STUDIES OF *H. PYLORI*-INDUCED HYPERGASTRINAEMIA

BY

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

I DEDICATE THIS THESIS

IN MEMORY OF MY PARENTS

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PREFACE

Over the last few years, I have had the good fortune to work with Professor Kenneth E.L. McColl. He introduced me to the exciting field of research into gastroenterology, especially in relation to *Helicobacter pylori* infection and peptic ulcer disease. His expert advice in the clinical and scientific field have been invaluable and his interest in my career was most encouraging.

Most of the work in this thesis has been published and a list of these publications is submitted with the thesis. Collaboration with a number of colleagues has been necessary as described in the Acknowledgement.

The work presented in this thesis has been carried out by myself at the University Department of Medicine & Therapeutics, Western Infirmary, Glasgow.

ACKNOWLEDGEMENT

The help of many individuals who were indispensable in the actual preparation of this thesis must be acknowledged. However, even before the writing began I already owed much. Firstly, I must mention Professor Kenneth E.L. McColl, Professor of Gastroenterology, University of Glasgow. I have had the privilege of working and conducting my research work under his supervision and excellent advice. His encouragement and enthusiasm has helped me tremendously to understand many aspects in the field of gastroenterology, and specifically in relation to the *Helicobacter pylori* infection. The thesis would not have been possible without his help, support and guidance.

I must also acknowledge the effort of my colleagues who helped me through all the work which is presented in the thesis. I owe special thanks to Dr. Joy Ardill, Dr. Craig Williams, Dr. Duncan Neithercut, Dr. Peter Rowe, Dr. Janet Mackenzie, Dr. Ravi Chittajallu and Dr. Ian Penman. Additional thanks are due to my colleague, Dr. Emad El-Omar, for his sage advice and special thanks to all in the Gastrointestinal Investigation Unit and the M.R.C. Dyspepsia Clinic at Gartnavel General Hospital.

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Above all, I would like to sincerely thank my dear wife, Sheona. Words are insufficient to express my appreciation for her understanding, encouragement and patience - especially in the final stage of preparing this thesis. Many thanks for those endless cups of tea!

CHAPTER ONE

HELICOBACTER PYLORI INFECTION AND ASSOCIATED DISEASES IN MAN

1.1 DISCOVERY AND NOMENCLATURE

Helicobacter pylori infection of the upper gastrointestinal mucosa is arguably the commonest chronic bacterial infection in man. The organism was first cultured in April 1982 in the Microbiology Department of the Royal Perth Hospital in Perth, Western Australia (Marshall et al 1984). It was initially called *Campylobacter pyloridis* but this was recognised to be grammatically incorrect and it was renamed *campylobacter pylori* in 1987 (Marshall et al 1987). Subsequent studies indicated that the ultrastructure and fatty acid composition of the bacterium were distinct from *campylobacter* organisms (Goodwin et al 1985). Further studies of rRNA sequence showed clearly that the organism did not belong to the genus *campylobacter* or any other previously reported genus (Romaniuk et al 1987) . For this reason, a new genus name was created - *Helicobacter* and the organism called *Helicobacter pylori* (Goodwin et al 1989). The name *Helicobacter* was chosen to reflect the two morphological appearances of the organism, *Helical in vivo* but often rodlike *in vitro*. It was called *Helicobacter pylori* as the bacteria are found in most abundance in the pre-pyloric region of the stomach (Dick et al 1989).

1.2 MICROBIOLOGY

H. pylori is a gram negative organism measuring 2-4um in length and 0.5-1.0um in diameter. It is a motile organism with 6-7 sheathed flagella (Jones 1985). *H. pylori* is a microaerophilic organism and requires to be cultured in vitro in a "microaerobic" atmosphere with a reduced oxygen content. If exposed for more than a short period of time to air with 21% oxygen, the organism dies (Skirrow 1990). *H. pylori* is a urease positive organism and indeed is remarkable on account of its high urease activity. This particular biochemical feature of *H. pylori* will be discussed more fully in the following Chapter.

1.3 EPIDEMIOLOGY

As already mentioned, *H. pylori* is one of the commonest infections in man. Its prevalence in the general community varies with age and between countries. In the United Kingdom, the prevalence approximately equals that of the age of the subject, being less than 10% in those under 10 years and more than 50% in those more than 50 years of age (Jones et al 1986). The prevalence in the United States is similar to that of the United Kingdom (Graham et al 1988). In the developing world, the prevalence of *H. pylori* is much higher and the infection is acquired much earlier in life (Holcombe et al 1992). For example, in the Ivory Coast, more than 50% of children are infected before reaching the age of 10 years (Megraud 1989). In spite of the high prevalence of the infection, very little is known about its mechanism of transmission.

1.4 THE NATURE OF THE INFECTION IN MAN

H. pylori infection is confined to gastric-type mucosa. The organism is found in most abundance in the antrum and, particularly, the pre-pyloric region of the stomach. It is also found in the mucosa of the body of the stomach. The infection is only found in the duodenum on patches of gastric metaplasia (Wyatt et al 1987). It has also been identified on islets of gastric metaplasia in Barret's oesophagus (Paull et al 1988), Meckel's diverticulum (Morris et al 1989) and in the rectum (Dye et al 1990). The specificity of *H. pylori* for gastric-type mucosa is thought to relate to its attachment to specific glycolipid receptors found only on gastric epithelial cells (Lingwood et al 1989).

The organism is found lying underneath the gastric mucus layer in close contact with the epithelial cells. It can usually be visualised on routine haematoxylin and eosin-stained biopsies, although a number of stains have been developed to aid its detection (Wyatt and Gray 1992). The Warthin-Starry Silver Stain (conventionally a technique for demonstrating spirochaetes in tissue sections) was used by Warren and Marshall in their original observation (Marshall et al 1987). It should be noted that none of the routine histochemical stains referred to above, is specific for *H. pylori* but instead only demonstrate the characteristic appearance of the organism. Strictly speaking, bacteria recognised only by histological methods should be referred to as "*Helicobacter*-like organisms" (Goodwin et al 1989).

1.4.1 ANTRAL GASTRITIS

H. pylori infection does not invade the gastric epithelium but does induce an inflammatory reaction in the underlying mucosa. The vast majority of subjects with *H. pylori* thus also have gastritis and *H. pylori* is now recognised as the major cause of gastritis in humans (Dixon 1992). The gastritis is usually more marked in the antral and pre-pyloric region of the stomach. It cannot be detected reliably by endoscopic appearances but is readily apparent on histological examination of mucosal biopsies. Epithelial erosions may be present and polymorphs may be visualised within the epithelium. However, the most pronounced feature is a marked infiltrate of the inflammatory cells within the lamina propria consisting of polymorphonuclear cells, plasma cells and lymphocytes (Figure 1). In the presence of *H. pylori*, lymphoid follicles also develop in the mucosa (Strickland and Fenoglio-Preiser 1991).

For some time, it remained unclear whether the gastritis was caused by *H. pylori* or whether the organism colonised an already inflamed mucosa. However, two pieces of evidence provide strong support for the gastritis

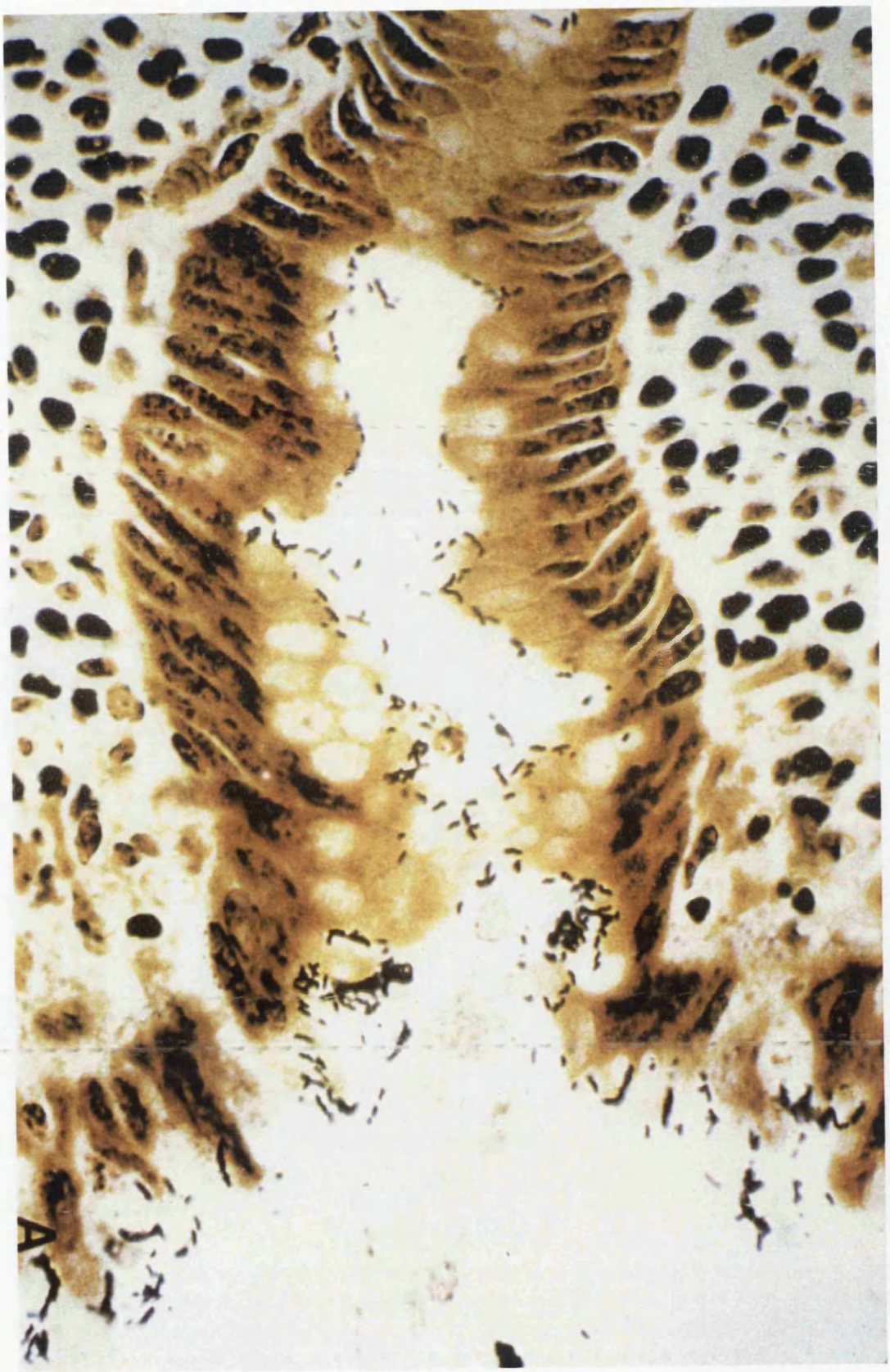

Figure 1

Gastric antral mucosal biopsy positive for *Helicobacter pylori* using Warthin-Starry stain, demonstrating chronic gastritis.

being due to *H. pylori* infection. The first is that eradicating the infection results in complete resolution of the gastritis (Rauws et al 1988). The second is that self-ingestion studies have shown that infection with the organism induces the characteristic changes in a previously normal mucosa (Morris and Nicholson 1987). The mechanism by which *H. pylori* induces the inflammatory response in the underlying mucosa is unclear. It has been suggested that it may be due to local damaging effects of ammonia produced by the organism's urease activity (Murakami et al 1988). However, an immune reaction to soluble bacterial proteins seem more likely.

1.4.2 HUMAN IMMUNE RESPONSE TO *H. PYLORI*

The great majority of patients colonised with *H. pylori* produce a measurable systemic antibody response to the organism. The immunoglobulin classes and sub-classes of these circulating anti-*H. pylori* antibodies are consistent with a prolonged chronic mucosal infection, with IgG and IgA predominating and IgM antibodies rarely seen (Kist 1991, Rathbone et al 1986, Rathbone 1986, Steer et al 1987). In the single, well-documented volunteer study, in which ingestion of *H. pylori* eventually led to chronic gastritis, IgG seroconversion occurred between 22 and 33 days post-infection (Kaldor et al 1985, Morris and Nicholson 1987). Subsequent studies showed that there was also an initial, short-lived, IgM response and a seroconversion of specific IgA (Morris and Nicholson 1989). Following eradication of *H. pylori* infection, there is a gradual fall in the circulating antibody levels (Vaira et al 1988, Newell et al 1990). In addition to the circulating antibodies (Perez-Perez et al 1988), there is also local production of IgA and IgG classes by immunoglobulin secreting B cells which make up a large proportion of the lymphoid cell infiltrate in the gastric mucosa (Kirchner et al 1990).

In addition to activation of the B cell limb of the immune response,

there is also infiltration of CD4- and CD8-positive T cells in *H. pylori* antral gastritis (Engstrand et al 1989, Wyatt and Rathbone 1988, Rathbone et al 1989). However, activated T cell clones, specific for *H. pylori* derived antigenic determinants, have still to be isolated from gastric mucosa and one cannot exclude these T cells being activated against other ingested antigens which are enabled to leak into the mucosa as a result of the *H. pylori*-induced gastritis. These cells are thought to be predominantly of the CD4 positive T cells ('helper' cells) have been classified into two main classes: TH1 and TH2 based on differential cytokine responses (Karttunen et al 1995). There is some evidence that the T cell immune response may be important in the eradication of the infection of *Helicobacter pylori*. *Helicobacter pylori* infection in mice can be eradicated by immunisation with white cell bacterial sonicated and these will lead to an active T cell response (Chen et al 1993).

1.5 *H PYLORI* AND OTHER UPPER GASTROINTESTINAL DISEASES IN MAN

1.5.1 DUODENAL ULCERATION

Duodenal ulceration is a common disease in the Western world with approximately 1 in 10 of the population being affected by it at some time in their life. Though rarely fatal, it causes a great burden on the Health Service resources due to the cost of investigations and therapy as well as time lost off work. In spite of the prevalence of the disorder, there has been little advance in the understanding of its pathogenesis apart from the observation that the parietal cell mass in patients with duodenal ulceration is approximately twice that of non-ulcer controls (Lam 1984). Most research into duodenal ulcer disease over the past 20 years has been concerned with the application of acid-controlling drugs which suppress acid hypersecretion but do not control the underlying disorder causing it. The possibility that duodenal ulcer disease might be associated with an

infection of the upper gastrointestinal mucosa, and that eradication of this infection might cure the disease, has revolutionised our thinking of this common disease.

The first piece of evidence linking *H. pylori* infection with duodenal ulcer disease is the high prevalence of the organism in patients with duodenal ulceration. The prevalence of *H. pylori* in subjects with duodenal ulceration is greater than 95% compared with a prevalence of less than 50% in a control population matched for age, sex and race (Marshall et al 1985, Borody et al 1991). Detailed studies have examined the very small proportion of patients with chronic duodenal ulceration unassociated with *H. pylori* infection. Approximately half of such patients can be explained by false negative results due to the poor sensitivity of single tests for detecting the presence of *H. pylori* (Borody et al 1991, Nensey et al 1991). Of the remaining patients who are truly negative for *H. pylori* infection but have chronic duodenal ulcer disease, approximately 50% are taking non-steroidal anti-inflammatory drugs. The very small number of remaining patients are an unusual sub-type of duodenal ulcer disease and include patients with Crohn's disease of the duodenum, Zollinger-Ellison syndrome, motility disorders and tumours (McColl et al 1993).

The second piece of evidence incriminating *H. pylori* infection in the pathogenesis of chronic duodenal ulceration is the fact that eradicating the infection markedly reduces the ulcer relapse rate. The relapse rate following a standard healing course of therapy which does not eradicate the infection is approximately 80% at one year, whereas eradication of the infection reduces the relapse rate to less than 20% (Marshall et al 1988, Coghlan et al 1987). Initially, it was unclear whether the reduced relapse rate might be due to an independent effect of the bismuth preparation used as part of the eradication drug regimen but patients who received this drug

but in whom the infection was not eradicated had a higher relapse rate than in those in whom it was eradicated (Coghlan et al 1987). In addition, recent studies have shown that one can reduce the duodenal ulcer relapse rate by eradicating the infection without any bismuth preparation (Rune et al 1992).

The mechanism by which *H. pylori* infection predisposes to duodenal ulceration has remained unclear. There are two main hypotheses. The first could be called the histological hypothesis (Wyatt et al 1987). It proposes that duodenal ulceration is due to local damage produced by the organism lying on islets of gastric metaplasia in the duodenum (Rune 1987). One problem about this hypothesis is that the infection with *H.* pylori *is most marked in the pre-pyloric region and if local mucosal* damage was the important mechanism, then one would expect a close correlation between *H. pylori* infection and pre-pyloric ulceration which has not been confirmed (Thomas et al, 1992).

The other main hypothesis could be referred to as the functional one (Thomas et al 1992). This suggests that the infection of the antrum somehow disturbs upper gastrointestinal physiology in such a way as to predispose to duodenal ulceration. The antrum is important in regulating both gastric acid secretion and gastric emptying and alterations in the control of either or both of these could predispose to excessive exposure of the duodenum to acid and thus lead to its ulceration. The G-cells in the antral mucosa are the major source of the hormone gastrin which is the major hormone regulating the gastric phase of acid secretion (Royton 1978). The release of gastrin is inhibited by intragastric acid. It is therefore possible that the ammonia produced by *H. pylori* urease activity, could block the feedback mechanism and thus result in increased gastrin release and consequently excessive acid secretion.

1.5.2 GASTRIC ULCERATION

The pathogenesis of gastric ulcer is multifactorial with physiological and environmental factors contributing (McIntosh et al 1985). Reduced mucosal defence appears to be the major abnormality involved in gastric ulcer formation and in some individuals gastric acid secretion may be lower than controls (Grossman et al 1963, Lanzon-Miller et al 1987, Deroda et al 1990). Chronic gastritis is of considerable importance in the pathogenesis of chronic gastric ulcer. *Helicobacter pylori* infection associated with gastric ulcer is less obvious than the association it has with duodenal ulcer. Between 50-80% cases of chronic gastritis are associated with *H. pylori* infection, and there is increasing evidence that this is a casual relationship (Marshall 1985, Rauws et al 1988). *Helicobacter pylori* infection leads to changes in the mucus lipoprotein which leads to the damage of the mucosal barrier and thus leads to gastric ulcer (Sidebotham 1990). The presence of free radicals due to the inflammatory process induced by *Helicobacter pylori* infection could be another factor.

Non-steroidal anti-inflammatory drugs are one of the important causes in the pathogenesis of gastric ulcer. Aspirin has similarly been linked to the development of gastric ulceration (Langman et al 1991). Many animal and volunteer studies have examined the pathogenesis of mucosal injury following both acute and chronic administration of non-steroidal anti-inflammatory drugs including aspirin. The damage induced by those drugs is a complex process and mutli-factorial including interference with mucus production, blood flow and bicarbonate production (Yeoman et al 1992).

1.5.3 *H. PYLORI* INFECTION AND GASTRIC CANCER

There is now considerable epidemiological evidence showing an association between *Helicobacter pylori* infection and the subsequent development of gastric carcinoma. Three separate case-control studies have indicated that *H. pylori* infection increases the risk of subsequently developing gastric carcinoma (Nomura et al 1991, Parsonnet et al 1991, Forman et al 1991). If this association is causal, then approximately 60% of gastric cancers worldwide may be explained by the infection (Correa 1991).

It is unclear why *H. pylori* in some patients results in duodenal ulceration which is associated with gastric acid hypersecretion, whereas in other patients results in gastric cancer which is a disorder associated with reduced acid secretion.

1.5.4 *H. PYLORI* INFECTION AND NON-ULCER DYSPEPSIA

There is considerable interest in a possible role of *H. pylori* infection in non-ulcer dyspepsia (Shallcross et al 1992). Several studies have shown that *H. pylori* infection is more common in patients with non-ulcer dyspepsia than in asymptomatic controls. However, it also has to be recognised that the majority of subjects with *H. pylori* infection are asymptomatic (Laffeld et al 1989). Studies of the value of eradicating *H. pylori* infection in patients with non-ulcer dyspepsia have produced conflicting results. It seems likely that *H. pylori* infection may play a role in a subgroup of patients with non-ulcer dyspepsia and much further work will be required to identify this group which may benefit from eradication of the infection.

1.6 DIAGNOSIS OF *H PYLORI* INFECTION IN MAN

As already mentioned, *H. pylori* infection can be diagnosed by microscopic examination of antral biopsies. In addition, it can be detected by culturing antral biopsies. Serology is also of some value but has a low sensitivity and specificity (Newell et al 1988, Newell et al 1989). *H. pylori* urease activity provides a valuable way of diagnosing the infection and this is used in the breath test and urease slide test which will be discussed more fully in the following chapter.

1.7 ERADICATION OF *H. PYLORI* INFECTION

Eradication as opposed to clearance of *H. pylori* infection is generally defined as being the inability to detect any infection at four weeks following completion of eradication therapy. At an early time point after completing eradication therapy, the infection may merely be suppressed and reappear as the effects of the therapy wear-off.

Eradication of *H. pylori* infection is difficult. Studies with single drugs or even dual therapy have proven relatively ineffective and at present the most reliable and effective means of eradicating the infection involves 2-3 weeks treatment with three anti-bacterial agents. The agents used in this triple therapy include tripotassium dicitratobismuthate, metronidazole and either amoxycillin, tetracycline or erythromycin (O'Riordan et al 1990). This triple therapy produces eradication rates of greater than 90% (Logan 1991). One of the main determinants of the success of the triple therapy is the sensitivity of the organism to metronidazole; patients with metronidazole-resistant organisms having a much reduced chance of eradication (Glupczynski et al 1990, Weil et al 1990, Becx et al 1990). In this country, resistance to metronidazole is present in less than 10% of the population but is more common in other European countries and in Africa the majority of strains of *H. pylori* are

metronidazole-resistant, possibly due to the frequent use of metronidazole or tinidazole for other infections (Glupczynski et al 1990). In addition to the problem of bacterial resistance, the current eradication regimen produces side-effects in a considerable proportion of subjects (Axon 1989). Nausea is experienced by most patients and diarrhoea may also occur. The patients also have to avoid alcohol during treatment because of the possible antabuse reaction with metronidazole. Due to these problems, patients undergoing eradication therapy for *H. pylori* infection must be highly motivated. Fortunately, re-infection following successful eradication is low (Borody et al 1992, Borody et al 1989).

At present, considerable work is being directed at discovering a more effective and more acceptable means of eradicating *H. pylori* infection. Particular attention is being paid to the possibility of using the combination of omeprazole with a single antibiotic. Omeprazole is a powerful inhibitor of gastric acid secretion and its elevation of intragastric pH may make the local environment less hospitable to *H. pylori* (Biasco et al 1989). In addition, its elevation of intragastric pH may help the antibiotic to reach the organism in higher concentrations. Omeprazole has also been shown to have some *in vitro* antibacterial properties (Bugnoli et al 1993, Sauerbaum et al 1990).

The present regimen for treating *Helicobacter pylori* consists of giving omeprazole 20mg twice per day plus amoxycillin 500mg t.i.d. and metronidazole 400mg t.i.d. for two weeks and the success rate is nearly 80% on this regime and the other regimen we can use is clarithromycin 500mg t.i.d. plus omeprazole 20mg twice per day plus amoxycillin 500mg t.i.d. for one week. This gives us an almost similar percentage of eradication and is effective as the triple therapy (Heatley 1992 and Logan et al 1994).

CHAPTER TWO

HELICOBACTER PYLORI AND ITS UREASE ACTIVITY

2.1 INTRODUCTION

H. pylori is remarkable on account of its high urease activity which is greater than that of any other urease-producing bacterial species (Molbey et al 1988). By means of its urease enzyme *H. pylori* splits urea into ammonia and carbon dioxide, with two molecules of ammonia being produced for each molecule of urea split. *In vivo*, the ammonia produced by *H. pylori* diffuses into the acidic gastric juice where it is trapped as the ammonium ion. The carbon dioxide, on the other hand, is absorbed into the blood stream and rapidly excreted in the breath. There is evidence that the high urease activity of *H. pylori* helps the organism to survive in the unusual intragastric environment and that it may also be involved in causing some of the pathological sequelae associated with the infection (Levi et al 1989, Chittajallu et al 1991).

2.2 LOCALISATION OF *H. PYLORI* UREASE ACTIVITY

The ultrastructural localisation of the bacterium's urease activity has been examined by several groups. In electron microscopic studies by McLean et al 1985 and Bode et al 1989, strains of *H. pylori* were incubated with sodium tetraphenylboron, an ammonia-precipitating agent in the presence of urea. In eleven of the twelve strains studied, there was urease activity in the periplasmic region and at the outer membrane, and in six of those strains there was also activity in the cytoplasm. The localisation of *H. pylori* urease activity has also been studied by Hawtin using monoclonal antibodies and an indirect immunogold technique. In these studies using both prefixed bacteria and ultrathin cryosectioned bacteria the enzyme was located on the cell surface and in material apparently shed from the organism (Hawtin et al 1990).

2.3 STRUCTURE OF *H. PYLORI* UREASE

The urease of *H. pylori* has been partly purified using fast protein liquid chromatography by Hawtin et al (1990). The material contained 10nm doughnut-like structures when examined by electron microscopy and comprised three major polypeptides (61KDa, 56KDa and 28KDa). The above material was examined further using polyarylamide gel electrophoresis and this showed absence of the 56KDa polypeptide. It therefore appears that the urease consists of only two major subunits. The 28KDa polypeptide appears to be the more important for urease activity as only monoclonal antibodies directed against it inhibited or captured urease activity.

The genes encoding the two structural subunits of *H. pylori* urease enzyme have now been cloned and sequenced (Clayton et al 1990). The derived amino acid sequences of the two *H. pylori* urease genes indicates 12/19 amino acids (61%) in common with *K* aerogenes urease and 15/21 amino acids (71%) in common with the *P.* mirabilis enzyme. *H. pylori* urease also shows close homology with jack bean and soybean ureases, with almost 57% residues being identical in the bacterial and plant enzymes.

2.4 SIGNIFICANCE OF UREASE ACTIVITY TO THE BACTERIUM

H. pylori expends a tremendous amount of its metabolic resources producing urease and the enzyme presumably confers significant advantages to the bacterium. The production of ammonia, a strong alkali, by *H. pylori* urease has been shown to facilitate the survival of the organism in acidic environments. Studies by Marshall et al (1990) showed that the organism could survive at pH < 2.5 in the presence of 2mmol/l urea but rapidly died at this pH in the absence of urea. The concentration

of urea in gastric juice is approximately 2mmol/l and it is likely that this allows the organism to survive in the acidic gastric juice when it is initially ingested and before it becomes established in the less acidic environment beneath the mucus layer.

H. pylori urease activity may also provide a means by which the organism can utilise urea as a nitrogen source. Urease is commonly found in spiral organisms in the distal small intestine of rodents where it is thought to be important for providing a source of nitrogen for the organism (Ferrero et al, 1988). Studies by Greig et al (1991) have shown that only a proportion of the ammonia produced the hydrolysis of urea by *H. pylori* is recovered in the incubating medium, and this is consistent with the ammonia being incorporated intracellularly. *H. pylori* possesses glutamate dehydrogenase activity which would allow incorporation of ammonia into amino acids following combination with alpha ketoglutarate.

2.5 ROLE OF UREASE ACTIVITY IN PATHOLOGICAL SEQUELAE OF *H. PYLORI* INFECTION

In addition to assisting the organism's survival, it is possible that *H. pylori* urease activity and ammonia production may be responsible for some of the pathological sequelae associated with the infection. As discussed in the preceding chapter, elevation of antral surface pH by the ammonia might be responsible for *H. pylori* related hypergastrinaemia and thereby duodenal ulceration. In addition, it has been suggested that the ammonia might result in local toxic damage to the gastric epithelial cells and be one means by which the organism produces gastritis (Murakami et al 1988). *H. pylori* urease may be involved in producing the gastritis independent of any direct toxic effects of the ammonia it produces. A significant proportion of the antibodies produced against the organism are directed against the protein of the urease enzyme (Hu et al 1990, Hawtin et

al 1990). The inflammatory infiltrate which characterises *H. pylori* gastritis can be considered to be at least partly due to sensitisation of the host's immune system to *H. pylori* urease protein (Wyatt et al 1989).

2.6 VALUE OF *H. PYLORI* UREASE ACTIVITY IN CLINICAL DIAGNOSIS OF THE INFECTION

The high urease activity of *H. pylori* provides a useful means of detecting the infection in man. Two diagnostic tests based upon the organism's urease activity are commonly employed in clinical practice.

2.6.1 THE UREASE SLIDE TEST

The basic test involves placing an antral gastric biopsy obtained at endoscopy in a small well containing urea broth or agar and phenol red. If urease activity is present in the biopsy specimen it will hydrolyse the urea and the ammonia produced will cause a rise in pH. This rise in pH is detected by the phenol red which changes colour from yellow/brown at pH 6.8 to pink at pH 8.4. A buffer is used to stabilise the test and the incubation is performed at 37°C to accelerate the reaction.

A commercially produced urease slide test has been produced by Marshall et al (1987). It is called the *campylobacter*-like organism test (CLO test) and manufactured by Delta-West Ltd., Perth, Western Australia (Fig. 2). After placing the biopsy in the well, adhesive is then used to cover the well and the slide is conveniently placed in the endoscopist's trouser pocket to allow it to incubate at near 37°C. The CLO test is reported to be almost 100% specific and to have a sensitivity of 70% after 1 hour's incubation and of 90% after 24 hours (McNulty et al 1989, Dye et al 1988, Schnell et al 1988).

2.6.2 THE UREA BREATH TESTS

The urea breath tests provide a non-invasive means of diagnosing *H. pylori* infection. The tests involve the oral administration of urea which

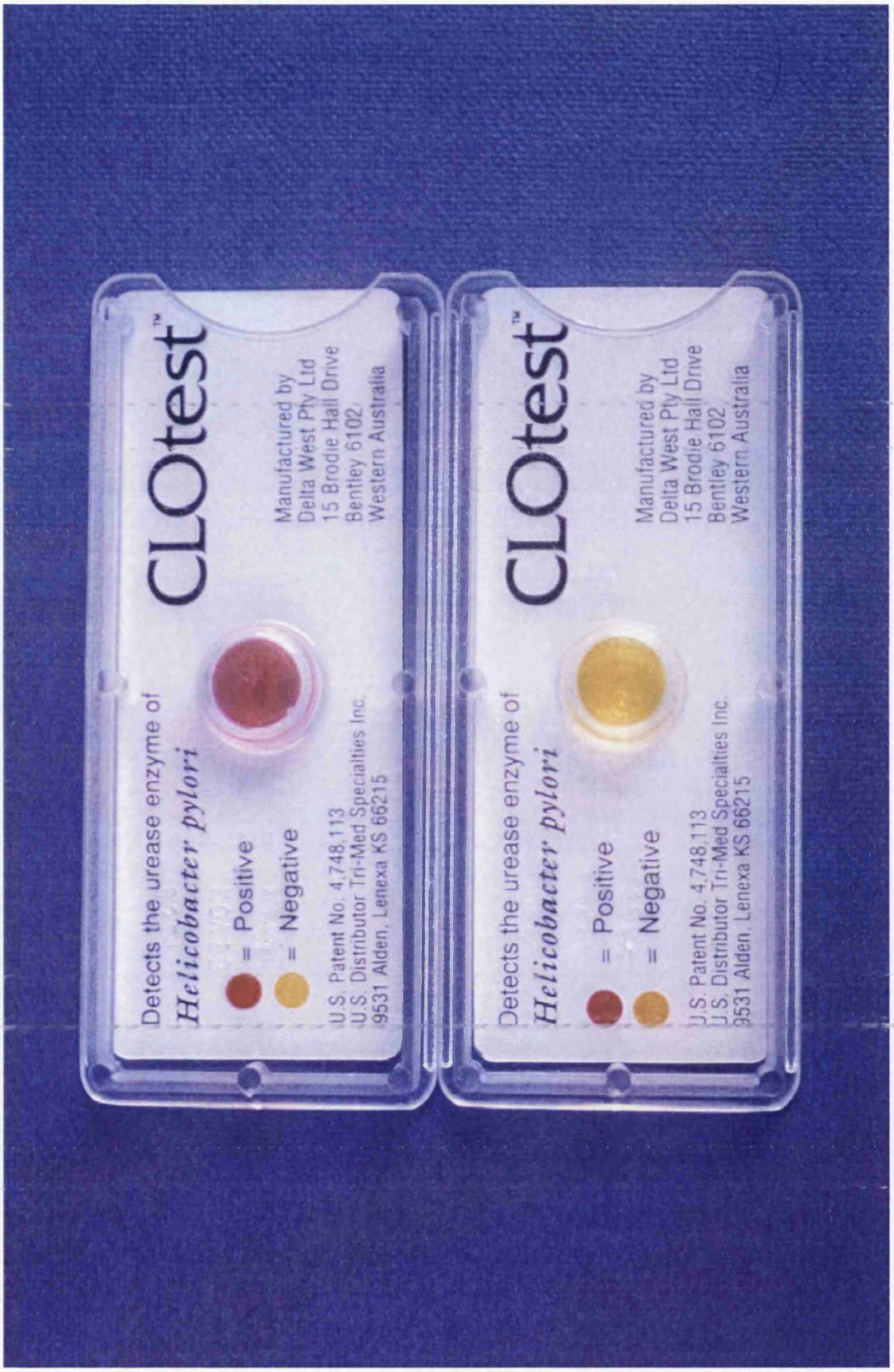

Figure 2: The CLO test rapid urease test for diagnosis of H. pylori in gastric biopsy specimens. Positive biopsies produce a red colour, usually within 1 hour. If the gel remains yellow 24hrs after insertion of the biopsy, then H. pylori is not present (sensitivity 95%).

has its carbon atom labelled isotopically. In the presence of *H. pylori*, the urea is hydrolysed in the stomach to ammonia and carbon dioxide and the latter absorbed and excreted in the breath where it can be detected on account of its isotopic label. Either ^{14}C- or ^{13}C-labelled urea may be employed. The urea breath tests have a specificity of and sensitivity of greater than 90% diagnosing *H. pylori* infection (Dill et al 1990, Logan et al 1991, Veldhuyzen et al 1990, Rauws et al 1989).

The ^{14}C-Urea Breath Test

In our own Radioisotope Department in the Western Infirmary, Glasgow, we use the ^{14}C-urea breath test as described below (Fig. 3).

Our patients are fasted overnight from 9p.m. the previous night. On arrival they are weighed with indoor clothes and shoes on. The patients then clean their teeth without swallowing any water, and discarding any rinsings into running water in the basin. This is performed to remove any urease producing bacteria present in the mouth. A basal sample of breath is then collected. Following this they drink 200ml of *Ensure Plus* (Abbott Laboratories Ltd., Queensborough, Kent), followed by 0.4MBq ^{14}C-labelled urea in 25ml of water in a disposable paper cup. The cup is flushed out twice with 25ml water which the patient also drinks. The *Ensure Plus* is a high lipid drink and is taken to delay gastric emptying and retain the ^{14}C-urea in the stomach. After drinking the isotope the patient again cleans his teeth to remove any labelled urea from the mouth which might be hydrolysed by urease positive bacteria in the mouth. Breath samples are then collected at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes after ingestion of ^{14}C-urea.

The breath samples are collected by blowing into a glass tube connected to a scintillator vial containing 2ml indicator solution which is 60mgm of thymolphthalein dissolved in 500ml of ethyl alcohol and 500ml of 1 molar hyamine hydroxide added. The glass tube contains anhydrous

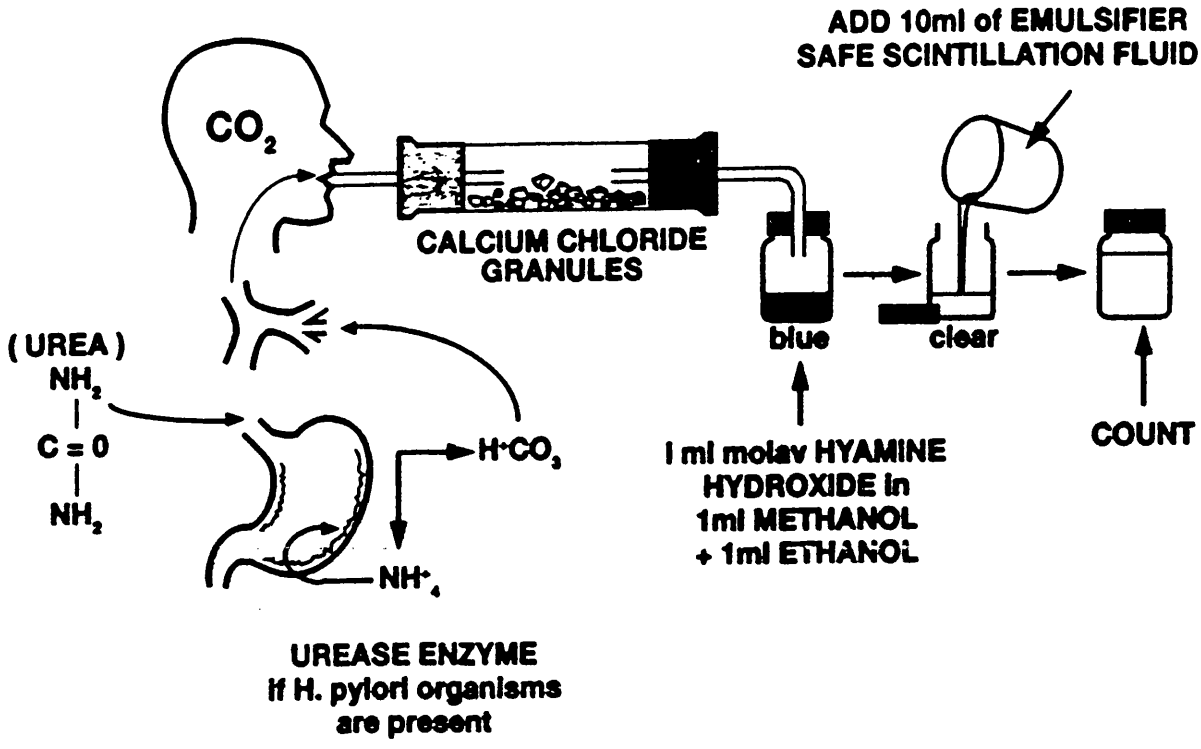

Figure 3

The principle of urea breath test is shown in this figure. If *H. pylori* urease is present in the gastric mucosa, isotope-labelled urea is hydrolysed forming $^{14}CO_2$ which is expired in the breath. Patients blow through to a liquid trap containing methanol, ethanol, hyamine and a pH indicator. The blue (alkaline) solution changes to colourless upon CO_2 saturation, scintillation fluid is added, then counted.

calcium chloride to dry the breath. The patient blows into the tube until the blue colour disappears completely i.e. the hyamine hydroxide is neutralised.

Two ml aliquots of the indicator solution are then counted on a liquid scintillation counter which has a ^{14}C quench correction curve.

The excretion of ^{14}C-CO_2 in the breath is then calculated as follows:-

Sample nett D.P.M. (disintegration per minute) x patient weight in kilogram x 100 x 100, divided by the given dose D.P.M. The result is expressed as kilogram % dose per mmol CO_2 x 100.

The results are plotted on a graph of time (min) versus Kgm % dose/mmol CO_2 x 100.

The ^{14}C-urea in the ^{14}C-urea breath test is either hydrolysed and expired as $^{14}CO_2$, or excreted unchanged as urea in the urine. The total dose which will accumulate as the radiation dose is very small and its effective half-life is a few hours to a few days. The ^{14}C-urea breath test could also be performed in a shorter period i.e. breath samples collected at 0 and 20 minutes after ingestion of ^{14}C-urea (Bell and Weil 1992).

Restrictions on the use of the ^{14}C-urea breath test are as follows:-

The ^{14}C-urea breath test should follow the general radiologic procedures and guidelines, it should not be given to pregnant women and the 10 day rule is observed.

^{13}-C-Urea Breath Test

The ^{13}C-urea breath test is similar to the ^{14}C-urea test except that the non-radioactive isotope is used and this means that the test can be applied without restrictions. Two disadvantages of the ^{13}C-urea breath test are the higher cost of the non-radioactive isotope and of measuring it by mass spectrometry. The results obtained by the ^{13}C- and ^{14}C-urea breath tests are equivalent (Graham et al 1988).

CHAPTER THREE

PHYSIOLOGY AND PATHOLOGY OF GASTRIN

3.1 INTRODUCTION

Gastrin was discovered in 1905 as a gastric acid stimulatory factor in the extraction of antral mucosa (Edkins 1905). It was subsequently purified as sulphated and non-sulphated Gastrin 17 hecadecapeptide (Gregory and Tracy 1964, Yalow and Berson 1970). Understanding of the physiology and pathology of gastrin increased after 1969 when it became possible to measure the hormone in serum by radioimmunoassay (McGuigan and Trudeau 1968, Yalow and Berson 1970).

3.2 GASTRIN BIOSYNTHESIS AND PROCESSING

The complex biosynthetic pathway of gastrin has recently been elucidated (Rehfeld and Hilsted 1992). The hormone is initially synthesised as preprogastrin which is 101 amino acids in length. This peptide is enzymatically cleaved to yield progastrin after which it is subjected to a sequence of further protolytic cleavages. The resulting carboxyl terminal glycine-extended intermediate is then carboxyamidated to produce the biologically active hormone (Rehfeld and Hilsted 1992, Walsh 1993). During the cleavage of the larger precursors into smaller fragments, various modifications can occur to individual amino acids including sulphation making the peptide more resistant to degradation and amidation which requires copper, oxygen, ascorbic acid and a pH around 5 (Eipper et al 1987, Hilsted 1991). The crucial step for biological activity is alpha-amidation and the common C-terminal tetrapeptide amide (-Trp-Met-Asp-Phe-NH_2) has traditionally been regarded as responsible for all of the biological effects of gastrin. In 1994, however, several groups presented preliminary evidence that progastrin-derived glycine-extended intermediates may possess both trophic (Seva et al 1994, Negré et al 1994) and acid regulatory properties (Kaise et al 1995).

After translation of gastrin mRNA (messenger ribonucleic acid) in the rough endoplasmic reticulum (RER) and co-translational removal of the N-terminal pre or signal peptide from pre-progastrin, the intact progastrin is transported to the Golgi apparatus. From the trans-Golgi apparatus, small vesicles carry the processing intermediates of progastrin towards the basal part of the G-cells (gastrin-producing cells), where gastrin peptides are stored (Håkanson et al 1982). As a result of the elaborate biosynthetic pathway, the normal antral G-cells in humans release a heterogeneous mixture of progastrin products from the mature secretory granules (Hilsted and Hansen 1988).

The three molecular forms of gastrin which have biological activity with respect to stimulating gastric acid secretion are: big gastrin (G34,) little gastrin (G17) and mini-gastrin (G14) (Gregory 1974). The Gastrin-17 (G17) is formed mainly in antral mucosa, accounting for 90% of the extractable gastrin. However, in the duodenum the majority of extractable gastrin is G34 (Berson and Yalow 1971). G17 and G34 half-lives in the circulation have been found to be approximately five and forty-two minutes respectively (Walsh et al 1976). The half-life of G14 is similar to that of G17 (Debas et al 1974).

3.3 HOMOLOGY OF HUMAN GASTRIN WITH OTHER PEPTIDES

Most biologically active peptides occur in peptide families whose members display a significant structural homology. The occurrence of peptide families reflects development by gene duplication and subsequent mutation from a single ancestral peptide gene for each family (Larsson et al 1977). Gastrin is a member of the peptide family which includes cholecystokinin (CCK). Both are isolated from the gastrointestinal tract

and shown to be present in the brain. Different forms of these peptides are released into the circulation by differential proteolysis. Gastrin and CCK share the same carboxyl terminal pentapeptide sequence. The carboxyl (COOH) terminus tetrapeptide represents the region responsible for biological activity of both hormones whereas the rest of the two molecules only modifies their selectivity and potency for different target cells. The main difference between gastrin and CCK is in the tyrosine residue which is present six amino-acids from the COOH terminus in gastrin, whereas CCK has a tyrosine on the seventh amino-acid. The tyrosine residues may be sulphated in various peptides of CCK but are only partially sulphated in gastrin. The presence of a sulphate residue increases the affinity of gastrin for its receptor and sulphated G17 was a nineteen fold higher affinity for gastrin/CCK_B receptors compared to non-sulphated G17 (Huang et al 1989). The peptides of gastrin/CCK family exert their effects by interaction with at least three different types or states of receptor (Hays et al 1980, Saito et al 1980, Soll et al 1984). These include pancreatic type (CCK_A), the cerebral colorectal type (CCK_B), and the gastrin receptors. The CCK and gastrin exert their effect on the same receptors, although the latter two types are now thought to be the same. Cholecystokinin exerts trophic effects via gastrin/CCK_B receptors with similar affinities (Hughes et al 1993).

3.4 THE G AND D CELLS WITHIN GASTRIC MUCOSA

In the normal mammalian organism, most gastrin is produced by the antral G cell (Larsson et al 1980) which are part of APUD cells, found throughout the gastrointestinal mucosa. Other cells in the same system include D-cells which secrete somatostatin. Gastrin and somatostatin are two potent regulatory peptides; both influence the secretion of gastric acid which is stimulated by gastrin and inhibited by somatostatin depending on

the pH value in the stomach and duodenum (Barros et al 1975, Polak et al 1978). G- and D-cells are concentrated in the antrum (Canese and Bussolati 1977). G-cells are often found in close contact with finger-like cytoplasmic processes of somatostatin cells (Larsson et al 1979) and with nerve fibres containing bombesin-like peptides (Holst et al 1987) such as gastrin releasing peptide (GRP), (Guo 1987, Holst et al 1987). The G-cell ultrastructure is characterised by cytoplasmic granules which are accumulated in the basal and paranuclear cytoplasm. The granules represent the main storage sites for hormonal peptides. The D-cells have long finger-like cytoplasmic processes that directly contact neighbouring cells i.e. parietal cells and G-cells. The morphological evidence for a paracrine mode of action to gastric D-cells is the presence of somatostatin receptors on their adjacent cells (Larsson 1979).

3.5 THE REGULATION OF GASTRIN RELEASE

Gastrin release is a complex process. Ingestion of a meal, especially one containing beef protein, is the most important stimulant of gastric release. Peptone and amino acids derived from partial digestion of protein are particularly potent (McArthur et al 1988), whereas fat and carbohydrates are ineffective. Similarly, gastrin is released when intragastric pH rises above three and conversely, antral acidification after a meal potently inhibits gastrin release (Schubert 1993).

Vagal innervation stimulates gastrin release, probably via release of gastrin-releasing peptide (GRP) from enteric nerve endings. Vagal reflexes can also inhibit gastrin release, although the mechanism of this is unclear (Walsh 1994). A number of gastrointestinal peptides also inhibit gastrin release, but somatostatin is by far the most important. Somatostatin cells are located in close proximity to gastrin cells of the

antrum and parietal cells of the fundus. Somatostatin exerts a continuous inhibitory paracrine effect on the secretion of gastrin and acid (Chiba and Kinoshita 1993). Other inhibitors of gastrin release include secretin, glucagon, vasoactive intestinal polypeptide (VIP), cholecystokinin, gastrin inhibitory peptide (GIP) (Rayford et al 1974) and calcitonin (Becker et al 1974, Gregory 1974). Others include prostaglandin E analogues in parietal cells which interact with inhibitory receptors coupled to adenylate cyclase (Chen et al 1988, Ippoliti et al 1976). All these inhibitors may be acting indirectly via stimulation of somatostatin release and their full physiological importance is still unclear.

3.6 EFFECTS OF GASTRIN ON GASTRIC ACID SECRETION

The major physiological role of gastrin is stimulation of gastric acid secretion. An ATP-dependent ion pump, situated in the secretory membrane of the stimulated parietal cell is directly responsible for acid secretion. Gastrin binding to the parietal cells using [^{125}I-Tyr] gastrin has been demonstrated by autoradiography (Nakamura et al 1987). Kuamamoto and colleagues demonstrated specific ^{125}I-G17 binding to gastric fundic mucosa from a duodenal ulcer patient (Kuamamoto et al 1989).

The activity of the parietal cell is controlled by receptors for acetylcholine, histamine and gastrin on the basolateral cell membrane (Nakamora et al 1987). Other receptors include A_1 adenosine, epidermal growth factor, enteroglucagon and adenergic compounds (Gerber et al 1985). Stimulation of these receptors modulate the level of protein kinase in the cell and brings about the change from resting to stimulating structure. In recent years, an increasing number of receptor antagonists for gastrointestinal peptides have become available as powerful tools with which to study many aspects of gastrointestinal function. At least eight

antagonists for gastrin $CCK/_B$ have been developed (Presti and Gardner 1993).

3.7 TROPHIC EFFECTS OF GASTRIN

The physiological trophic effect of gastrin on the gastrointestinal mucosa was first observed clinically in patients with Zollinger-Ellison syndrome. This disease results in a gastrin secretory endocrine tumour of the digestive tract and patients are noted to have thickened gastric rugae, gastric mucosal hyperplasia and an increased parietal cell mass (Ellison et al 1967). Patients with low circulating gastrin concentration due to antrectomy, demonstrate mucosal atrophy (Lees et al 1968).

In 1969, Crean and Johnson were the first to report trophic effects of gastrin on the gastric mucosa of rats following injection of pentagastrin (Crean et al 1969, Johnson et al 1969). Since these studies, evidence for a physiological important trophic role for gastrin in the stomach has steadily accumulated.

Johnson (1977) found that intraperitoneal injection of pentagastrin ($250\mu g/kg^{-1}$) had no effect on [^{3}H] thymidine incorporation of antral mucosa but stimulated oxyntic mucosal thymidine uptake by 150 times compared to control rats. Others have confirmed these findings (Balas et al 1985) although Lehy et al (1979) found gastrin to be equally effective at stimulating [^{3}H] thymidine uptake in both glandular and antral portions of the rat stomach. However, the weight of evidence would suggest that antrum is not regulated trophically by gastrin. Johnson pointed out that this is perhaps not surprising, given the neuroendocrine function of the antrum (Johnson 1977).

3.8 GASTRIN AND ENTEROCHROMAFFIN-LIKE (ECL) CELLS

ECL cells constitute the major endocrine cell population in the acidopeptic mucosa and are small, irregularly shaped and heavily aggrophilic with different methods of staining (Capella et al 1991, Solcia et al 1975). ECL cells are poorly represented in the cardia and are absent from the pyloric gland area. In the oxyntic gland, ECL cells are scattered in the deep and intermediate regions with few observed in the neck and none in the surface epithelium (Capella et al 1971). Histamine is the only messenger/mediator which has been demonstrated to restore the ECL cells of all species (Hakanson et al 1986). These cells produce and store histamine along with a yet undefined peptide(s). The first detailed description of non-tumour endocrine growth arising in the oxyntic mucosa were of patients with pernicious anaemia and chronic atrophic gastritis (Rubin 1969). In human pathology, variable patterns of ECL cell hyperplasia are observed in conditions of longstanding hypergastrinaemia evoked by two different mechanisms, achlorhydria in chronic active gastritis and gastrin cell tumour in the Zollinger-Ellison syndrome with or without multiple endocrine neoplasia syndrome. The key role of gastrin in determining ECL cell growth is shown by the observation that in chronic active gastritis patients, the withdrawal of hypergastrinaemia following antrectomy induces a dramatic shrinking of proliferated ECL cells, eventually leading to complete disappearance of hyperplastic changes (Richards et al 1987, Olbe et al 1988, Hirschowitz et al 1992). Sustained hypergastrinaemia induces either by continuous infusion of gastrin (Rybert et al 1990) or by chronic treatment with inhibition of acid secretion such as ranitidine (Havu et al 1990) or omeprazole (Havu 1986, Ekman et al 1985), results in ECL cell hyperplasia with continued treatment. Larsson showed ECL carcinoid tumours developed in the stomach of treated

(Larsson et al 1988). The role of gastrin in stimulating proliferation of ECL cells has been reviewed recently (Håkanson and Sundler 1991, Berlin 1991). The main explanation of the proliferation pressure on the ECL cell hyperplasias is not fully understood. Clinical evidence suggests that other factors both genetic and environmental are required to induce ECL cell transformation and carcinoid tumour (Solcia et al 1993). Further work is required to establish the exact relation between hypergastrinaemia and the ECL cells.

3.9 CAUSES OF HYPERGASTRINAEMIA

Treatment with inhibitors of acid secretion such as ranitidine or omeprazole will cause hypergastrinaemia (Havu 1986, Lamberts et al 1993). The role of gastrinoma Zollinger-Ellison syndrome is well established and the subject has been reviewed by Isenberg et al (1973) and Walsh and Grossman (1975). Other conditions include antral G-cell hyperplasia and isolated retained antrum (Berson and Yalow 1972). Basal and meal-stimulated gastrin were both observed to be higher in patients with pyloric stenosis compared with both normal subjects and non-stenotic ulcer patients (Tani and Shimazu 1977), while other workers (Hamburg et al 1979) found no difference in the fasting and post-feeding serum gastrin. A large proportion of patients with chronic renal failure have increased serum gastrin concentration. This appears to be related to the severity of the renal failure as affected by the serum creatinine level (Hansky et al 1975). Serum gastrin levels remain high in patients undergoing chronic haemodialysis and were less elevated in patients who had renal transplantation (Doherty et al 1978). The increase in serum gastrin levels may also be a reflection of atrophic gastritis and gastric hypo-acidity (Mitchell et al 1979). A direct correlation was found between G-cell density and parathyroid function in patients with chronic renal failure,

suggesting that secondary hyperparathyroidism may play a role in the gastrin elevation (Crivelli et al 1979).

Fasting gastrin also increased after vagotomy in duodenal ulcer patients (Walsh and Grossman 1975) and in non-ulcer patients (Lam et al 1978). Hypergastrinaemia has been reported in rheumatoid arthritis (Rowden et al 1978), cirrhosis of the liver (Lam 1976) and in patients with intestinal resection (Straus et al 1974).

AIM OF THE THESIS

The aims of this thesis are to study:

(1) The effect of *H. pylori* infection on serum gastrin concentrations.

(2) The role of bacterial ammonia production in producing hypergastrinaemia.

(3) The effect of *H. pylori* status on serum gastrin during proton pump inhibitor therapy.

(4) The effect of proton pump inhibitor therapy on *H. pylori* infection.

CHAPTER FOUR

EFFECT OF *H. PYLORI* INFECTION ON BASAL AND MEAL-STIMULATED PLASMA GASTRIN CONCENTRATIONS

4.1 INTRODUCTION

There is increasing evidence that *Helicobacter pylori* (*H. pylori*) infection is important in the development and relapse of duodenal ulceration (Graham 1989). However, the mechanism by which this chronic bacterial infection predisposes to ulceration of the duodenum remains unclear. As the infection mainly involves the gastric antrum where the gastrin producing G-cells are located, it could interfere with gastrin release and thereby affect gastric acid secretion. In order to investigate this we have examined basal and meal stimulated plasma gastrin concentrations and gastric acid status before and one and seven months after eradication of *H. pylori* infection in duodenal ulcer patients.

4.2 PATIENTS

Ten patients (9 men, 1 woman, median age 49 years; range 29-65) confirmed endoscopically to have duodenal ulceration within the previous year were studied. Seven were workers. None had evidence of active ulceration at endoscopy performed within one month of entry to the study. Antral biopsies obtained at this examination showed gastritis associated with *H. pylori*-like organisms and gave a positive reaction to the rapid urease test (CLO test) described by Marshall et al (1987). All the patients had been treated with H_2 antagonists but none had undergone gastric surgery.

4.3 METHODS

On entry to the study each patient had a ^{14}C-breath test and a combined study of daytime intragastric pH and plasma gastrin. Six patients also had studies of night-time acid output. These tests were repeated along with repeat antral biopsies 4 weeks after completing a one month course of

tripotassium dicitrato bismuth (De-Nol tab) 120mg four times a day, metronidazole 400mg three times a day and amoxycillin 250mg three times a day. Seven months after completing eradication therapy nine patients had repeat breath tests and combined studies of daytime intragastric pH and plasma gastrin concentrations and six of them also had repeat studies of night-time acid output.

4.3.1 COMBINED INTRAGASTRIC pH/GASTRIN STUDIES

The combined studies of intragastric pH and plasma gastrin concentrations were performed at least four weeks after withdrawal of any acid inhibitory agents. The patients reported fasted at 0830h and a combined glass electrode (Radiometer, GK2802C) was passed perorally into the body of the stomach where its position was confirmed radiologically. The electrode was connected to a digital recorder (Digitrapper MKII, Synectics Medical) which registered the pH every 4 seconds from 0830h until 2100h. At the start and completion of each recording the electrodes were calibrated using standard buffers of pH 1.07 (Synectics 5002) and 7.01 (Synectics 5001). During the first 30 minutes of the pH recording and with the patient still fasted, 10ml venous blood samples were taken at 15 minute intervals for gastrin determination. The patient then took a standard OXO breakfast (40kcal) consisting of two beef cubes (OXO Ltd., Croydon, England), dissolved in 200ml water at 50°C and further venous blood samples were taken at 10 minute intervals for 90 minutes. On each study day patients were given standard meals (700kcal each) at 1230h and 1730h. Smokers documented their cigarette consumption on their first study day and smoked an identical number on subsequent study days.

Seven of the patients studied had participated in a four week H_2 antagonist therapy study 3-6 months earlier. The pre-treatment day of that study employed the identical protocol to the present study. It was

therefore possible to compare these patients' basal and OXO-meal stimulated gastrin values when they had first been studied in this way with the results of their pre-treatment study day for the present study. In this way, it was possible to assess whether repeating the test in itself could affect the gastrin values.

4.3.2 NIGHT-TIME ACID OUTPUT

In the patients who also had studies of night-time acid output, the pH electrode was removed at 2100h and replaced by a size 14F vented gastric tube (Andersen Inc., New York). Constant aspiration was performed throughout the night (2300h-0800h) and the gastric juice analysed as hourly aliquots. The volume and pH of each collection were recorded and hydrogen ion concentration measured by titration with 0.1N Na OH to pH7 using an autotitrator (Radiometer ETS 822). During the night-time acid output studies performed before therapy and one month post treatment further venous blood samples for gastrin determination were obtained at 2 hourly intervals from 2300h until 0700h.

4.3.3 ^{14}C-UREA BREATH TEST

The ^{14}C breath tests were performed within 5 days of the combined acid and gastrin studies. After an overnight fast the patients drank 250ml Ensure Plus (Abbot Laboratories, England) immediately followed by 0.4MBq ^{14}C-urea (Amersham International) in 25ml water. Breath samples were collected at 10 min intervals for 2 hours and analysed as described by Marshall and Surveyor (1988). As the 20 min sample has been shown to be the most discriminating (Bell et al 1987), this was selected for assessing the effectiveness of the eradication therapy.

4.3.4 HISTOLOGY

Antral biopsies were fixed in formol saline, embedded in paraffin wax, sectioned at 4um and stained with Haematoxylin and Eosin. They were all

examined blind by a single pathologist (SD) and given a gastritis score of between 0 and 10. Chronic inflammatory infiltrate in the mucosa was scored as 0-2, lamina propria polymorph infiltrate as 0-3, intraepithelial polymorph infiltrate as 0-3 and mucosal erosions as 0-2 as described by Rauws et al (1988).

4.3.5 GASTRIN DETERMINATION

The venous blood samples were immediately centrifuged at 4°C and the plasma stored at -20°C. Gastrin determination was performed by radioimmunoassay using antibody R98 which has a sensitivity of 5ng/l (Ardill 1973). The pre- and one month post-treatment samples were assayed in the same batch. When the seven month post treatment samples were assayed, stored samples from each patient's pre- and one month post treatment study were re-assayed along with them and their values were within 5% of the original in each case.

The basal gastrin value for each patient was determined by taking the median of the three samples obtained at 15 min intervals prior to the OXO meal. The night-time gastrin value was taken as the median of the 5 samples obtained at 2h intervals through the night. The integrated gastrin response to the OXO meal was taken as the area under the plasma gastrin time curve calculated by the trapezoid method.

4.3.6 INTRAGASTRIC pH ANALYSIS

The intragastric pH data were transferred from the Digitrapper recorder to an IBM Compatible computer (Amstrad PC 1512 HD20) and analysed using the GastrograpH program (Ver 5.0, 1987, Gastrosoft Inc). For each patient the median value of all their daytime recordings (0900-2100h) was calculated and called their daytime pH. More detailed analysis was performed of the intragastric pH response to the OXO meal as this was the meal during which plasma gastrin was monitored. This included the

measurement of the peak pH achieved and the time the pH was above 2 in the hour following this meal. For the patients in whom *H. pylori* was successfully cleared, integrated median daytime pH profiles prior to and following eradication were created, by combining individual median values at 10 minute intervals using he Statphac program (Ver 2.07, 1987, Gastrosoft Inc).

Statistical comparison of values before and after eradication of *H. pylori* were performed using the two-sided Wilcoxon's paired samples ranking test.

The study was approved by the Hospital Ethical Committee and all patients gave written informed consent before entry.

4.4 RESULTS

The one month course of triple therapy eradicated *H. pylori* in 9 of the 10 patients. This was confirmed by the absence of the organism in repeat antral biopsy, a negative CLO test and normalisation of the ^{14}C-urea breath test. In these 9 patients the 20 min breath test values (percentage ^{14}C dose per mmol CO2 x 100 x kg body weight) ranged from 72-171 (median 135) prior to eradication therapy and were 0-5 (median 2), one month following therapy ($p < 0.01$). In each of the 8 of these patients who had repeat breath tests seven months after completing therapy, the values were again less than 5. In the one patient in whom the bacterium was still present in the antral biopsy and whose CLO test remained positive one month after therapy, the 20 min breath test value was 60 pre-treatment, 40 one month post treatment and 59 seven months post treatment.

4.4.1 HISTOLOGY

In the 9 patients in whom the bacterium was eradicated, the median gastritis score prior to treatment was 5 (range 1-8) and fell to 1 (range 0-1) one month post treatment ($p < 0.01$). In the patient in whom the organism was not cleared, the score was 5 before therapy and 5 following it.

4.4.2 PLASMA GASTRIN CONCENTRATIONS

In the 9 patients cleared of *H. pylori* their median basal gastrin concentration fell from 30ng/l (range 20-60) to 20 (range 5-35) at one month following eradication ($p < 0.02$) and their integrated gastrin response to the OXO meal from 3,650ng/l.min (range 1,875 - 6,025) to 1,800 (range 1,200 - 3,075) ($p < 0.01$) (Fig. 4). In each of the 5 patients cleared of the organism and studied overnight, there was a fall in their night-time gastrin concentration; the median night-time concentration being 40ng/l (range 20-55) compared with 25 (range 15-30) one month following therapy. In the eight patients studied seven months after eradication of the organism their median basal gastrin concentration (median 15, range 5-20) and their integrated gastrin response (median 1,312, range 875 - 2,025) were similar to the respective values at one month post treatment. In the single patient in whom *H. pylori* was not eradicated the integrated gastrin response values before, at one month and at seven months after therapy were 5,075ng/l.min; 5,450ng/l.min and 3,800ng/l.min respectively.

In the 7 patients who had undergone an identical pre-treatment study day 3-6 months previously, the gastrin values on that occasion were similar to their values on the pre-treatment day of the current study. Their median basal gastrin concentration from the previous study was 42ng/l (range 30-55) compared with 35ng/l (20-60) for the current study, and their median integrated gastrin response was 4,075ng/l.min (range 3,350-5,225) compared with 4,975 (range 1,875 - 6,025).

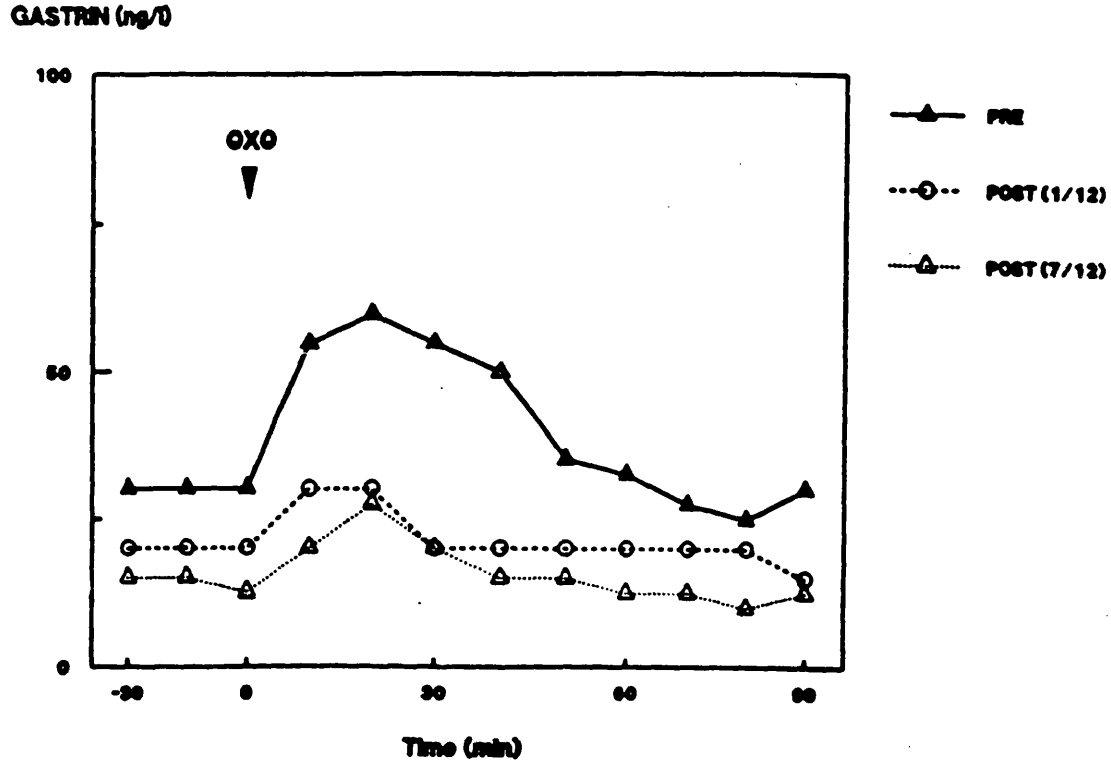

Figure 4

Basal and OXO meal-stimulated plasma gastrin concentrations in duodenal ulcer patients before and at 1 and 7 months after eradication of *H. pylori* infection. The values are medians of nine patients except for the 7 months post-treatment time, when only eight patients were examined.

4.4.3 INTRAGASTRIC pH

In the 9 patients in whom *H. pylori* was eradicated there was no significant difference between their overall daytime pH values before treatment (median 1.4, range 1.1 - 2.1) and one month post treatment (1.4, 1.1 - 2.3) (Fig. 5) or seven months post treatment (1.4, 1 - 2.1) (Fig. 5). In these patients, however, the peak pH reached in response to the buffering effect of the peptide meal was higher one month following eradication (median 4.2, range 3 - 5.7) than before treatment (3.4, 1.5 - 4.5) ($p < 0.02$). In addition, the time the pH was above 2 following this meal was longer one month after treatment (median 11 min, range 2-15) than before (30 seconds, 0 - 11 min) ($p < 0.03$). At seven months post-eradication, the peak pH reached after the peptide meal (2.9, 1.8 - 7) and the time pH was above 2 (6 min, 0 - 15 min) were not significantly different from the pre-eradication values.

4.4.4 NIGHT-TIME ACID OUTPUT

Night-time acid output (mmol/10h) was similar before (median 86, range 52-114), one month after eradication (76, 50-143) and seven months after eradication (94, 63-106) (Figure 6).

4.5 DISCUSSION

This study demonstrates that eradication of *H. pylori* is associated with a marked and sustained fall in both basal and meal stimulated plasma gastrin concentrations in duodenal ulcer subjects. The observation that the gastrin values from an identical pre-treatment study day 3 - 6 months previously were similar to those on entry to this study indicates that the fall in gastrin was not merely due to exaggerated levels on the first test day of this study. A similar fall in fasting gastrin concentration has been noted

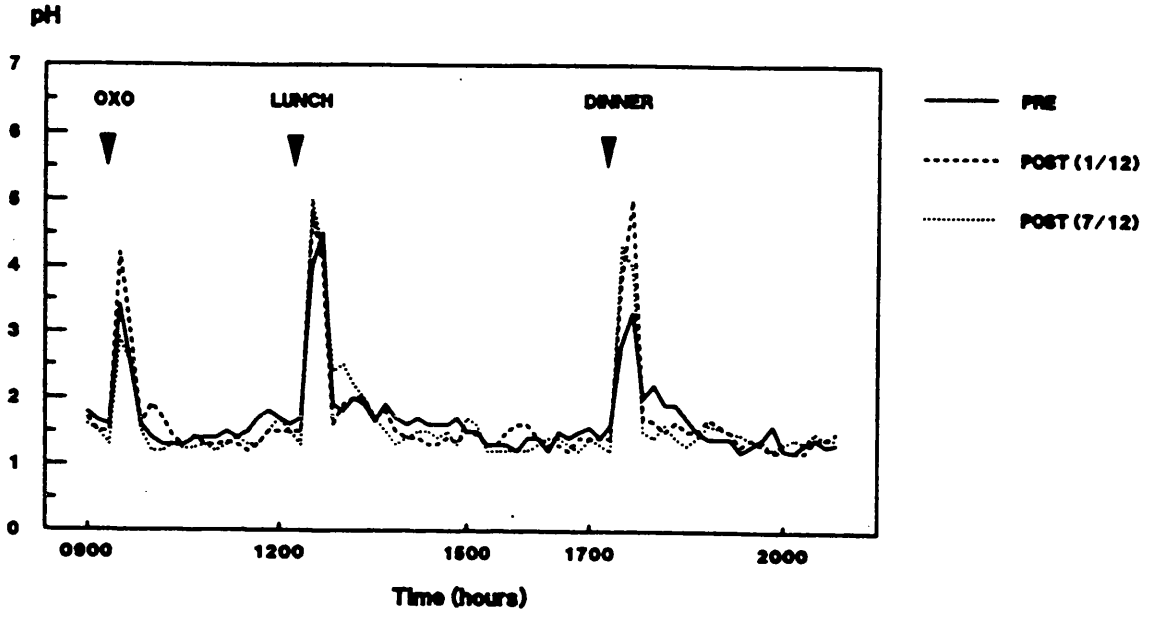

Figure 5

Median daytime intragastric pH of nine duodenal ulcer patients before (n=9) and at 1 month (n=9) and 7 months (n=8) after eradication of *H. pylori* infection.

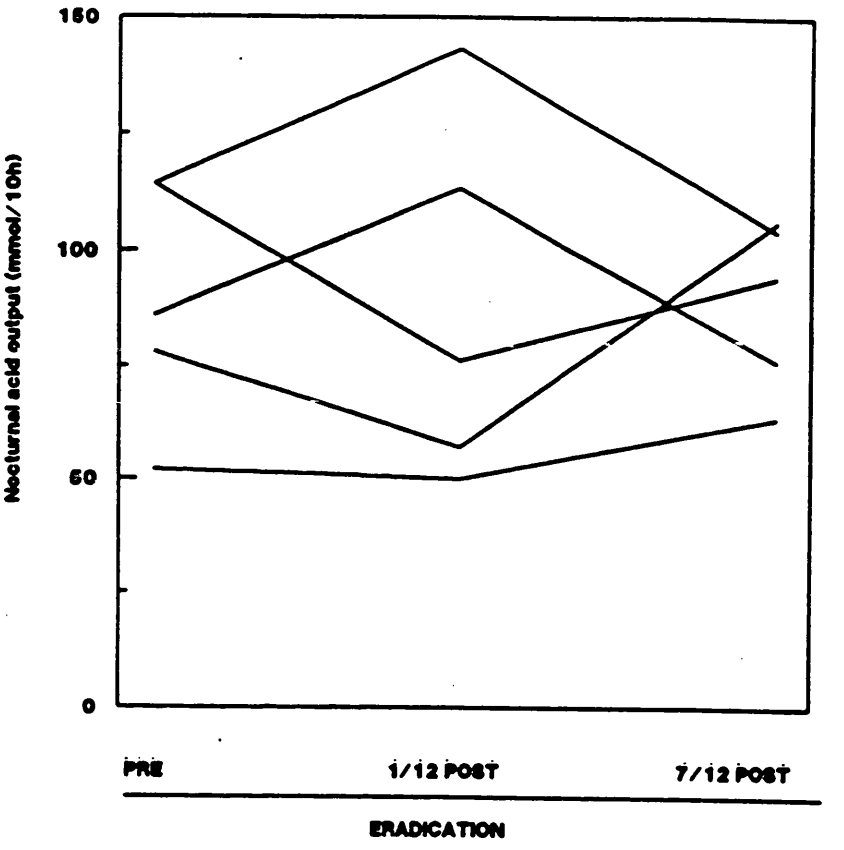

Figure 6

Nocturnal acid output (2300-0800h) in five duodenal ulcer patients before and at 1 and 7 months after eradication of *H. pylori*.

following eradication of the organism in children with a variety of upper gastrointestinal disorders (Oderda et al 1989). Levi et al (1989) noted a similar fall in meal stimulated gastrin concentrations but not in basal concentrations in duodenal ulcer subjects two days after completing *H. pylori* eradication therapy. Healthy volunteers found to have *H. pylori* also have elevated 24h gastrin concentrations (Smith et al 1989). There is convincing evidence, therefore, that chronic infection with *H. pylori* results in increased circulating levels of gastrin. In view of the high prevalence of the bacterium in healthy subjects, the currently accepted normal range for plasma gastrin and its physiological control require to be re-assessed in non-infected subjects.

The mechanism by which *H. pylori* increases gastrin levels is unclear. Levi et al (1989) have proposed that it is due to the ammonia produced by the organism's urease enzyme raising the pH in the vicinity of the antral G cells. In this way, the organism would interfere with the physiological suppression of gastrin release by gastric acid leading to inappropriate circulating levels of the hormone. An alternative explanation for the hypergastrinaemia is that it is due to the chronic inflammation which the bacterium induces in the antral mucosa where the G cells are located.

In spite of the marked lowering of gastrin levels the overall daytime intragastric pH and night-time acid output were unaltered following eradication of the organism. This does not exclude a reduction in meal-stimulated acid secretion as in situ pH monitoring registers only the hydrogen ion concentration of the gastric contents and not the volume of juice secreted. The major physiological role of circulating gastrin may be in regulating meal-stimulated acid secretion. The more marked pH rise in response to the buffering effect of the OXO meal one month after eradicating *H. pylori* would be consistent with reduced acid secretion in response to this meal. The major difference between the intragastric pH

profile of duodenal ulcer patients and controls is reduced buffering effect of meals in the former (Bumm & Blum 1987).

In addition to directly stimulating parietal cells to secrete acid, gastrin also exerts a trophic effect on the oxyntic mucosa (Willems & Lehy 1975) and prolonged hypergastrinaemia produces parietal cell hyperplasia (Crean et al 1969). Loss of the trophic effect on the parietal cells due to the fall in circulating gastrin would be expected to result in a reduction in both basal and meal stimulated acid secretion. The half-life of parietal cells is 23 days in mice (Ragins et al 1968) and presumably longer in man and, therefore, removal of a trophic influence might not be apparent for several months. However, we were unable to detect any evidence of lowering of gastric acid status seven months following eradication and indeed at this time there was not even a significant difference in the pH response to the buffering effect of the OXO meal.

It is possible that the lack of any convincing change in gastric acid status accompanying the highly significant lowering of circulating gastrin is due to the insensitivity of intragastric pH monitoring at detecting changes in meal-stimulated secretion. However, it is necessary to consider other reasons for the lack of change in acid status. One explanation is that the gastrin released in the presence of *H. pylori* infection is of reduced biological activity. This could occur as a result of the chronic inflammation of the antral mucosa resulting in damage to the G cells and leakage of biologically inactive but immunologically recognisable fragments of the hormone.

Another clue to the reason for the discrepancy between the change in plasma gastrin and gastric acidity could be the observation that acute infection with *H. pylori* results in marked hypochlorhydria which may persist for several months (Marshall et al 1985, Graham et al 1988) and which may be explained by a direct inhibitory effect of the bacterium on

the parietal cells (Defize et al 1988). Some inhibition of parietal cell function may persist in patients with chronic infection and counteract the effect of the increased gastrin. On eradicating the infection there would be both lowering of gastrin and removal of the inhibition of parietal cells producing no net effect on acid status. It is even possible that the increased gastrin level is itself in part a secondary response to inhibition of parietal cell function by *H. pylori.*

This study clearly demonstrates that eradication of *H. pylori* results in a marked fall in gastrin levels in duodenal ulcer subjects. However, the inability to detect any convincing change in acid status casts doubt on a major role for gastrin in the link between *H. pylori* infection and duodenal ulceration. It is possible that subjects with *H. pylori* also have abnormalities of antral hormones involved in the regulation of gastro-duodenal motility and which could explain their predisposition to duodenal ulceration.

CHAPTER 5

EFFECT OF *HELICOBACTER PYLORI* INFECTION ON INTRAGASTRIC UREA AND AMMONIA CONCENTRATION - VALUE IN DIAGNOSIS

5.1 INTRODUCTION

Helicobacter pylori (*H. pylori*) infection of the gastric antral mucosa is present in the vast majority of duodenal ulcer patients and eradication of the infection markedly reduces the ulcer relapse rate (Marshall et al 1988). The mechanism by which the infection predisposes to duodenal ulcer is likely to be related to its stimulation of increased gastrin release. The mechanism by which the infection stimulates gastrin release is unknown but may be related to production of ammonia by the bacterium. In this chapter we examine the effect of *H. pylori* on the concentration of urea and ammonia in gastric juice.

In addition to furthering our understanding of the mechanism by which *H. pylori* may raise serum gastrin, the measurement of urea and ammonia in gastric juice may be of value in the clinical diagnosis of *H. pylori* infection. At present there are two measurement of the rate of ammonia formation following incubation of an antral biopsy in a urea containing medium (Marshall et al 1987). There are several variations of this method and most detect the ammonia formed by means of the change in colour of a pH indicator such as phenol red. The time from incubation of the biopsy until the change in colour is apparent and varies from 3-24 hours. The second method involves the oral administration of ^{14}C or ^{13}C labelled urea and subsequent analysis of labelled CO_2 excreted in the breath (Bell et al 1987, Marshall et al 1988, Graham et al 1987). This test takes 1-2 hours to perform and the final result is not usually available until the following day at the earliest.

In this chapter we assess the effect of *H. pylori* on gastric juice urea and ammonia concentrations and the value of their measurement in diagnosing *H. pylori* status.

5.2 PATIENTS AND METHODS

Twenty seven patients (21 men, age range 18-64 years) with a history of endoscopically proved duodenal ulceration were examined. Two of them were taking ranitidine but none had been prescribed omeprazole. All had normal renal function. Twelve were examined on one occasion only, having never received any anti-*H. pylori* treatment. Twelve were examined before and one month after completing a four week course of tripotassium dicitrato bismuthate (120mg t.i.d.)., metronidazole (400mg t.i.d.), and amoxycillin (250mg t.i.d.). Three patients were examined only one month after the same treatment. At each time point examined, an upper gastrointestinal endoscopy and ^{14}C urea breath test were performed. All the patients had fasted for 16 hours before endoscopy.

During the endoscopy and just after entering the stomach, 2ml of gastric juice were aspirated through the suction channel of the endoscope and collected in a trap inserted in the suction line. Routine inspection of the upper gastrointestinal tract was then performed and after this an antral biopsy specimen was obtained for histological examination. The specimen was fixed in formalin before staining with haematoxylin and eosin. The severity of histological gastritis was scored as described by Rauws et at and the presence of *H. pylori* noted.

The gastric juiced samples were stored frozen at -20°C until analysed. Before analysis, the samples were centrifuged at 3000g for 10 minutes to remove the mucus. The concentration of ammonium was measured in the supernatant after dilution in 0.2mol/l phosphate buffer pH 7.4 using an enzymatic method (Sigma Chemical Co., Dorset, U.K.) adapted for the Cobas Bio (Roche, Welwyn Garden City, U.K.) Studies were performed to assess the reliability of this method for determining ammonium concentrations in gastric juice. Urea concentrations were measured by a urease enzymatic method (SMAC 1, Technicon, Basingstoke, U.K.).

Within five days of each endoscopic examination, a ^{14}C urea breath test was performed. After a 14 hour overnight fast, the patients drank 240ml of Ensure Plus (Abbott, Maidenhead, U.K.) to delay gastric emptying, followed by 0.4MBq ^{14}C urea in 20ml water. Samples of breath CO_2 were collected every 10 minutes for 30 minutes before administration o the isotope and for 120 minutes afterwards. The appearance of ^{14}C labelled CO_2 in the breath samples was followed by liquid scintillation counting. The area under the curve of the breath test was calculated using the trapezoide rule for the time period 0-30 minutes, 0-40 minutes, 0-60 minutes, and 0-120 minutes of the test.

5.3 RESULTS

In the 24 duodenal ulcer patients who had not received anti-*H. pylori* treatment, the organism was present in each on examination of their antral biopsy specimen. In each of these patients antral gastritis was also present and their median score for severity of gastritis was 5 (range 2-8). In 14 of the 15 patients examined one month after completing a course of anti-*H. pylori* therapy, no *H. pylori* like organisms were identified and none had gastritis scores greater than 1. In one patient the treatment failed to eradicate the infection and the bacterium was still present on the mucosa and associated with persisting antral gastritis with a severity score of 5. This patient's pretreatment aspirate ammonium and urea concentrations were 2.5mmol/l and 0.7mmol/l respectively, while after treatment they were 2.7mmol/l and 0.7mmol/l respectively.

Analysis of the area under the breath test curve two hours after isotope administration showed clear separation of the patients with histological evidence of the infection and who had never received anti-*H. pylori* treatment (Table 1).

The values in the former were all less than 20 (range 3-18) and in the

Area Under the Breath Test Curve (Median) (Range) (% Dose/mmool/CO_2xkgxmin)					
UNITS	**0 - 30 min.**	**0 - 40 min.**	**0 - 60 min.**	**0 - 120 min.**	**Peak of 10 and 20 min breath test value % dose/mmol Co_2 Kg**
Pre eradication of *H. pylori* (n = 24)	25 (6-42)	41 (12-69)	76 (23-127)	152 (63-267)	137 (39-225)
Post eradication of *H. pylori* (n = 14)	0.6 (0.1-1.1)	0.7 (0.3-2.5)	1.7 (0.8-7.2)	5.0 (3.0-18.0)	2.6 (0-5.1)

TABLE 1: AREA UNDER THE BREATH TEST CURVE BEFORE AND AFTER ERADICATION OF *H. PYLORI*.

latter all greater than 60 (range 63-267). In the one patient with histological evidence of failure of eradication the value was 114 before treatment and 90 after treatment. She was therefore included only as a positive for *H. pylori* in the further analysis. There was also complete separation of the two groups with the calculated area under the curve for the first 30 minutes, 40 minutes, and 60 minutes of the breath test. Even the higher single value of either of the 10 minute or 20 minute breath test results was also found to separate the patients clearly into the two groups, indicating that a shortened 20 minute breath test could be as useful as the complete two hour test.

The method of ammonium measurement in the gastric juice samples was assessed. The intra-assay coefficient of variation at 10mmol/l ammonium concentration was 1.0% while the inter-assay coefficient of variation was 2.0%. Using samples from patients, the intra-assay coefficient of variation, including dilution, varied from 8.5% at an ammonium concentration of 2.3mmoll to 1% at an ammonium concentration of 13.0mmol/l. The measured ammonium concentration in patients' samples was linear with serial dilution. The detection limit for the assay was 30μmol/l. Samples kept in a freezer at -20°C also showed no significant change in ammonium concentration over a 21 day period.

The median (range) gastric juice urea concentration was 0.8mmol/l (0.5-2.9mmol/l) in those with the infection compared with 2.1mmol/l (1.0-3.7mmol/l) in the patients in whom it had been eradicated ($p<0.001$) (Fig. 7). The median (range) gastric juice ammonium concentration was 3.4mmol/l (1.0-13.0mmol/l) in infected subjects and 0.64mmol/l (0.02-1.4mmol/l) after eradication ($p<0.001$) (Fig. 7). Though there was considerable overlap between the two groups in respect of their urea and ammonium concentrations there was complete separation of the groups when the urea: ammonium ratio was considered. The median ratio in

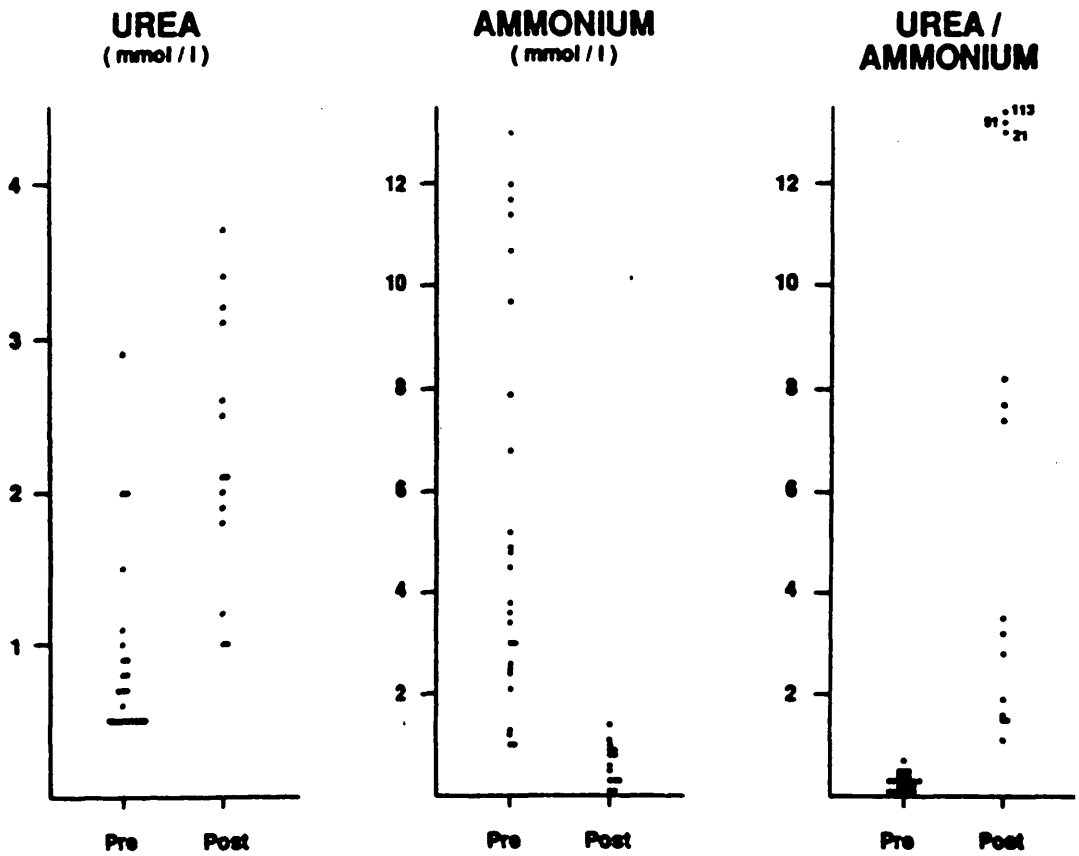

Figure 7

Urea and ammonium concentrations (mmol/l) and the urea: ammonium ratio in gastric juice, samples from subjects before and after eradication of *Helicobacter pylori*.

gastric juice of infected subjects was 0.26 (0.04-0.7) compared with 3.4 (1.1-113) in those cleared of the infection ($p<0.001$). Thus, all subjects with *H. pylori* infection had a urea:ammonium ratio of less than 0.8 and all those in whom the infection had been eradicated had a ratio of more than 1.0.

In those with the infection, there was no correlation of the area under the two hour breath test values curve and the gastric urea concentration, ammonium concentration, or urea: ammonium ratio (Fig. 8).

5.4 EFFECT OF H_2 RECEPTOR ANTAGONISTS

In the 10 patients studied before and after seven days' ranitidine treatment, there was no change in the gastric juice concentrations of urea and ammonium or in their ratio (Table 2). Taking urea:ammonium ratios of 0.8 or below as indicative of infection, the H_2 antagonist treatment did not change the *H. pylori* classification of any of the subjects.

5.5 DISCUSSION

The unusually high urease activity of *H. pylori* has been used to rapidly detect the presence of the organism in the gastric antral mucosa in several different ways. The ^{14}C urea breath test relies on the detection of exhaled radio-labelled CO_2 formed during the hydrolysis of urea. This test is reproducible and is regarded as a sensitive method of detecting infection of the antral mucosa with the organism (Bell et al 1987, Marshall et al 1988). However, it does have several disadvantages. The collection of the breath samples takes 2 h to complete using the standard protocol although a shortened breath test relying on a 40 min breath sample has been proposed (Weil and Bell 1989). Our data indicates that the higher value of the 10 min and 20 min value can distinguish between those with the infection from those in whom it has been eradicated. This would allow

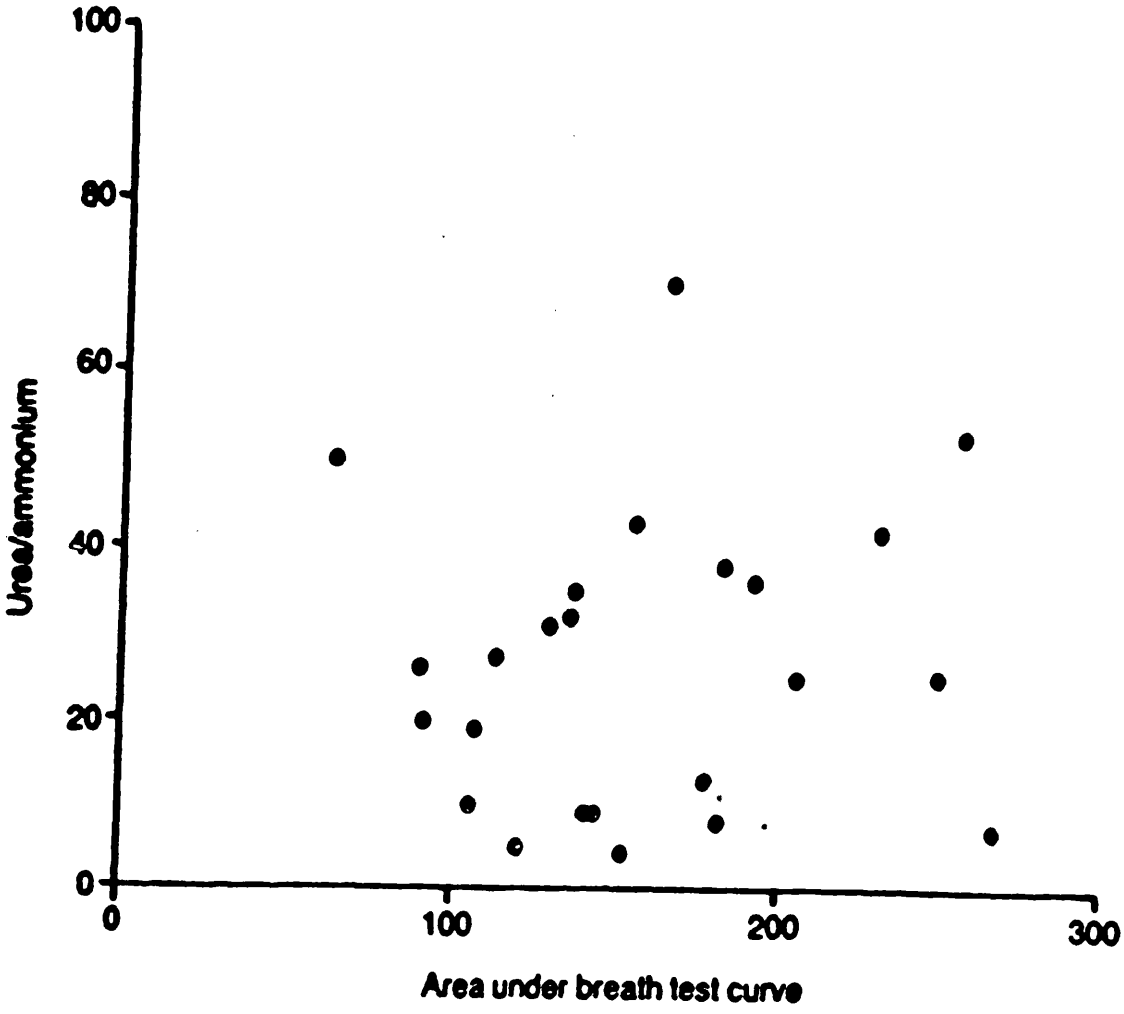

Figure 8

Correlation of the area under the 120 minute breath test curve (% dose/mmol CO_2/kg body weight x min) with the urea: ammonium ratio in gastric juice (r=0.004).

H. pylori -ve Patients	Pre-Treatment Ammonium	Urea	Ratio	During Ranitidine Treatment Ammonium	Urea	Ratio
1	2.0	2.9	1.4	1.9	2.6	1.3
2	2.2	3.1	1.4	2.9	2.6	0.9
3	2.0	4.3	2.1	2.5	3.0	1.2
4	0.8	2.2	2.6	2.0	2.7	1.3
5	1.6	2.5	1.6	2.8	3.0	1.1
6	1.4	2.0	1.4	1.2	1.9	1.6
7	2.2	2.1	0.9	2.5	2.6	1.0
H. pylori **+ve Patients**						
1	6.1	1.2	0.2	5.7	1.4	0.2
2	8.8	2.0	0.2	5.2	1.6	0.3
3	7.2	0.6	0.1	8.4	0.2	0.1

TABLE 2: **EFFECT OF 7 DAYS TREATMENT WITH RANITIDINE 300mg NOCTE ON THE CONCENTRATIONS OF UREA AND AMMONIUM AND THEIR RATIO IN GASTRIC JUICE.**

the test to be performed within 30 min. Unfortunately, the analysis of the breath samples is also time consuming making it difficult to obtain the result on the same day. The breath test involves the administration of a small dose of radioactivity which might make the test unsuitable for the investigation of children and premenopausal women and for multiple studies. The ^{13}C urea breath test eliminates the risk of administering a radioisotope but because mass spectrometry is required for the detection of ^{13}C CO_2 is unlikely to be introduced into general use (Graham et al 1987).

The rapid urease (CLO-test) uses the change in colour of a pH indicator dye in response to the urease activity in a biopsy sample when inserted into the gel to detect the organism. The colour change in the gel usually takes 3 hr to complete but may take up to 24 hr. The disadvantages of this test are that an antral biopsy is required and the investigator is also required to remember to read the gel at the correct time. Again, one cannot rely on having the result on the same day as the test is performed.

We have found that the urease activity of the organism results in low gastric juice urea concentrations and raised gastric juice ammonia concentrations. The inverse relationship between the concentrations of those two analytes in gastric juice of those with the organism means that their ratio allows the clear separation of patients with the infection from those in whom it has been eradicated. In those with the infection there was no correlation between the urease activity assessed by the urea breath test and the concentrations of either urea or ammonia or of their ratio in gastric juice. This may be explained by the rate of ammonia production in infected individuals being mainly dependent on the availability of urea. We have previously noted that intragastric infusion of urea results in a marked increase in the ammonia concentration in gastric juice indicating that it is the substrate availability which determines the ammonia production (Chittajallu et al 1991).

Finally, the concentration of urea in gastric juice of patients with the organism may, on occasion, be below the detection limit for accurate determination.

The analysis of the concentrations of ammonium and urea in gastric juice could be completed more rapidly than either the urea breath test or the rapid urease test. With modern analytical equipment the measurement of their concentrations in a sample of gastric juice obtained at endoscopic examination might be completed within 10 to 15 minutes. Our studies indicate that dosing with the H_2 antagonist ranitidine on the evening before the endoscopic examination does not alter the discriminating value of the urea: ammonium ratio or indeed the concentrations of urea and ammonium in gastric juice.

In conclusion, these studies indicate that *H. pylori* markedly increases the concentration of ammonia in gastric juice and this avid production of ammonia may be involved in the production of *H. pylori*-related hypergastrinaemia. In addition, we have shown that the measurement of the ratio of the concentrations of urea and ammonium in gastric juice samples from fasted subjects provides a simple method of determining the *H. pylori* status of duodenal ulcer patients.

CHAPTER SIX

THE ROLE OF AMMONIA IN THE PATHOGENESIS OF THE GASTRITIS, HYPERGASTRINAEMIA AND HYPERPEPSINOGENAEMIA I CAUSED BY *HELICOBACTER PYLORI* INFECTION

6.1 INTRODUCTION

It is now generally acknowledged that *Helicobacter pylori* (*H. pylori*) infection is the major cause of antral gastritis (McNulty 1989). However, the bacterium does not penetrate the gastric epithelium and the mechanism by which it induces inflammation of the underlying mucosa is unknown. The organism has very high urease activity (Marshall & Langton 1986) and it has been suggested that the production of high concentrations of ammonia at the epithelial surface could cause mucosal damage (Murakami et al 1988). Though the acidic gastric juice will rapidly convert ammonia to less toxic ammonium ions, the high pH at the site of ammonia production underneath the mucus layer could allow it to remain in its unionised noxious state predispose to mucosal damage by denaturing the structure of the protective mucus layer (Sidebotham & Baron 1990).

H. pylori infection has also been shown to increase the serum concentration of gastrin and pepsinogen I (Oderda et al 1989, Chittajallu et al 1992). Levi et al proposed that the hypergastrinaemia was related to bacterial ammonia production raising antral surface pH (Levi et al 1989), but studies from our own group do not support this (Chittajallu et al 1991, Nujumi et al 1991, Chittajallu et al 1991, Chittajallu et al 1992, McColl et al 1992).

In order to investigate the role of ammonia in the histological and biochemical changes accompanying *H. pylori* infection we have examined patients with chronic renal failure. The high intragastric urea concentrations in such patients greatly increases *H. pylori* ammonia production which should accentuate any ammonia-related effects.

6.2 PATIENTS AND METHODS

Gastric juice ammonium concentration, antral histology, and serum concentrations of gastrin and pepsinogen I were examined in 9 *H. pylori*

positive and 9 *H. pylori* negative normal uraemic patients. These results were compared with those from age and sex matched control patients with normal renal function with (n=9) and without (n=9) *H. pylori* infection. Each of the patients examined had been referred for upper gastrointestinal endoscopy on account of dyspeptic symptoms. None of the patients was receiving antibiotic therapy or had taken acid-inhibitory agents within the previous week. In addition none of the patients had been treated with bismuth preparations, antibiotic therapy or had taken acid inhibitory agents within the previous week.

The median age of the *H. pylori* positive uraemic patients was 51 years (range 34-73) compared with 49 years (range 27-67) in those uninfected. Three of the *H. pylori* positive patients were on maintenance haemodialysis, four on continuous ambulatory peritoneal dialysis and two had not yet commenced dialysis. One of the *H. pylori* negative patients was on maintenance haemodialysis and eight on continuous ambulatory peritoneal dialysis. The median duration of dialysis was similar in the *H. pylori* positive (15 months, range 0-100) and negative (20.5 months, range 0-142) patients.

All patients were examined between 0900h and 1100h after an overnight fast. Upper gastrointestinal endoscopy was performed after a venous blood sample had been removed for determination of serum concentrations of urea, gastrin and pepsinogen I. Immediately after passing the instrument 10ml of gastric juice was collected by means of a trap in the suction line. Following inspection of the upper gastrointestinal tract, two biopsies were taken from the greater curvature of the antrum 2cm from the pylorus.

The *H. pylori* status of patients and controls was determined by microscopy of antral biopsy for Helicobacter-like organisms, rapid urease slide test (CLO test) of antral biopsy (Marshall et al 1987), and ^{14}C-urea

breath test. These tests have been shown to be reliable in detecting *H. pylori* infection in patients with and without renal failure (Nujumi et al 1991).

6.3 ANALYSES

In the patients with chronic renal failure the pH of the gastric juice was determined using a combined glass electrode (Radiometer ETS 822) prior to storage at -20°C. Gastric juice urea and ammonium concentrations were determined in all subjects. For this the samples were thawed and centrifuged at 3000g for 10 min to remove the mucus. The concentration of ammonium was measured in the supernatant following dilution in 0.2M phosphate buffer pH 7.4, using an enzymatic method (Sigma, Dorset, U.K.) adapted for the Cobas Bio (Roche, Welwyn Garden City, U.K.) as previously described (Neithercut et al 1991).

The antral biopsies were fixed in formalin and processed routinely. Paraffin sections were cut at three levels and stained with haematoxylin and eosin. An extra section from level two was stained with Cresyl Fast Violet for detection of *H. pylori* (Burnett et al 1987). They were examined by a single pathologist (S.D.) unaware of their clinical details. The severity of antral gastritis was scored using the method of Rauws et al (1988) and which we have found to be a sensitive method for assessing the severity of *H. pylori*-induced gastritis (McColl et al 1991, Chittajallu et al 1991, Neithercut et al 1991). Chronic inflammatory infiltrate in the lamina propria was scored as 0, 1 or 2, lamina propria polymorph infiltrate as 0, 1, 2 or 3 intra-epithelial polymorph infiltrate as 0, 1, 2 or 3 and mucosal erosions as 0, 1, or 2. The scores for these individual components of *H. pylori*-related gastritis are then added to give a cumulative gastritis score ranging from 0-10.

The intra-observer variation in the scoring of the severity of the

gastritis was assessed. This was performed by randomly selecting 15 slides scored by the pathologist (S.D.) at least one year earlier and having him rescore them unaware that he had previously examined them. The mean cumulative gastritis score for the group of 15 slides was 3.2 (range 0-6) when first scored compared with 3.3 (range 0-8) when rescored. The mean absolute difference in the cumulative gastritis score between the two assessments was 0.66 (range 0-2) and the co-efficient of variation was 21%. This indicates that our pathologist would have a greater than 95% chance of detecting a difference in mean cumulative gastritis score of 1 when comparing two groups of nine subjects.

The serum gastrin concentration was determined by radioimmunoassay using antibody R98 (Ardill 1973). This detects both G17 and G34 and uses G17 as standard. Serum pepsinogen I was measured using commercial radioimmunoassay kits obtained from Incstar Ltd. (Berkshire).

Statistical significance of differences between groups was assessed by the Mann Whitney U test.

The study was approved by the Western Infirmary Ethical Committee and all patients gave written, informed consent.

6.4 RESULTS

The serum urea concentration was elevated to a similar extent in the renal failure patients with (median 20mmol/l, range 12-31) or without (23, 11-32) *H. pylori* infection (normal range 2.5 - 7.5mmol/l).

The median (range) gastric juice ammonium concentration in the *H. pylori* positive uraemic patients was 19mmol/l (11-43) which was approximately four-fold greater than that in the *H. pylori* positive non-uraemic patients (5, range 1-11) ($p < 0.005$) (Fig. 9). Gastric juice ammonium concentrations were similar in the *H. pylori* negative uraemic patients (median 3, range 0.5-11) and *H. pylori* positive non-uraemic

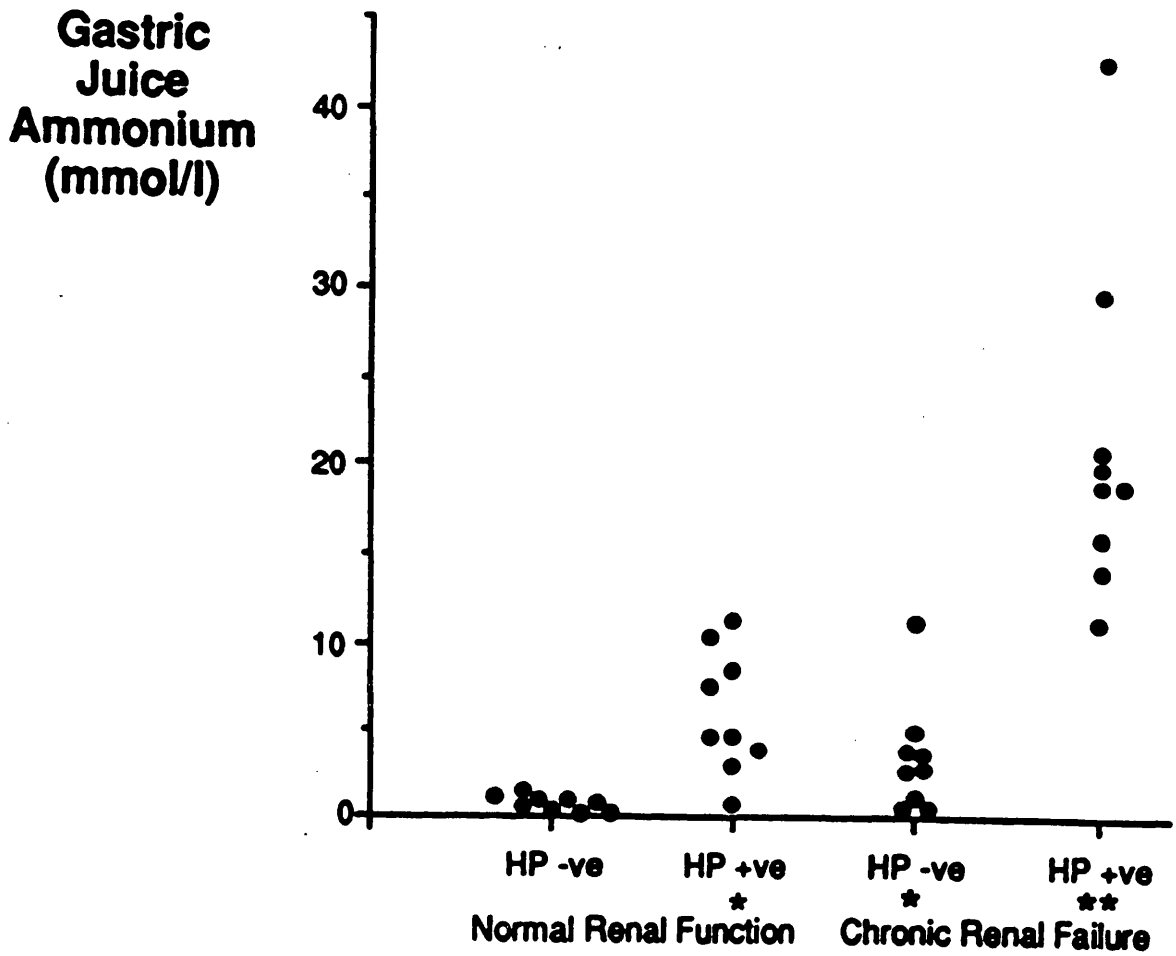

Figure 9

Effect of uraemia and *Helicobacter pylori* (*HP*) status on intragastric ammonium concentrations. *Indicates higher than *H. pylori* -ve non-uraemic patients at $p<0.02$. **Indicates higher than the *H. pylori* +ve non-uraemic patients at $p<0.005$.

patients (5, range 1-11) ($p = 0.2$), and both were significantly higher than the values for the *H. pylori* negative non-uraemic patients (0.7, range 0.1-1.4) ($p < 0.02$ for each). Intragastric pH was similar in the *H. pylori* positive and negative uraemic patients, with a median value of 3.0 (range 1.0 - 7.1) in the former and 2.1 (1.2 - 6.6) in the latter.

In spite of the marked difference in intragastric ammonium concentration, the severity of histological gastritis was similar in the *H. pylori* positive patients with (median cumulative gastritis score = 5, range 3-6) or without (5, range 3-7) renal failure (Fig. 10). Combining the *H. pylori* positive patients with and without renal failure provided a wide range of gastric juice ammonium concentrations (1-43 mmol/l) but there was no correlation between this and the severity of the *H. pylori*-related antral gastritis (Table 3). In spite of the *H. pylori* negative uraemic patients having intragastric ammonium concentrations similar to the *H. pylori* positive non-uraemic patients, the former had cumulative gastritis scores of 1 or less which was equivalent to those in the *H. pylori* negative patients with normal renal function.

There was no difference in the endoscopic appearance of the upper gastrointestinal tract in the renal failure patients with and without *H. pylori* infection. Of the 9 *H. pylori* positive uraemic patients, two had oesophagitis, two had scattered petechiae in the stomach and duodenum, and one had erosive duodenitis. Of the 9 *H. pylori* negative uraemic patients, two had oesophagitis, and one had scattered petechiae in stomach and duodenum. Endoscopy in the 9 *H. pylori* positive non-uraemic patients showed oesophagitis in one patient and active duodenal ulcer in another. In the 9 *H. pylori* negative non-uraemic patients one had erosive duodenitis and another a deformed duodenum.

The median serum pepsinogen I concentration (ng/ml) was higher in the *H. pylori* positive uraemic patients (352, range 280-653) than in the

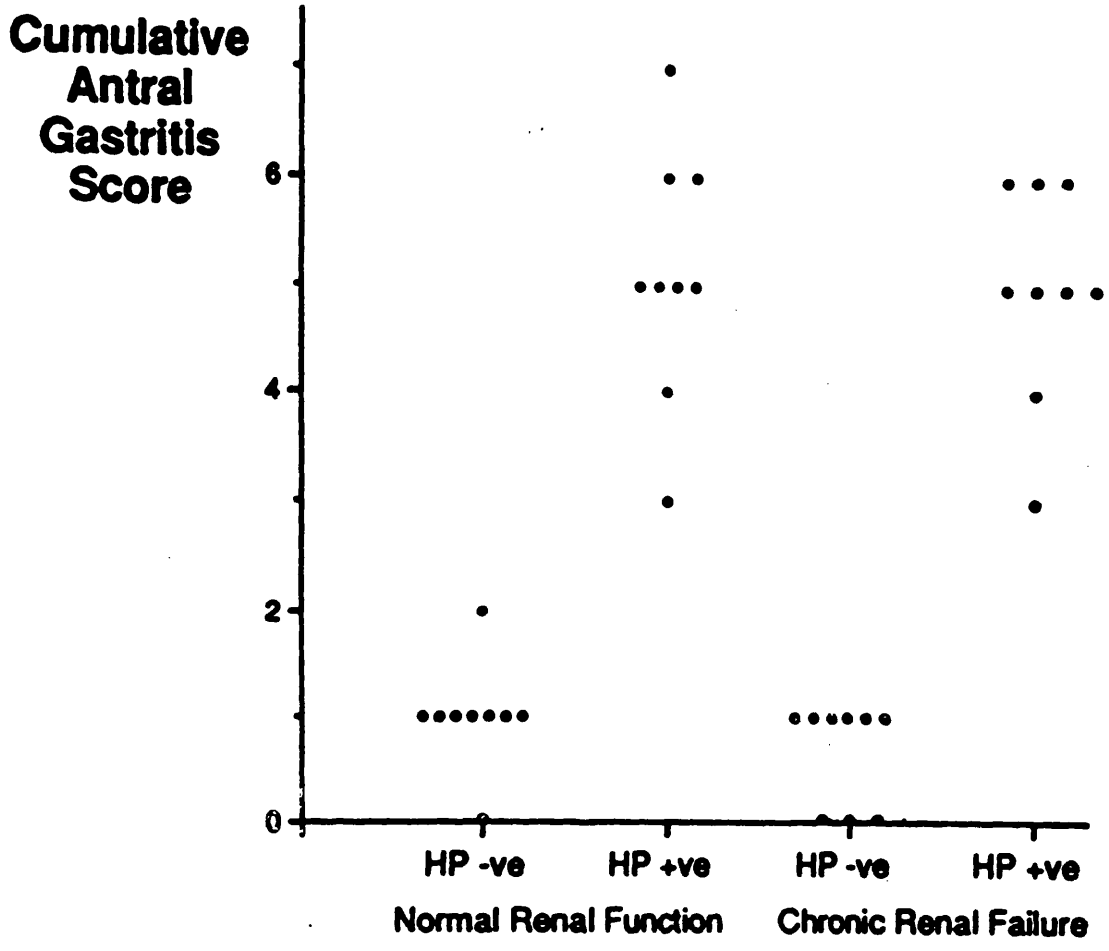

Figure 10

Cumulative antral gastritis scores in patients with and without chronic renal failure and of different *Helicobacter pylori* status.

DESCENDING ORDER OF GASTRIC JUICE AMMONIUM CONCENTRATION.

Gastric Juice Ammonium (mmol/l)	Chronic Renal Failure	Lamina Propria Chronic Infiltrate Score (0-2)	Intra-Epithelial Polymorph Infiltrate Score (0-3)	Lamina Propria Polymorph Infiltrate Score (0-3)	Epithelial Erosions Score (0-2)	CUMULATIVE GASTRITIS SCORE (0-10)
43	+	2	1	1	0	4
30	+	2	1	2	0	5
21	+	2	2	1	0	5
20	+	2	2	2	0	6
19	+	2	2	1	0	5
19	+	2	2	2	0	6
16	+	1	1	1	0	3
14	+	2	2	2	0	6
11	+	1	1	1	2	5
11	-	2	2	1	0	5
10	-	2	2	2	1	7
8	-	2	1	2	0	5
7	-	2	0	1	0	3
5	-	2	1	2	0	5
5	-	2	1	1	0	4
4	-	2	1	2	0	5
3	-	2	2	2	0	6
1	-	2	2	2	0	6

TABLE 3: GASTRIC JUICE AMMONIUM CONCENTRATIONS AND ANTRAL GASTRITIS SCORES IN THE *H. PYLORI* POSITIVE INDIVIDUALS WITH (+) AND WITHOUT (-) CHRONIC RENAL FAILURE. THEY ARE LISTED IN DESCENDING ORDER OF GASTRIC JUICE AMMONIUM CONCENTRATION.

H. pylori negative uraemic patients (165, range 86-337) ($p < 0.01$) and both these groups had higher values than the non-uraemic patients of corresponding *H. pylori* status ($p < 0.05$ for each) (Fig. 11). Serum pepsinogen I values were similar in the non-uraemic patients with (median = 103, range 40-170) or without (92, 35-127) *H. pylori* infection ($p = 0.3$). There was no relationship between the serum pepsinogen I concentration and type of renal replacement treatment.

The median serum gastrin concentration (pmol/l) in the non-uraemic patients was higher in those with *H. pylori* (17, range 7-24) than in those without the infection (10, range 7-14) ($p < 0.05$) (Fig. 12). Compared with these non-uraemic patients, the gastrin concentrations were markedly elevated in the uraemic patients and there was no difference between the latter patients with (median = 95, range 52-333) or without (114, range 47-533) *H. pylori* infection. There was no relationship between the serum gastrin concentration and type of renal replacement treatment.

6.5 DISCUSSION

This study demonstrates that the gastric juice ammonium concentration is markedly affected by both uraemia and *H. pylori* infection. The grossly elevated intragastric ammonium concentration in the renal failure patients with *H. pylori* infection can be explained by the combination of their high gastric juice urea concentration (Lieber & Lefeure 1959) and the high urease activity of the organism. We have previously demonstrated that the intragastric production of ammonia by *H. pylori* is controlled by the availability of urea in gastric juice (Chittajallu et al 1992).

The reason for the gastric juice ammonium concentration being higher in the *H. pylori* negative uraemic patients than in the *H. pylori* negative non-uraemic patients is not clear. However, a variety of urease producing bacteria are present in the mouth and swallowed in the saliva (Bowden et

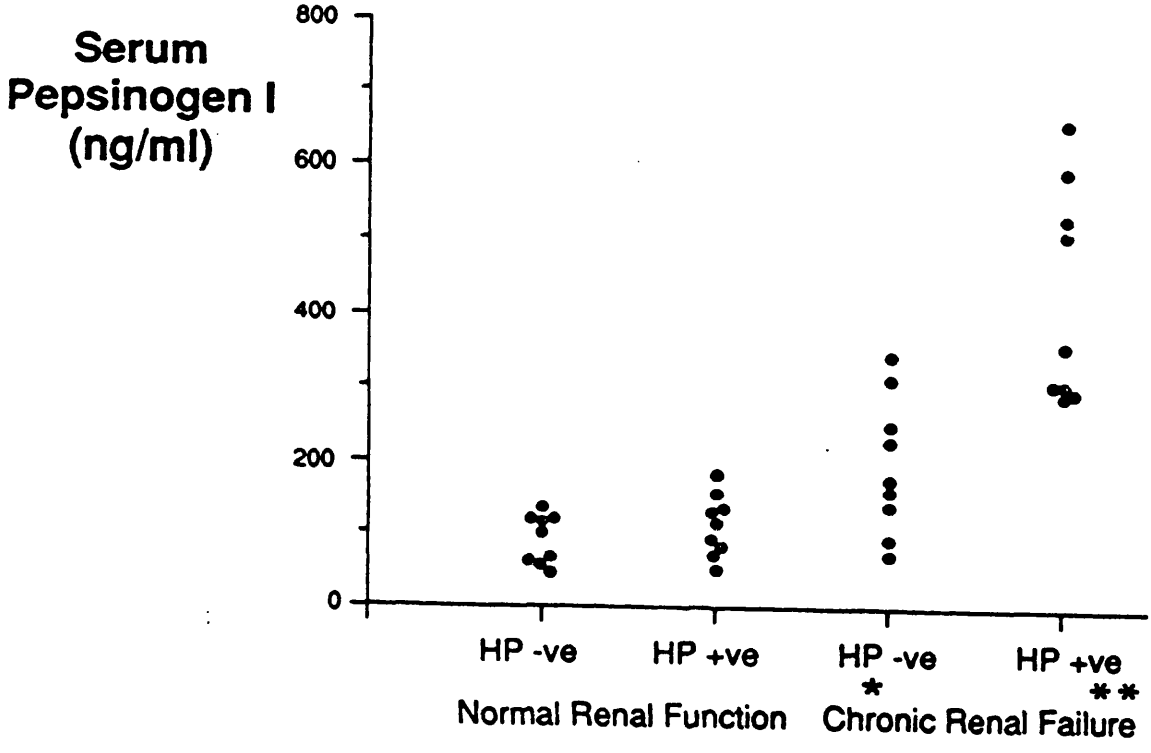

Figure 11

Serum pepsinogen I concentrations in patients with and without chronic renal failure and of different *Helicobacter pylori HP* status. *Indicates higher than non-uraemic patients of corresponding *H. pylori* status at $p<0.05$. **Indicates higher than *H. pylori* status at $p<0.05$. **Indicates higher than *H. pylori* -ve uraemic patients at $p<0.001$.

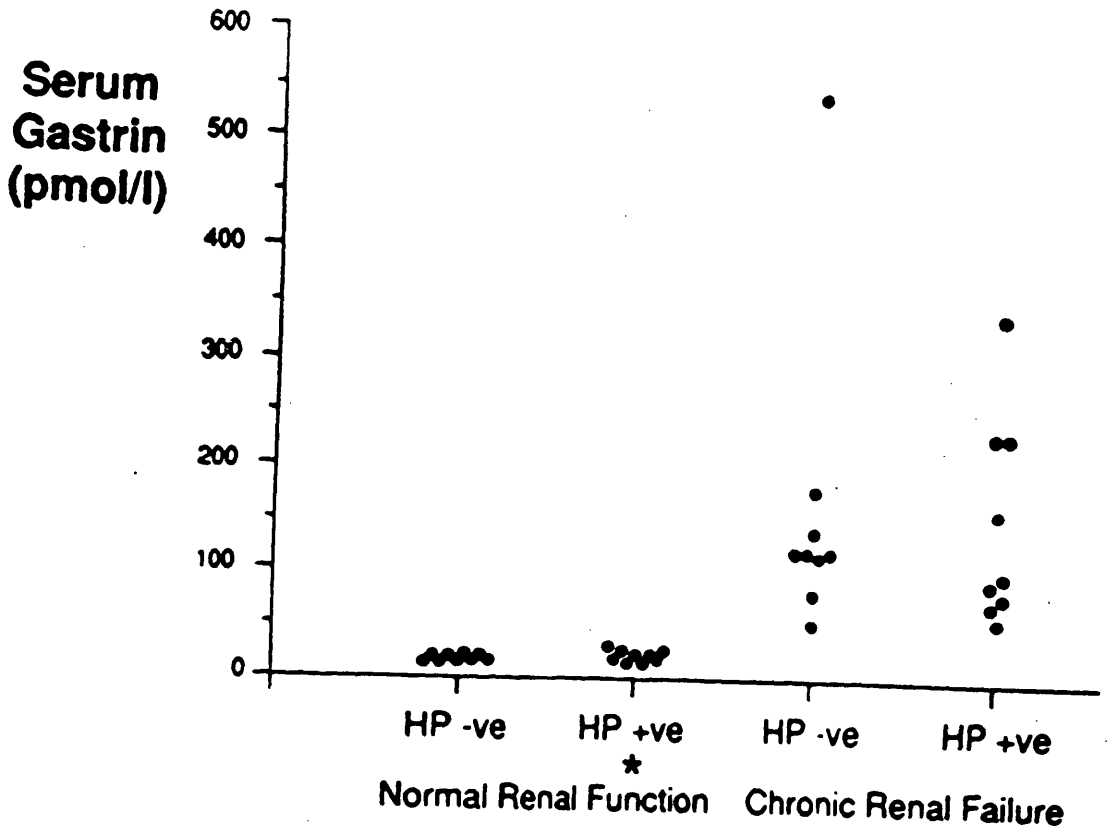

Figure 12

Effect of chronic renal failure and *Helicobacter pylori* (*HP*) status on fasting serum gastrin concentrations. *Indicates higher than *H. pylori* -ve non-uraemic patients at $p<0.05$.

al 1979). Though such bacteria have lower urease activity than *H. pylori*, they may produce significant amounts of ammonia in the presence of high gastric juice concentrations of urea. In addition, it is possible that the high gastric juice urea concentration encourages the colonisation of the upper gastrointestinal tract by urease positive organisms. We have previously observed that *H. pylori* negative uraemic patients have higher urease activity assessed by the ^{14}C-urea breath test than *H. pylori* negative non-uraemic patients (Nujumi et al 1991).

Some in vitro and experimental animal studies have suggested that the antral gastritis induced by *H. pylori* may be caused by the ammonia produced by the organism's high urease activity (Murakami et al 1988, Smoot et al 1990, Xu et al 1990, Hazell 1990). Murakami et al showed that ammonia produced by the administration of urea plus urease to rats can cause microscopic injury to their gastric mucosa (Murakami 1988). In vitro studies by Smoot et al demonstrated that ammonia produced by *H. pylori* is cytotoxic to cultures of human gastric epithelial cells (Smoot et al 1990, Xu et al 1990) and similar studies by Xu et al found that *H. pylori* ammonia production causes vacuolization of Vero cell lines (Xu et al 1990). In addition to a direct toxic effect of ammonia, it has been proposed that ammonium ions may interact with neutrophil-produced hypochlorous acid to produce the highly toxic mono-N-chloramine, NH_2Cl (Hazell 1990). It has also been proposed that *H. pylori* ammonia production will lead to mucosal damage by denaturing the protective mucus layer (Sidebotham & Baron 1990). In spite of the findings with animal models and in vitro experiments, the present study does not support a role for ammonia production in the causation of the antral gastritis in man. This was demonstrated by the absence of any correlation between the severity of the gastritis or epithelial surface damage and the bacterial ammonia production. Examining patients with and without uraemia

enabled us to look at the gastric mucosal damage over a very wide range (43 fold) of in vivo ammonia production making it unlikely that any association was missed. The abnormalities related to ammonia administration noted in the earlier in vitro studies and animal models may be explained by the high pH employed which would have increased the proportion of unionised ammonia.

Triebling et al have recently claimed that ammonia production does play a pathogenic role in the development of *H. pylori*-related gastritis in man (Triebling et al 1991). However, this conclusion is not supported by their own data. They measured gastric juice ammonium and the severity of gastritis in 5 patients with chronic renal failure and *H. pylori*, before and after treatment with ampicillin, and in 5 uninfected patients with renal failure. A positive correlation was observed between the gastric juice ammonium and severity of gastritis. This correlation, however, can be explained simply by the presence or absence of *H. pylori* in their patient group and does not provide evidence of a causal association between *H. pylori* ammonia production and gastritis. Within their small group of 5 patients with *H. pylori* there was no correlation between gastric juice ammonium and severity of gastritis which is consistent with the findings of our present study in which we examined 18 such patients. Our conclusion that ammonia does not play a pathogenic role in the development of *H. pylori*-related gastritis in man is also consistent with the recent ultrastructural studies by Thomsen et al (1990). They noted that there was no correlation between the location of the organism and the morphological damage to adjacent epithelial cells or the degree of subjacent inflammatory cell infiltrate.

The markedly elevated serum pepsinogen I concentration in the patients with chronic renal failure is consistent with previous reports (Samloff et al 1975) and can be explained by its impaired renal clearance

(Waldrum 1982). The elevation of serum pepsinogen I was particularly marked in the renal failure patients with *H. pylori* with its concentration being double that in the renal patients without the infection. It has previously been reported that *H. pylori* infection raises serum pepsinogen I in non-uraemic patients but only by about 25% (Oderda et al 1989, Chittajallu et al 1992). The fact that *H. pylori* infection raises serum pepsinogen I to a greater extent in uraemic than non-uraemic subjects would be consistent with bacterial ammonia production raising the serum concentration of the zymogen. However, little is known about the mechanism by which pepsinogen I reaches the serum or about the mechanism of its renal excretion. If it is excreted by a saturable process then in patients with renal failure the same degree of increased delivery of pepsinogen I into the serum could produce a more marked increase in its serum concentration.

As previously reported, the serum gastrin concentration was found to be elevated in the renal failure patients compared to those with normal renal function (Muto et al 1985, El Ghonaimy et al 1981, Wesdrop et al 1981, Ala-Kailan et al 1989). Serum gastrin concentration is also known to be raised in patients with *H. pylori* infection (Oderda et al 1989, Levi et al 1989, McColl et al 1991, Graham et al 1990, Smith et al 1990) and this is seen in the non-uraemic patients in the present study. It has been suggested that the hypergastrinaemia induced by *H. pylori* is due to the ammonia produced by its urease raising antral surface pH and thereby blocking the suppression of gastrin release by luminal acid (Levi et al 1989). However, we have previously found that neither increasing (Chittajallu et al 1991) inhibiting (Nujumi et al 1991) nor completely abolishing (Chittajallu et al 1990) *H. pylori* urease activity in man alters serum gastrin. We have also found that *H. pylori*-related hypergastrinaemia cannot be explained by ammonia altering mucosal

surface pH (Chittajallu et al 1992, McColl et al 1992). The finding that the gastrin concentration is similar in the *H. pylori* positive and negative uraemic patients despite the much higher rate of ammonia production in the former is further evidence against the hypergastrinaemia being due to bacterial ammonia production.

Ala-Kaila et al recently observed that patients with chronic renal failure could be divided into two statistically distinct groups according to their degree of hypergastrinaemia (Ala-Kaila et al 1989). The differences in gastrin could not be explained by differences in acid secretion or severity of renal failure and they postulated an unknown mechanism causing enhanced synthesis of gastrin. The present study indicates that *H. pylori* infection is not the unknown factor.

In conclusion, this study demonstrates that the very high rate of *H. pylori* ammonia production in uraemic patients is not associated with more marked gastritis or hypergastrinaemia. These findings support our previous work that *H. pylori* release of ammonia is unlikely to be responsible for either the hypergastrinaemia or the gastritis caused by this bacterium.

CHAPTER SEVEN

EFFECT OF INHIBITION OF *HELICOBACTER PYLORI* UREASE ACTIVITY BY ACETOHYDROXAMIC ACID ON SERUM GASTRIN IN DUODENAL ULCER PATIENTS

7.1 INTRODUCTION

Eradication of *Helicobacter pylori* infection of the gastric antrum results in a lowering of the circulating gastrin concentration (Oderda et al 1989, McColl et al 1989, Levi et al 1989, Graham et al 1990, Chittajallu et al 1991). The fasting concentration falls by 27%-33% and the integrated gastrin response to a meal by 30-58%. This stimulation of gastrin release by *H. pylori* may be relevant to the role of the organism in duodenal ulcer disease. The mechanism by which chronic infection of the antral mucosa with *H. pylori* results in increased gastrin release is not known.

H. pylori is remarkable because of its high urease activity by which it hydrolyses urea to ammonia and carbon dioxide (Marshall & Langton 1986). As a result of this, patients with the infection have reduced concentrations of urea and increased concentrations of ammonium in their gastric juice (Chittajallu et al 1991). The production of ammonia by the bacterium at the antral epithelial surface could increase gastrin release by any of three theoretical mechanisms. Ammonia is a strong alkali and could therefore prevent the physiological inhibition of gastrin release by gastric acid (Walsh et al 1975). In addition, elevation of antral surface pH by ammonia would facilitate the entry of dietary amines into the antral G cells and thereby their stimulation of gastrin release (Lichtenberger et al 1986). Thirdly, ammonia could directly stimulate gastrin release as has been shown to occur in the rat (Lichtenberger et al 1982).

In an attempt to elucidate the mechanism of the hypergastrinaemia associated with *H. pylori* infection, we have examined the effect of inhibiting the bacterium's urease activity and ammonia production on serum gastrin in duodenal ulcer patients.

7.2 PATIENTS AND METHODS

7.2.1 STUDIES IN PATIENTS WITH *H. PYLORI* INFECTION

Six patients confirmed endoscopically to have duodenal ulceration within the previous year but currently in clinical remission were studied. Their median age was 39 years (range 26-52) and three were females. In each patient, antral biopsy obtained endoscopically within the preceding three months had shown gastritis associated with *H. pylori*-like organisms.

The patients reported fasted and a venous blood sample was removed at 0800 hours for gastrin determination. Immediately following this, they drank 50ml water and further blood samples were taken at 30 min intervals over the following 2 hours. At 1000 h they took a standard meal consisting of 2 beef cubes (OXO Ltd., Croydon, England) dissolved in 200 ml water at 50°C. Further blood samples were taken at 10 minute intervals for 70 minutes and a final one at 90 minutes following the OXO drink. Immediately following this sample a ^{14}C-urea breath test was performed to measure *H. pylori* urease activity. For this they drank 250 ml Ensure Plus (Abbott Laboratories, England) to delay gastric emptying followed by 0.4 MBq ^{14}C-urea (Amersham International) in 25 ml water. Breath samples for ^{14}C-CO2 analysis were obtained at 10 min intervals for 90 mins.

On the following day, the study was repeated in an identical fashion except that the patients received 750 mg acetohydroxamic acid (Lithostat, Mission Pharmacol, U.S.A.) with a 50 ml drink of water at 0800 hrs.

Ten days later a third ^{14}C-urea breath test was performed to determine whether the temporary inhibition of urease activity had resulted in clearance of the infection.

7.2.2 STUDIES IN PATIENTS WITHOUT *H. PYLORI* INFECTION

Two male patients (aged 25 and 52 years) with a past history of duodenal ulcer disease, but in whom *H. pylori* had been eradicated within

the previous year, were studied in an identical fashion to that described above. This was performed in order to exclude the possibility that acetohydroxamic acid might have a direct effect on gastrin release.

7.2.3 STUDY IN HEALTHY VOLUNTEER WITH *H. PYLORI* INFECTION

The effect of acetohydroxamic acid on the concentrations of urea and ammonium in gastric juice was examined in a single healthy volunteer (A.N. aged 32 years) with *H. pylori* infection. Endoscopic antral biopsy obtained three months earlier had demonstrated gastritis and *H. pylori*-like organisms. A ^{14}C-urea breath tests performed one month prior to the study gave a 30 min value of 109 percentage dose/mmol CO_2 x kg body wt x 100 which is in the middle of our range for infected subjects (35-225).

He reported fasted at 0800 h and a nasogastric tube was passed perorally. The resting gastric juice was aspirated and discarded and then constant suction applied. At 30 min the suction was temporarily discontinued and a 5 ml sample of gastric juice obtained by manual aspiration. A further such sample was obtained at 60 min. Immediately following this 750 mg acetohydroxamic acid was taken orally with 50 ml water and no suction applied until 1 hour later when all the resting volume was aspirated and a 5 ml aliquot retained for analyses. Suction was then recommenced but temporarily discontinued every 30 min. over the following 3.5 hrs to allow manual aspiration of 5 ml samples of gastric juice.

7.3 ANALYSES

Gastrin concentrations in serum were determined by a standard radioimunoassay kit (C.I.S. U.K. Ltd.). Each patient's Day 1 and Day 2 samples were assayed in the same batch. The concentration of urea in gastric juice was determined by a prospective analyser (American

Monitor, West Sussex, U.K.) and the concentration of ammonium by an enzymatic method (Sigma, Dorset, U.K.). Preliminary studies confirmed that acetohydroxamic acid did not interfere with the analysis of urea or ammonium in gastric juice. The integrated gastrin response to the OXO meal was assessed by calculating the area under the serum gastrin concentration time curve using the trapezoid method.

Statistical analysis was performed by means of the Wilcoxon signed rank-sum test. The study was approved by the Western Infirmary Ethical Committee and each patient gave written informed consent.

7.4 RESULTS

The excretion of ^{14}C-CO2 in the breath was markedly lower following the administration of acetohydroxamic acid (Fig. 13). On the first study day, the median 30 min breath test value was 152 percentage dose/mmol CO_2 x kg body wt x 100 (range 111-335) compared with 22 (range 14-95) following acetohydroxamic acid ($p < 0.03$). The median value 10 days after the administration of acetohydroxamic acid was 149 (range 126-257) which was similar to the pre-treatment value (Fig. 14).

In spite of the marked suppression of *H. pylori* urease activity, there was no difference between the two study days with respect to the basal or meal-stimulated serum gastrin concentrations (Fig. 15). The median integrated gastrin response to the OXO meal was 78 ng/l.h (range 21-222) on Day 1 and 79 (range 33-207) on Day 2 (Fig. 16).

In the two patients without *H. pylori* infection their basal and meal-stimulated gastrin levels were also unaffected by the acetohydroxamic acid.

In the healthy volunteer with *H. pylori* infection the administration of acetohydroxamic acid resulted in a rise in the urea concentration and a fall in the ammonium concentration in gastric juice (Fig. 17). This was

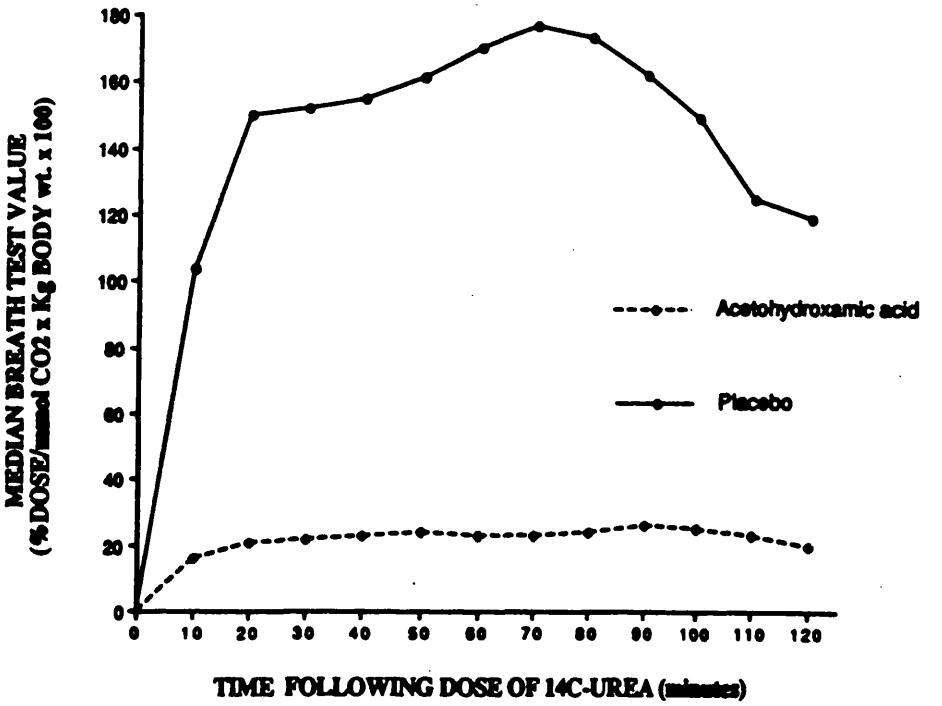

Figure 13

Effect of acetohydroxamic acid on *Helicobacter pylori* urease activity assessed by the ^{14}C urea breath test. The values are medians of six patients.

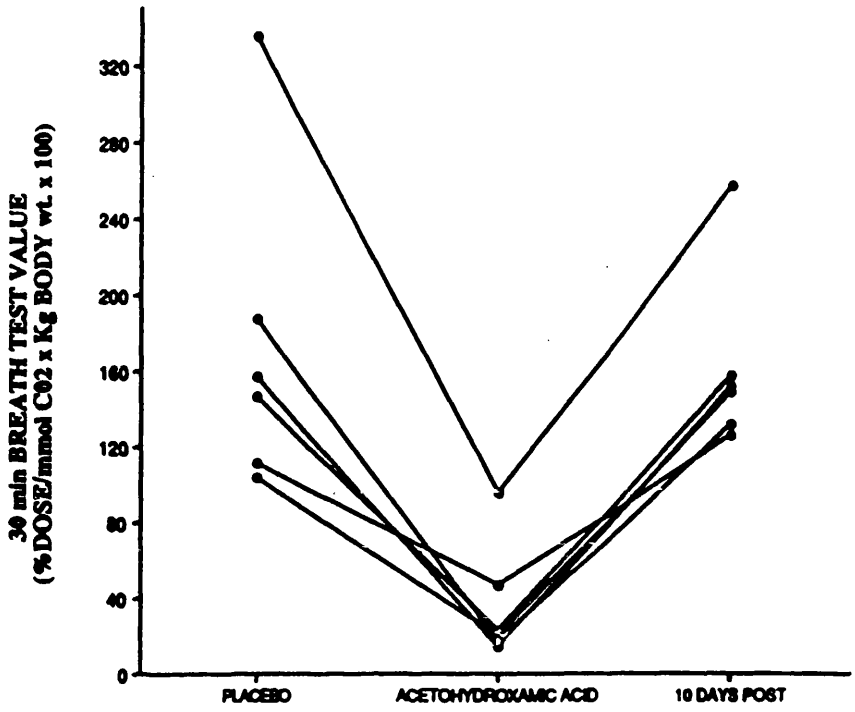

Figure 14

Subjects 30 minute ^{14}C urea breath test values on placebo, 3-5 hours after single 750 mg dose of acetohydroxamic acid, and 10 days later.

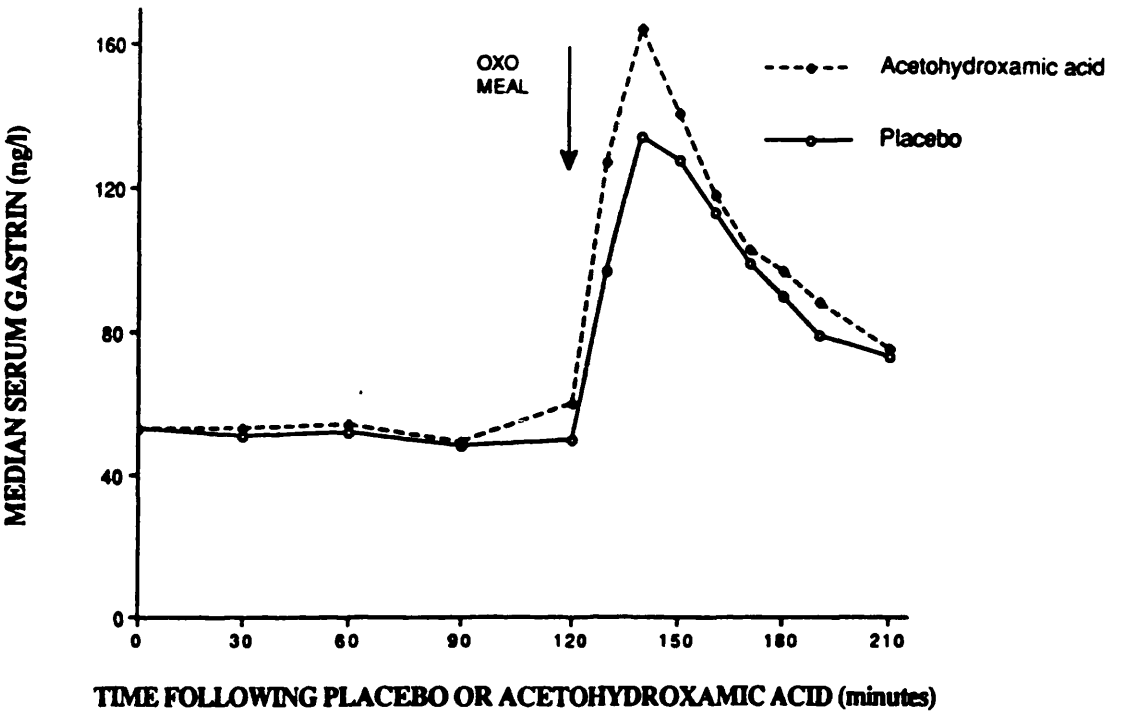

Figure 15

Basal and meal stimulated serum gastrin concentrations on placebo and after 750mg acetohydroxamic acid. The values are medians of six patients.

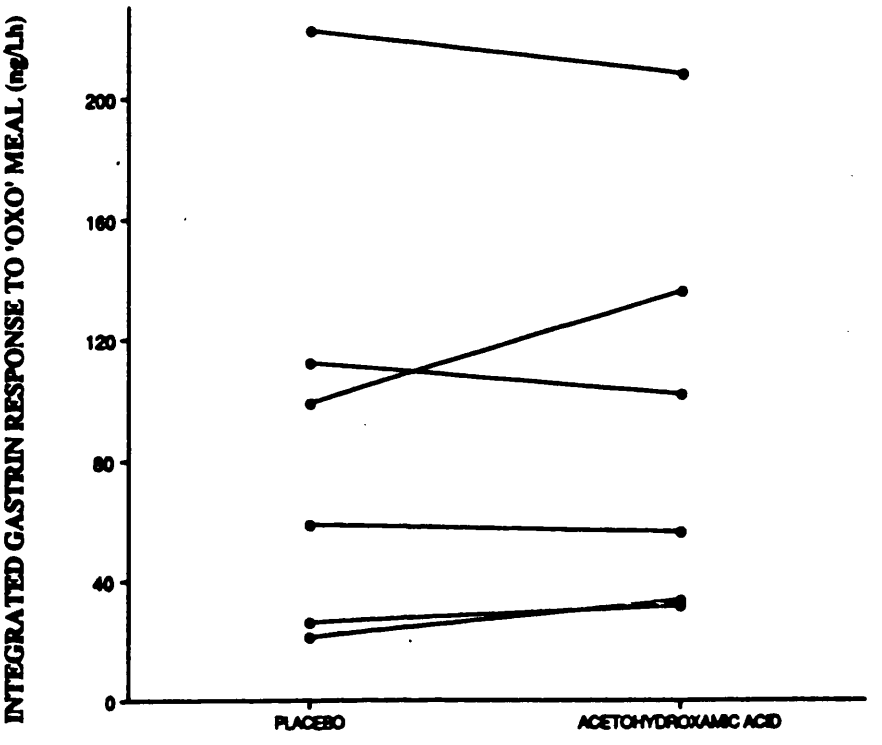

Figure 16

Subjects integrated gastrin response the OXO meal on placebo and acetohydroxamic acid.

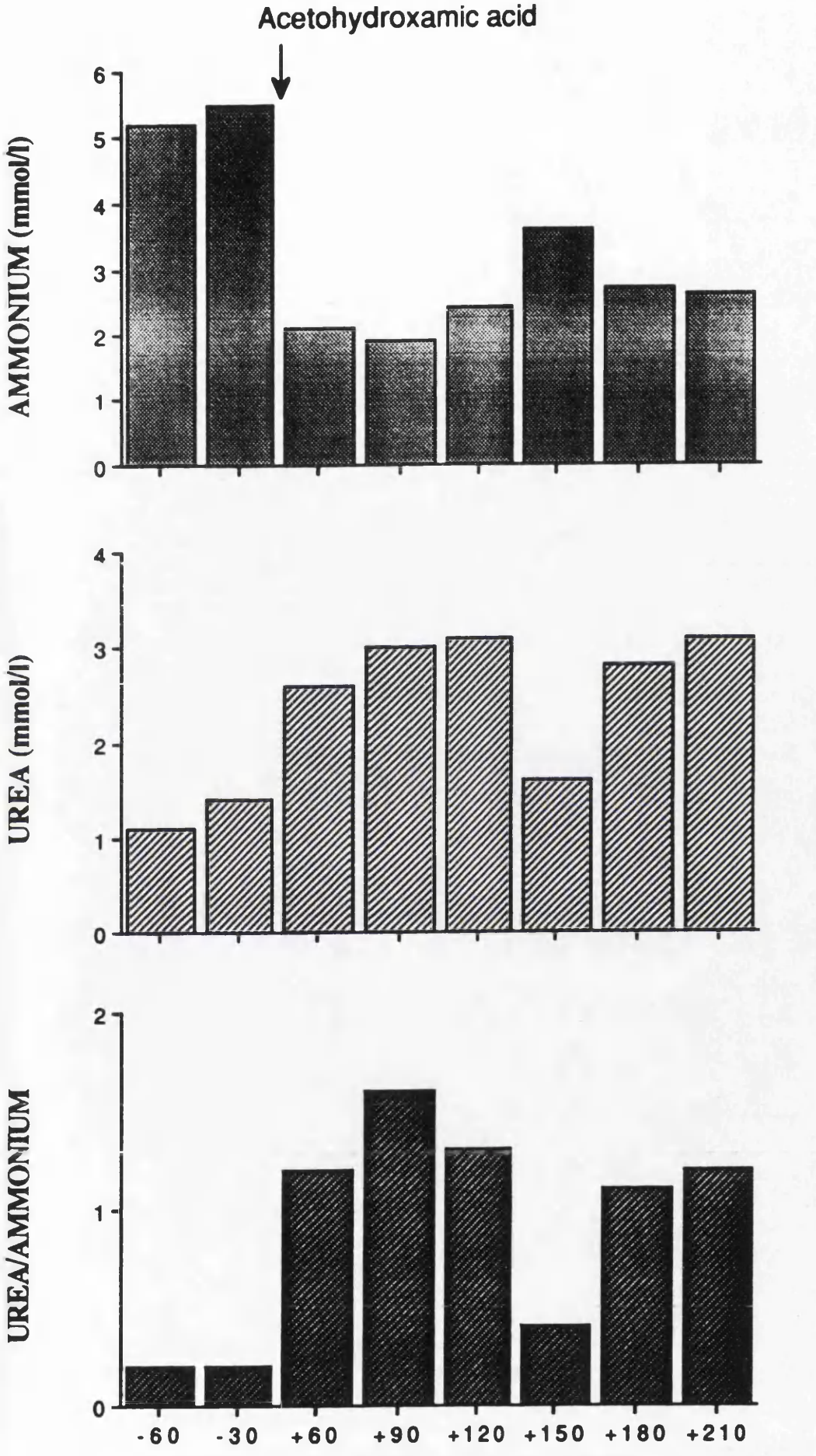

Figure 17

Effect of 750mg acetohydroxamic acid on gastric juice concentrations of ammonium and urea and their ratio in healthy volunteers with *Helicobacter pylori* infection.

evident in the first sample of juice which was collected one hour following the administration of the urease inhibitor and persisted for the full 3.5 hours studied. The ammonium concentrations in gastric juice prior to the inhibitor were 5.2 and 5.5 mmol/l and following the inhibitor ranged from 1.9-3.6 mmol/l. The ratio of urea/ammonium in gastric juice prior to the urease inhibitor was 0.2 whereas 5 of the 6 following the inhibitor were more than 1.0.

None of the patients experienced any side-effects following the administration of acetohydroxamic acid.

7.5 DISCUSSION

Acetohydroxamic acid is a specific urease inhibitor which has been used to reduce bacterial ammonia production in patients with renal calculi secondary to chronic urinary tract infection (Williams et al 1984). Reducing the ammonia production lowers the pH of the urine and thereby lessens the tendency for calculi formation. In this situation the drug has been prescribed in a dose of 250mg four times a day. We administered the drug as a once-off dose of 750mg in order to achieve rapid and effective inhibition of urease activity.

Inhibition of *H. pylori* urease activity was demonstrated by the 5-fold reduction in the 30 minute values of the ^{14}C-urea breath test. The concentrations of urea and ammonium in the gastric juice of the volunteer also confirmed inhibition of urease activity. We have previously noted that though there is overlap of *H. pylori* negative and positive subjects with respect to their gastric juice ammonium concentration, the urea/ammonium ratio in gastric juice provides clear separation of the two groups (Neithercut et al 1991). The median urea/ammonium ratio prior to the urease inhibitor was 0.2 which is within our range for infected subjects (0.04-0.7), and that following administration of the inhibitor

was 1.2 which is just within our range for patients eradicated of the infection (1.1-113). The finding of altered urea and ammonium concentrations in the first sample of juice examined and its persistence throughout the remainder of the 3.5 hours observed is consistent with the fact that acetohydroxamic acid is rapidly absorbed from the upper gastrointestinal tract reaching peak plasma concentrations at 60 minutes and with a plasma half-life of 3.5-5 hours (Feldman et al 1978). It is not clear whether the inhibition of *H. pylori* urease activity by orally administering acetohydroxamic acid is due to a topical or systemic effect or both.

In spite of the suppression of *H. pylori* urease activity there was no accompanying fall in the basal gastrin concentration or in the gastrin response to the OXO meal. There was also no change in gastrin concentration in the *H. pylori* negative subjects which excluded the possibility that a fall in gastrin in those with the infection had been masked by a direct gastrin stimulating effect of the drug.

It has been proposed that the hypergastrinaemia in patients with *H. pylori* infection is due to the ammonia produced by the bacterium raising antral surface pH (Levi et al 1989). This would prevent the physiological suppression of gastrin release by intragastric acid, resulting in inappropriate release of the hormone. If this were the mechanism of the hypergastrinaemia, the gastrin level should fall rapidly following inhibition of bacterial urease activity. In patients with hypergastrinaemia secondary to achlorhydria the gastrin falls within 5-15 minutes of intragastric instillation of hydrochloric acid (Yalow and Berson 1970). Likewise, in healthy subjects the intragastric administration of acid at the same time as a meal markedly suppresses or abolishes the gastrin response to the meal (Walsh et al 1975). The lack of a fall in serum gastrin even 3.5 hours after suppression of urease activity indicates that the hypergastrinaemia is unlikely to be due to disruption of the acid-inhibitory

feedback mechanism by bacterial ammonia. However, we cannot exclude the possibility that the degree of inhibition of urease activity with the acetohydroxamic acid might have been insufficient to allow restoration of the acid-inhibitory mechanism.

We have previously reported that increasing *H. pylori* ammonia production three-fold for four hours by intragastric infusion of urea does not raise serum gastrin (Chittajallu et al 1991). The present finding that inhibition of *H. pylori* ammonia production does not lower serum gastrin provides further evidence against the hypergastrinaemia being directly linked with bacterial urease activity.

Other mechanisms to explain the hypergastrinaemia need to be considered. It could be related to the chronic inflammatory cell infiltrate which the infection induces in the underlying antral mucosa where the G cells are located. 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). Recent observations by Wyatt et al indicate that hypergastrinaemia correlates more closely with inflammation of the antral mucosa than with infection with *H. pylori* (Wyatt et al 1989).

This study also demonstrates that normal urease activity is not essential for *H. pylori* to survive within the human stomach. It has been suggested that the organism produces ammonia in order to create an alkaline micro-environment and thus shield itself from the acidic gastric juice (Goodwin et al 1986). In spite of marked suppression of urease activity the infection was not cleared in any of our patients as shown by the repeat breath test 10 days later. Ammonia production may be important in protecting the bacterium from luminal acid when the organism is first ingested but less important when the infection becomes established in the less acidic environment of the deep mucus layer.

CHAPTER EIGHT

HELICOBACTER PYLORI AND HYPERGASTRINAEMIA DURING PROTON PUMP INHIBITOR THERAPY.

8.1 INTRODUCTION

Most acid inhibitory agents increase the circulating concentration of gastrin (Londong et al 1983, Walan et al 1979, Forrest et al 1979, Mahachai et al 1985, Lanzon-Miller et al 1987). This effect is most marked with the proton pump inhibitor omeprazole which is the most powerful inhibitor of acid secretion currently available (Koop et al 1990, Lind et al 1988, Jansen et al 1990, Larsson et al 1988). Jansen et al noted that during maintenance treatment with 20mg omeprazole, 25% of their patients had fasting gastrin concentrations of more than six times the upper limit of normal (Jansen et al 1990). The effects of this degree of hypergastrinaemia in man are unclear but animal studies have shown that prolonged drug-induced hypergastrinaemia causes ECL cell hyperplasia and carcinoid tumours of the stomach and colon (Larsson et al 1988, Larsson et al 1986, Havu 1986, Poynter et al 1985). The degree of increase in circulating gastrin during omeprazole therapy varies considerably from patient to patient and does not correlate closely with the degree of acid inhibition (Koop et al 1990, Lind et al 1988). This suggests that the gastrin response is influenced by factors other than the degree of acid inhibition.

In addition to increasing the serum gastrin concentration, omeprazole also increases the serum pepsinogen I concentration (Jansen et al 1990, Ten Kate et al 1988, Festen et al 1984). The mechanism of this effect is unclear.

We and others have recently demonstrated that the circulating gastrin concentration is raised in patients with chronic *Helicobacter pylori* (*H. pylori*) infection and falls by approximately 50% following eradication therapy (McColl et al 1989, Levi et al 1989, Levi et al 1989, Graham et al 1990, Oderda et al 1989, Smith et al 1990, McColl et al 1991). The mechanism by which *H. pylori* raises serum gastrin is unknown but it has

been proposed that it is due to its ammonia production elevating antral surface pH and thus a similar mechanism to that of proton pump inhibitory agents (Levi et al 1989). Chronic *H. pylori* infection has also been shown to raise the serum pepsinogen I concentration (Oderda et al 1989).

In the present study we investigate the influence of chronic *H. pylori* infection on the rise in serum gastrin and serum pepsinogen I during treatment with the proton pump inhibitor pantoprazole (SmithKline Beecham).

8.2 SUBJECTS AND METHODS

8.2.1 SUBJECTS

Sixteen male patients with a history of endoscopically confirmed duodenal ulceration within the previous two years were studied. In 8 of them, *H. pylori* infection had been successfully eradicated 2-14 months (median 4 months) prior to entering the present study. This had been achieved by four weeks therapy with a combination of tri-potassium dicitrato bismuthate (De-Nol tab.) 120mg t.i.d., metronidazole 400mg t.i.d. and amoxycillin 250mg t.i.d. The eradication of their *H. pylori* infection resulted in a median fall of 27% in their basal gastrin concentration and of 46% in their integrated gastrin response to a peptone meal and this data has been previously reported (McColl et al 1989, McColl et al 1991).

The two groups of patients of different *H. pylori* status were similar with respect to age and smoking habits. Their *H. pylori* status was confirmed within the month prior to entering the study by histology of antral biopsy, rapid urease test (CLO-test) (Marshall et al 1987) and ^{14}C-urea breath test. All the *H. pylori* positive patients had 20 minute ^{14}C-urea breath test values of more than 50 per cent ^{14}C dose per mmol CO_2 x kg body weight x 100 and those in whom the infection had been eradicated had values of less than 15. Each patient discontinued any acid

inhibitory therapy at least one month prior to entering the study. None of them had received any antibiotics or bismuth preparations within the previous two months.

8.2.2 DESIGN

Each patient was studied prior to commencing pantoprazole and on the fifth day of treatment with 40mg per day. The drug was taken as a single daily dose in 100ml water at 0925h which was immediately before breakfast. On both study days intragastric pH and serum gastrin concentrations were measured. In the 8 patients who were *H. pylori* positive, ^{14}C-urea breath tests were performed 10 days after completing the course of therapy to exclude the possibility that the proton pump inhibitor had cleared the infection.

The patients reported fasted at 0830h and a combined glass electrode (Radiometer, GK2802C) was passed perorally so that its recording tip was 50cm from the incisor teeth. It was connected to a digital recorder (Digitrapper MKII, Synectics Medical) which registers the pH every 4 seconds. At the start and completion of each recording, the electrodes were calibrated using standard buffers.

The content and timing of the meals was identical on each study day. Breakfast was taken at 0930h and consisted of 20g Crunchy Nut Cornflakes (Kellog Company) with 200ml semi-skimmed milk, one roll lightly spread with butter and one cup of tea. At 1300h they had a peptone meal consisting of two beef cubes (OXO Ltd., Croydon) dissolved in 200ml water at 55°C. A further cup of tea was taken at 1545h. Dinner was taken at 1800h and consisted of lentil soup with one roll lightly spread with butter, followed by Lasagne (447kcal, Marks and Spencer plc) with additional whole carrots (Heinz, Middlesex) and a chocolate coated ice cream (Safeway Foodstores Ltd., Kent) to finish. They also had a cup of tea immediately after dinner and a further cup with a single oat cake

biscuit at 2200h. Patients who smoked recorded the timing and number of cigarettes on the first study day and precisely reproduced this smoking habit on the subsequent day.

A venous blood sample was taken for fasting serum gastrin determination at 0830h which was prior to passing the pH electrode and taking the drug. Further samples were taken for determining the pre-prandial gastrin concentrations at 1230h, 1245h and 1255h. The OXO meal was commenced at 1300h and further samples for gastrin determination taken every 15 minutes for 75 minutes.

Serum pepsinogen I concentrations were determined in the fasting serum sample and also in the 1245h pre-prandial serum sample. It was not determined in the post-prandial samples as serum pepsinogen I concentration rises only very slightly following eating (Samloff et al 1975).

The venous blood samples for gastrin and pepsinogen I were placed in plain containers and allowed to stand for 15 minutes to allow clotting. The serum was then separated by centrifugation at 4°C for 10 minutes and stored at -20°C.

8.2.3 INTRAGASTRIC pH ANALYSIS

The intragastric pH data were transferred from the Digitrapper recorder to an IBM compatible computer (Amstrad PC 1512 HD20) and analysed using the GastrograpH program (Ver 5.0, 1987, Gastrosoft Inc). For each patient the median value of all their recordings from 0900h-2300h was calculated and called their daytime pH.

8.2.4 BIOCHEMICAL ANALYSIS

Serum gastrin was measured using commercial radioimmunoassay kits obtained from CIS (UK) Ltd., (Bucks). The gastrin assay reacted 100% with gastrin-17 and 72% with gastrin-34 in molar terms. The minimum

detection limit of the assay was 20ng/l (CV - 22%) and the interassay CV was <10% from 40-700ng/l. All samples for each patient were measured in the same batch. The median value of the 3 samples obtained over the 30 minutes prior to commencing the OXO meal was calculated for each patient and called their preprandial gastrin. The highest gastrin concentration in response to the OXO meal was called the peak prandial gastrin.

Serum Pepsinogen I was measured using commercial radioimmunoassay kits obtained from Incstar Ltd. (Berkshire). This assay reacted with Group I pepsinogens and had a minimum detection limit of 1ng/ml and an interassay CV of < 10% from 2-30ng/ml. Patient samples were diluted 1:10 or 1:20 prior to analysis as appropriate to obtain values within the range of the standard curve.

8.2.5 ^{14}C-UREA-BREATH TEST

For the ^{14}C-urea breath tests the patients drank 250ml of Ensure Plus immediately followed by 0.4MBq ^{14}C-urea (Amersham International) in 25ml water. Breath samples were collected every 10 minutes for 30 minutes and analysed as described by Marshall and Surveyor (Marshall & Surveyor 1988). The 20 minute value was used as the determinant of the urease activity (Bell et al 1987).

8.2.6 STATISTICS

Statistical analysis of differences between the two groups was performed using the Mann-Whitney U test and of differences within each group by the Wilcoxon's Signed Rank-Sum Test. The data was expressed as medians and ranges because of its non-parametric distribution.

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

8.3 RESULTS

The repeat ^{14}C-urea breath tests performed in the *H. pylori* positive patients after completing the 5-day course of pantoprazole were similar to the pre-treatment values indicating that the infection had not been eradicated in any of these patients by the proton pump inhibitor therapy.

8.3.1 INTRAGASTRIC pH

In the *H. pylori* eradicated patients their median (range) daytime pH rose from 1.9 (1.4 - 2.8) pre-treatment to 3.7 (1.9 - 6.1) on treatment ($p = 0.03$) and in the *H. pylori* positive patients from 1.7 (1.3-2.2) to 4.4 (3.1-6.2) ($p = 0.02$). There was no significant difference between the *H. pylori* eradicated and *H. pylori* positive groups with respect to their intragastric pH values either pre-treatment or during treatment.

8.3.2 SERUM GASTRIN

The median serum gastrin concentrations for the *H. pylori* positive and *H. pylori* eradicated patients before and during treatment are shown in Figure 18.

Pre-treatment, the *H. pylori* positive and eradicated patients did not differ significantly with respect to either their fasting or pre-prandial gastrin concentrations. However, the pre-treatment median peak prandial gastrin concentration (ng/l) was higher in the *H. pylori* positive patients (63 range 42-225) than in the *H. pylori* eradicated patients (42, range 30-93) ($p < 0.05$).

In the *H. pylori* eradicated patients their median fasting serum gastrin concentration rose from 37 (27-43) pre-treatment to 45 (33-52) on treatment ($p = 0.02$), their pre-prandial gastrin from 38 (27-51) to 51 (36-172) ($p < 0.01$) and their peak prandial gastrin from 42 (30-93) to 76 (39-221) ($p < 0.01$). In the *H. pylori* positive patients their median fasting gastrin concentration was 47 (34-113) pre-treatment and 59 (41-88) on

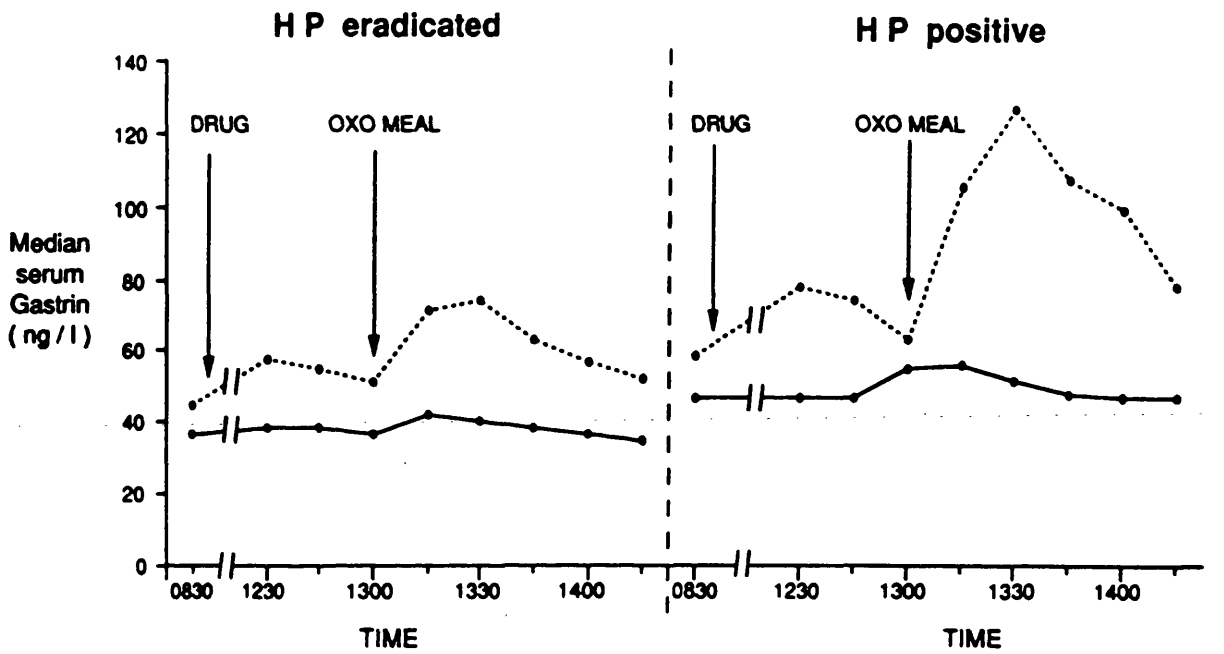

Figure 18

Median serum gastrin concentration before (——) pantoprazole and on the 5th day of treatment (- - - -) with 40mg/day in patients with *Helicobacter pylori* infection and in patients in whom it had been eradicated.

treatment (n.s., p = 0.25). Their pre-prandial gastrin rose form 47 (28-97) pre-treatment to 73 (39-285) on treatment ($p < 0.01$) and their peak prandial gastrin from 63 (42-225) to 124 (72-309) ($p < 0.01$) (Figs. 19 and 20).

The median percentage increase in serum gastrin on treatment was similar in the *H. pylori* eradicated and *H. pylori* positive patients being 45 (2-377) and 41 (21-194) respectively for pre-prandial values and 69 (15-152) and 81 (37-223) for peak prandial values. Consequently, the peak prandial concentrations during treatment were significantly higher in the *H. pylori* positive than in the eradicated patients ($p < 0.05$).

8.3.3 SERUM PEPSINOGEN I

Pre-treatment, there was no significant difference between the *H. pylori* positive and eradicated patients with respect to either their fasting or pre-prandial serum pepsinogen I concentrations.

In the *H. pylori* eradicated patients their median fasting serum pepsinogen I concentration (ng/ml) rose from 92 (41-182) pre-treatment to 121 (53-196) ($p = 0.02$) on treatment and the pre-prandial values from 99 (48-142) to 135 (52-197) ($p = 0.01$). In the *H. pylori* positive patients their median fasting serum pepsinogen I rose from 89 (63-171) to 191 (115-555) ($p < 0.01$) and their pre-prandial values from 104 (70-179) to 191 (125-575) ($p < 0.01$) (Fig. 21).

The median percentage increase in fasting serum pepsinogen I on treatment was greater in the *H. pylori* positive patient (151, range 23-454) than in the *H. pylori* eradicated patients (29, range -4 to +51) ($p < 0.02$). Likewise, the percentage increase in pre-prandial serum pepsinogen I was higher in the *H. pylori* positive patients (114, range 27-418) than eradicated patients (8, range -22 to 203) ($p < 0.05$). Consequently, during treatment the serum pepsinogen I concentrations were significantly higher in the *H. pylori* positive patients than in the eradicated patients ($p < 0.05$).

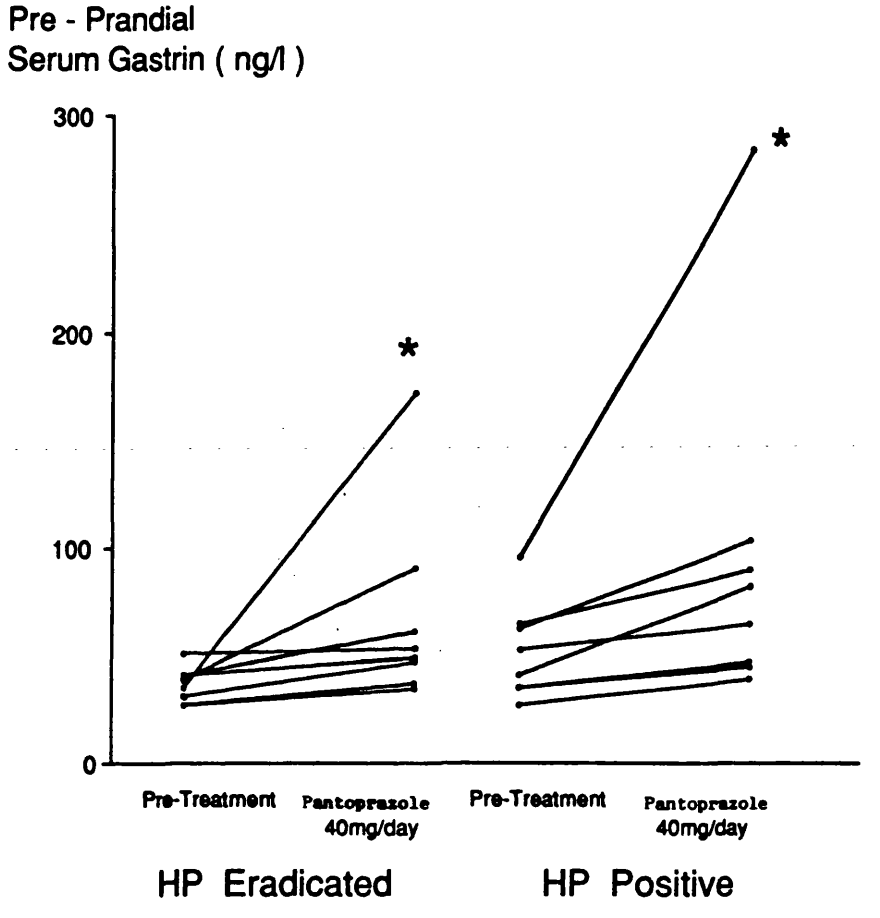

Figure 19

Effect of 5 days therapy with pantoprazole on pre-prandial serum gastrin concentrations in the patients with different *H. pylori* status. *Indicates higher than corresponding pre-treatment values at $p<0.01$.

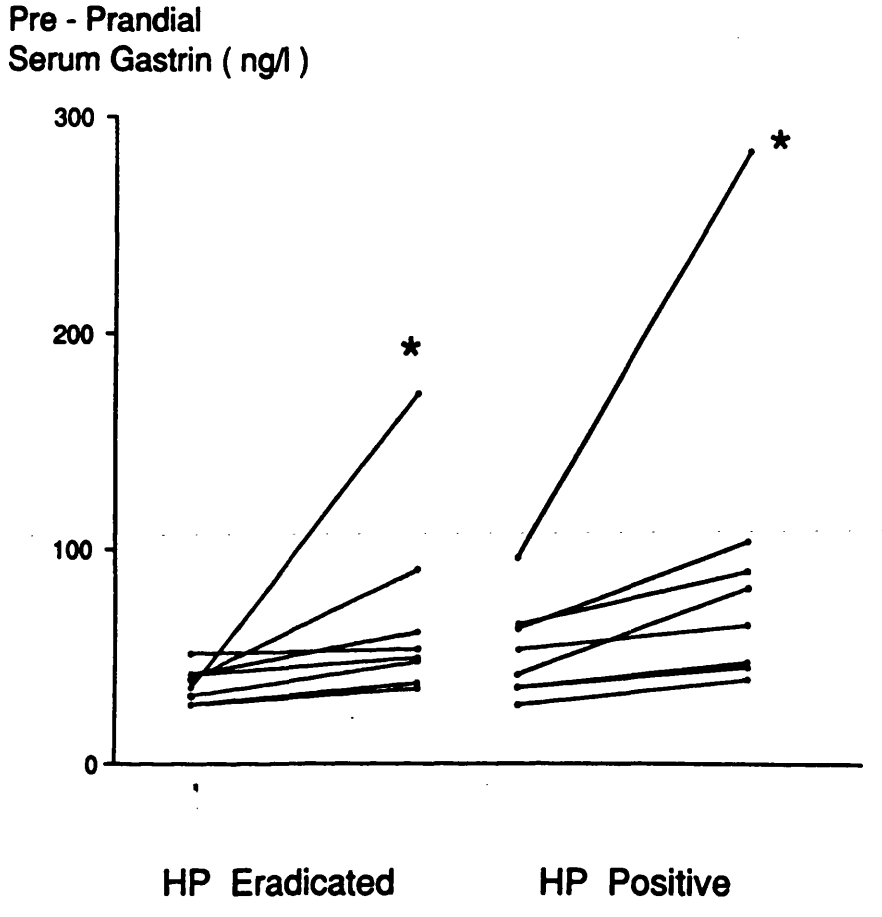

Figure 20

Effect of pantoprazole on peak prandial serum gastrin concentrations in patients with different *Helicobacter pylori* status. *Indicates higher than corresponding pre-treatment values at $p<0.01$. **Indicates higher than values for *H. pylori* eradicated patients receiving pantoprazole at $p<0.05$.

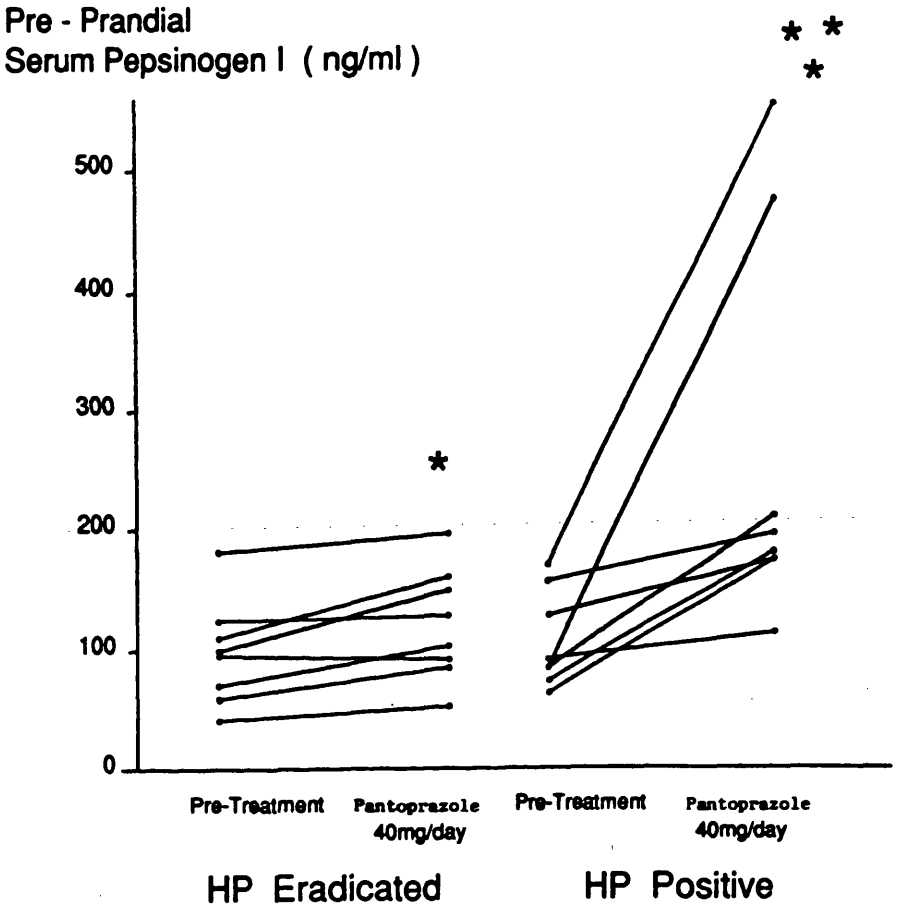

Figure 21

Effect of pantoprazole on pre-prandial serum pepsinogen I concentrations in the patients with different *H. pylori* status. *Indicates higher than corresponding pre-treatment values at p<0.01. **Indicates higher than values for *H. pylori*-eradicated patients receiving pantoprazole at p<0.05.

8.4 DISCUSSION

The finding pre-treatment of higher meal-stimulated gastrin concentrations in the *H. pylori* positive patients is consistent with the previous reports that the infection stimulates gastrin release (McColl et al 1989, Levi et al 1989, Levi et al 1989, Graham et al 1990, Oderda et al 1989, Smith et al 1990, McColl et al 1991). Levi et al have proposed that the increased gastrin concentration stimulates excessive acid secretion and thereby explains the link between the infection and duodenal ulcer disease (Levi et al 1989). However, this study does not support the hypothesis as the increased serum gastrin concentration in the *H. pylori* positive patients was not accompanied by increased intragastric acidity. We have previously shown that intragastric pH and nocturnal acid output are unchanged even seven months after eradication of *H. pylori* infection in duodenal ulcer patients (McColl et al 1991). The role, if any, of hypergastrinaemia in the link between chronic *H. pylori* infection and duodenal ulcer disease is unclear.

One of the side-effects of marked suppression of intragastric acidity with proton pump inhibitory agents is the resulting rise in serum gastrin. This study has shown that the percentage increase in serum gastrin on treatment is similar in *H. pylori* positive and *H. pylori* eradicated patients. Consequently, patients with *H. pylori* infection have higher serum gastrin concentrations during proton pump inhibitor therapy than similar patients in whom the infection has been eradicated.

The findings of this study are relevant to the mechanism by which *H. pylori* infection induces hypergastrinaemia. Levi et al proposed that it is due to the ammonia produced by the bacterium's urease raising antral surface pH and thereby preventing the inhibition of gastrin release by gastric acid (Levi et al 1989). If the hypergastrinaemia in *H. pylori*

infection were due to elevated antral pH then the rise in gastrin induced by elevation of intragastric pH by pantoprazole should be less in *H. pylori* positive patients. In other words, if the inhibitory influence of intragastric acid on gastrin release is already blocked by *H. pylori* infection, then reducing intragastric acidity with the proton pump inhibitor should have little further effect. However, we found that the percentage rise in serum gastrin was the same in those with and without *H. pylori* infection. Our finding in this study is consistent with our recent observation that the increase in serum gastrin during antral alkalinization is similar in *H. pylori* positive and negative subjects (Chittajallu et al 1992). These findings demonstrate that *H. pylori* hypergastrinaemia cannot be explained by elevation of antral surface pH by the bacterium's urease activity.

The findings of the present study are also consistent with our previous studies indicating that *H. pylori*-induced hypergastrinaemia is not linked to the bacterium's urease activity. Neither increasing *H. pylori* ammonia production by intragastric infusion of urea (Chittajallu et al 1991) nor inhibiting it with acetohydroxamic acid (Nujumi et al 1991), nor completely suppressing it with twenty four hours of triple therapy (Chittajallu et al 1991), altered the serum gastrin concentration.

Pre-treatment serum pepsingogen I concentrations were not significantly different in the *H. pylori* positive and eradicated patients though there was a trend in favour of higher values in the latter. Oderda et al observed that the mean pepsinogen I concentration fell by 32% after eradicating *H. pylori* in children with a variety of upper gastrointestinal disorders (Oderda et al 1989). The mechanism by which the infection raises serum pepsinogen I is not known but could be due to the accompanying inflammation resulting in leakage of the zymogen from chief cells and mucus neck cells. The higher serum pepsinogen I concentrations reported in children with *H. pylori* infection may be

explained by them having a more acute and widespread gastritis involving the gastric body where most of the pepsinogen secreting cells are located. In the adult duodenal ulcer patients examined in the present study, the infection is mainly confined to the gastric antrum.

The observation that the serum pepsinogen I concentration increased on pantoprazole is consistent with reports with the other proton pump inhibitor omeprazole (Jansen et al 1990, Ten Kate et al 1988, Festen et al 1984). The mechanism by which treatment with proton pump inhibitors raises serum pepsinogen I is unclear. It may be due to passive back-diffusion into the systemic circulation resulting from increased concentrations of the zymogen in the gastric glands secondary to low volume secretion (Ten Kate et al 1988). Our observation that the percentage increase in serum pepsinogen I was higher in the patients with *H. pylori* infection is new and the reason for their exaggerated response is unclear.

The finding that eradication of *H. pylori* reduces the hypergastrinaemia during proton pump inhibitor therapy has important clinical implications. The proton pump inhibitor omeprazole is the most effective medical agent for treating severe oesophagitis. However, there is reluctance to maintain patients on this therapy because of the potential adverse effects of long-term hypergastrinaemia. The present study suggests that eradicating *H. pylori* infection may be a means of reducing hypergastrinaemia in such patients. There is no evidence incriminating *H. pylori* infection in the pathogenesis of oesophagitis and therefore eradicating the infection would not remove the need for the proton pump inhibitor therapy. Studies are required to assess whether the serum gastrin concentration remains lower in *H. pylori* negative patients even during long-term treatment with proton pump inhibitors.

CHAPTER NINE

EFFECT OF ERADICATING *H. PYLORI* ON HYPERGASTRINAEMIA DURING LONG TERM OMEPRAZOLE THERAPY

9.1 INTRODUCTION

Hypergastrinaemia is a well recognised side-effect of treatment with proton pump inhibitor drugs (Koop et al 1990). This occurs due to the marked suppression of gastric acid secretion and thus removal of the acid mediated inhibition of gastrin release (Banerjee et al 1995, Lind et al 1991). The clinical significance of such hypergastrinaemia in patients requiring long term proton pump inhibitor therapy is unclear.

In rats, long term omeprazole treatment is associated with argyrophil cell hyperplasia within the oxyntic mucosa (Creutzfeldt et al 1986, Larsson et al 1988, Koop et al 1987) and the development of gastric carcinoid tumours in up to 30% of female animals (Ekmann et al 1985, Betton et al 1988). These changes in the rats have been shown to be a consequence of the drug induced hypergastrinaemia (Eissele et al 1992). In man, long term treatment with omperazole also results in argyrophil cell hyperplasia which correlates with the degree of hypergastrinaemia (Creutzfeldt et al 1991). Gastrin also exerts trophic effects on gastric and colonic mucosa and upon certain carcinoma cell lines derived from these tissues (Kusyk et al 1986, Ishizuka et al 1992). The possible role of hypergastrinaemia in the development and growth of certain gastrointestinal tumours is uncertain but continues to attract research interest (Watson et al 1993, Rehfeld 1995). Due to the uncertainty about the clinical significance of long term hypergastrinaemia in man, it would seem prudent to minimise the disruption of gastrin physiology induced by proton pump inhibitor drugs if possible.

Another common cause of chronic hypergastrinaemia in humans is *H. pylori* infection (Chittajallu et al 1992). This is present in about 50% of the adult population Veldhuyzen van Zanten 1994) and in a similar or higher proportion of patients who will be prescribed proton pump inhibitor drugs for management of their dyspeptic disease. There is little

information concerning the influence of *H. pylori* status on the gastrin response to proton pump inhibitor therapy. In the present study, we have examined this and in particular assessed whether eradicating *H. pylori* infection prior to commencing omeprazole therapy could be a means of reducing the hypergastrinaemia during the proton pump inhibitor therapy.

9.2 MATERIALS AND METHODS

9.2.1 PATIENTS

Thirty three patients with endoscopically confirmed disease were studied. Their median age was 45 years (range 26-60). Twenty-two were males. Their endoscopic diagnosis included duodenal ulcer (20), oesophagitis (6), prepyloric ulcer (3), oesophagitis + DU (3), prepyloric ulcer + DU (1). Each of the patients had evidence of *H. pylori* infection confirmed by microscopy of antral biopsy and ^{14}C-urea breath test.

9.2.2 STUDY DESIGN

On entry to the study all patients had their serum gastrin concentration assessed fasted and in response to a standardised meal. A ^{14}C-urea breath test was performed simultaneously. Any acid inhibitory drugs were stopped at least 4 weeks prior to this assessment. Following this they were randomised to receive either *H. pylori* eradication therapy consisting of 3 weeks treatment with tripotassium dicitratobismuthate 120g t.i.d., metronidazole 400mg t.i.d. and amoxycillin 250mg t.i.d. plus gastrocote (alginic acid plus aluminium hydroxide plus magnesium trisilicate plus sodium bicarbonate, Boehringer Mannheim, Livingstone, U.K.) as required for symptoms or gastrocote alone. Four weeks after completing this therapy all patients had repeat serum gastrin studies and ^{14}C-urea breath test and endoscopic examination.

Subjects then received four weeks treatment with omeprazole 40mg/day followed by six months treatment with omeprazole 20mg/day.

The doses of omeprazole were taken before breakfast. At the end of the one month course of the omeprazole 40mg/day and again the end of the six months omeprazole 20mg/day the serum gastrin measurements and endoscopic examination were repeated. After the 6 months of omeprazole 20mg/day the proton pump inhibitor was stopped and patients maintained if required on Gastrocote for 4 weeks before having a further ^{14}C-urea breath test.

9.2.3 COMBINED ^{14}c-UREA BREATH TEST AND GASTRIN ASSESSMENT

Patients reported between 09:00h and 10:00h following an overnight fast. Subjects cleaned their teeth and then an intravenous cannula was inserted. Three x 20ml venous blood samples obtained at 15 min intervals for fasting gastrin determination. A breath sample was collected for baseline ^{14}C-CO_2 determination. The subject then drank over 5 minutes 250ml Ensure Plus (Abbott Labs. Ltd., Maidenhead, U.K.) which contains 300calories/200ml, 12.5g of protein, 40g carbohydrate, 10g fats, and vitamins including Vitamin A, Vitamin D, Vitamin K, Vitamin C and Vitamin E, pholic acid and B_1, B_2, B_6 plus elements of sodium, potassium and calcium.

Immediately following this they drank 100ml water containing 0.4MBq ^{14}C-urea. Further 20ml blood samples for serum gastrin were obtained at 15 min intervals for 90 mins. A further breath sample for $^{14}CO_2$ determination was obtained at 20 min, 40 min and 100 min following administration of the ^{14}C-CO_2.

When the combined gastrin/breath tests were performed at the end of the omeprazole treatment periods the omeprazole medication was taken that morning two hours prior to commencing the test.

9.2.4 ENDOSCOPIC EXAMINATION

During each endoscopic examination 3 biopsies were taken from the antrum of stomach for histology, CLO-test (Marshall et al 1987) for *H. pylori* and culture for *H. pylori*. Gastric antral biopsies were taken and transported to the laboratory in 0.9% sterile saline, all biopsies were cultured within 4 hours.

9.3 COMPLIANCE

Compliance with the omeprazole therapy was assessed by performing capsule counts at each visit. Missing >25% of the total tablets over the relevant treatment period was considered to be unacceptable compliance. In addition, patients were contacted by telephone at regular intervals during the study to encourage compliance. Patients were also contacted 7 days and 24 hours prior to the assessment visits during the omeprazole therapy to further ensure compliance.

9.4 ANALYSES

Serum gastrin concentration was measured by radioimmunoassay using antibody 198 which detects G17 and G34 with similar affinity (Mulholland et al 1993). Fasting gastrin was calculated as the median of the 3 samples obtained at 15 min intervals prior to commencing the meal. The meal-stimulated gastrin was calculated as the median of the 3 samples obtained at +15, +30 and +45 min. This time was chosen as it represented the time showing the nominal gastrin response to the meal.

The ^{14}C-urea breath test and culture of *H. pylori* were performed. The gastric antral biopsies were taken and transported to the laboratory in 0.9% sterile saline (Veenendaal et al 1993). All biopsies were cultured within four hours. The biopsies were homogenised and inoculated onto a blood agar base no. 2 (Oxoid) agar plate (Jones et al 1984) containing 10% v/v

horse blood and Skirrows selective supplement (Oxoid). The plates were incubated for 72 hours at 37°C in a microaerophilic atmosphere (BBL Campypak). Typical colonies which were oxidase and urease positive were identified as *Helicobacter pylori.*

The statistical significance of changes following the various treatments was assessed by the Wilcoxon paired samples ranking test. The statistical significance of differing responses between the *H. pylori* eradicated and non-eradicated groups was assessed.

The study was approved by the West Glasgow Hospitals University NHS Trust Ethics Committee.

9.5 RESULTS

Of the thirty-three patients initially recruited, poor compliance with the protocol resulted in eight being excluded from the analysis after the one month of omeprazole 40mg and eleven after the six months of omeprazole 20mg.

Of the thirty-three patients recruited, fifteen were randomised to *H. pylori* eradication therapy and thirteen of them were *H. pylori* negative when reassessed four weeks following discontinuation of the therapy. Each of those thirteen had a 20 minute ^{14}C-urea breath test value of less than 14kg percentage dose/mmol CO_2 x 100 a negative antral CLO-test and no *H. pylori* on microscopy or culture of antral biopsies. Both of those still positive had a 20 minute ^{14}C-urea breath test value of more than 362 positive antral CLO-test and positive microscopy and culture of antral biopsies. Of the eighteen subjects who received only symptomatic therapy, all remained positive for *H. pylori* one month post-treatment by each of the above tests.

9.5.1 ENDOSCOPIC FINDINGS

Of the thirteen patients in whom *H. pylori* was subsequently eradicated, their endoscopic diagnoses on initial presentation included duodenal ulceration in eight, oesophagitis in three and duodenal ulceration plus oesophagitis in one and one further patient with duodenal ulcer plus pre-pyloric ulcer. The only endoscopic abnormality in those patients at one month after triple therapy was mild oesophagitis in one patient. Of the two patients in whom the triple therapy failed to eradicate *H. pylori*, one had duodenal ulceration and one had prepyloric ulceration on initial presentation, but both showed complete healing one month after triple therapy.

Of the eighteen patients who received symptomatic therapy, endoscopic findings at initial presentation included duodenal ulceration in ten, prepyloric ulceration in two, oesophagitis in three, duodenal ulceration plus oesophagitis in two and duodenal ulceration plus gastritis in one patient. On reassessment, after two months symptomatic therapy alone, endoscopic examination showed duodenal ulceration in nine, oesophagitis in four, prepyloric ulceration in two, duodenal ulcer and gastritis in two and one patient showed no persisting abnormality.

Endoscopic examination was normal in all but one subject after one month of omeprazole 40mg per day. This one exception was a patient with persisting prepyloric ulceration and who had originally received symptomatic therapy. Endoscopic examination was normal in each of the subjects reassessed after six months treatment with omeprazole 20mg per day.

9.5.2 *H. PYLORI* ASSESSMENT DURING OMEPRAZOLE THERAPY

Of the thirteen patients who were successfully eradicated of *H. pylori* when examined one month after triple therapy, each had negative antral CLO tests and negative antral culture and negative histology for *H. pylori*

when reassessed after one month omeprazole 40mg/day and six months of omeprazole 20mg/day.

Of the twenty patients who remained *H. pylori* positive after symptomatic (n=18) or triple therapy (n=2), fifteen were reassessed after one month omeprazole 40mg per day. At this time point, six had negative antral CLO tests and three of these six showed normal antral histology and no microscopic evidence of *H. pylori*. One further patient had a positive antral CLO test but had normal histology and no evidence of bacteria on microscopy. Eight of the fifteen patients had negative culture of antral biopsy.

Of these twenty patients who were *H. pylori* positive after symptomatic or triple therapy, 12 were reassessed after six months of omeprazole 20mg per day. Four had both negative antral CLO test and no evidence of *H. pylori* on microscopy of antral biopsy and two of these patients showed resolution of antral gastritis. One further patient had no evidence of *H.P* on antral microsocpy. Seven of them were negative for *H.P* on culture (Tables 4,5 and 6).

The ^{14}C-urea breath tests performed 4 weeks after completing the omeprazole 20mg were negative in all those initially eradicated of the infection by triple therapy and positive in all those who had the unsuccessful triple therapy or no triple therapy.

9.5.3 SERUM GASTRIN

On initial presentation prior to treatment with triple/symptomatic therapy, the patients in whom *H. pylori* was subsequently eradicated had similar fasting (median = 43ng/l, range 17-226) and meal-stimulated

PATIENT	CLO TEST	HISTOLOGY	CULTURE
1	Negative	Normal	Negative
2	"	"	"
3	"	"	"
4	"	"	"
5	"	"	"
6	"	"	"
7	"	"	"
8	"	Mild gastritis	"
9	"	Mild gastritis	"

TABLE 4: EFFECT OF OMEPRAZOLE ON 40mg FOR ONE MONTH ON THE *H. PYLORI* ERADICATED PATIENTS

NO.	CLO TEST	HISTOLOGY	CULTURE
1	+	Ch. gastritis *H.P* +ve	+
2	+	"	+
3	-	"	-
4	+	"	+
5	+	"	-
6	-	NAD *H.P* -ve	-
7	+	Ch. gastritis *H.P* +ve	+
8	-	NAD *H.P* -ve	-
9	-	"	-
10	+	Ch. gastritis *H.P* +ve	+
11	+	NAD *H.P* -ve	-
12	-	Ch. gastritis *H.P* +ve	-
13	-	"	-
14	+	Mild gastritis *H.P* -ve	-
15	+	Ch. gastritis *H.P* +ve	+

TABLE 5: EFFECT OF OMEPRAZOLE 40mg FOR FOUR WEEKS ON THE *H. PYLORI* NON-ERADICATED PATIENTS

NO.	CLO TEST	HISTOLOGY	CULTURE
1	-	Ch. gastritis	*H.P.* -ve
2	+	Ch. gastritis	*H.P.* -ve
3	+	Ch. gastritis	*H.P.* +ve
4	-	Ch. gastritis *H.P.* -ve	*H.P.* +ve
5	+	Ch. gastritis *H.P.* +ve	*H.P.* -ve
6	-	NAD *H.P* -ve	*H.P.* -ve
7	-	NAD *H.P* -ve	*H.P.* -ve
8	+	Ch. gastritis *H.P* +ve	*H.P.* -ve
9	+	"	*H.P.* +ve
10	+	"	*H.P.* +ve
11	+	"	*H.P.* -ve
12	+	"	*H.P.* +ve

TABLE 6: EFFECT OF OMEPRAZOLE 20mg FOR SIX MONTHS ON *H. PYLORI* NON-ERADICATED PATIENTS

serum gastrin concentrations (median = 93, range 31-286) compared with the patients in whom the infection was not subsequently eradicated (fasting median = 38, range 19-114; meal-stimulated median = 75, range 24-162).

On re-assessment at $^{1}/_{12}$ following failed triple therapy or symptomatic therapy there was no change in fasting or meal-stimulated gastrin concentrations (Fig. 22). In contrast, the patients in whom *H. pylori* was eradicated showed a 36% median fall in fasting gastrin and 48% median fall in meal-stimulated gastrin ($p<0.01$ for each) (Fig. 23).

In the patients who were not eradicated of *H. pylori* their median fasting gastrin at the end of the one month course of 40mg omeprazole was 145% higher than that immediately prior to commencing the proton pump inhibitor ($p < 0.001$) and their meal-stimulated gastrin 43% higher ($p<0.005$) (Fig. 24). In these subjects their median fasting gastrin at the end of 6 months 20mg omeprazole was 78% higher than that immediately prior to omeprazole ($p<0.005$) though their median meal-stimulated gastrin was only 2% higher which was not statistically significant (Fig. 25).

In the patients eradicated of *H. pylori* their median fasting gastrin at the end of the one month course of 40mg omeprazole was 38% higher than that immediately prior to commencing the proton pump inhibitor ($p<0.05$) and their meal stimulated gastrin was 112% higher ($p<0.02$) (Fig. 26). In these subjects their median fasting gastrin at the end of the 6 months of 20mg omeprazole was 31% higher than that immediately prior to starting omeprazole though their meal-stimulated gastrin was only 6% higher which was not statistically significant (Fig. 27).

When taking the gastrin values immediately prior to omeprazole as the starting values the change with respect to both fasting and meal-stimulated

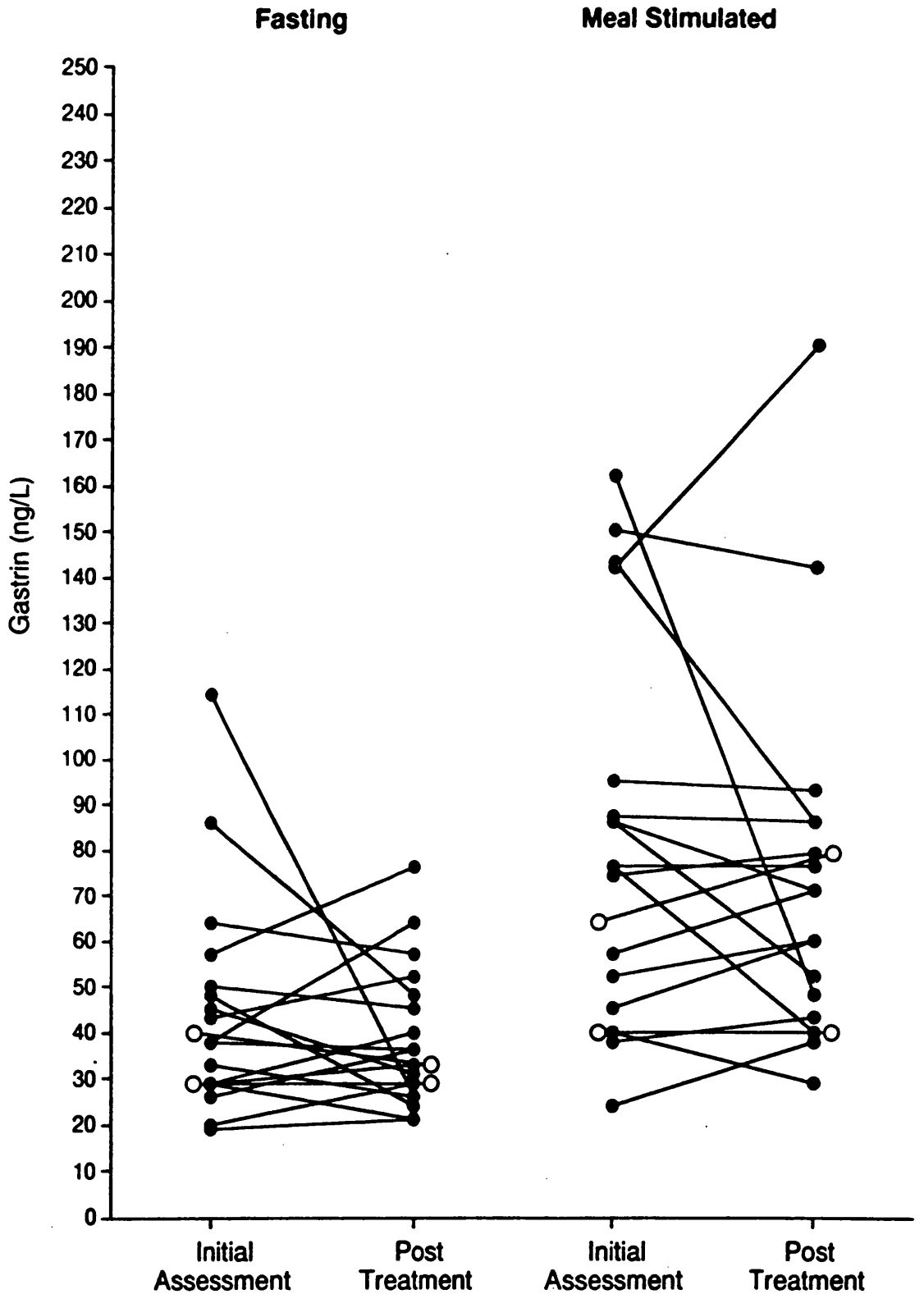

Figure 22

Fasting and meal-stimulated gastrin concentrations on initial assessment and after 6 weeks of symptomatic treatment (•) or failed anti-*H. pylori* therapy (O).

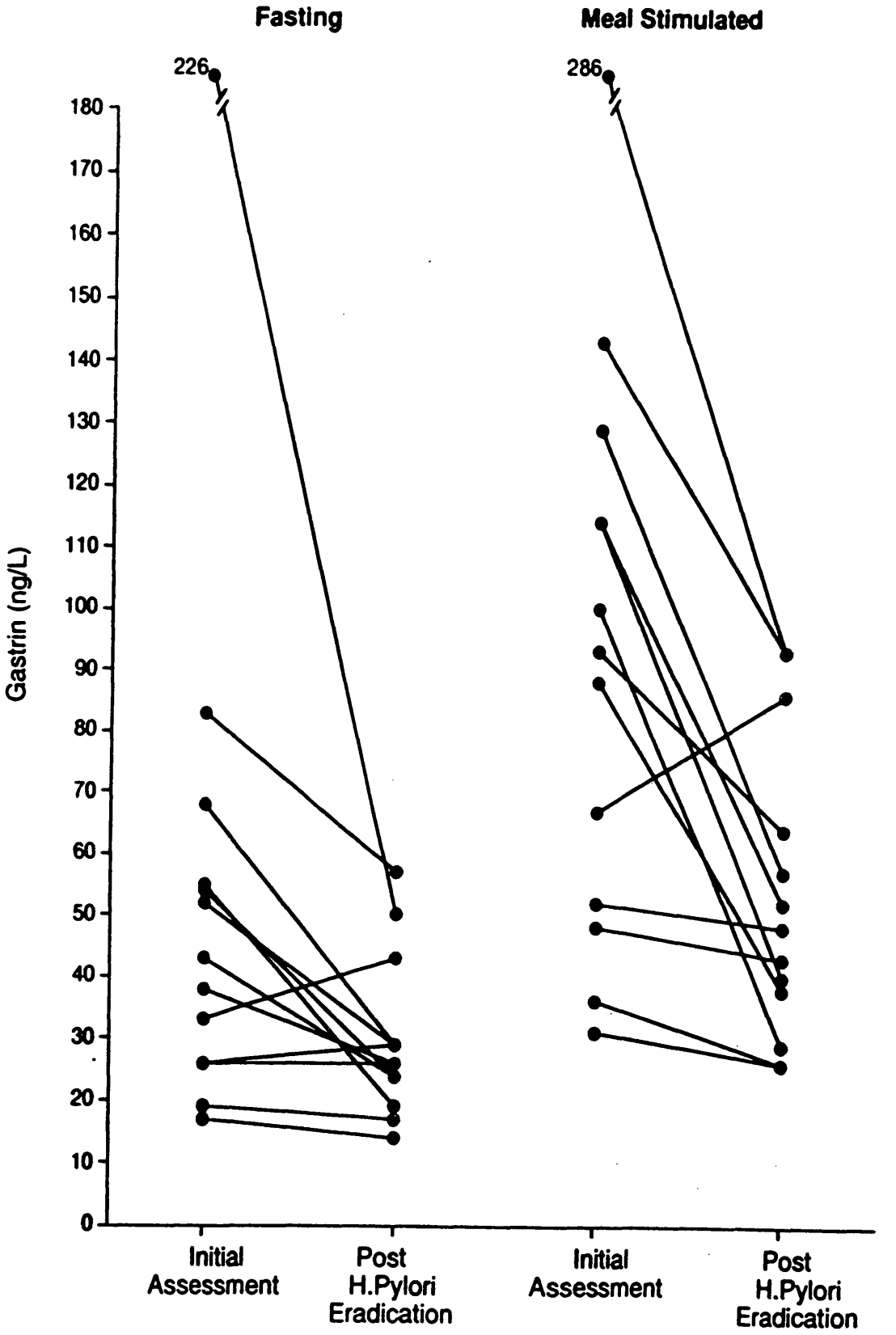

Figure 23

Fasting and meal-stimulated gastrin concentrations on initial assessment and 1 month after completing successful anti-*H. pylori* therapy.

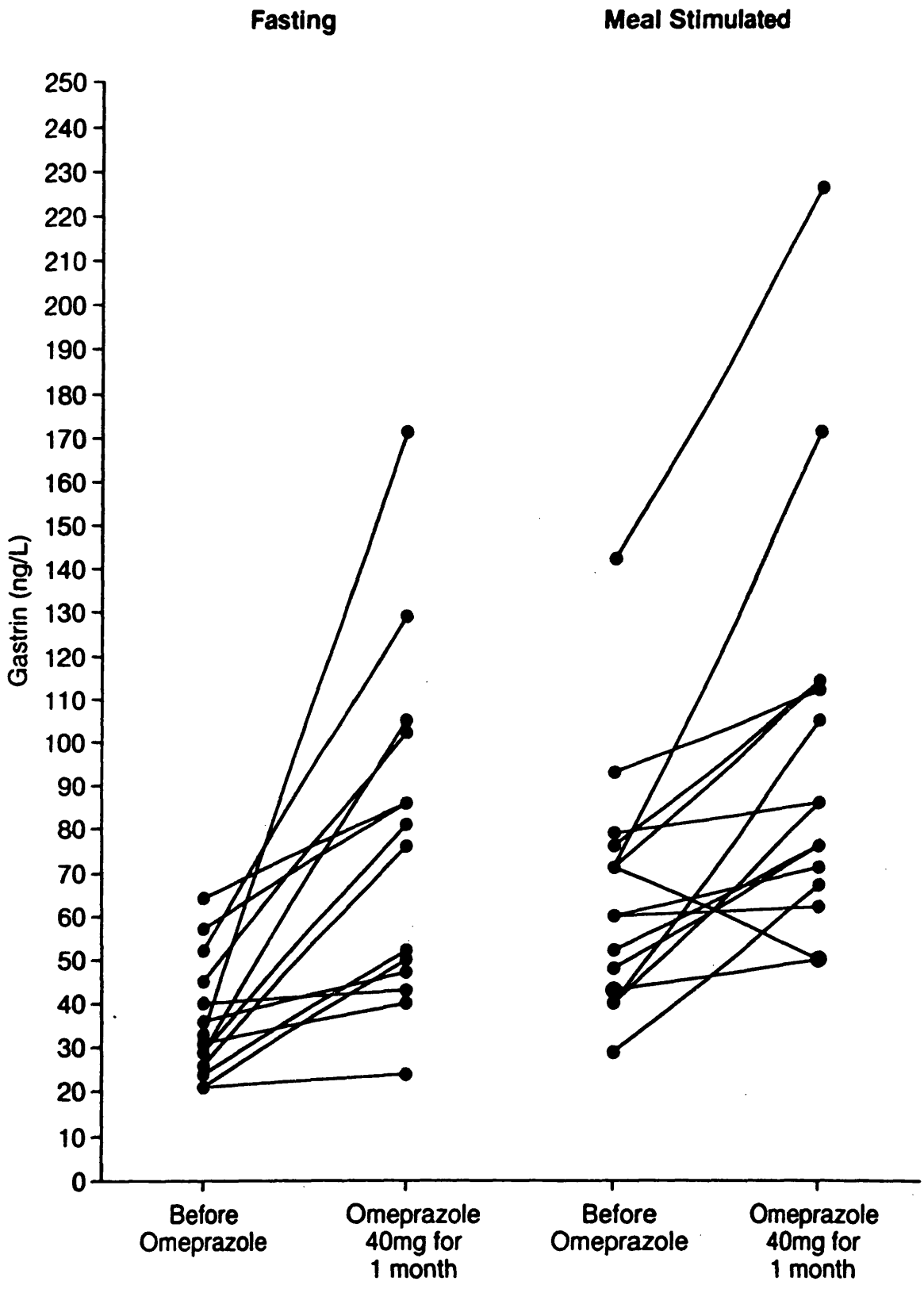

Figure 24

Fasting and meal-stimulated gastrin concentrations before commencing any omeprazole and after 1 month omeprazole 40mg/day in the patients who were not eradicated of their *H. pylori* therapy prior to commencing the proton pump inhibitor.

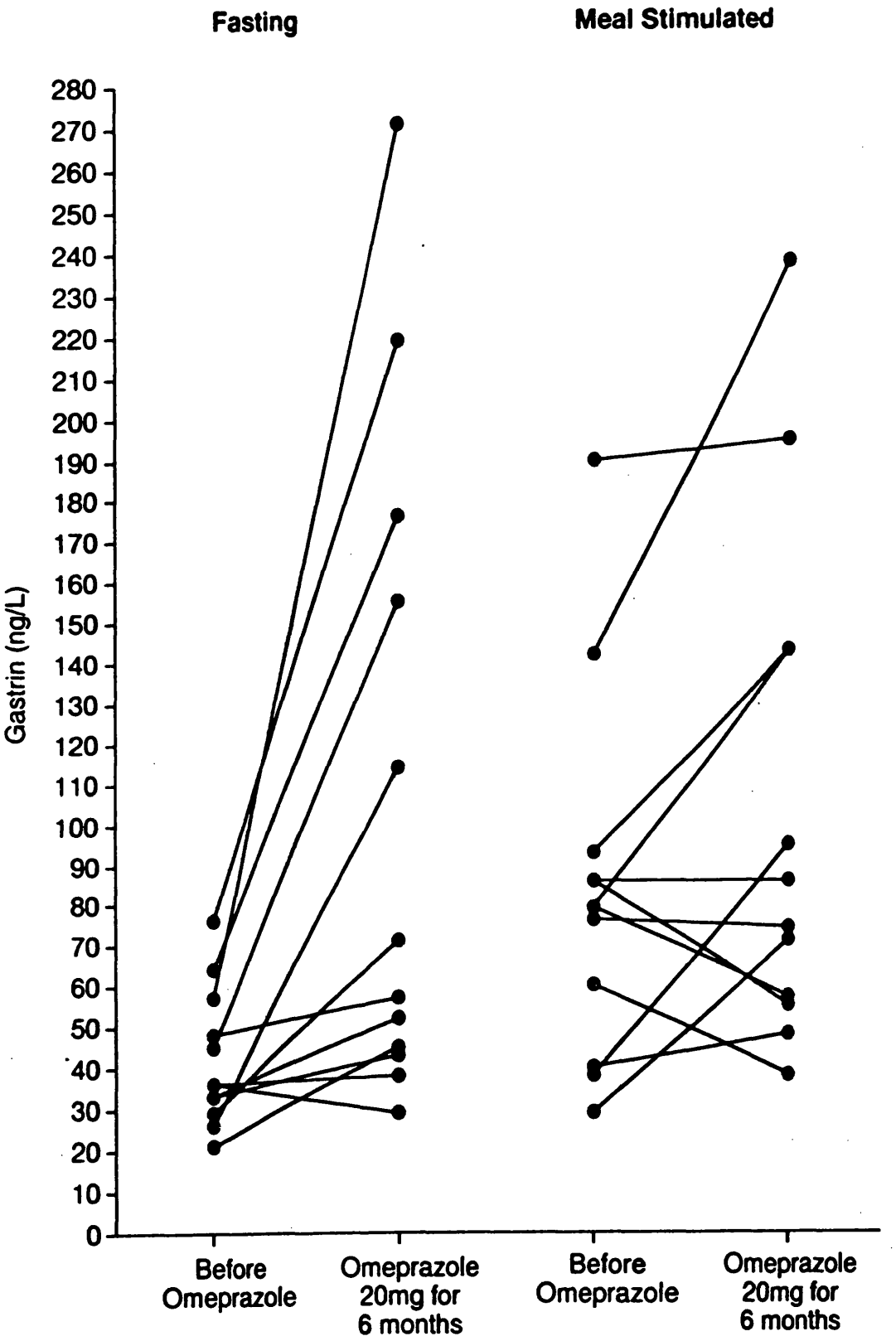

Figure 25

Fasting and meal-stimulated gastrin concentrations before commencing any omeprazole and after 6 months of omeprazole 20mg/day in the patients who were not eradicated of their *H. pylori* infection.

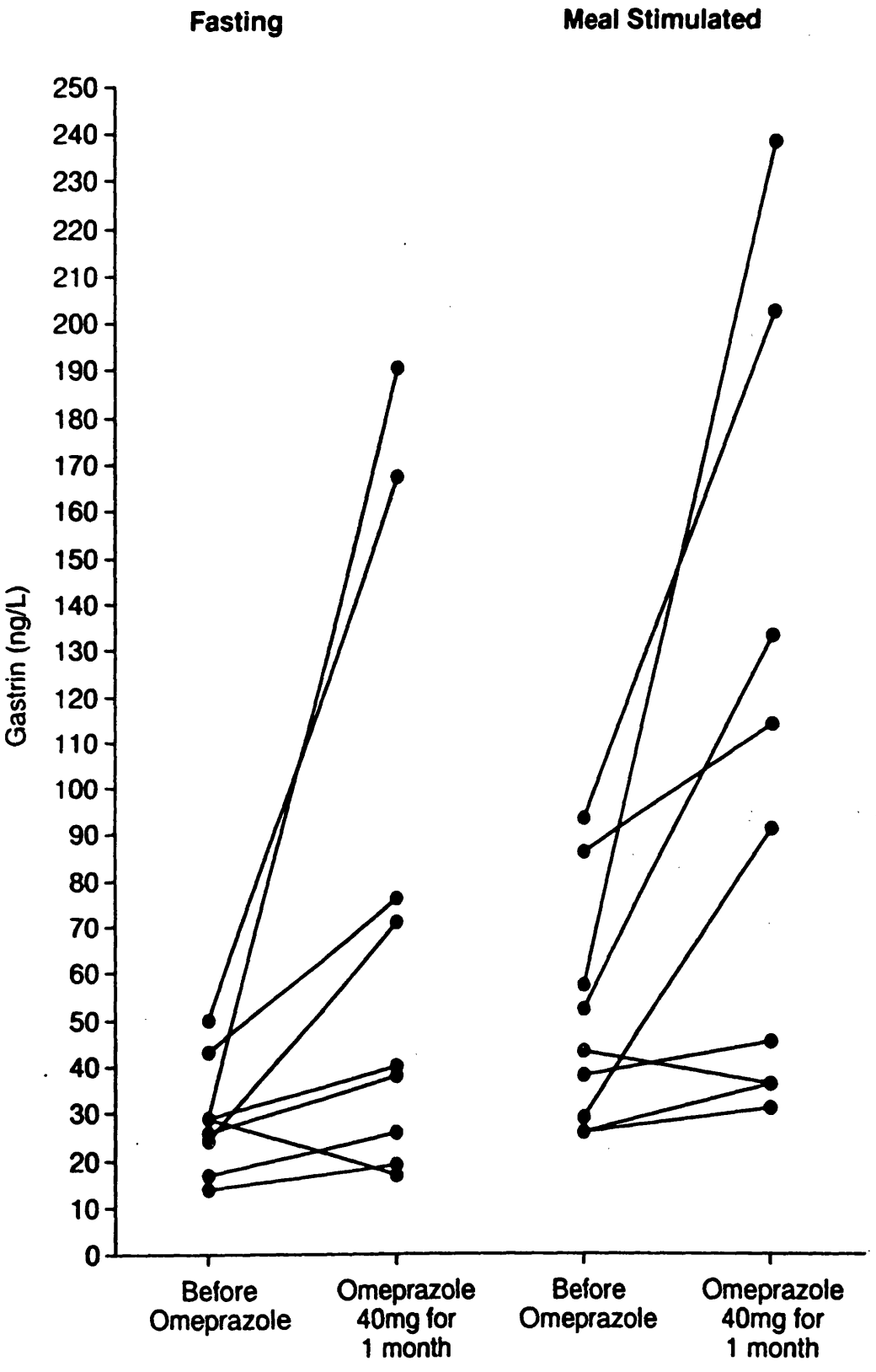

Figure 26

Fasting and meal-stimulated gastrin concentrations before and after 1 month omeprazole 40mg/day in the patients who had been eradicated of their *H. pylori* infection. The *before* gastrin assessment was performed 4 weeks after completing the *H. pylori* eradication therapy.

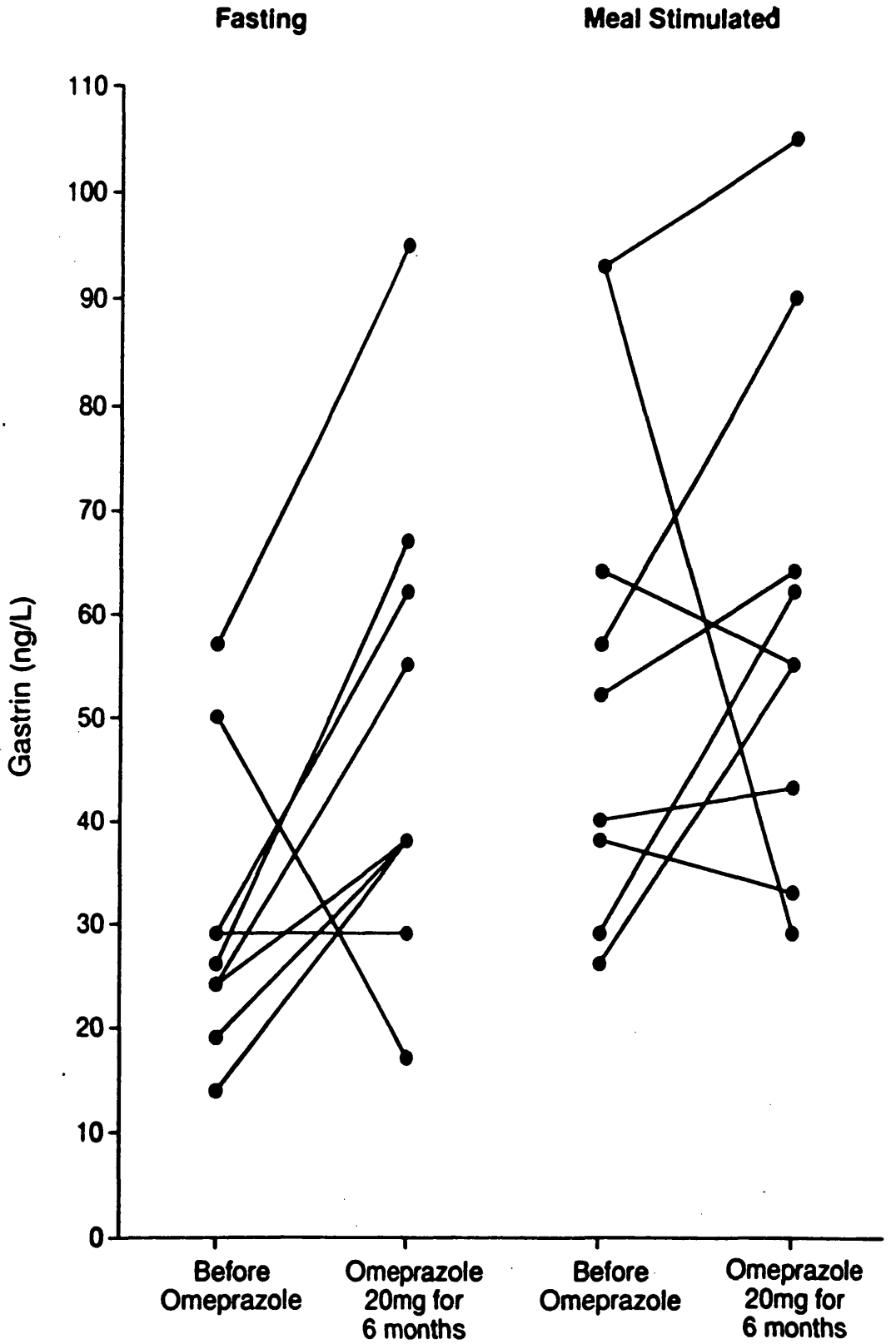

Figure 27

Fasting and meal-stimulated gastrin concentrations before commencing any omeprazole and after 6 months of omeprazole 20mg/day in the patients who

gastrin in response to omeprazole was similar in the *H. pylori* eradicated and *H. pylori* non-eradicated patients.

Compared to their values at the initial assessment the median fasting gastrin concentrations in those not eradicated of *H. pylori* was increased by 100% after 1 month omeprazole 40mg and by 68% after 6 months omeprazole 20mg ($p<0.005$ for both) (Fig. 28). In contrast, in the patients who were eradicated of *H. pylori* pre-omeprazole their fasting gastrin concentrations after both one month 40mg omeprazole and 6 months 20mg omeprazole were similar to those at initial assessment (Fig. 29).

In the patients not eradicated of *H. pylori* their median postprandial gastrin concentration after one month omeprazole 40mg was 16% higher than that on initial presentation ($p<0.05$), but their value after 20mg for 6 months was similar to that on initial presentation (Fig. 30). In the patients eradicated of *H. pylori* pre-omeprazole their median postprandial gastrin concentration after 1 month omeprazole 40mg was similar to that on initial presentation, however their median value after 6 months omeprazole 20mg was 52% lower than on initial presentation (Fig. 31).

9.5.4 ^{14}C UREA BREATH TEST

The results for the ^{14}C-urea breath test in the two groups, *H. pylori* positive and *H. pylori* negative as shown in Figures 32 and 33. The 40mg omeprazole dose lowered ^{14}C-urea breath test value in the *H. pylori* positive subjects and increased it in the *H. pylori* negative subjects, resulting in a loss of statistical difference between the two groups. The effect was less marked on the 20mg omeprazole for 6 months and resolved within four weeks of stopping the proton pump inhibitor. The ^{14}C-urea breath test nearly reversed to the initial value after four weeks without the proton pump inhibitor (Fig. 34).

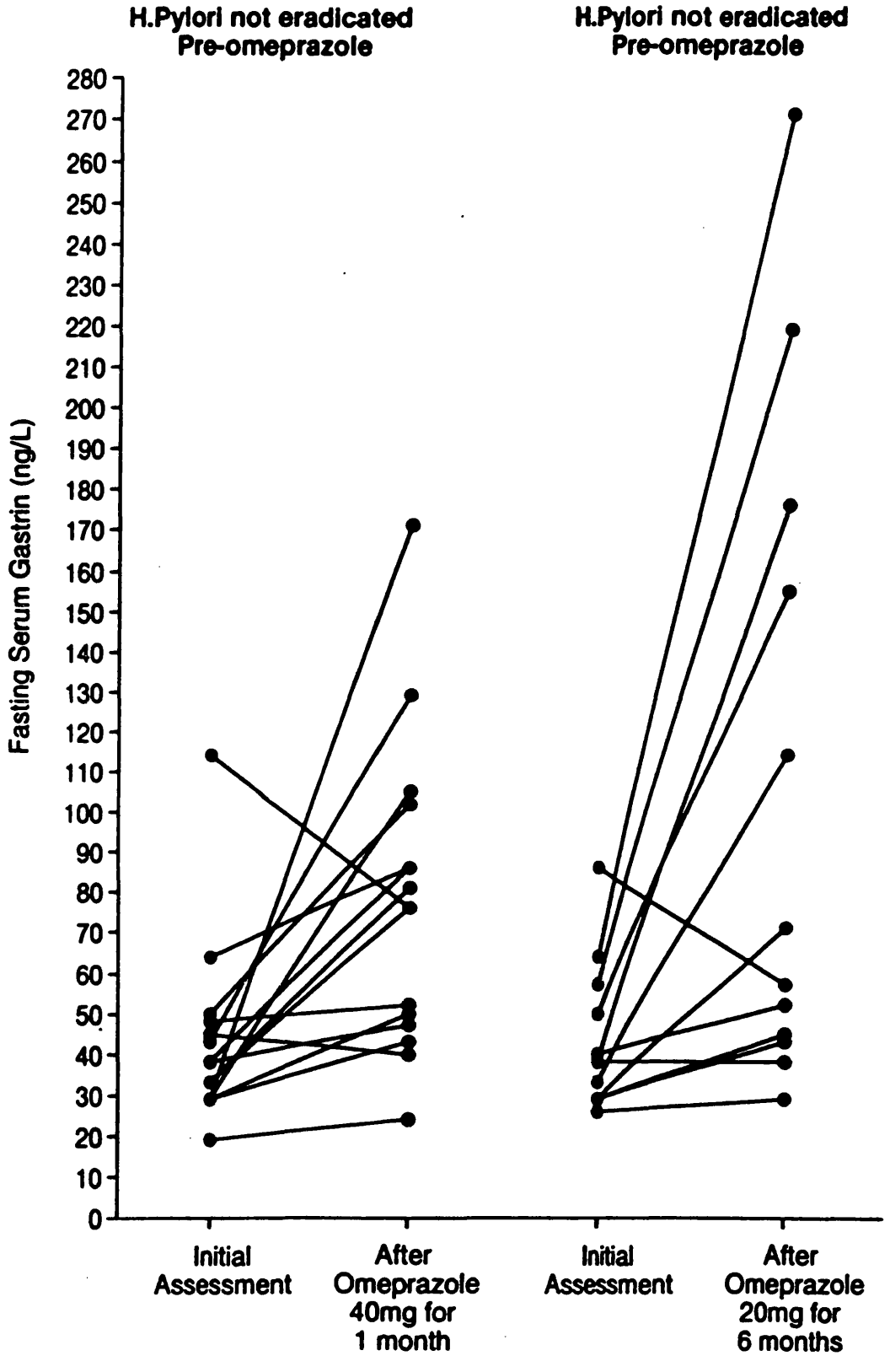

Figure 28

Fasting serum gastrin on initial presentation and after 1 month omeprazole 40mg and 6 months omeprazole 20mg/day in those not eradicated of their *H. pylori* infection prior to commencing the proton pump inhibitor.

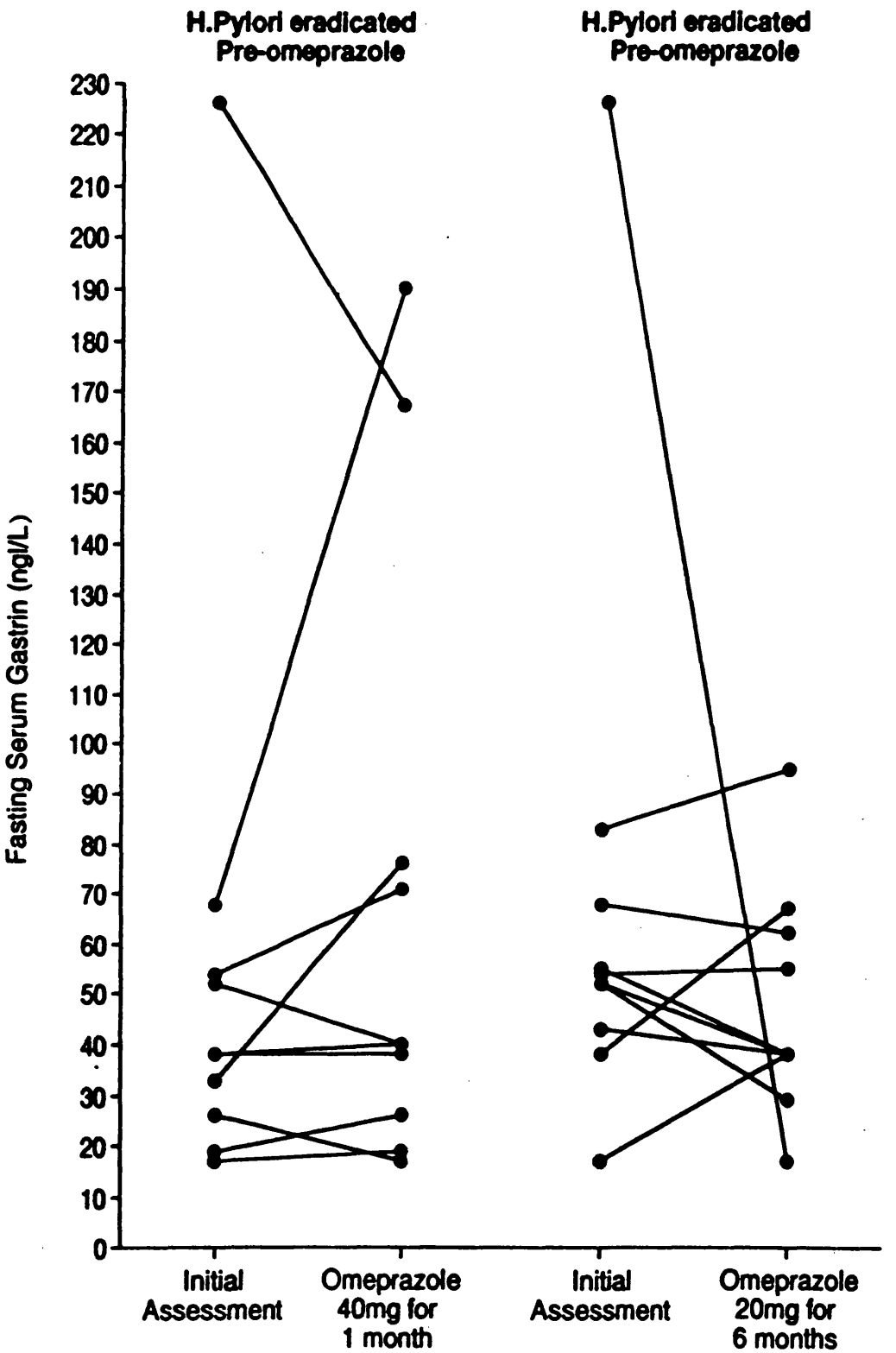

Figure 29

Fasting serum gastrin on initial presentation and after 1 month omeprazole 40g/day 6 months omeprazole 20mg/day in those eradicated of their *H. pylori* prior to commencing the proton pump inhibitor.

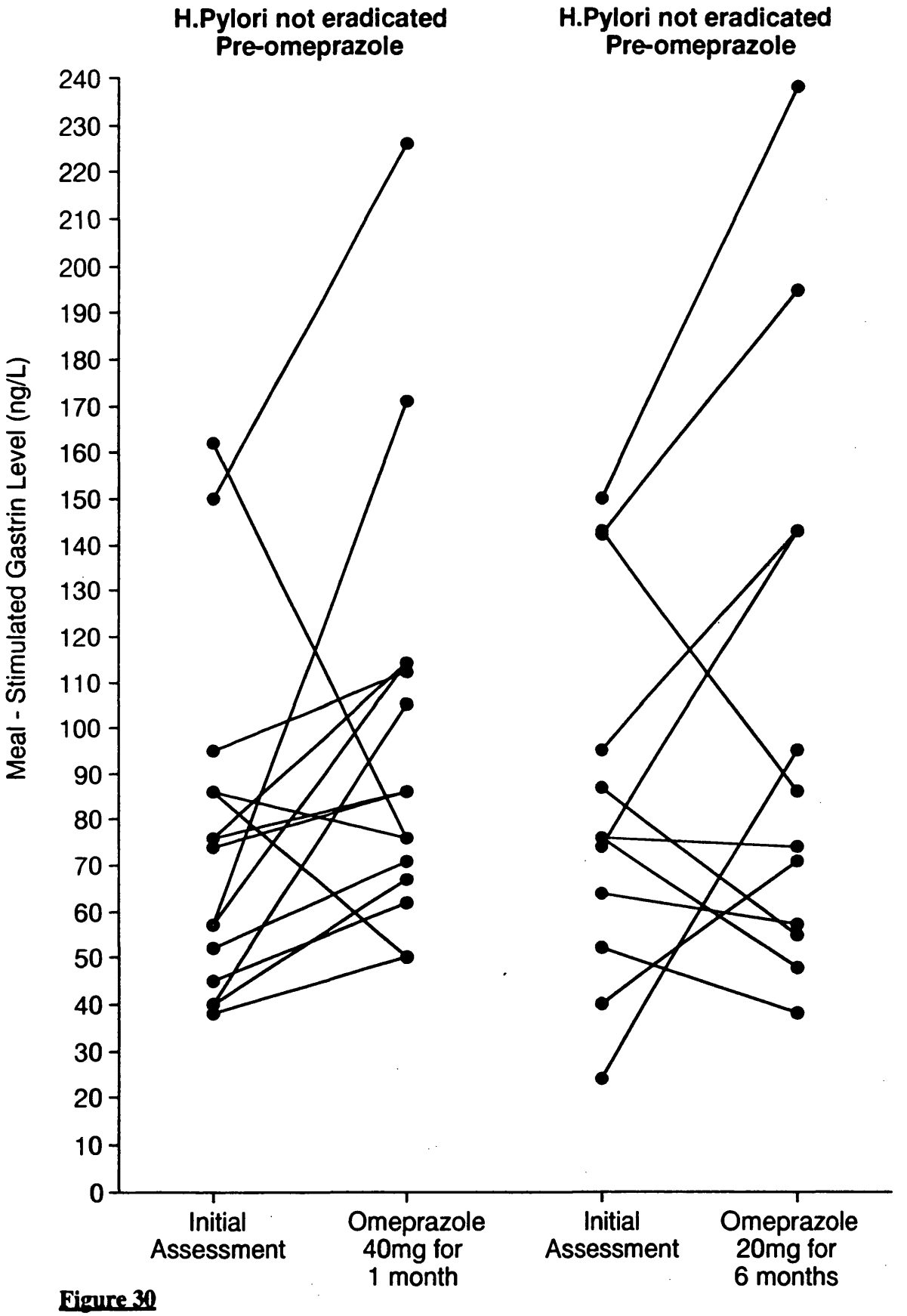

Figure 30

Meal-stimulated gastrin on initial presentation and after 1 month omeprazole 40g/day and 6 months omeprazole 20mg/day in those not eradicated of *H. pylori* prior to commencing the proton pump inhibitor.

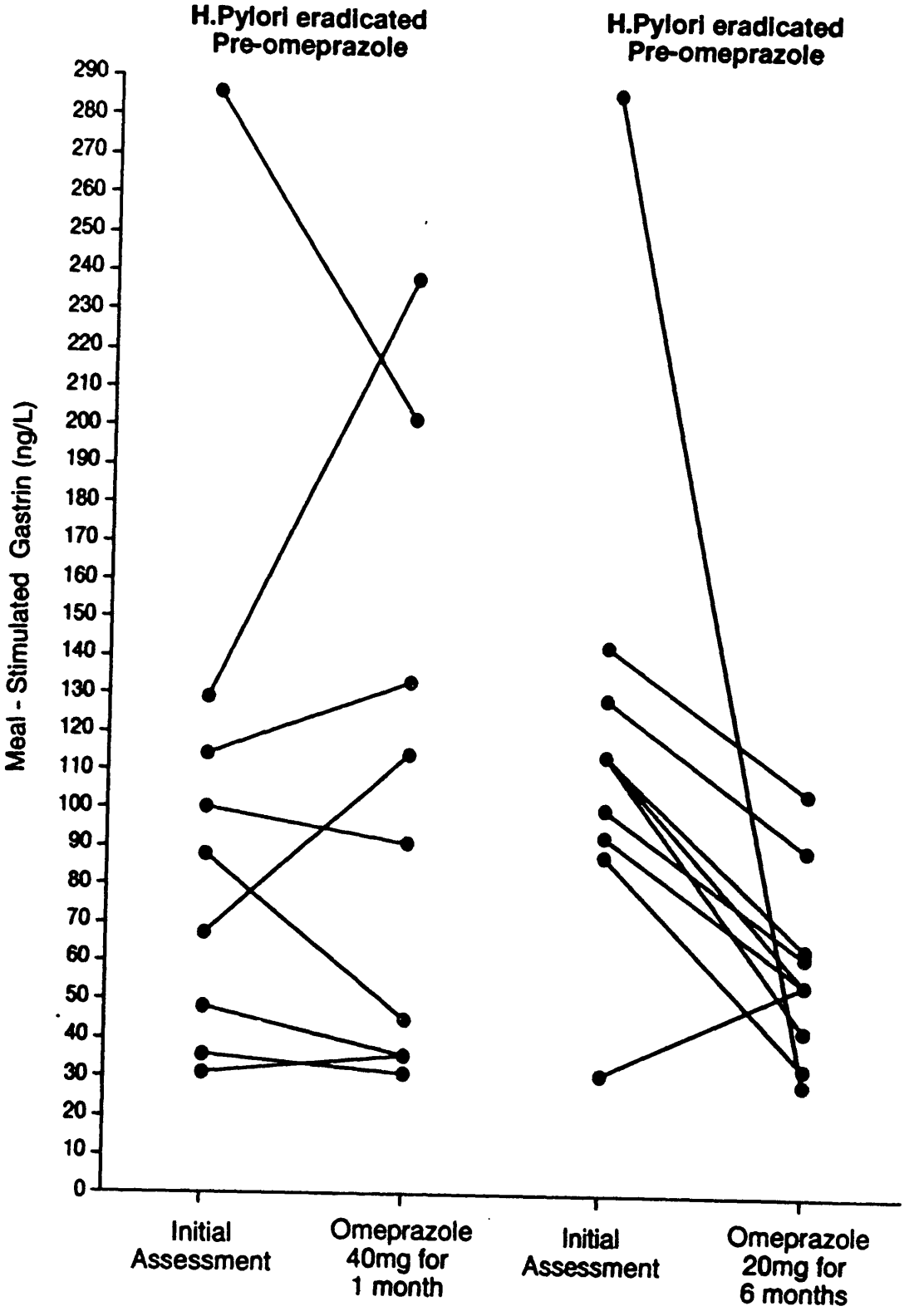

Figure 31

Meal-stimulated gastrin on initial presentation and after 1 month omeprazole 40mg/day and 6 months of omeprazole 20mg/day in those eradicated of *H. pylori* prior to commencing the proton pump inhibitor.

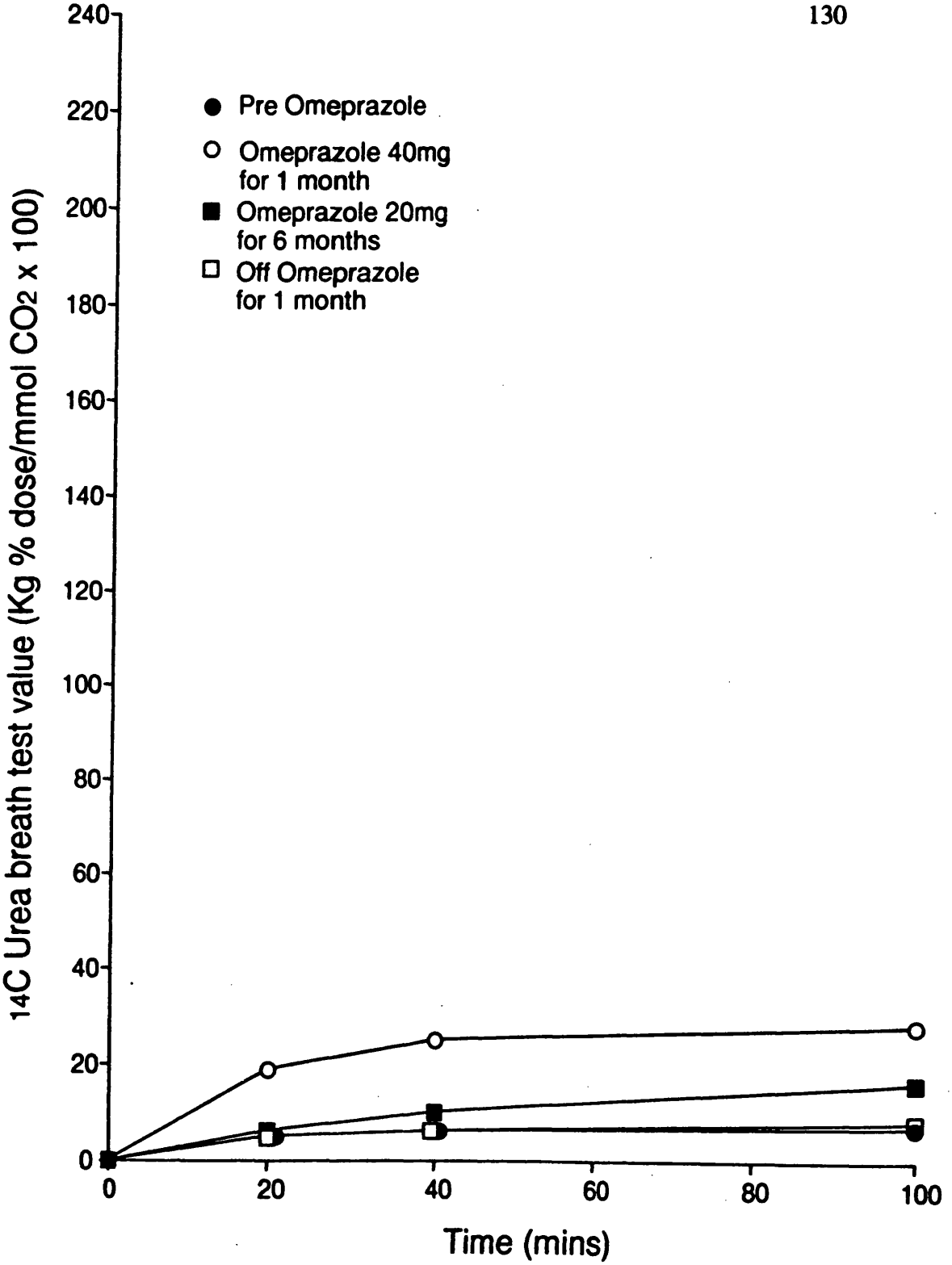

Figure 32

The effect of omeprazole on ^{14}C-urea breath test in eradicated patients with *H. pylori*.

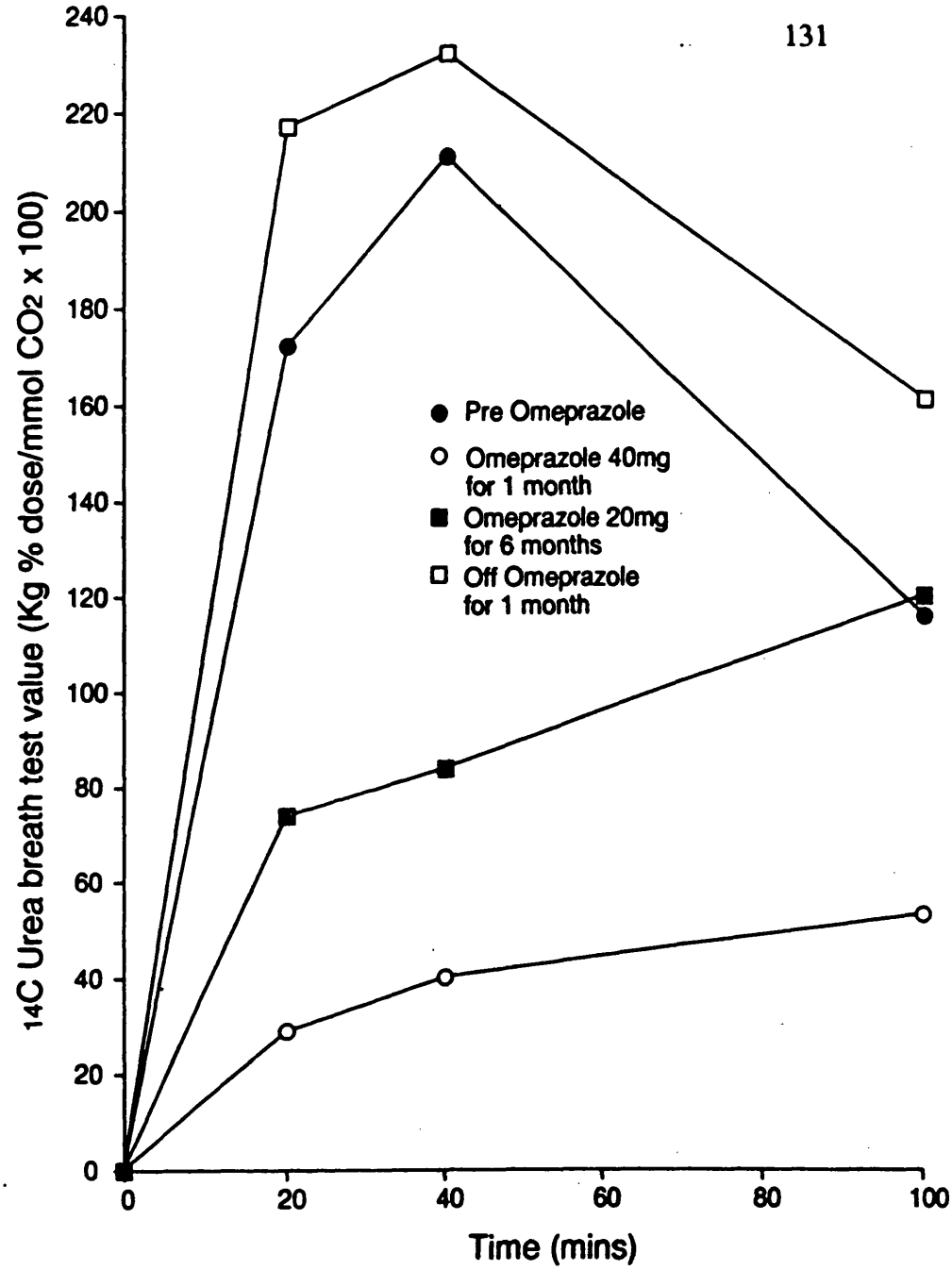

Figure 33

The effect of omeprazole on ^{14}C-urea breath test in the non-eradicated patients with *H. pylori*.

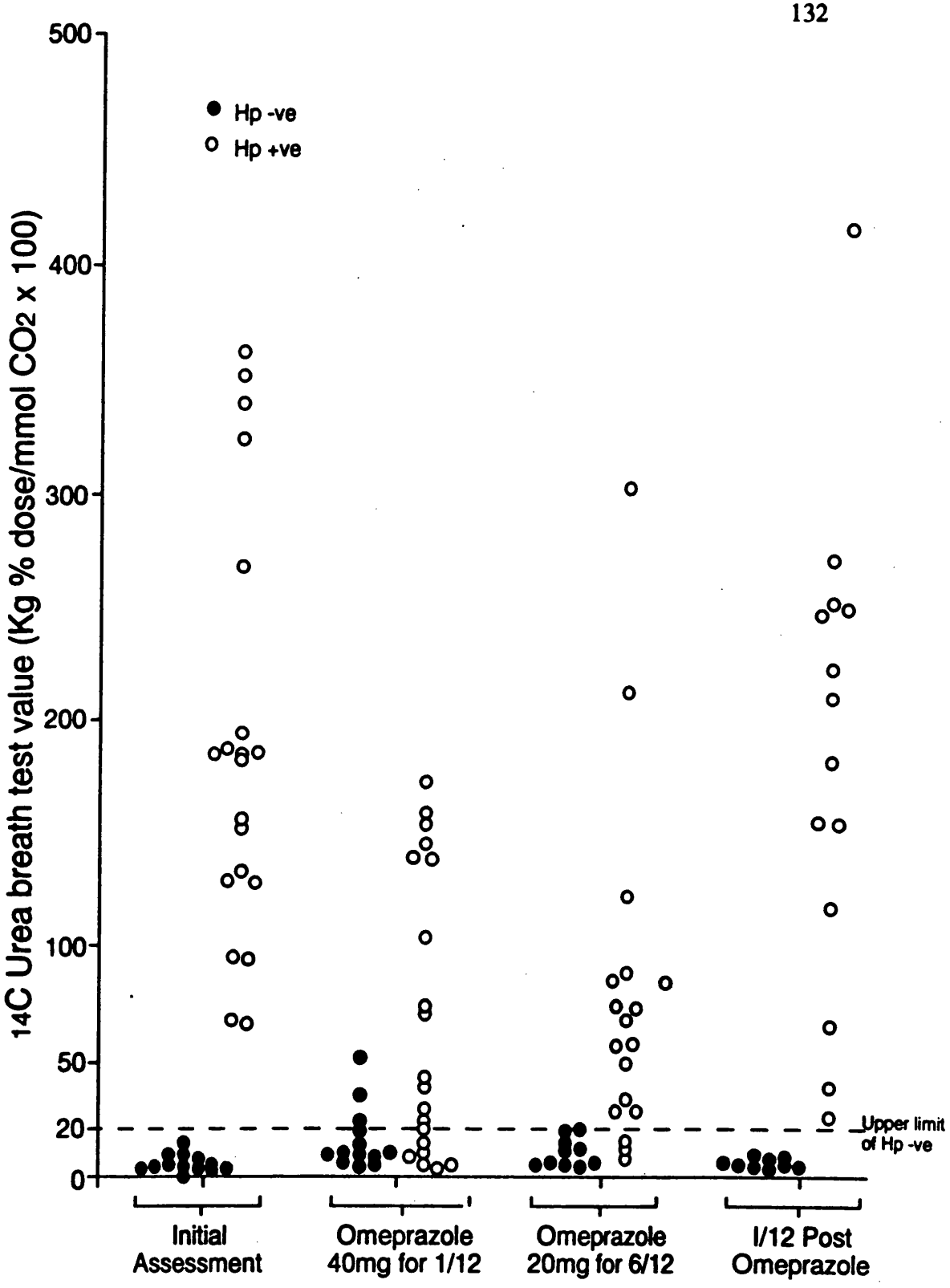

Figure 34

The effect of omeprazole on the ^{14}C-urea breath test (20 mins. value) on the eradicated and non-eradicated patients with *H. pylori*.

9.6 DISCUSSION

Proton pump inhibitor drugs and *H. pylori* infection are both important causes of hypergastrinaemia (Koop et al 1990, Chittajallu et al 1992). Due to the high prevalence of *H. pylori* infection, a considerable proportion of subjects prescribed long term proton pump inhibitor therapy will be *H. pylori* positive and there is therefore interest in the combined effects of the two on serum gastrin. The present study indicates that the percentage increase in gastrin induced by proton pump inhibitors at least as high in *H. pylori* positive compared to negative subjects. Consequently patients who have a higher gastrin value prior to commencing omeprazole to *H. pylori* infection also have a higher gastrin during omeprazole therapy. This observation is consistent with our previous study with 5 days treatment with another proton pump inhibitor pantoprazole (McColl et al 1992). Our findings are also consistent with studies prior to the recognition of *H,. pylori* which noted that subjects with higher pre-treatment gastrin also had higher values on omeprazole (Koop et al 1990, Jansen et al 1990). The present study also demonstrates that eradicating *H. pylori* infection prior to commencing the proton pump inhibitor is an effective means of reducing the hypergastrinaemia on treatment.

Though both *H. pylori* infection and proton pump inhibitor therapy cause hypergastrinaemia, the present studies indicate that they differ in the degree to which they raise fasting and meal-stimulated gastrin concentrations. Eradication of *H. pylori* infection was associated with a more profound fall in meal-stimulated than fasting gastrin concentrations and this is consistent with many previous studies showing that the infection predominantly increases meal-stimulated gastrin concentrations (McColl et al 1991). In contrast, in the present study, the proton pump inhibitor therapy resulted in a greater percentage rise in fasting gastrin

levels than meal-stimulated levels and this was apparent in both *H. pylori* positive and *H. pylori* eradicated subjects. The relative efficacy of omeprazole and *H. pylori* in raising meal-stimulated gastrin levels explains how the combination of omeprazole + *H. pylori* eradication actually lowered meal-stimulated gastrin, the *H. pylori* eradication gastrin-lowering effect being greater than the omeprazole gastrin-elevating effect. The reason for omeprazole having a more marked effect on fasting than meal-stimulated gastrin may be explained by the difference in fasting and fed intragastric pH. Fasting pH is usually considerably less than 3 and at this intragastric acidity gastrin release is inhibited (Penston et al 1990). Omeprazole usually raises fasting pH above pH4 thus disinhibiting gastrin release (Lind et al 1991). Following a meal intragastric pH may already be above pH4 due to the buffering effect of the food and thus gastrin release will already be disinhibited. Consequently, any additional increase in pH due to omeprazole may have relatively little effect on the post meal gastrin level.

The resultant effect on gastrin of proton pump inhibitor therapy plus *H. pylori* infection will depend upon the mechanism by which each independently produces hypergastrinaemia. As stated above, the former is thought to cause hypergastrinaemia by removing the inhibition of gastrin release exerted by low intragastric pH (Banerjee et al 1995, Lind et al 1991). Hypergastrinaemia associated with *H. pylori* infection is thought to be secondary to depletion of antral somatostatin which exerts paracrine inhibitory control on gastrin release by the antral G cells (Odum et al 1994, Kaneko et al 1992, Moss et al 1992). Somatostatin is largely responsible for mediating the inhibition of gastrin release exerted by low intragastric acid pH (Lucey et al 1989) and impaired inhibition of gastrin release at low pH occurs in the presence of *H. pylori* infection (Tarnasky et al 1993). Thus, both *H. pylori* infection and acid inhibitory therapy are

thought to cause hypergastrinaemia by disrupting the inhibition of gastrin release normally exerted by gastric acid. If that is the case, then one might expect that acid inhibitory therapy would result in a greater percentage increase in gastrin in *H. pylori* negative subjects who start with their gastrin normally suppressed by acid than in *H. pylori* positive subjects in whom the infection has already disinhibited gastrin release. However, in our present study we found that the percentage increase in gastrin during omeprazole therapy was at least as great in the presence versus absence of *H. pylori* infection. This disparity may be explained by additional factors influencing the combined effect of *H. pylori* plus omeprazole on serum gastrin.

The first of these additional factors is the recent observation that the degree of elevation of intragastric pH caused by omeprazole is dependent upon *H. pylori* status (Verdu et al 1995, Verdu et al 1995). Verdu et al observed that omeprazole 20mg/day raised daytime pH from 1.3 to 5.4 in *H. pylori* positive subjects but from 1.4 to only 2.9 in *H. pylori* negative subjects. Likewise, it raised nighttime pH from 1.1 to 4.7 in *H. pylori* positive subjects, but from 1.1 to only 1.9 in *H. pylori* negative subjects. The mechanism by which *H. pylori* infection accentuates the antacid effects of omeprazole is unclear. However, it is known that there is an increase in severity of corpus gastritis (Logan et al 1995) in *H. pylori* subjects during omeprazole therapy and this may impair the acid secreting capacity of the oxyntic mucosa. Whatever its mechanism, this accentuated pH elevation produced by omeprazole in *H. pylori* positive subjects will result in more complete disinhibitor of gastrin release.

Recent studies indicate that there is progression of corpus atrophy during long term proton pump inhibitor therapy in *H. pylori* positive subjects which does not occur in uninfected subjects (Kuipers et al 1994, Kuipers et al 1995). The development of atrophy during longer term

omeprazole treatment will further reduce intragastric acidity and consequently further increase gastrin levels in *H. pylori* positive subjects.

Another factor influencing the combined effect of omeprazole and *H. pylori* on gastrin is the observation that omeprazole treatment reduces the density of the infection present in the gastrin-producing antral region of the stomach (Logan et al 1995, Weil et al 1991, Hui et al 1991). In the present study, *H. pylori* infection was cleared from the antral mucosa in 33% of the infected subjects. This suppression of *H. pylori* in the antral region by omeprazole will tend to reduce hypergastrinaemia by reducing the *H. pylori* induced hypergastrinaemia. This may explain the variations in degree of hypergastrinaemia seen in *H. pylori* positive subjects during omeprazole.

Though the interacting mechanisms involved in the combined effects of *H. pylori* and omeprazole on gastrin are complex, the present study clearly shows that eradicating *H. pylori* effectively reduces gastrin levels during subsequent omeprazole therapy. In view of the uncertainty about some of the possible longterm sequelae of hypergastrinaemia in man, it may be appropriate to screen for *H. pylori* prior to commencing longterm proton pump inhibitory therapy and eradicate it when present. A similar policy has recently been advocated in order to prevent the development of corpus atrophy during longterm proton pump inhibitory therapy (Kuipers et al 1994; Kuipers et al 1995). Whether such a policy will also reduce the clinical efficacy of the proton pump inhibitor due to reducing its pH elevating effect is unknown.

CHAPTER TEN

SUMMARY AND CONCLUSIONS

In this thesis we have studied the effect of *H. pylori* infection on serum gastrin and the possible role of bacterial ammonia production producing hypergastrinaemia. We have also studied the interacting effects of *H. pylori* infection and proton pump inhibitor therapy on serum gastrin concentrations.

In Chapter 4 it was noted that *H. pylori* infection increased both basal and meal-stimulated serum gastrin concentrations. Eradication of the infection produced resolution of the hypergastrinaemia. The increased serum gastrin concentrations associated with *H. pylori* infection in the D.U. patient was accompanied by a less marked rise in intragastric pH in response to the buffering effect of a meal. This is consistent with the hypergastrinaemia producing increased gastric acid secretion in response to the meal. However, it was appreciated that the measurement of intragastric pH was a relatively insensitive means of measuring changes in acid secretion due to *H. pylori* induced hypergastrinaemia and tests of actual gastric acid secretion would be more appropriate.

We then proceeded to investigate the mechanism of the hypergastrinaemia induced by *H. pylori* infection. A possible mechanism was that the ammonia produced by *H. pylori* urease activity was increasing antral surface pH and thus inhibiting the physiological suppression of gastrin release exerted by low intragastric pH. We approached this hypothesis by first of all examining in Chapter 5 the effect of *H. pylori* on gastric juice, urea and ammonia concentrations. This showed a marked lowering of urea concentration and marked elevation of ammonia concentrations in subjects with *H. pylori* infection compared with uninfected controls. This work also suggested that the measurement of the ratio of urea to ammonia in gastric juice might provide a rapid means of diagnosing the presence of the infection during the endoscopic examination.

We then proceeded to investigate the relationship of *H. pylori* ammonia production and serum gastrin. In Chapter 6, we examined patients with uraemia due to chronic renal failure. Such patients with *H. pylori* infection had markedly increased gastric juice ammonia concentrations compared to uninfected uraemic patients. This allowed us to determine whether very high levels of ammonia production in these patients was associated with more marked hypergastrinaemia. However, we were unable to show any association between the degree of *H. pylori* ammonia production and the degree of hypergastrinaemia.

We proceeded to further investigate the relationship between *H. pylori* ammonia production and serum gastrin in Chapter 7 by inhibiting *H. pylori* ammonia production and determining whether this produced a fall in serum gastrin. This was done using the urease inhibitor acetohydroxamic acid. The use of this drug markedly inhibited *H. pylori* ammonia production but this was not associated with any change in serum gastrin.

We then proceeded to further test the hypothesis that *H. pylori* was raising serum gastrin as a result of the bacterium elevating antral surface pH. If this hypothesis were true, then elevation of intragastric pH by proton pump inhibitor therapy should produce a smaller percentage increase in serum gastrin in *H. pylori* positive subjects than in *H. pylori* negative subjects. This would be the case because *H. pylori* infection would already have interrupted the inhibition of gastrin due to intragastric acid and therefore removing intragastric acid would have little effect. In contrast, in those without *H. pylori* infection, their gastrin would be suppressed by the intragastric acid and removing it by proton pump inhibitory therapy would therefore cause a significant rise in gastrin. We initially tested this hypothesis in Chapter 8 using one weeks treatment with a proton pump inhibitor and found that the rise in gastrin was similar in those with and without *H. pylori* infection.

We then proceeded to a longer term study in Chapter 9 in which we examined the effect of proton pump inhibitors on serum gastrin in patients with *H. pylori* infection and another group of patients in whom we have rendered *H. pylori* negative by eradication therapy. We studied serum gastrin in these patients at initial presentation after treatment with eradication therapy or placebo, and then after treatment with the proton pump inhibitor for one month and for six months. We found that the percentage rise in serum gastrin during the treatment with the omeprazole was similar in those who are *H. pylori* positive compared to those who are *H. pylori* eradicated. This again failed to provide support for the hypergastrinaemia being due to *H. pylori* induced elevation of antral surface pH.

One clinically relevant observation from the above study is that eradication of *H. pylori* infection is an effective means of reducing the degree of hypergastrinaemia induced by long term proton pump inhibitor therapy. There is concern that hypergastrinaemia on such therapy may lead to ECL-cell hyperplasia and even carcinoid tumours and consequently the ability to limit the hypergastrinaemia by eradicating *H. pylori* infection seems indicated. We would therefore recommend that patients requiring long term proton pump inhibitory therapy should be tested for *H. pylori* infection and have the infection eradicated if present.

Overall studies have found little support for the hypothesis that *H. pylori* induced hypergastrinaemia is related to bacterial ammonia production. Other mechanisms need to be considered including the effect of cytokines and other inflammatory mediators induced by the bacterial infection.

In the course of these studies we have made two observations with important clinical implications. The first is the finding that eradicating *H. pylori* infection is effective in lowering serum gastrin concentration and, in particular, in reducing the marked hypergastrinaemia associated with long term proton pump inhibitor therapy. In view of the fact that hypergastrinaemia induces ECL cell

hyperplasia, it would seem appropriate to eradicate *H. pylori* prior to long-term proton pump inhibitor therapy. The second important observation is that omeprazole markedly interferes with the accuracy of most *H. pylori* diagnostic tests. This is due to temporary suppression of the infection without true eradication. An increasing number of dyspeptic patients are receiving omeprazole and this will be confusing the results of their *H. pylori* tests.

LIST OF REFERENCES

Ala-Kailan, A., Kekki, M., Paronen, I. and Poakkalo, T.
Serum gastrin in chronic renal failure: its relation to acid secretion, G-cell density and upper gastrointestinal findings.
Scand. J. Gastroenterol., 1989; 24: 939-948.

Ardill, J.E.S.
The measurement of gastrin by radioimmunoassay.
Queen's University, Belfast, [PhD Thesis], 1973.

Axon, A.T.R.
Campylobacter pylori - therapy review.
Scand. J. Gastroenterol., 1989; 24 (suppl 160): 35-38.

Balas, D., Senagas-Balab, F., Pradayrol, L., Vayssette, J., Bertrand, C., Ribet, A.
Long term comparative effect of cholecystokinin and gastrin on mouse stomach antrum, intestine and exocrine pancreas.
Am. J. Anat., 1985; 174; 27-43.

Banerjee, S., Ardill, J.E.S., Beattie, A.D. and McColl, K.E.L.
Effect of omeprazole and feeding on plasma gastrin in patients with achlorhydria.
Aliment. Pharm. Ther., 1995; 9: 507-512.

Barros D'Sa, A.A.J., Bloom, S.R., Baron, J.H.
Direct inhibition of gastric acid by growth hormone in dogs.
Lancet, I: 1975; 886-887.

Becker, H.D., Reeder, D.D. and Scurry, M.T.
Inhibition of gastrin release and gastric secretion by calcitonin in patients with peptic ulcer.
American Journal of Surgery, 1974; 127: 71-75.

Becx, M.C.J.M., Janssen, A.J.H.M., Clasener, H.A.L. and De Koning, R.W.
Metronidazole-resistant *Helicobacter pylori.*
Lancet, March 3rd 1990; 335: 539-540.

Bell, G.D. and Weil, J.
Detection of *Helicobacter pylori* by the ^{14}C-urea breath test.
Helicobacter pylori and gastroduodenal disease.
Edited by B.J. Rathbone and R.V. Heatley, 2nd edition, 1992; 74-87.

Bell, G.D., Weil, J., Harrison, G., Morden, A., Jones, P.H., Gant, P.W., Trowell, J.E., Yoong, A.K., Daneshmend, T.K. and Logan, R.F.A.
^{14}C-urea breath analysis, a non-invasive test for *Campylobacter pylori* in the stomach.
Lancet, 1987; 1367-1368.

Berlin, R.G.
Omeprazole, gastrin and gastric endocrine cell data from clinical studies.
Dig. Dis. Sci., 1991; 36: 129-136.

Berson, S.A. and Yalow, R.S.
Radioimmunoassay in gastroenterology.
Gastroenterology, 1972; 62: 1061-1084.

Berson, S.A. and Yalow, R.S.
Nature of immunoreactive gastrin extracted from tissues of gastrointestinal tract.
Gastroenterology, 1971; 60: 215-222.

Betton, G.R., dormer, C.S., Wells, T., Pert, P., Price, C.A. and Buckley, P.
Gastric ECL-cell hyperplasia and carcinoids in rodents following chronic administration of H_2-antagonists SK&F 93479 and oxmetidine and omeprazole.
Toxicol Pathol, 1988; 16: 288-298.

Biasco, G., Miglioli, M., Barbara, L., Corinaldesi, R. and Di Febo, G.
Omeprazole, *Helicobacter pylori*, gastritis and duodenal ulcer.
Lancet, December 1989; 1403.

Bode, G., Malfertheiner, P., Nilius, M., Lehnhardt, G. and Ditschuneit, H.
Ultrastructural localization of urease in outer membrane and periplasm of *Campylobacter pylori*.
J. Clin. Pathol., 1989; 42: 778-779.

Borody, T.J. George, L.L., Brandl, S., Andrews, P., Ostapowicz, N., Hyland, L. and Devine, M.
Helicobacter pylori-negative duodenal ulcer.
Am. J. Gastroenterol., 1991; 86: No. 9: 1154-1157.

Borody, T.J., Brandly, S., Andrews, P., Jankiewicz, E. and Ostapowicz, N.
Helicobacter pylori-negative gastric ulcer.
Am. J. Gastroenterol., 1992; 87: No. 10: 1403-1406.

Borody, T., Andrews, P., Mancuso, N., Jankiewicz, E. and Brandi, S.
Helicobacter pylori reinfection 4 years post-eradication.
Lancet, 1992; 339: 1295.

Borody, T.J., Cole, P., Noonan, S. et al.
Recurrence of duodenal ulcer and *(Campylobacter) Helicobacter pylori* infection after eradication.
Med. J. Aust., 1989; 151: 431-435.

Bowden, G.H.W., Ellwood, D.S. and Hamilton, I.R.
Microbial ecology of the oral cavity.
In: Advances in Microbial Ecology, Vol. 3.
M. Alexander, Ed. Plenum Press, New York, 1979; 135-192.

Bugnoli, M., Bayeli, P.F., Rappuoli, R., Pennatini, C., Figura, N., Crabtree, J.E.
Inhibition of *Helicobacter pylori* urease by omeprazole.
Eur. J. Gastroenterol. & Hepatol., 1993; 5: 683-685.

Bumm, R. and Blum, A.L.
Lessons from prolonged gastric pH monitoring.
Aliment. Pharmacol. Therap., 1987; 1: 518S-526S.

Burnett, R.A., Brown, I.L. and Findlay, J.
Cresyl fast violent staining method for *campylobacter*-like organisms.
J. Clin. Path., 1987; 40: 353.

Canese, M.G., Bussolati, G.
Immuno-electron-cytochemical localization of the somatostatin cells in the human antral mucosa.
J. Histochem. Cytochem., 1977; 25: 1111-1118.

Capella, C., Finzi, G., Cornaggia, M. et al.
Ultrastructural typing of gastric endocrine cells.
In: Håkanson, R. and Sundler F. (eds).
The Stomach as an Endocrine Organ, 1991; 27-51, Amsterdam, Elsevier.

Capella, C., Vassallo, G. and Solcia, E.
Light and electron microscopic identification of the histamine-storing argyrophil (ECL) cell in murine stomach and of its equivalent in other mammals.
Zeitachrift fur Zelforschung, 1971; 118: 68-84.

Chen, M.C., Lee, A., Hazell, S.
Immunisation against gastric infection with *Helicobacter* species: First step in the prophylaxis of gastric cancer.
Inf. J. Med. Microbiol. Virolo. Parasitol. & Inf. Dis., 1993; 280: 102.

Chen, M.C., Amirian, D.A., Toomey, M., Sanders, M.J. and Soll, A.H.
Prostanoid inhibition of canine parietal cells: mediation by the inhibitory guanosine triphosphate-binding protein of adenylate cyclase.
Gastroenterology, 1988; 94: 1121-1129.

Chiba, T., Kinoshita, Y.
Interaction between D cells and G cells.
In: J.H. Walsh, (ed).
Gastrin, Raven Press Ltd., New York, 1993; 115-127.

Chittajallu, R.S., Ardill, J.E.S. and McColl, K.E.L.
The degree of hypergastrinaemia induced by *Helicobacter pylori* is the same in duodenal ulcer patients and asymptomatic volunteers.
Eur. J. Gastroenterol., & Hepatol., 1992; 4: 49-53.

Chittajallu, R.S., Neithercut, W.D., Ardill, J.E.S. and McColl, K.E.L.
Helicobacter pylori-related hypergastrinaemia is not due to elevated antral surface pH. Studies with antral alkalinisation.
Scand. J. Gastroenterol., 1992; 27: 218-222.

Chittajallu, R.S., Dorrian, C.A., Ardill, J.E.S. and McColl, K.E.L.
Effect of *Helicobacter pylori* on serum pepsinogen I and plasma gastrin in duodenal ulcer patients.
Scand. J. Gastroenterol., 1992; 27: 20-25.

Chittajallu, R.S., Dorrian, C.A., Neithercut, W.D., Dahill, S. and McColl, K.E.L.
Is *Helicobacter pylori* associated hypergastrinaemia due to the bacterium's urease activity or the antral gastritis?
Gut, 1991; 32: 1286-1290.

Chittajallu, R.S., Neithercut, W.D., Macdonald, A.M.I. and McColl, K.E.L.
Effect of increasing *Helicobacter pylori* urease activity by acetohydroxamic acid on serum gastrin in duodenal ulcer subjects.
Gut, 1991; 32: 21-24.

Chittajallu, R.S., Ardill, J.E. and McColl, K.E.L.
The degree of hypergastrinaemia induced by *Helicobacter pylori* is the same in duodenal ulcer patients and asymptomatic volunteers.
Eur. J. Gastroenterol. Hepatol., 1992; 4: 49-53.

Chittajallu, R.S., Neithercut, W.D., Macdonald, A.M.I. and McColl, K.E.L.
The effect of increasing *Helicobacter pylori* ammonia production by urea infusion on plasma gastrin concentrations.
Gut, 1991; 32: 21-24.

Clayton, C.L., Pallen, M.J. Kleanthous, H., Wren, B.W. and Tabaqchali, S.
In: P. Malfertheiner, H. Ditschuneit (Eds).
Helicobacter pylori, gastritis and peptic ulcer.
Springer-Verlag, Berlin, 1990; pp. 74-80.

Coghlan JG, Gilligan D, Humphries H, McKenna D, Dooley C, Sweeney E, Keane C and O'Morain C.
Campylobacter pylori and recurrence of duodenal ulcers - A 12 month follow-up study.
Lancet, November 1987; 1109-1111.

Correa P.
Is gastric carcinoma an infectious disease?
N. Engl. J. Med., 1991; Oct. 17: 1170-1171.

Crean, G.P., Marshall, M.W. and Rumsey, R.D.E.
Parietal cell hyperplasia induced by the administration of pentagastrin (ICI 50, 123) to rats.
Gastroenterology, 1969; 57: 147-155.

Creutzfeldt, W. and Lamberts, R.
Is hypergastrinaemia dangerous to man?
Scand. J. Gastroenterol., 1991; 180 (Suppl.): 179-191.

Creutzfeldt, W., Stockmann, F., Conlon, J.M., Folsch, U.R., Bonatz, G. and Wulfrath, M.
Effect of short and long-term feeding of omeprazole on rat gastric endocrine cells.
Digestion, 1986; 35(Suppl. 1): 84-97.

Crivelli, O., Pera, A., Lombardo, L., Vernero, S., Varetto, H., Frutten, B., Giovcelli, G., Babando, G. and Verme, G.
Antral G and D cell counts in chronic renal failure.
Scand. J. Gastroenterol, 1979; 14: 327-331.

Davenport, A., Shallcross, T.M., Crabtree, J.E. et al.
Prevalence of *Helicobacter pylori* in patients with end stage renal failure and renal transplant recipients.
Nephron, 1991; 59: 597-601.

Debas, H.T., Walsh, J.H. and Grossman, M.I.
Pure human mini-gastrin: secretion, potency and disappearance rate.
Gut, 1974; 15: 686-689.

Defize, J., Goldic, J. and Hunt, R.H.
Effect of *Campylobacter pylori* on acid production by isolated guinea pig parietal cells.
Gut, 1988; 29: A1435.

Deroda, J.K., Howden, C.W., Burget, D.W., Hunt, R.H.
Twenty four hour intragastric acidity and nocturnal gastric secretion in gastric ulcer patients: The effect of cimetidine.
Alim. Pharm. Ther., 1990; 4: 275-282.

Dick, E., Lee, A., Watson, G. and O'Rourke, J.
Use of mouse for the isolation and investigation of stomach-associated spiral/helical shaped bacteria from man and other animals.
J. Med. Microbiol, 1989; 29: 55-62.

Dill, S., Payne-James, J.J., Misiewicz, J.J. et al.
Evaluation of ^{13}C-urea breath test in the detection of *Helicobacter pylori* and in monitoring the effect of tripotassium dicitratobismuthate in non-ulcer dyspepsia.
Gut, 1990; 31: 1237-1241.

Dixon, M.F.
Helicobacter pylori and chronic gastritis.
In: Rathbone, B.J. and Heatley, R.V. (eds).
Helicobacter pylori and gastroduodenal disease, 2nd edition.
Blackwell Scientific Publications, London, 1992; pp. 124-139.

Doherty, C.C., Buchanank, K.D., Ardil, J. and McGeown, M.G.
Elevations of gastrointestinal hormone in chronic renal failure.
Proc. Eur. Dialysis Transplant Assoc., 1978; 15: 456-465.

Dye, K.D., Marshall, B.J., Frierso, H.F., Barrett, L.J., Guerrant, R.L. and McCallum, R.W.
Is CLO test alone adequate to diagnose *Campylobacter pylori*?
Am J Gastroenterol, 1988; 83: 1032.

Dye, K.R., Marshall, B.J., Frierson, H.F. et al.
Campylobacter pylori colonizing heterotopic gastric tissue in the rectum.
Am. J. Clin. Pathol., 1990; 93: 144-147.

Edkins, J.S.
On the chemical mechanism of gastric secretion.
Proc. Royal Soc. London, 1905; 76: 376.

Eipper, B.A., Park, L.P., Dickenson, I.M., Kentmann, H.T., Thiele, E.A., Rodriquez, H., Schofield, P.R. and Mains, R.E.
Structure of the precursor to an enzyme mediating COOH-terminal.
Amidation in peptide biosynthesis.
Mol. Endocrinol., 1987; 1: 777-790.

Eissele, R., Patberg, H., Koop, H., Krack, W., Lorenz, W., McKnight, A.T. and Arnold, R.
Effect of gastrin receptor blockade on endocrine cells in rats during achlorhydria.
Gastroenterology, 1992; 103: 1596-1601.

Ekman, L., Hansson, E., Havu, N., Carlsson, E. and Lundberg, C.
Toxicological studies on omeprazole.
Scand. J. Gastroenterol., 1985; 20: (Suppl. 108): 53-69.

El-Ghonaimy, E., Barsoum, R., Soliman, M., El-Fikky, A., Rashwan, S., El-Rouby, O., Haddad, S., Abou-Zeid, M. and Hassaballah, N.
Serum gastrin in chronic renal failure: morphological and physiological correlations.
Am. J. Surg., 1981; 141: 334-338.

Ellison, E.H. and Wilson, S.D.
Further observations on factors influencing the symptomatology manifest by patients with Zollinger-Ellison syndrome.
In: T.K. Shnitka, J.A.L. Gilber, R.C. Harrison (eds).
Gastric Secretion, Pergamon, New York, 1967; 363-369.

Engstrand, L., Scheynius, A., Pahlson, C., Grimelius, L., Schwan, A. and Gustavsson, S.
Association of *Campylobacter pylori* with induced expression of class II transplantation antigens on gastric epithelial cells.
Infect. Immun., 1989; 57: 827-832.

Feldman, S., Putcha, L. and Griffith, D.P.
Pharmacokinetics of acetohydroxamic acid.
Preliminary investigations.
Invest. Urol., 1978; 15: 498-500.

Ferrero, R.L., Hazell, S.L. and Lee, A.
The urease enzymes of Campylobacter pylori are a related bacterium.
J. Med. Microbiol., 1988, 27: 33-40.

Festen, H.P.M., Thys, J.C., Lamers, C.B.H.W., Jansen, J.B.M.J., Pals, G., Frants, R.R., Defize, J. and Meuwissen, S.G.M.
Effects of oral omeprazole on serum gastrin and serum pepsinogen I levels.
Gastroenterology, 1984; 87: 1030-1034.

Forman D, Newell D G, Fullerton F, Yarnell J W G, Stacey A R, Wald N and Sitas F.
Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation.
B.M.J., June 1991; 302: 1302-1305.

Forrest, J.A.H., Fettes, M.R., McLoughlin, G.P. and Heading, R.C.
Effects of long-term cimetidine on gastric acid secretion, serum gastrin and gastric emptying.
Gut, 1979; 20: 404-407.

Gerber, J.G., Nies, A.S. and Payne, N.A.
Adenosine receptors on canine parietal cells modulate gastric acid secretion to histamine.
Journal of Pharmacology and Experimental Therapeutics, 1985; 233: 623-627.

Glupezynski, Y., Burette, A., De Koster, E., Nyst, J.F., Deltenre, M., Cantranel, S., Bourdeaux, L. and De Vos, D.
Metronidazole resistance in *Helicobacter pylori.*
Lancet, 1990; 21: 335(8695): 976-977.

Goodwin, C.S., Armstrong, J.A., Chilvers, T., Peters, M., Collins, M.D., Sly, L., McConnell, W. and Harper, W.E.S.
Transfer of *Campylobacter pylori* and *Campylobacter* mustelae to *Helicobacter pylori* comb. now., respectively.
Int. J. Systematic Bacteriol., 1989; 39: 397-405.

Goodwin, C.S., Armstrong, J.A. and Marshall, B.J.
Campylobacter pyloridis, gastritis and peptic ulceration.
J. Clin. Pathol., 1986; 39: 353-365.

Goodwin, C.S., McCulloch, R.K., Armstrong, J.A. and Wee, S.H.
Unusual cellular fatty acids, and distinctive ultrastructure in a new spiral bacterium (*Campylobacter pyloridis*).
J. Med. Microbiol., 1985; 19: 257-267.

Graham, D.Y., Malaty, H.D., Evans, D.G. et al.
Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Effect of age, race, and socioeconomic status.
Gastroenterology, 1991; 100: 1495-1501.

Graham, D.Y., Opekum, A., Lew, G.M., Evans, D.J., Klein, P.D. and Evans, D.G.
Ablation of exaggerated meal-stimulated gastrin release in duodenal ulcer patients after clearance of *Helicobacter pylori* infection.
Am. J. Gastroenterol., 1990; 85: 394-398.

Graham, D.Y.
Campylobacter pylori and peptic ulcer.
Gastroenterology, 1989; 96: 615-625.

Graham, D., Alpcot, L., Smith, J. and Yoshimura, H.
Intragastric *Campylobacter pylori* as a cause of epidemic achlorhydria.
Am. J. Gastroenterol., 1988; 83: 976-980.

Graham, D.Y., Klein, P.D., Opekun, A.R. and Boutton, T.W.
Effect of age on the frequency of active *Campylobacter pylori* infection diagnosed by the ^{13}C-urea breath test on normal subjects and patients with peptic ulcer disease.
J. Infect. Dis., 1988; 157: 777-780.

Graham, D.Y., Evans, D.J., Alpert, L.C. et al.
Campylobacter pylori detected non-invasively by the ^{14}C-urea breath test.
Lancet, 1987; i: 1174-1177.

Gregory, R.A.
The gastrointestinal hormones: a review of recent advances.
J. Physiology (London), 1974; 241: 1-32.

Gregory, R.A. and Tracy, H.J.
The constitution and properties of two gastrins extracted from hog antral mucosa.
Gut, 1964; 5: 103-117.

Greig, M.A., Neithercut, W.D., Hossack, M. and McColl, K.E.L.
Harnessing of urease activity of *Helicobacter pylori* to induce self-destruction of the bacterium.
J. Clin. Pathol., 1991; 44: 157-159.

Grossman, M.I., Kirsner, J.B. and Gillespie, I.E.
Basal and histology stimulated gastric secretion in control subjects and in patients with peptic ulcer or gastric cancer.
Gastroenterology, 1963; 45: 14-26.

Guo, Y.S., Mok, L., Cooper, G.W., Greeley, G.H. Jr., Thompson, J.C., Singh, P.
Effect of gastrin-releasing peptide analogues on gastrin and somatostatin release from isolated rat stomach.
Am. J. Physiol., 1987; 253: G206-G210.

Håkanson, R. and Sundler, F.
Histamine-producing cells in the stomach and their role in the regulation of acid secretion.
Scand. J. Gastroenterol, 1991; 180: 88-94.

Håkanson, R., Bottcher, G., Ekblad, E. et al.
Histamine in endocrine cells in the stomach: a survey of several species using a panel of histamine antibodies.
Histochemistry, 1986; 86: 5-17.

Håkanson, R., Rehfeld, J.F., Ekekind, M. and Sundler, F.
The life cycle of gastrin granule.
Cell Tissue Res., 1982; 222: 479-481.

Hambourg, M.A., Mignon, M., Ricour, C., Accary, J. and Pellerin, D.
Serum gastrin levels in hypertrophic pyloric stenosis of infancy. Response to a gastrin secretion test.
Arch. Dis. Child., 1979; 54: 208-212.

Hansky, J., King, R.W. and Holdsworth, S.
Serum gastrin in chronic renal failure.
In: Gastrointestinal Hormones (ed). Thompson, J.C. 1975; pp. 115-124. Austin and London University of Texas Press.

Havu, N., Mattsson, H., Ekman, L., Carlsson, E.
Enterochromaffin-like cell carcinoids in the rat gastric mucosa following long term administration of ranitidine.
Digestion, 1990; 45(4): 189-195.

Havu, N.
Enterchromaffin-like cell carcinoids of gastric mucosa in rats after life-long inhibition of gastric secretion.
Digestion, 1986; 35 (suppl. 1): 42-55.

Hays, S.E., Beinfeld, M.C., Jensen, R.T., Goodwin, T.I.C., Paul, S.M.
Demonstration of a purative receptor site for cholecystokinin in rat brain.
Neuropeptides, 1980; 1: 53-62.

Hawtin, P.R., Stacey, A.R. and Newell, D.G.
Investigation of the structure and localization of the urease of *Helicobacter pylori* using monoclonal antibodies.
J. Gen. Microbiol., 1990; 136: 1995-2000.

Hazell, S.L.
Urease and catalase as virulence factors of *Helicobacter pylori.*
In: *Helicobacter pylori* 1990.
Menge H (Ed). Springer-Verlag, Berlin, 1991; 3-14.

Heatley, R.V.
Review article: the treatment of *Helicobacter pylori* infection.
Al. Pharm. Ther., 1992; 6: 291-303.

Hilsted, L.
Glycine-extended gastrin precursors.
Regul. Peptides, 1991; 36: 323-343.

Hilsted, L. and Hansen, C.P.
Co-release of amidated and glycine-extended antral gastrins after a meal.
Am. J. Physiol., 1988; 255: G665-G669.

Hirschowitz, B.I., Griffith, H., Pellegrin, D. and Cummings, O.W.
Rapid regression of ECL-cell gastric carcinoids in 3 patients with pernicious anaemia after elimination of hypergastrinemia by antrectomy.
Gastroenterology, 1992; 102: 1409-1418.

Holcombe, C., Omotare, B.A., Eldridge, J. and Jones, D.H.
H. pylori, the most common bacterial infection in Africa.
A random serological study.
Am. J. Gastroenterol., 1992; 87: 28-30.

Holst, J.J., Knuhtsen, S. Orskov, C., et al.
GRP-producing nerves control antral somatostatin and gastrin secretion in pigs.
Am. J. Physiol., 1987; 253: G767-G774.

Hu, L.T. and Mobley, H.L.T.
Purification and N-terminal analysis of urease from *Helicobacter pylori*.
Infect. Immun., 1990; 58: 992-998.

Huang, S-C., Yu, D.H., Wank, S.A., Mantley, S., Gardner, J.D., Jensen, R.T.
Importance of sulfation of gastrin or cholecystokinin (CCK) on affinity for gastrin and CCK receptors.
Peptides; 1989; 10: 785-789.

Hughes, J., Woodroff, G., Horwell, D., McKnight, A., Hill, D.
Gastrin-cholecystokinin/B receptor pharmacology.
In: J.H. Walsh, Gastrin- Raven Press, New York, 1993; 169-188.

Hui, W.M., Lam, S.K., Ho, J., Lok, A.S.F., Ng M.M.T., et al.
Effect of omeprazole on duodenal ulcer-associated antral gastritis and *Helicobacter pylori*.
Dig. Dis. Sci., 1991; 36: 577-582.

Ippoliti, A.F., Isenberg, J.I., Maxwell, V. and Walsh, J.H.
The effect of 16,16-dimethyl prostaglandin E_2 on meal-stimulated gastric acid secretion and serum gastrin in duodenal ulcer patients.
Gastroenterology, 1976; 70: 488-491.

Isenberg, J.I., Walsh, J.H. and Grossman, M.I.
Zollinger-Ellison syndrome.
Gastroenterology, 1973; 65: 140-165.

Ishizuka, J., Martinez, J., Townsend, C.M. and Thompson, J.C.
The effect of gastrin on growth of human stomach cancer cells.
Ann. Surg., 1992; 215: 528-535.

Jansen, J.B.M.J., Klinkenberg-Knol E.C., Meuwissen, S.G.M., De Bruijne, J.W., Festen, H.P.M., Snel, P., Luckers, A.E.G., Biemond, I, and Lamers, B.H.W.
Effect of long-term treatment with omeprazole on serum gastrin and serum group A and C pepsinogens in patients with reflux esophagitis.
Gastroenterology, 1990; 99: No. 3: 621-628.

Johnson, L.R.
New aspects of the trophic action of gastrointestinal hormone.
Gastroenterology, 1977; 72: 788-792.

Johnson, L.R., Aures, D. and Yuen, L.
Pentagastrin-induced stimulation of protein synthesis in the gastrointestinal tract.
Am. J. Physiol., 1969b; 217: 251-254.

Jones, D.M., Eldridge, J., Fox, A.J., Sethe, P. and Whorwell, P.J.
Antibody to the gastric Campylobacter-like organism (*Campylobacter pyloridis*): clinical correlations and distribution in the normal population.
J. Med. Microbiol., 1986; 22: 57-62.

Jones, D.M., Curry, A. and Fox, A.J.
An ultrastructural study of the gastric *campylobacter*-like organism *Campylobacter pyloridis*.
J. Gen. Microbiol., 1985; 131: 2335-2441.

Jones, D.M., Lessels, A.M., Eldridge, J.
Campylobacter-like organisms on the gastric mucosa: culture, histological and serological studies.
J. Clin. Pathol., 1984; 37: 1002-1006.

Kaise, M., Muraoka, A., Seva, C., Takeda, H., Dickinson, C.J., Yamada, T.
Glycine-extended progastrin processing intermediates induce H^+,$K(^+)$-ATPase alpha-subunit gene expression through a novel receptor.
J. Biol. Chem., 1995; 12: 270(19): 11155-11160.

Kaldor, J., Tree, W., McCarthy, P. and Dwyer, B.
Immune response to *Campylobacter pyloridis* in patients with peptic ulcerations.
Lancet, 1985; i: 921.

Kaneko, H., Nakada, K., Mitsuma, T., Uchida, K., Furusawa, A., Maeda, Y. and Morise, K.
Helicobacter pylori infection induces a decrease in immunoreactive-somatostatin concentrations of human stomach.
Dig Dis Sci, 1992; 37: No. 3: 409-416.

Karttunen, R., Karttunen, T., Ekre, H-P.T., MacDonald, T.T.
Interferon gamma and interleukin 4 secreting cells in the gastric antrum in *Helicobacter pylori* positive and negative gastritis.
Gut, 1995; 36: 341-345.

Kirchner, T., Melber, A., Fischblach, W., Heilmann, K.L. and Hermelink, H.K.
Immunohistological patterns of the local immune response in *Helicobacter* gastritis.
In: Malfertheiner, P. and Ditschuneit, H. (eds). *Helicobacter pylori, Gastritis and Peptic Ulcer.*
Springer-Verlag, Berlin, 1990; pp. 213-222.

Kist, M.
Immunology of *Helicobacter pylori.*
In: Marshall, B.J., McCallum, R.W. and Guerrant, R.L. (eds).
Helicobacter pylori in peptic ulceration and gastritis, 1991; 92-110.

Koop, H., Klein, M. and Arnold, R.
Serum gastrin levels during long-term omeprazole treatment.
Aliment. Pharmacol. Therap., 1990; 4: 131-138.

Koop, H., Willemer, S., Steinbach, F., Eissele, R., Tuch, K. and Arnold, R.
Influence of chronic drug-induced achlorhydria by substituted benzimidazoles on the endocrine stomach of rats.
Gastroenterology, 1987; 92: 406-413.

Kuamamoto, T., Sumii, K., Haruma, K., Tari, A., Tanaka, K., Kajiyuma, G.
Gastrin receptors in the human gastrointestinal tract and pancreas.
Gastroenterol. Jpn., 1989; 26: 109-114.

Kuipers, E.J., Lee, A., Klinkenberg-Knol, E.C. and Meuwissen, S.G.M.
Review article: the development of atrophic gastritis - *Helicobacter pylori* and the effects of acid suppressive therapy.
Aliment. Pharmacol. Ther., 1995; 9: 331-340.

Kuipers, E.J., Bloemena, E., Hazenberg, H.J.A., Lindeman, J., Klinkenberg-Knol, E.C. and Meuwissen, S.G.M.
Check Title ...
Gastroenterology, 1994; 106: No. 4: Part 2: A112.

Kusyk, C.J., McNeil, N.O. and Johnson, L.R.
Stimulation of growth of a colon cancer cell line by gastrin.
Am. J. Physiol., 1986; 25: 9597-9601.

Laffeld, R.J.L.F., Stobberinges, E., Flendrig, J.A., van Speernwell, J.P. and Arends, J.W.
Diagnostic value of an immunoassay to detect anti-*Campylobacter pylori* antibodies in non-ulcer dyspepsia.
Lancet, 1989; 1: 1182-1185.

Lam, S.K.
Pathogenesis and Pathophysiology of Duodenal Ulcer.
Clin. Gastroenterol., 1984; 13: 447-472.

Lam, S.K., Chan, P.K.W., Wong, J. and Ong, G.B.
Fasting and postprandial serum gastrin levels before and after highly selective gastric vagotomy, truncal vagotomy with pyloroplasty and truncal vagotomy with antrectomy: is there a cholinergic antral gastrin inhibitory and releasing mechanism?
B.J.S., 1978; 65: 797-800.

Lam, S.K.
Hypergastrinaemia in cirrhosis of liver.
Gut, 1976; 17: 700-708.

Lamberts, R., Creutzfeldt, W., Struber, H.G., Brunner, G. and Solcia, E.
Long-term omeprazole therapy in peptic ulcer disease: gastrin, endocrine cell growth and gastritis.
Gastroenterology, 1993; 104: 1356-1370.

Langman, M.J., Brooks, P., Hawkey, C.J., Silverstein, F., Yeomans, N.
Non-steroidal anti-inflammatory drugs associated ulcer: epidemiology, causation and treatment.
J. Gastroenterol. Hepatol., 1991; 6(5): 442-449.

Lanzon-Miller, O., Pounder, R.E., Hamilton, M.R., Chronos, N.A.F., Ball, S., Raymond, F., Olausson, M. and Cederberg, C.
Twenty-four hour intragastric acidity and plasma gastrin concentration before and during treatment with either ranitidine or omeprazole in healthy subjects and patients with duodenal ulcer or gastric ulcer or pernicious anaemia.
Aliment. Pharmacol. Therap., 1987; 1: 225-237.

Larsson, H., Carlsson, E., Håkanson, R., Mattsson, H., Nilsson, G., Seensalu, R., Wallmark, B. and Sundler, F.
Time-course of development and reversal of gastric endocrine cell hyperplasia after inhibition of acid secretion.
Gastroenterology, 1988; 95: 1477-1486.

Larsson, H., Carlsson, E., Håkanson, R. et al.
Time-course of development and reversal of gastric endocrine cell hyperplasia after inhibition of acid secretion. Studies with omeprazole and ranitidine in intact and antrectomized rats.
Gastroenterology, 1988a; 95: 1477-1486.

Larsson, H., Carlsson, E., Mattsson, H., Lundell, L., Sundler, F., Sundell, G., Wallmark, B., Watanabe, T. and Håkanson, R.
Plasma gastrin and gastric enterochromaffin like cell activation and proliferation. Studies with omeprazole and ranitidine in intact and antrectomized rats.
Gastroenterology, 1986; 90: 391-399.

Larsson, L.I.
Gastrointestinal cells producing endocrine, neurocrine and paracrine messengers.
Clin. Gastroenterol., 1980; a: 485-516.

Larsson, L.I., Gottermann, N., De Magistris, L., Rehfeld, J.F., Schwartz, T.W.
Somatostatin cell process as pathways for pancreatic secretion.
Science, 1979; 205: 1393-1395.

Larsson, L.I. and Rehfeld, J.F.
Evidence for a common evolutionary origin of gastrin and cholecystokinin.
Nature (London), 1977; 269: 335-338.

Lees, F.
The gastric and jejunal mucosae in healthy patients with partial gastrectomy.
Arch. Intern. Med., 1968; 101: 9437-9451.

Lehy, T., Dubrasquet, M., Bonfils, S.
Effect of somatostatin on normal and gastric-stimulated cell proliferation in the gastric and intestinal mucosae of the rat.
Digestion, 1979; 19: 99-109.

Levi, S., Beardshall, K., Haddad, G., Playford, R., Ghosh, P. and Calam, J.
Campylobacter pylori and duodenal ulcers: the gastrin link.
Lancet, 1989; i: 1167-1168.

Levi, S., Bearsdhall, K., Swift, I., Foulkes, W., Playford, R., Ghosh, P. and Calam, J.
Antral *Helicobacter pylori*, hypergastrinaemia and duodenal ulcers: effect of eradicating the organism.
Brit. Med. J., 1989; 299: 1504-1505.

Lichtenberger, L.M., Nelson, A.A. and Graziani, L.A.
Amine trapping: Physical explanation for the inhibitory effect of gastric acidity on the postprandial release of gastrin.
Gastroenterology, 1986; 90: 1223-1231.

Lichtenberger, L.M., Graziani, L.A. and Dubinsky, W.D.
Importance of dietary amines in meal-induced gastrin release.
Am. J. Physiol., 1982; 243: G341-347.

Lieber, C.S. and Lefevre, A.
Ammonia as a source of gastric hypoacidity in patients with uremia.
J. Clin. Invest., 1959; 1271-1277.

Lind, T., Cederberg, C., Idstrom, J.P., Lonroth, H., Olbe, L. and Lundell, L.
24-Hour intragastric acidity and plasma gastrin during long-term treatment with omeprazole or ranitidine in patients with reflux esophagitis.
Scand. J. Gastroenterol., 1991; 26: 620-626.

Lind, T., Cederberg, C., Forssel, H., Olausson, M. and Olbe, L.
Relationship between reduction of gastric acid secretion and plasma gastrin concentration during omeprazole treatment.
Scand. J. Gastroenterol., 1988; 23: 1259-1266.

Lingwood, C.A., Law, H., Pellizzari, A., Sherman, P. and Drumm, B.
A novel gastric glycolipid as a receptor for *Campylobacter pylori*.
Gastroduodenal disease and *Campylobacter pylori*.
Charlottesville Va., 1989; May 10-12.

Logan, R.P.H., Walker, M.M., Misiewicz, J.J., Gummett, P.A., Karim, Q.N. and Baron, J.H.
Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole.
Gut, 1995; 36: 12-16.

Logan, R.P.H., Gummett, P.A., Schantelberger, H.D., Greaves, R.R.F.H., Mendelson, G.M., Walker, M.M., Thomas, P.H., Baron, J.H and Misiewicz, J.J.
Eradication of *H. pylori* with clarithromycin and omeprazole.
Gut, 1994; 35: 323-326.

Logan, R.P.H., Polson, R.J., Misiewicz, J.J. et al.
Simplified single sample ^{13}carbon urea breath test for *Helicobacter pylori*.
Comparison with histology, culture and ELISA serology.
Gut, 1991; 32: 1461-1464.

Logan, R.P.H., Gummett, P.A., Schautelberger, H.D., Greaves, R.R.F.H., Mendelson, G.M., Walker, M.M., Thomas, P.H., Baron, J.H., Misiewicz, J.J.
Eradication of *H. pylori* with clarithromycin and omeprazole.
Gut, 1994; 35: 323-326.

Logan, R.P.H., Polson, R.J., Baron, J.H. and Misiewicz, J.J.
Follow-up after anti-*Helicobacter pylori* treatment.
Lancet, 1991; 337: 562-563.

Londong, W., Londong, V., Cederberg, C. and Steffen, H.
Dose-response study of omeprazole on meal-stimulated gastric acid secretion and gastrin release.
Gastroenterology, 1983; 85: 1373-1378.

Lucey, M.R. and Yamada, T.
Biochemistry and physiology of gastrointestinal somatostatin.
Dig. Dis. Sci., 1989; 34: 5S-13S.

Lucey, M.R.
Endogenous somatostatin and the gut.
Gut, 1986; 27: 457-467.

Mahachai, V., Walker, K. and Thomson, A.B.R.
Comparison of cimetidine and ranitidine on 24-hour intragastric acidity and serum gastrin profile in patients with oesophagitis.
Dig. Dis. Sci., 1985; 30: 321-328.

Marshall, B.J., Barrett, L.J., Prakash, C., McCallum, R.W. and Guerrant, R.L.
Urea Protects *Helicobacter (Campylobacter) pylori* from the Bactericidal Effect of Acid.
Gastroenterology, 1990; 99(3): 697-702.

Marshall, B.J. and Surveyor, I.
Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori*-associated gastritis.
J. Nucl. Med., 1988; 29: 11-16.

Marshall BJ, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, Phillips M, Waters TE and Sanderson CR.
Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter Pylori.*
Lancet, December 1988; 1437-1441.

Marshall, B.J., Warren, R., Francis, G.J., Langton, S.R., Goodwin, C.S. and Blincow, E.D.
Rapid urease test in the management of *Campylobacter pyloridis*-associated gastritis.
Am. J. Gastroenterol., 1987; 82: No. 3: 200-210.

Marshall, B.J. and Goodwin, C.S.
Revised nomenclature of *Campylobacter pyloridis.*
Int. J. Syst. Bacteriol., 1987; 37: 68.

Marshall, B. and Langton, S.
Urea hydrolysis in patients with *Campylobacter pyloridis* infection.
Lancet, 1986; i: 965-966.

Marshall, B., Armstrong, J., McGechie, D. and Glancy, R.
Attempt to fulfil Koch's postulates for pyloric campylobacter.
Med. J. Aust., 1985; 142: 436-439.

Marshall, B.J., Joyce, H., Anwar, D.I., Goodwin, C.S., Pearmans, J.W., Warren, J.R. and Armstrong, J.A.
Original isolation of *Campylobacter pyloridis* from human gastric mucosa.
Microbios. Lett., 1984; 25: 83-88.

Megraud, F., Brassens-Rabbe, M-P., Denis, F., Belbouri, A. and Hoa, D.Q.
Seroepidemiology of *Campylobacter pylori* infection in various populations.
J. Clin. Microbiol., 1989; 27: 1870-1873.

Mitchell, C.J., Jewell, D.P., Lewin, M.R., McLaughlin, J.F., Moorehead, J.F.
Gastrin function and histology in chronic renal failure.
J. Clin. Pathol., 1979; 32: 208-213.

Molbey, H.T., Cortesia, M.J., Rosenthal, L.E. and Jones, B.D.
Characterisation of urease from Campylobacter pylori.
J. Clin. Micriobol., 1988; 26: 831-836.

Morris, A. and Nicholson, G.
Campylobacter pylori: human ingestion studies.
In: Rathbone, B.J. and Heatley, R.V. (eds).
Campylobacter pylori and Gastroduodenal Disease.
Blackwell Scientific Publications, Oxford, 1989; pp. 185-189.

Morris, A., Nicholson, G., Zwi, J. et al.
Campylobacter pylori infection in Meckel's diverticula containing gastric mucosa.
Gut, 1989; 30: 1233-1235.

Morris, A. and Nicholson, G.
Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting pH.
Am. J. Gastroenterol., 1987; 82: 192-199.

Moss, S.F., Legon, S., Bishop, A.E., Polak, J.M. and Calam, J.
Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease.
Lancet, 1992; 340: 930-932.

Mulholland, G., Ardill, J.E.S., Fillmore, D., Chittajallu, R.S., Fullarton, G.M. and McColl, K.E.L.
Helicobacter pylori related hypergastrinaemia is the result of a selective increase in gastrin 17.
Gut, 1993; 34: 757-761.

Murakami, M., Yoo, J.K., Inada, M. and Miyake, T.
Effect of ammonia on the gastric mucosa in rats: Pathophysiological importance of urease in gastric ulcer disease.
Jpn. J. Pharmacol., 1988; 47: 330-332.

Muto, S., Murayama, N., Asano, Y., Hosado, S. and Moyata, M.
Hypergastrinaemia and achlorhydria in chronic renal failure.
Nephron, 1985; 40: 143-148.

McArthur, K.E., Walsh, J.H. and Richardson, C.T.
Soy protein meals stimulate less gastric acid secretion and gastrin release than beef meals.
Gastroenterology, 1988; 95(4): 920-926.

McColl, K.E.L., El-Nujumi, A.M., Chittajallu, R.S., Dahill, S.W., Dorrian, C.A., El-Omar, E., Penman, I., Fitzsimons, E.J., Drain, J., Graham, H., Ardill, J.E.S. and Bessent, R.
A study of the pathogenesis of *Helicobacter pylori*-negative chronic duodenal ulcer disease.
Gut, 1993; 34: 762-768.

McColl, K.E.L., Nujumi, A.M. El., Dorrian, C.A., Macdonald, A.M.I., Fullarton, G.M. and Harwood, J.
Helicobacter pylori and hypergastrinaemia during proton pump inhibitor therapy.
Scand. J. Gastroenterol., 1992; 27: 93-98.

McColl, K.E.L., Fullarton, G.M., Chittajallu, R., El Nujumi, A.M., Macdonald, A.M.I., Dahill, S.W. and Hilditch, T.E.
Plasma gastrin, daytime intragastric pH and nocturnal acid output before and at 1 and 7 months after eradication of *Helicobacter pylori* in duodenal ulcer subjects.
Scand. J. Gastroenterol., 1991; 26: No. 3: 339-346.

McColl, K.E.L., Fullarton, G.M., Nujumi, A.M., Macdonald, A.M., Brown, I.L. and Hilditch, T.E.
Lowered gastrin and gastric acidity after eradication of *Campylobacter pylori* in duodenal ulcer.
Lancet, 1989; ii: 499-500.

McGuigan, J.E. and Trudeau, W.L.
Immunochemical measurement of elevated levels of gastrin in the serum of patients with pancreatic tumours of the Zollinger-Ellison variety.
N.E.J.M., 1968; 278: 1308-1313.

McIntosh, J.H., Byth, K, and Peper, D.W.
Environmental factors in the aetiology of chronic gastric ulcer: a case control exposure variable before the first symptom.
Gut, 1985; 26: 789-798.

McLean, R.J.C., Cheng, K.J., Gould, W.D. and Costerton, Y.W.
Cytochemical localization of urease in a rumen staphylococcus sp. by electron microscopy.
Appl. Environ. Microbiol., 1985; 49: 253-255.

McNulty, C.A.M.
Detection of *Helicobacter pylori*: the biopsy urease test.
Helicobacter pylori and Gastroduodenal Disease.
Edited by Rathbone, B.J. and Heatley, R.V., 2nd edition, 1992; 58-63.

McNulty, C.A.M., Dent, J.C., Uff, J.C., Gear, M.W.L. and Wilkinson, S.P.
Detection of Campylobacter pylori by the biopsy urease test; an assessment in 1445.
Gut, 1989; 30: 1058-1062.

McNulty, C.A.M.
Pathogenicity of *Campylobacter pylori* - A causative factor in gastritis?
J. Gastroenterol., 1989; 24 (Suppl. 160): 3-6.

Nakamura, M., Oda, M., Kaneko, K. et al.
Autoradiographic demonstration of gastrin binding sites in rat gastric mucosa.
Peptides, 1987; 8: 391-398.

Neithercut, W.D., Milne, A., Chittajallu, R.S., El Nujumi, A.M. and McColl, K.E.L.
The detection of *Helicobacter pylori* infection of the gastric mucosa by measurement of gastric aspirate ammonium and urea concentrations.
Gut, 1991; 32: 973-976.

Negré, F., Fagog-Revurat, P., Vaysse, N., Rehfeld, G.F., Pradayrol, L.
Progastrin induced autocrine/intracrine proliferative effects on pancreatic rat tumoral cells.
Gastroenterology, 1994; 6: A309.

Nensey, Y.M., Schubert, T.T., Bologna, S.D. and Ma, C.K.
Helicobacter pylori-negative duodenal ulcer.
Am. J. Med., 1991; 91: 15-18.

Newell, D.G., Bell, G.D., Weil, J., Jones, P., Grant, P. and Harrison, G.
The effect of treatment on circulating anti-*Helicobacter pylori* antibodies - a two year follow-up study.
In: Malfertheiner, P. and Ditschuneit, H. (eds).
Helicobacter pylori, Gastritis and Peptic Ulcer. Springer-Verlag, Berlin, 1990; pp. 172-175.

Newell, D.G., Bell, G.D., Weil, J., Jones, P., Grant, P. and Harrison, G.
The effect of treatment on circulating anti-*Helicobacter pylori* antibodies - a two year follow-up study.
In: Malfertheiner, P. and Ditschuneit, H. (eds).
Helicobacter pylori, Gastritis and Peptic Ulcer.
Springer-Verlag, Berlin. 1989; pp. 172-175.

Newell, D.G. and Rathbone, B.J.
The serodiagnosis of *Campylobacter pylori* infection - a review.
Serodiagnosis Immunother., 1989; 3: 1-6.

Newell, D.G., Johnston, B.J., Ali, M.H. and Reed, P.I.
An enzyme-linked immunosorbent assay for the serodiagnosis of *Campylobacter pylori* associated gastritis.
Scand. J. Gastroenterol., 1988; 23: suppr. 142: 53-57.

Niemela, S., Karttunen, T. and Kerola, T.
Chronic gastritis in patients with gastric ulcer; a 10 year follow up.
Scan. J. Gastroenterol, 1995; 30(5): 428-433.

Nomura, A., Stemmermann, G.N., Chyou, P.H., Kato, I., Perez-Perez, G.I. and Blaser, M.J.
Helicobacter pylori infection and gastric carcinoma among Japanese American in Hawaii.
N. Eng. J. Med., 1991; 325(16): 1132-1136.

Nujumi, A.M. El., Rowe, P., Dorrian, C.A. and McColl, K.E.L.
Value of ^{14}C-urea breath test to diagnose *Helicobacter pylori* in uraemic patients.
Gut, 1991; 32: A1220.

Nujumi, A.M. El., Dorrian, C.A., Chittajallu, R.S., Neithercut, W.D. and McColl, K.E.L.
Effect of inhibition of *Helicobacter pylori* urease activity by acetohydroxamic acid on serum gastrin in duodenal ulcer subjects.
Gut, 1991; 32: 866-870.

Oderda, G., Vaira, D., Holton, J., Ainley, C., Altare, F. and Ansaldi, N.
Amoxycillin plus tinidazole for *Campylobacter pylori* gastritis in children: Assessment by serum IgG antibody, pepsinogen I and gastrin levels.
Lancet, 1989; i: 690-692.

Odum, D., Petersen, H.D., Andersen, I.B., Hansen, B.F. and Rehfeld, J.F.
Gastrin and somatostatin in *Helicobacter pylori* infected antral mucosa.
Gut, 1994; 35: 615-618.

Olbe, L., Lundell, L., Sundler, F., Simonnson, J. and Håkanson, R.
Antrectomy in a patient with multiple ECL-cell gastric carcinoids and pernicious anaemia.
Gastroenterology International, 1988; 1: 340.

O'Riordan, T., Mathai, E., Tobin, E., McKenna, D., Keane, C., Sweeney, E. and O'Morain, C.
Adjuvant antibiotic therapy in duodenal ulcers treated with colloidal bismuth subcitrate.
Gut, 1990; 31: 999-1002.

Parsonnet J, Friedman G D, Vandersteen D P, Chang Y, Vogelman J H, Orentreich N and Sibley R K.
Helicobacter pylori infection and the risk of gastric carcinoma.
N. Engl. J. Med., 1991; Oct. 17: 325: 16: 1127-1131.

Paull, G. and Yardley, J.H.
Gastric and oesophageal *Campylobacter pylori* in patients with Barrett's oesophagus.
Gastroenterology, 1988; 95: 216-218.

Penston, J.G., Dixon, J.S., Selway, S.A.M. and Wormsley, K.G.
Gastric histology and plasma gastrin response to a meal in patients with duodenal ulcer disease after five years treatment with ranitidine.
Aliment. Pharmacol. Therap., 1990; 4: 381-392.

Perez-Perez, G.I., Divorkin, B.M., Chodos, J.E. and Blaser, M.J.
Campylobacter pylori antibodies in humans.
Ann. Intern. Med., 1988; 109(1): 11-17.

Polak, J.M., Bloom, S.R., Bishop, A.E., McCrossan, M.V.
D cell pathology in duodenal ulcers and achlorhydria.
Metabolism, 1978; (suppl. 27): 1239-1242.

Poynter, D., Pick, C.R., Harcourt, R.A., Selway, S.A.M., Ainge, G., Harman, I.W., Spurling, N.W., Fluck, P.A. and Cook, J.O.
Association of long-lasting unsurmountable histamine H_2 blockade and gastric carcinoid tumours in the rat.
Gut, 1985; 26: 1284-1295.

Presti, M.E., Gardner, J.D.
Receptor antagonists for gastrointestinal peptides.
Am. J. Physiol., 1993; 264(3 Pt. 1): G399-406.

Ragins, H., Wincze, F., Liu, S.M. and Dittbrenner, M.
The origin and survival of gastric parietal cells in the mouse.
Anat. Rec., 1968; 162: 99-110.

Rathbone, B.J., Wyatt, J.I. and Heatly, R.V.
Local response of the host to *Campylobacter pylori.*
Gastroenterol. Clin. Biol., 1989; 13: 75B-77B.

Rathbone, B.J., Wyatt, J.I., Worsley, B.W., Shires, S.E., Trejdosiewicz, L.K., Heatley, R.V. and Losowsky, M.S.
Systemic and local antibody responses to gastric *Campylobacter pyloridis* in non-ulcer dyspepsia.
Gut, 1986; 27: 642-647.

Rathbone, B.J., Wyatt, J., Tompkins, D., Heatly, R.V. and Losowsky, M.S.
Diagnostic IgG ELISA for Gastric *Campylobacter pyloridis* infection using serum samples.
Gut, 1986; 27: A607.

Rauws, E.A., Langenberg, W., Houthoff, H.J., Zanen, H.C. and Tytgat, G.N.
Campylobacter pyloridis-associated chronic acute antral gastritis. A prospective study of its prevalence and the effects of antibacterial and anti-ulcer treatment.
Gastroenterology, 1988; 94: 33-40.

Rayford, P.L., Villar, H.V., Reeder, D.D. and Thompson, J.C.
Effect of GIP and VIP in gastrin release and gastric secretion.
Physiologist, 1974; 17: 319.

Rehfeld, J.F.
Gastrin and colorectal cancer; a never-ending dispute?
Gastroenterology, 1995; 108: 1307-1309.

Rehfeld, J.F., Hilsted, L.
Gastrin and Cancer.
Adv. Clin. Chem., 1992; 29: 239-262.

Rehfeld, J.F.
Four basic characteristics of gastrin-cholecystokinin system.
Am. J. Physiol., 1981; 240: G255-G266.

Richards, A.T., Hindler, R.A. and Harrison.
Gastric carcinoid tumors associated with hypergastrinaemia and pernicious anaemia. Regression of tumors by antrectomy.
South African Medical Journal, 1987; 72: 51-53.

Romaniuk, P.J., Zoltouska, B., Trust, T.J., Lane, D.J., Olsen, G.J., Pace, N.R. and Stahl, D.A.
Campylobacter pylori, a spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp.
J. Bacteriol., 1987; 169: 2137-2141.

Royton, C.M.S., Polak, J., Bloom, S.R., Cooke, W.M., Russell, R.C.G., Pearse, A.G.E., Spencer, J., Welbourn, R.B., Baron, J.H.
G cell population of the gastric antrum, plasma gastrin, and gastric acid secretion in patients with and without duodenal ulcer.
Gut, 1978; 19: 689-698.

Rowden, D.R., Taylor, I.L., Richter, J.A., Pinals, R.S. and Levine, R.A.
Is hypergastrinaemia associated with rheumatoid arthritis?
Gut, 1978; 19: 1064-1067.

Rubin, W.
Proliferation of endocrine-like (enterochromaffin) cells in atrophic gastric mucosa.
Gastroenterology, 1969; 57: 641-648.

Rune, S.J.
Helicobacter pylori, peptic ulcer disease and inhibition of gastric acid secretion.
Digestion, 1992; 51: (suppl. 1): 11-16.

Rune, S.J., Justesen, T., Hansen, J.M. et al.
Campylobacter pyloridis and acid-induced gastric metaplasia in the pathogenesis of duodenitis.
J. Clin. Pathol., 1987; 40: 841-848.

Ryberg, B., Tielemans, Y., Axelson, J. et al (1990).
Gastrin stimulates the self-replication rate of enterochromaffin-like cells in the rat stomach.
Gastroenterology, 1990; 99: 935-942.

Saito, A., Sankaran, H., Goldfine, I.D., Williams, J.A.
Cholecystokinin receptors in the brain.
Science, 1980; 208: 1155-1156.

Samloff, I.M., Liebman, W.M. and Panitch, N.M.
Serum group I pepsinogens by radioimmunoassay in control subjects and patients with peptic ulcer.
Gastroenterology, 1975; 69: 83-90.

Schnell, G.A., Schubert, T.T., Barnes, W.G. and Rupani, M.K.
Comparison of urease, H&E and culture tests, for *Campylobacter pylori.*
Gastroenterology, 1988; 94(5): A410.

Schubert, M.L.
Neural and paracrine regulation of gastrin secretion.
In: J.H. Walsh (ed), Gastrin, Raven Press Ltd., New York, 1993; 129-137.

Seva, C., Dickinson, C.J., Yamada, T.
Growth-promoting effects on glycine-extended progastrin.
Scient, 1994; 15: 265(5170): 410-412.

Shallcross, T.M., Rathbone, B.J. and Heatley, R.V.
In: Rathbone, B.J. and Heatley, R.V. (Eds).
Helicobacter pylori and gastroduodenal disease. 2nd edition.
Blackwell Scientific Publications, Oxford, 1992; pp. 165-176.

Sidebotham, R.L. and Baron, J.H.
Hypothesis: *Helicobacter pylori*, urease mucus and gastric ulcer.
Lancet, 1990; 335: 193-195.

Sitas, F., Forman, D., Yarnell, J.W.G., et al.
Helicobacter pylori infection rates in relation to age and social class in a population of Welsh men.
Gut, 1991; 32: 25-28.

Skirrow, M.B.
Campylobacter, Helicobacter and other motile gram-negative rods.
In: Parker, M.T. and Duerden, B.I. (eds).
Topley and Wilson's Principles of Bacteriology, Virology and Immunity. Vol. II: Edward Arnold Ltd., London, 1990; pp. 532-549.

Smith, J.T.L., Pounder, R.E., Nwokolo, C.U., Lanzon-Miller, S., Evans, D.G., Graham, D.Y. and Evans, D.J. (Jr).
Inappropriate hypergastrinaemia in asymptomatic healthy subjects infected with *Helicobacter pylori.*
Gut, 1990; 31: 522-525.

Smith, J.T.L., Pounder, R.E., Evans, D.J., Graham, D.Y. and Evans, D.G.
Inappropriate 24 hour hypergastrinaemia in asymptomatic *C. pylori* infection.
Gut, 1989; 30: A732-733.

Smoot, D.T., Mobley, H.L.T., Chippendale, G.R., Lewison, J.F. and Resau, J.H.
Helicobacter pylori urease activity is toxic to human gastric epithelial cells.
Infect. Immun., 1990; 58: 1992-1994.

Solcia, E., Rinidi, G., Silini, E., Villani, L.
Enterochromaffin-like (ECL) cells and their growths: relationship to gastrin, reduced acid secretion and gastritis.
Baillier's Clinical Gastroenterology, 1993; 7: No. 1: 149-165. ed. N.D. Yeomans.

Solcia E, Capella C, Vassallo G and Buffa, R.
Endocrine cells of the gastric mucosa.
International Review of Cytology, 1975; 42: 223-286.

Soll, A.H., Yamada, T., Park, J., Thomas, L.P.
Release of somatostatin-like immunoreaction from canine fundic mucosal cells in primary cultures.
Am. J. Physiol., 1984; 247: 9558-9566.

Steer, H.W., Hawtin, P.R. and Newell, D.G.
An ELISA technique for the serodiagnosis of *Campylobacter pyloridis* infection in patients with gastritis and benign duodenal ulceration.
Serodiagnosis Immunother., 1987; I: 253-259.

Straus, E., Yalow, R.S. and Berson, S.A.
Differential diagnosis in hyperchlorhydric hypergastrinaemia.
Gastroenterology, 1974; 66: 867.

Strickland, R.G. and Fenoglio-Prieser, C.M.
Gastritis-classification and histology then and now.
In: Marshall, B.J., McCallam, R.W., Guerrant, R.L. (eds).
Helicobacter pylori in peptic ulceration and gastritis.
Blackwell Scientific Publications, Oxford, 1991; pp.1-18.

Suerbaum, S., Leving, H., Hemmerele, B., Klemm, K., Opferkuch, W.
Antibacterial activity of pantoprazole, omeprazole, and other (H^+/K^+) ATPase inhibitors against *Helicobacter pylori.*
Rev. Esp. Enf. Digest., 1990; 78(suppl. 1): 134.

Tani, M. and Shimazu, H.
Meat-stimulated gastrin release and acid secretion in patients with pyloric stenosis.
Gastroenterology, 1977; 73: 207-210.

Tarnasky, P.R., Kovacs, T.O.G., Sytnik, B. and Walsh, J.H.
Asymptomatic *H. pylori* infection impairs pH inhibition of gastrin and acid secretion during second hour of peptone meal stimulation.
Dig. Dis. Sci., 1993; 38: No. 9: 1681-1687.

Teichmann, R.K., Pratschke, E., Grab, J., Hammer, C. and Brendel, W.
Gastrin release by interleukin-2 and Y-interferon in vitro.
Can. J. Physiol. Pharmacol., 1986; 64: suppl: 62.

Ten Kate, R.W., Tuynman, H.A.R.E., Festen, H.P.M., Pals, G. and Meuwissen, S.G.M.
Effect of high dose omeprazole on gastric pepsin secretion and serum pepsinogen levels in man.
Eur. J. Clin. Pharmacol., 1988; 32: 173-176.

Thomas, E., Farnum, J.B., Rohrbach, M., Moham, A.T. and Palaniswamy, K.R.
Antral gastritis, *Helicobacter pylori* and gastrin mucosal sensitivity.
Gastroenterology, 1992; 102(3): 108.

Thomsen, L.L., Gavin, J.B. and Tasman-Jones, C.
Relation of *Helicobacter pylori* to the human gastric mucosa in chronic gastritis of the antrum.
Gut, 1990; 31: 1230-1236.

Triebling, A.J., Korstein, M.A., Dlugosz, J.W., Paronetto, F. and Lieber, C.
Severity of *Helicobacter*-induced gastrin injury correlates with gastric juice ammonia.
Dig. Dis. Sci., 1991; 36: 1089-1096.

Vaira, D., Cairns Holton, J.Sr., Falzon, H., Polydorous, A., Dowsett, J.F. and Salmon, P.R.
Antibodies titres to *Campylobacter pylori* after treatment for gastritis.
Br. Med. J., 1988; 297-397.

Vaira, D., Holton, J., Londei, M., Beltrandi, F., Salmon, P.R. D'Anastasio, C., Dowsett, J.F., Bertoni, F., Grauenfels, P. and Gandolfi, L.
Campylobacter pylori in abattoir workers: is it a zoonosis?
Lancet, 1988; ii: 725-726.

Veenendaal, R.A., Lichtendal-Bernards, A.T., Pena, A.S., Endtz, H.P., van Boven, C.P., Lamers, C.B.
Effect of transport medium and transportation time on culture of *Helicobacter pylori* from gastric biopsy specimens.
J. Clin. Pathol., 1993; 46(6): 561-563.

Veldhuyzen van Zanten, S.J.O., Pollak, P.T., Best, L.M., Bezansons, G.S. and Marrie, T.
Increasing prevalence of *Helicobacter pylori* infection with age: continuous risk of infection in adults rather than cohort effect.
Journal of Infectious Diseases, 1994; 169: 434-437.

Veldhuyzen van Zanten, S.J., Tytgat, K.M., Hollingsworth, J. et al.
^{14}C-urea breath test for the detection of *Helicobacter pylori*.
Am. J. Gastroenterol., 1990; 85: 399-403.

Verdu, E.F., Armstrong, D., Fraser, R., Viani, F., Idstrom, J-P., Cederberg, C. and Blum, A.L.
Effect of *Helicobacter pylori* infection on intragastric pH during treatment with omeprazole.
Gut, 1995; 37: 743-748.

Verdu, E.F., Armstrong, D., Fraser, R., Viani, F., Idstrom, J-P., Cederberg, C. and Blum, A.L.
Effect of *Helicobacter pylori* status on intragastric pH during treatment with omeprazole.
Gut, 1995; 36: 539-543.

Walan, L., Madsen, T., Brandsborg, O. and Larsen, N.E.
The influence of cimetidine on basal gastro-oesophageal sphinctor pressure, intragastric pH and serum gastrin concentration in normal subjects.
Scand. J. Gastroenterol., 1979; 14: 349-353.

Waldrum, H.L., Jorde, R. and Gunnes, P.
Renal excretion of, and the effect of, posture on serum group I pepsinogens.
Scand. J. Gastroenterol., 1982; 17: 253-255.

Walsh, J.H.
Gastrin.
In: J.H. Walsh and G.J. Dockray (eds), Gut Peptides: Biochemistry and Physiology. Raven Press Ltd., New York, 1994; 75-121.

Walsh, J.H.
Gastrointestinal hormones: past, present and future.
Gastroenterology, 1993; 105(3): 653-657.

Walsh, J.H., Isenberg, J.I., Ansfield, J. and Maxwell, V.
Clearance and acid-stimulating action of human big and little gastrins in duodenal ulcer subjects.
J. Clin. Invest., 1976; 57: 1125-1131.

Walsh, J.H. and Grossman, M.I.
Gastrin.
N.E.J.M., 1975; 292: 1324-1332.

Walsh, J.H., Richardson, C.T. and Fordtran, J.S.
pH dependence of acid secretion and gastrin release in normal and ulcer subjects.
J. Clin. Invest., 1975; 55: 462-468.

Watson, S.A. and Steele, R.J.C.
Gastrin antagonists in the treatment of gastric cancer.
Anti-Cancer Drugs, 1993; 4: 599-604.

Weil, J., Bell, D.G., Powell, K., Morden, A., Harrison, G., Gants, P.W. et al.
Omeprazole and *Helicobacter pylori* temporary suppression rather than true eradication.
Aliment. Pharmacol. Ther., 1991; 5: 309-313.

Weil, J., Bell, G.D., Powell, K., Jobson, R., Trowell, J.E., Gant, P. and Jones, P.H.
Helicobacter pylori and metronidazole resistance.
Lancet, 1990; 336: 1445.

Weil, J. and Bell, G.D.
Detection of *Campylobacter pylori* by the ^{14C} breath test in *Campylobacter pylori* and gastroduodenal disease.
Rathbone, B.J., Heatley, R.V. eds. London: Blackwell Scientific, 1989; 83-93.

Wesdorp, R.I., Falcao, H.A., Banks, P.B., Martino, J. and Fisher, J.E.
Gastrin and gastric acid secretion in renal failure.
Am. J. Surg., 1981; 141: 334-338.

Willems, G. and Lehy, T.
Radioautographic and quantitative studies on parietal and peptic cell kinetics in the mouse. A selective effect of gastrin on parietal cell proliferation.
Gastroenterology, 1975; 69P 416-426.

Williams, J.J., Rodman, J.S. and Peterson, C.M.
A randomized double-blind study of acetohydroxamic acid in struvite nephrolithiasis.
N. Eng. J. Med., 1984; 311: 760-764.

Wyatt, J.I. and Gray, S.F.
Detection of *Helicobacter pylori* by histology.
In: Rathbone, B.J. and Heatley, R.V. (eds).
Helicobacter pylori and gastroduodenal disease.
2nd edition, Blackwell Scientific Publications, London, 1992; pp. 51-63.

Wyatt, J.I., Rathbone, B.J., Green, D.M. and Primrose, J.
Rapid fasting serum gastrin in chronic gastritis is independent of *Campylobacter pylori* status and duodenal ulceration.
Gut, 1989; 30: A1483.

Wyatt, J.I. and Rathbone, B.J.
Immune response of the gastric mucosa to *Campylobacter pylori.*
Scand. J. Gastroenterol., 1988; 23(Suppl. 142): 44-49.

Wyatt, J.I., Rathbone, B.J., Dixon, M.F. and Heatley, R.V.
Campylobacter pyloridis and acid-induced gastric metaplasia in the pathogenesis of duodenitis.
J. Clin. Pathol., 1987; 40: 841-848.

Xu, J.K., Goodwin, C.S., Cooper, M. and Robinson, J.
Intracellular vacuolisation caused by the urease of *H. pylori.*
J. Infect. Dis., 1990; 161: 1302-1304.

Yalow, R.S. and Berson, S.A.
Size and change distinctions between endogenous human plasma gastrin in peripheral blood and heptadecapeptide gastrins.
Gastroenterology, 1970; 58: 609-615.

Yalow, R.S. and Berson, S.A.
Radioimmunoassay of gastrin.
Gastroenterology, 1971; 58: 1-14.

Yalow, R.S. and Berson, S.A.
Radioimmunoassay of gastrin.
Gastroenterology, 1970; 58: 1: 1-14.

Yeomans, N., Skeljo, M.V., Giraud, A.S.
The role of acid regulation in the treatment of NSAID-induced mucosal damage.
Digestion, 1992; 51: (suppl. 1): 3-10.

PUBLICATIONS

Papers

Nujumi, A.M. El., Rowe, P.A., Dahill, S., Dorrian, C.A., Neithercut, W.D., McColl, K.E.L.
Role of ammonia in the pathogenesis of the gastritis, hypergastrinaemia, and hyperpepsinogenaemia I caused by *Helicobacter pylori* infection.
Gut, 1992; 33: 1612-1616.

Nujumi, A.M. El., Dorrian, C.A., Chittajallu, R.S., Neithercut, W.D., McColl, K.E.L.
Effect of inhibition of *H. pylori* urease activity by acetohydroxamic acid on serum gastrin in duodenal ulcer subjects.
Gut, 1991; 32: 866-870.

Nujumi, A.M. El., Fullarton, G.M., Cuthbert, G.F., Bessent, R.G., Gray, H.W., McColl, K.E.L.
Effect of *H. pylori* on gastric-emptying of liquids and solids in DU patients.
Gut, 1991; 32: 556.

Nujumi, A.M. El., Rowe, P., Dorrian, C.A., McColl, K.E.L.
Value of ^{14}C-urea breath test to diagnose of *Helicobacter pylori* in uraemic patients.
Gut, 1991; 32: 1220.

Fullarton, G.M., **Nujumi, A.M. El.,** McColl, K.E.L.
Gastroduodenal pH and duodenal ulcer pain.
Gut, 1992; 33: Suppl. No. 1: T194.

McColl, K.E.L., **Nujumi, A.M. El.,** Chittajallu, R.S., Dahill, S.W., Dorrian, C.A., El-Omar, E., Penman, I., Fitzsimons, E.J., Drain, J., Graham, H., Ardill, J.E.S., Bessent, R.
A study of the pathogenesis of *Helicobacter pylori* negative chronic duodenal ulceration.
Gut, 1993; 34: 762-768.

McColl, K.E.L., **Nujumi, A.M. El.,** Chittajallu, R.S., Omar, E. El., Dahill, S., Bessent, R., Gray, H.
A study of chronic duodenal ulcer disease in patients without *H. pylori* infection.
Gut, 1992; 33: Suppl. No. 1: T162.

McColl, K.E.L., **Nujumi, A.M. El.,** Dorrian, C.A., Macdonald, A.M.I., Fullarton, G.M., Harwood, J.
Helicobacter pylori and hypergastrinaemia during proton pump inhibitor therapy.
Scand. J. Gastroenterol., 1992; 27: 93-98.

Neithercut, W.D., **Nujumi, A.M. El.,** McColl, K.E.L.
Measurement of urea and ammonium concentrations in gastric juice.
Journal of Clinical Pathology, 1993; 46: 462-464.

Rowe, P.A., **Nujumi, A.M. El.,** Junor, B.J.R., Briggs, J.D., McColl, K.E.L.
Helicobacter pylori infection in uraemic patients.
Nephrology, Dialysis and Transplantation, (in press), (Abstract).

McColl, K.E.L., Fullarton, G.M., **Nujumi, A. El.,** Brown, I.L., Hilditch, T.E.
Lowered gastrin and gastric acidity after eradication of *Campylobacter pylori* in duodenal ulcer.
Lancet, 1989; ii: 499-500.

Neithercut, W.D., Rowe, P.A., **Nujumi, A.M. El.,** Dahill, S., McColl, K.E.L.
Effect of *Helicobacter pylori* infection on intragastric urea and ammonium concentrations in patients with chronic renal failure.
Journal of Clinical Pathology, 1993; 46: 544-547.

McColl, K.E.L., Fullarton, G.M., Chittajallu, R.S., **Nujumi, A.M. El.,** Macdonald, A.M.I., Dahill, S.W., Hilditch, T.
Plasma gastrin, daytime intragastric pH and nocturnal acid output before and at one and seven months following eradication of *Helicobacter pylori* in duodenal ulcer subjects.
Scand. J. Gastroenterol., 1991; 26: 339-346.

Neithercut, W.D., Milne, A., Chittajallu, R.S., **Nujumi, A.M. El.,** McColl, K.E.L.
Detection of *Helicobacter pylori* infection of the gastric mucosa by measurement of gastric aspirate ammonium and urea concentrations.
Gut, 1991; 32: 973-977.

Abstracts

Nujumi, A.M. El., Ardill, J.E.S., McColl, K.E.L.
Eradicating *H. pylori* reduces hypergastrinaemia associated with long term omeprazole therapy.
Gastroenterology, (in press).

Nujumi, A.M. El., Hilditch, T.D., McColl, K.E.L.
Effect of omeprazole therapy on determination of *Helicobacter pylori* status by ^{14}C-urea breath test.
Gut, 1996; suppl. 1: 38: A12.

Fullarton, G.M., **Nujumi, A.M. El.,** McColl, K.E.L.
Evaluation of the role of gastroduodenal pH in duodenal ulcer pain.
Gastroenterology, 1992; 102: A72.

Greig, M.A., Neithercut, W.D., Hossack, M., Macdonald, A.M.I., **Nujumi, A.M. El.,** McColl, K.E.L.
Suicidal destruction of *H. pylori* mediated by its urease activity.
Gut, 1990; 31: A600.

McColl, K.E.L., **Nujumi, A.M. El.,** El-Omar, E. Dickson, A.
Evidence supporting a *H. pylori* test and treat strategy for simple dyspepsia.
American Gastroenterological Association, May 1996.