculated as the apparent or intrinsic sensitivity.

Studies comparing parietal cell sensitivity with gastrin in duodenal ulcer patients and healthy

volunteers have produced conflicting conclusions. Some have demonstrated an increased sensitivity (ISENBERG et al 1975, LAM et al 1980) whereas others have found no difference (PETERSEN et al 1975, KOFFMAN et al 1977, HIRSCHOWITZ 1984, HIRSCHOWITZ et al 1985). The present study indicates that these conflicting findings cannot be attributed to varying H.pylori status of the subjects studied. This study, however, cannot exclude the possibility that the H.pylori infection produces an alteration in parietal cell sensitivity which does not resolve within 1 month of eradicating the infection. It does, however, exclude the possibility that the increased gastrin is due to reduced parietal cell sensitivity, as the gastrin fell within the time period of the study.

Although my study indicates that H.pylori infection does not reduce parietal cell sensitivity to gastrin in duodenal ulcer patients, we cannot exclude that this is also the case in all subjects with the infection. The reason why only a small proportion of individuals with H.pylori develop duodenal ulcers is unclear. As described earlier, the degree of hypergastrinaemia induced by H.pylori is similar in duodenal ulcer patients and healthy volunteers, yet acid secretion is known to be higher in the former. It is possible that healthy volunteers with H.pylori have

a more marked body gastritis, impairing their acid response to the hypergastrinaemia and thus protecting them from duodenal ulceration. In contrast, duodenal ulcer subjects with H.pylori have both an elevated gastrin and an unimpaired acid response to gastrin, as shown by the present study.

The mechanism by which H.pylori infection raises plasma gastrin remains unclear. LEVI et al (1989) postulated that it was due to the elevation of antral surface pH by the organism's ammonia production, but my earlier studies have indicated that this is not the case. The present study indicates that the hypergastrinaemia in duodenal ulcer patients with H.pylori is not a compensatory response to impaired parietal cell function.

In the light of these studies the most likely explanation for the hypergastrinaemia is that it is caused by the inflammation the infection induces in the antral mucosa. Studies using isolated perfused canine antrum have shown that the T-lymphocyte products interleukin-2 and gamma-interferon stimulate gastrin release (TEICHMANN et al 1986). The inflammatory process could cause the hypergastrinaemia by a direct stimulatory effect on the antral G cells or indirectly by inhibiting the release of somatostatin by the antral D cells.

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CHITTAJALLU R.S., ARDILL J.E.S., McCOLL K.E.L. The degree of hypergastrinaemia induced by Helicobacter pylori is the same in duodenal ulcer patients and asymptomatic volunteers.
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CHITTAJALLU R.S., NEITHERCUT W.D., MacDONALD A.M.I., McCOLL K.E.L. Effect of increasing Helicobacter pylori ammonia production by urea infusion on plasma gastrin concentration.
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COMMUNICATIONS TO LEARNED SOCIETIES

The effect of increasing H.pylori ammonium production on plasma gastrin concentration in duodenal ulcer subjects.

Glasgow Gut Club	1989
British Society of Gastroenterology	March 1990
Digestive Diseases Week, Texas	May 1990

Is H.pylori associated hypergastrinaemia due to the bacterium's urease activity or the antral gastritis?

British Society of Gastroenterology	Sept 1990
III Workshop Gastroduodenal pathology and Campylobacter (Helicobacter) pylori, Toledo, Spain.	Nov 1990
Caledonian Society, Scotland	Nov 1990

H.pylori related hypergastrinaemia is not due to elevation of antral surface pH.

British Society of Gastroenterology	Apr 1991

RECENT DEVELOPMENTS

Research in the field of *Helicobacter pylori* infection is proceeding at a very rapid rate. Due to this there have been several key developments since the completion of the work in this thesis and these are therefore appended below.

(1) More effective eradication regimens have become available for treating *H pylori* infection. It is now possible to achieve eradication rates of more than 90% with a single week's treatment. The currently most commonly used eradication regimen consists of omeprazole 40mg per day plus metronidazole 400mg t.i.d. and amoxycillin 500mg t.i.d., all given for seven days[1].

(2) There have been rapid advances as a result of the application of molecular biology to the field of gastrin metabolism. In particular, the gene for the gastrin receptor has now been cloned[2].

(3) Several recent publications have demonstrated that *H pylori* lowers the concentration of somatostatin in the antral mucosa and also lowers the levels of somatostatin messenger RNA in antral mucosa[3-7]. The release of gastrin is under physiological suppression by somatostatin and thus the increased gastrin release is likely to be explained by these decreased synthesis of somatostatin. The mechanism by which *H pylori* lowers antral somatostatin remains unclear.

(4) Recent studies have highlighted the importance of the *H pylori* induced hypergastrinaemia by demonstrating that it is associated with markedly increased gastric acid secretion[8]. These recent studies have been performed by using gastrin releasing peptide as the stimulant to gastric acid secretion. This neuropeptide stimulates release of gastrin from the G-cells in the antral mucosa and this gastrin then circulates and stimulates the parietal cells in the body of the stomach to secrete acid. Gastrin releasing peptide also stimulates the release of a variety of inhibitory peptides which regulate the degree of gastrin release and also of the acid response to the gastrin. Gastrin releasing

peptide stimulates the response to a meal by measuring the combined functional response of the antrum and body of the stomach and also by activating both stimulatory and inhibitory control pathways. Studies employing gastrin releasing peptides have indicated that duodenal ulcer patients with *H pylori* infection have a six-fold increased acid response compared to uninfected controls. In addition, eradication of the infection results in complete normalisation of the acid response[9].

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