THE EFFECT OF *HELICOBACTER PYLORI* INFECTION ON GASTRIC ACID SECRETION IN MAN

BY

EMAD MUNIR ABDEL-GABBAR EL-OMAR

BSc (HONS), MB CHB (GLASGOW), MRCP (UK)

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IN THE NAME OF GOD

THE MOST GRACEFUL

AND THE MOST MERCIFUL

THIS THESIS IS DEDICATED TO MY

BELOVED FATHER AND MOTHER

PREFACE

Over the past eleven years, I have been extremely fortunate to know and work with Professor Kenneth McColl. Under his kind and expert guidance, I was introduced to the world of scientific research. I regard his faith in me and his mentorship with the greatest affection.

Some of the work presented in this thesis has been published and a list of published papers and abstracts is included at the end. Collaboration with some colleagues has been essential as described in the formal acknowledgements. Except where indicated, the work presented has been carried out by myself.

The writing of this thesis is entirely my own work.

CONTENTS

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I would like to end by expressing my everlasting love and gratitude to my parents who installed in me the desire to search for the truth, to my wife for her love and support and finally to my son Munir for giving us all so much joy and for making everything worthwhile.

SUMMARY

Helicobacter pylori (*H pylori*) infection is the commonest chronic bacterial infection world-wide. The work carried out in this thesis has sought to explore the effect of the infection on gastric secretory function in man.

We developed a new test of gastric acid secretion using the substance gastrin releasing peptide (GRP) as the stimulus. Acid secretion measured in response to intravenous infusion of GRP reflects the combined functional response of the antrum and body of the stomach. It activates both stimulatory and inhibitory controls of acid secretion and in these respects it simulates the response to eating. The reproducibility of the GRP test was assessed and was found to be high for both gastrin and acid secretion.

Using this new tool we studied acid secretion in a variety of subjects with and without *H pylori* infection. We have found that GRP stimulated acid secretion is increased six fold in DU patients with the infection compared to *H pylori* negative healthy volunteers (true normals). This exaggerated acid response is likely to represent a key pathophysiological defect which underlies DU disease. We have demonstrated for the first time that eradication of *H pylori* infection leads to normalisation of basal and GRP stimulated acid secretion in DU patients. These novel findings have shed considerable light on

the understanding of the pathophysiology of DU disease and the role of *H pylori* infection in it.

We have also shown for the first time that GRP stimulated acid secretion is increased two to three fold in *H pylori* positive healthy volunteers compared to true normals. This secretory abnormality also fully resolves following eradication of the infection. Since half the world's population is colonised with *H pylori*, it is essential that all future gastric secretory studies ensure that their control subjects are negative for the infection. The presence of the secretory abnormality in healthy volunteers may also be of relevance to other upper GI diseases such as reflux disease.

We proceeded to investigate the mechanism of the exaggerated acid response to GRP in DU patients and presented evidence compatible with this being due to impaired inhibitory control of acid secretion.

We proceeded to examine the effect of the most commonly prescribed medication for dyspepsia, i.e. ranitidine, on acid secretion in healthy volunteers with and without *H pylori* infection. We showed that a two month course of ranitidine leads to a doubling of basal acid output and a 68% increase in GRP stimulated acid output two days after withdrawing treatment. This rebound acid hypersecretion is not associated with any significant change

in gastrin concentrations and fully resolves within ten days of stopping ranitidine. These findings may offer an explanation for the common clinical problem of rapid resurgence of dyspeptic symptoms following discontinuation of acid suppressive therapy.

Finally, we used the GRP test to study acid secretion in patients with non ulcer dyspepsia (NUD) and *H pylori* infection. We showed that a significant proportion of NUD patients had an exaggerated acid response to GRP similar to DU patients i.e. they displayed the DU diathesis. This raises the exciting possibility that this group of NUD patients may also be cured by eradication of their *H pylori* infection.

CHAPTER ONE

A GENERAL INTRODUCTION ON *HELICOBACTER PYLORI* INFECTION

1.1. HISTORICAL BACKGROUND

Spiral organisms in the human stomach have been described by Bottcher as far back as 1874 (1) and various workers identified spirochaetes in animal and human stomachs during the first half of this century. In 1975, Steer noted spiral bacteria closely apposed to the gastric mucus-secreting cells, but culture only yielded *Pseudomonas aeroginosa* (2). In 1979, Fung et al, working at the Royal Perth Hospital in Western Australia, published histological and ultrastructural studies of the gastric mucosa in which spiral bacteria were seen, but these were judged irrelevant because they did not invade the mucosa (3). Warren, the histopathologist working at the same hospital, did however correlate them with the presence of polymorphonuclear leukocytes (4).

In 1981, a young Gastroenterologist by the name of Barry Marshall, was asked to review with the help of Warren, the patients in whom large numbers of gastric spiral bacteria had been seen. One of these patients had been treated fortuitously with tetracycline and he reported resolution of his dyspeptic symptoms and subsequent endoscopic biopsy showed that the antral gastritis had also resolved (5). This finding prompted the subsequent work in which gastric biopsies from 100 consecutive patients were examined. Gastric biopsies were examined by culture, Gram staining and histology. Among the first 34 specimens, six were positive by Gram staining. Culture, however, did

not yield any growth because the incubation period was limited to 48 hours. Fortunately, however, the 35th specimen was left incubating for five days as this coincided with an Easter holiday. When the plates were finally examined, a pure growth of 1mm transparent colonies was seen. These proved to be *H pylori* and the date of this significant advance was April 14th, 1982 (1).

The bacteria were initially named *Campylobacter pyloridis* by Skirrow (6) but the rules of Latin grammar required the changing of the name to *Campylobacter pylori.* Subsequent studies indicated that the *H pylori* had different taxonomic features from the genus *Campylobacter* and a new genus "*Helicobacter*" was created to accommodate it. The name *Helicobacter* was proposed by Goodwin and was accepted in October, 1989 (1).

In the relatively short period since its discovery, *H pylori* has literally revolutionised our thinking about upper GI Pathology. A massive amount of research has been conducted on all aspects of the infection and it has now been linked with gastritis, duodenal ulcer disease, gastric ulcer disease, non ulcer dyspepsia and gastric cancer. *H pylori* has become the most topical subject in Gastroenterology and arguably the most exciting breakthrough in Medicine in recent years. It promises to enhance our understanding not only of pathophysiologic mechanisms in upper GI disease but, perhaps more

importantly, as a model of the effect of an infection on the elaborate neuro-endocrine interactions in other organs.

1.2 EPIDEMIOLOGY OF *H PYLORI* INFECTION

1.2.1 Prevalence

H pylori is perhaps the commonest chronic bacterial infection world-wide with more than half the world's population carrying the organism. The prevalence of *H pylori* in Western countries is approximately 20% in persons below the age of 40 and 50% of those above the age of 60 years. *H pylori* is uncommon in young children and low economic status predicts *H pylori* infection. Immigration accounts for the isolated areas of high prevalence in some Western countries (7). The apparent increase in the prevalence of *H pylori* with age lead some to believe that the infection is commonly acquired in adulthood and an incidence of 1-2% per annum has been estimated. However, more recent epidemiological data indicate that *H pylori* epidemiology is compatible with a cohort effect in that cohorts born before 1940 have a far greater incidence of infection than subsequent ones (7).

Generally speaking, the prevalence of *H pylori* is much higher in developing countries compared to Western countries (8). Furthermore, the

infection is acquired at a much earlier age so that by the time subjects reach early adulthood, the majority will have acquired the infection. The reason for the higher prevalence of *H pylori* in developing countries is thought to be a reflection of the poor living standards and hygiene which prevail over large sections of these populations. Transmission through the oral-oral and/or faecal-oral route facilitate the rapid spread of the infection under circumstances of overcrowding, lack of hot water supply, and poor hygiene (9).

1.2.2. Duration of Infection and Re-infection with *H pylori*

Once acquired, *H pylori* infection persists lifelong with a spontaneous eradication rate of <1% per year (10). Studies which examine re-infection following eradication of *H pylori* suggest that the recurrence is rare. During a total 314 patient years of follow-up, Borody et al, had only two cases of re-infection following eradication treatment (11). When the recurrence occurs, it is usually diagnosed within six months of ending treatment. It is likely that cases of reinfection are probably recrudescence and not re-infection.

1.2.3 Source and Mode of Transmission

H pylori has never been cultured from the environment although it is able to survive for several days in distilled water, saline, and sea water if these are kept cool (12). Transmission is most probably by close contact between humans. The oral-oral and faecal-oral routes are thought to be involved in the

transmission of *H pylori* between humans. *H pylori* can be cultured from gastric juice (13). It has also been cultured from dental plaque of asymptomatic volunteers (14). It has recently become possible to culture *H pylori* from faeces (15).

1.3. DIAGNOSIS OF *H PYLORI* INFECTION

1.3.1. Histological Diagnosis

Warren demonstrated *H pylori* on gastric epithelium using the Warthin-Stary stain, a silver staining technique used for detection of spirochaetes in tissue section (4). Simplified histochemical stains have been introduced, the most widely employed being a modification of the Giemsa stain (16). There is little to choose between the many staining methods currently employed for detection of *H pylori*. What is more important, however, is a diligent, enthusiastic and well informed histopathologist.

1.3.2 Microbiological Tests

Helicobacters are microaerophilic bacteria and for their isolation *in vitro*, a microaerophilic atmosphere is required where the oxygen content is reduced to around 5%. These conditions are thought to reflect those found in the gastric mucosa. *H pylori* colonies are not visible before 2-3 days of incubation and may take 7 or more days to appear. A variety of methods have been used

to transport gastric biopsies, preserving *H pylori* prior to culture. These include Stewart's transport medium, saline, 20% glucose solution and various broth media.

Culture of biopsies for *H pylori* is relatively expensive and is unnecessary unless antibiotic sensitivities are required. Culture to detect live bacteria and the new ultrasensitive PCR tests will assist in the interpretation of therapeutic trials and epidemiological studies.

1.3.3. Urea Breath Tests

Kline and Graham wrote that "the principle of carbon breath tests is based on isotopic carbon tracers which exploit the concept of a target bond that links a low molecular weight, isotopically labelled group to the remainder of the substrate molecule. Cleavage of the target bond releases the labelled moiety that may be, or may undergo, subsequent conversion to carbon dioxide which is exhaled in the breath. The presence of the labelled carbon dioxide in breath signals the presence of an enzyme which acts on the target form, and the rate and extent of isotopic CO_2 production can be used to estimate the quantity of enzyme present" (17).

In the case of *H pylori*, the enzyme is a urease which is capable of splitting urea into CO_2 and ammonia. The urea can be labelled with either ^{14}C

or ^{13}C. Once the labelled urea is ingested, it is rapidly hydrolysed in the presence of *H pylori* urease to $^{13/14}CO_2$ and ammonia. The labelled CO_2 is then absorbed and excreted in the exhaled breath which would be collected and analysed. If *H pylori* is not present, hydrolysis does not occur and labelled CO_2 is not produced. Serial sample collection and analysis permit the construction of excretion curves. The ^{13}C is a natural non-radioactive isotope and can be measured relative to ^{12}C by gas isotope ratio mass spectroscopy. In recent years, mass spectrometers specifically designed for ^{13}C gas analysis have been developed. ^{14}C, the radioactive isotope of carbon, is measured by liquid scintillation counting.

Urea breath tests have certain advantages over other methods of diagnosing *H pylori*. Unlike serology, urea breath tests reflect current infection status and become negative immediately after suppression or eradication of *H pylori*. Breath tests have an advantage over endoscopic biopsy in sampling a much larger surface area of the stomach with less risk of a sampling error. The ^{13}C urea breath test does not involve any radiation risk but requires an expensive mass spectrometer for analysis. The ^{14}C urea breath test is relatively inexpensive but involves the administration of a small dose of radioactivity which has been equated to the dose received from a standard

chest x-ray. The urea breath tests are regarded by many as the most useful means of assessing *H pylori* status.

1.3.4. Serological Tests

Serology provides the easiest and most convenient approach for the diagnosis of *H pylori* infection. A number of serological methods have been developed, but the enzyme-linked immunosorbent assay (ELISA) has the advantage of simplicity, reliability and cost. Generally, such tests have sensitivities and specificities of >90% and the recent identification of appropriate antigens has lead to significant improvements in these test criteria.

Serology is of great value in epidemiological studies and has played an important role in defining the association between *H pylori* infection and a variety of gastroduodenal diseases, especially gastric cancer. Serology may also be of great value in pre-screening of selected groups with dyspeptic symptoms who will benefit from eradication therapy and will reduce the endoscopic workload. The major disadvantage of serology is the slow fall in titre which occurs following eradication therapy. It is, therefore, not ideal for assessing patients who have received eradication therapy. In addition, treatment of seropositive patients, without prior endoscopy, should be considered only with caution.

1.4. TREATMENT OF *HELICOBACTER PYLORI* INFECTION

Helicobacter pylori is a common bacterial infection world-wide. In spite of the availability of a large number of subjects for therapeutic trials and the extensive interest both from the scientific and pharmaceutical communities, eradication remains difficult. The difficulty in eradicating *H pylori* stems from the nature of this organism, its ecological environment and the host response to it. Unlike the majority of infectious agents, once *H pylori* has successfully colonised the stomach it persists, and unless an antibiotic regimen is able to completely eradicate it, cessation of therapy is followed by rapid recrudescence of the infection to pre-treatment levels within one month. An antibiotic regimen therefore has to abolish all bacteria present and not simply reduce the bacterial load to allow the host factors to eliminate the pathogen.

H pylori resides differentially within the stomach on the surface of the epithelial cells and beneath the mucus layer. This position is protected from gastric acid by the underlying secretion of alkali. The mucus layer also acts to interfere with the diffusion of antibiotics. The low intragastric pH also inactivates many antibiotics rendering them ineffective against *H pylori*. In addition, the constant emptying of the stomach reduces the effective antibiotic concentration necessary to act on the bacteria.

The ability of *H pylori* to cope with an acid environment has largely lead to its success as a parasite. Its powerful urease enzyme splits urea present in the gastric juice to ammonium hydroxide, an alkaline solution, which then surrounds the organism while it is present in the lumen of the stomach. Subsequent to that, the organism is able to penetrate the submucous layer which overlies the gastric epithelial cells where the pH is close to neutral. The possession of the urease enzyme therefore enables *H pylori* to colonise an inhospitable environment and gives it an advantage over other organisms which have not developed this ability to cope with acid.

Colloidal bismuth subcitrate was the first antimicrobial compound found to be effective against *H pylori in vivo*. The observation that treatment of duodenal ulcer with this drug led to a reduced recurrence rate compared with other compounds lead indirectly to the discovery that *H pylori* eradication prevented ulcer relapse. Used alone, bismuth compounds are only successful in 10-30% of individuals. Combination with other antibiotics led to higher eradication rates and the concept of triple therapy evolved. Triple therapy has become the treatment of choice in recent years (18). Most regimens use colloidal bismuth subcitrate together with metronidazole and tetracycline or amoxycillin. The doses and duration of treatment with these three drugs are widely variable and there is no standard regimen which has been found to be superior to the others. In our own Unit in Glasgow we have had a 90%

eradication rate with triple therapy consisting of De-Nol 120mg t.i.d., metronidazole 400mg t.i.d. and amoxycillin 500mg t.i.d. The three drugs are given for a total of three weeks. In patients who are allergic to penicillin, tetracycline at a dose of 250mg t.i.d. is given instead.

In recent years a new approach to the eradication of *H pylori* emerged with the use of an acid inhibitor plus one or two antibiotics. The commonest prescribed regimen combines omeprazole at a dose of 20mg b.d. with amoxycillin 500mg t.i.d. Eradication rates of between 30-80% have been reported. An eradication rate of 78% was reported with omeprazole 20mg b.d. and clarithromycin 500mg t.i.d. (19). The majority of patients will respond to either triple therapy or omeprazole in high dose plus an antibiotic. In patients who have a metronidazole resistant organism, it is logical to start with the omeprazole/amoxycillin or clarithromycin combination. If a patient fails to respond to either regimen it is advisable to try the other if indication is still present. It is important, however, to emphasise the importance of compliance and the risk of inadequate treatment with the emergence of resistant strains. Most patients tolerate triple therapy and other regimens reasonably well with only minor side-effects, the commonest of which being diarrhoea, nausea and occasional dyspepsia. Serious complications such as pseudomembranous colitis are exceedingly rare.

1.5. DISORDERS ASSOCIATED WITH *H PYLORI* INFECTION

1.5.1. Chronic Gastritis

Helicobacter pylori is now well established as the cause of Type B antral gastritis. This causal relationship is supported by a number of facts which include:- (1) a strong association of chronic gastritis with *H pylori* infection in 60-90% of patients; (2) the absence of *H pylori* in the autoimmune forms of chronic gastritis and in normal gastric mucosa; (3) the possession of *H pylori* of a number of virulence factors capable of initiating gastritis; (4) the fulfilment of Koch's postulate in that volunteers who ingested *H pylori* later developed gastritis and dyspeptic symptoms. *H pylori* colonisation of the gastric mucosa was demonstrated in those cases (20,21); (5) experimental studies in animals; (6) the resolution of the histological changes of gastritis including reduction in the inflammatory activity in the gastric mucosa and healing of the morphological lesions following suppression and/or eradication of the infection by antibiotics.

H pylori associated chronic gastritis is thought to start from the antral region of the stomach and progressively spread to the mucosa of the gastric body. Chronic gastritis induced by the infection is characterised by a neutrophil granulocyte infiltrate, which is detected within the epithelium and the lamina

propria. While *H pylori* is detectable in the active form of chronic gastritis, it disappears in the inactive form which is considered to be a later stage in the evolution of *H pylori* gastritis. Progression of chronic gastritis is accompanied by varying degrees of atrophy (22). *H pylori* infection induces a characteristic histological appearance and for this reason a new system of classifying chronic gastritis relying on aetiology, topography and morphology has been suggested (the Sydney System) (23).

1.5.2. Gastric Ulcer

The association between *H pylori* and gastric ulcer is less frequent than between *H pylori* and DU and is approximately 60-70%. This is largely due to the fact that a large proportion of gastric ulcers are caused by non-steroidal anti-inflammatory drugs. Sidebotham and Barron, 1990, proposed that *H pylori* infection predisposes to gastric ulceration through alteration of the mucus glycoprotein composition with influence on bicarbonate entrapment, allowing excessive backdiffusion of hydrogen ions (H^+) with breakdown of the mucosal barrier (24). The dense intra-epithelial infiltration of neutrophils, probably followed by an increase in free oxygen radicals, may be another element in the pathogenesis of gastric ulcer.

1.5.3 Gastric Cancer

According to the Lauren classification, gastric adenocarcinoma can be subdivided into the intestinal (the most prevalent) and the diffuse forms. The intestinal form typically arises in the antral region of the stomach. Due to the well established role of *H pylori* infection in chronic antral gastritis, an association has been sought between the infection and the intestinal type of gastric cancer.

In support of this association is the finding that *H pylori* induced antral gastritis progresses to atrophy after 10-20 years (25). Intestinal metaplasia, which is considered to be a precancerous condition follows 10-20 years after the appearance of atrophic changes (26,27). A precancer-cancer sequence has therefore been hypothesised which suggests that *H pylori* induced chronic superficial gastritis evolves through chronic gastritis with progressive atrophy to intestinal metaplasia, dysplasia and gastric cancer. This sequence of histological changes requires the acquisition of *H pylori* infection in early childhood.

Epidemiological studies have shown that there is a geographic association between those areas of the world with high rates of gastric cancer and those with a high prevalence of *H pylori* infection (28,29), although there are important exceptions to this general pattern, most notably in Africa (30). The

most convincing epidemiological evidence in support of an association is that from three prospective studies (31-33). In these studies blood was taken from healthy subjects and stored, and *H pylori* antibody status was later assessed in those who went on to develop gastric cancer. In all three studies, infection was significantly more common in the gastric cancer patients than in appropriately matched control groups.

1.5.4. Non Ulcer Dyspepsia (NUD)

The prevalence of *H pylori* infection in patients with NUD is higher than in normal age-matched controls. The reported prevalence varies from 50-87% depending on the series. Due to the controversial definition of NUD and the complexity of symptoms, the association with *H pylori* has been difficult to define. In particular it is difficult to define a particular symptom complex which could be linked to the infection although a higher symptom score has been reported by Lambert et al (34). Rathbone et al showed that belching is more frequent in *H pylori* positive patients (35), and Rokkas et al demonstrated a positive relationship between *H pylori* infection and postprandial bloating (36).

Studies which examined the effect of eradicating *H pylori* infection on symptoms in NUD patients also yielded conflicting results but the consensus seems to be that eradication therapy improves symptoms in a subpopulation of patients.

The major challenge in this field is to define this subpopulation of patients whose symptoms are caused by the infection and who are therefore likely to benefit from its eradication. This area is of relevance to the work presented in this thesis and will be discussed in greater detail later.

1.5.5. Duodenal Ulcer Disease

The highest prevalence of *H pylori* infection is found in duodenal ulcer patients. It is now generally accepted that more than 95% of DU patients have the infection. In all these patients the presence of the infection is associated with the presence of chronic gastritis which profusely involves the antral mucosa, often with mucosal lymphoid follicles. The body type mucosa is normal or only mildly inflamed and does not show any atrophy of acid secreting glands. This is the diffuse antral pattern of gastritis described by Correa, which is now known to be very closely associated with *H pylori* colonisation. The proportion of *H pylori* positive patients who have this pattern of gastritis varies and is thought to be in the region of 35%.

The importance of the role of *H pylori* in the aetiology of duodenal ulcer is most convincingly demonstrated by the absence of ulcer relapse in patients in whom *H pylori* has been eradicated, an effect which is independent of any mucosal protective effect of bismuth compounds. A number of important

studies have now shown that eradication of *H pylori* infection is associated with a dramatic lowering of the ulcer relapse rate (37-40) adding strong support to the causal relationship between *H pylori* infection and DU disease. This association is now so well established that a group of scientists who met at the NIH in Bethesda, USA, have recently issued a consensus statement in which they recommend that all patients with DU or GU disease who have *H pylori* infection should now be treated with eradication therapy regardless of whether they are suffering from the initial presentation of the disease or a relapse (41).

There are two main schools of thought regarding the role of *H pylori* in the pathogenesis of duodenal ulcer disease. The first argues that *H pylori* causes duodenal ulcers through a direct effect on the patches of gastric metaplasia which are frequently found in the duodenal mucosa of patients with duodenal ulcer. Duodenal ulcer patients are known to be hypersecretors of acid and this leads to the development of gastric metaplasia in the duodenum either as a direct result of epithelial damage caused by the acid/peptic attack or as a result of the inflammation secondary to this damage. These patches of gastric metaplasia are later colonised by *H pylori* organisms gaining access from the antral region. The presence of *H pylori* in these metaplastic patches leads to further inflammation and further metaplasia with a vicious cycle developing in which continued acid/peptic activity together with inflammation

act to overwhelm the normal protective mechanisms in the form of mucus-bicarbonate secretion and rapid cell replication and repair. When any of these repair mechanisms are overwhelmed by these or superimposed factors, such as NSAIDs or smoking, the balance will ultimately be tipped towards ulcer formation. However, against this hypothesis is the finding that prepyloric ulcers are found with equal frequency in patients with and without *H pylori* infection, suggesting that the presence of the infection is not necessary for the development of the actual ulcer.

The second school of thought argues that *H pylori* infection predisposes to duodenal ulceration through its disturbance of gastric physiological processes. Since this subject is of major relevance to the work presented in this thesis, it will be discussed in more detail in chapter 3 of the thesis.

In the next chapter a more detailed account of DU disease is given to set the background to the majority of work undertaken in this thesis.

CHAPTER TWO

DUODENAL ULCER DISEASE

2.1. INTRODUCTION

Peptic ulcers were once so common in industrialised nations that they were virtually considered stigmata of civilisation. Despite the huge volume of literature dealing with the different aspects of peptic ulcer disease, the exact pathogenesis of this condition remained largely unknown. The discovery and successful culture of *H pylori* in the early *80's* has revolutionised our thinking about peptic ulcer disease.

Peptic ulcers are chronic, most often solitary lesions that occur in any level of the gastrointestinal tract. Approximately 98-99% of peptic ulcers occur in either the duodenum or the stomach in a ratio of about 4:1. About 10-20% of patients with gastric ulcers have a concurrent duodenal lesion. Wherever they occur, chronic peptic ulcers have a fairly standard, virtually diagnostic gross and microscopic appearance. Despite this uniform morphology, gastric and duodenal ulcers may represent two somewhat distinctive diseases. Different influences are thought to be involved in their pathogenesis and there are further contrasts in their genetic linkages. The work presented in this thesis is largely concerned with the pathogenesis of duodenal ulcer disease and for this reason all subsequent discussions will be limited to DU disease.

2.2 EPIDEMIOLOGY OF DUODENAL ULCER DISEASE

2.2.1. Prevalence and Incidence

Prevalence: A number of well designed and conducted studies and surveys show that in developed countries approximately 10% of the population are likely to be affected by peptic ulcer during their lifetime, with the point prevalence for active ulcer disease about 1% (42).

Incidence: Several studies performed over three decades point to a yearly incidence of peptic ulcer of between fifteen and thirty cases per one thousand.

2.2.2. Trends in Ulcer Occurrence

Peptic ulcer disease was infrequently recognised before the beginning of the 20th century. The occurrence of DU disease then increased steadily until 1960. Over the last 20 years, however, a number of indirect indicators such as rates of hospitalisation, operation, and death suggested a marked decrease in DU and possibly in GU in England, Europe and the United States (42). The apparent decline in the number of hospitalisations for uncomplicated ulcer contrasts with trends regarding ulcer complications. Rates of hospitalisation for ulcer haemorrhage fell only slightly for DU while perforations were unchanged.

Death rates for ulcer disease complications appeared to decline for men but remained stable or possibly increased for women (42).

In the past, DU disease was commoner in males than in females. For example, the male to female ratios for DU hospitalisation and mortality rate were respectively 1.8:1 and 2.4:1 in 1970. Both ratios decreased in 1983 to 1.3:1 (43). The trends in ulcer occurrence reflect declining rates, particularly for younger men, and increasing rates, particularly for older women. Age-cohort phenomena account for these changes with at least three age and gender dependent environmental elements. The first is the fact that prevalence of *H pylori* infection increases with age. This apparent increase as mentioned previously reflects an age-cohort phenomenon. Improved hygiene and sanitation in developed countries predicts decreased exposure at young age and probably a decreased incremental infection rate as people age. The second element is the fact that complications from NSAID ulcers increase as a function of age, in parallel with an increased rate of prescription. The third element relates to smoking which is declining in younger people, particularly male, and is increasing in women, possibly affecting the declining male to female ratio.

2.2.3. Regional and Societal Variables

It is widely believed that DU affects highly stressed professionals and executives. However, the available evidence suggests that DU is slightly more common among unskilled labourers (44). There is an inverse relationship between peptic ulcer and family income (45), matched by a similar relationship between *H pylori* infection and family income.

There is some variation in the prevalence of peptic ulcer disease between geographic regions. For example, the prevalence of DU is higher in Scotland and Northern England compared to Southern England (44). Several factors may account for this regional variation including diet and the regional distribution of *H pylori* infection. It is interesting to note that in the Aboriginal population of Australia, peptic ulcer disease is virtually unknown in parallel with a very low prevalence of *H pylori* (46). In contrast, some areas of Ethiopia have a very high prevalence of *H pylori* seropositivity matched by a high prevalence of DU disease (47). On the other hand, populations in Northern Nigeria have a high serology prevalence of *H pylori* with a low prevalence of ulcer disease (48). This suggests that other variables are clearly involved in the pathogenesis of duodenal ulcer disease.

2.2.4. Diseases Associated With Duodenal Ulcer Disease

Reflux Oesophagitis and Barrett's oesophagus

DU disease is commonly associated with reflux oesophagitis and the link is thought to involve acid hypersecretion. Because of the association of DU and oesophagitis, an association may also exist with Barrett's oesophagus and oesophageal adenocarcinoma.

Chronic Pulmonary Diseases

There is an association between peptic ulcer disease and chronic obstructive airways disease. Up to 30% of patients with chronic lung disease have peptic ulcers and the frequency of chronic lung disease in peptic ulcer patients is increased 2-3 fold (42). Gastric ulcers appear to be more closely associated with lung disease than DU but both are associated. The major factor underlying this association is thought to be smoking which affects both diseases.

Evidence is available for the association of peptic ulcer disease and alpha-1-anti trypsin deficiency and cystic fibrosis. The relative risk for peptic ulcer and alpha-1-antitrypsin deficiency is estimated to be 1.5:3 times greater than the general population (49). In a study by McColley et al, sixty cystic fibrosis patients were studied (50). Forty of these were white and twenty were

black and 13% of the black patients but none of the white patients developed peptic ulcer disease. It is unclear whether the difference between the two groups could be accounted for by the higher incidence of *H pylori* in the black patients. The low incidence of DU in cystic fibrosis in the white patients, however, suggests that decreased pancreatic bicarbonate secretion *per se* is not a major risk factor for ulcer disease.

Cirrhosis

The incidence and prevalence of DU appears to be increased in cirrhotic patients. A study by Rabinovitz et al examined endoscopically 216 males being evaluated for liver transplantation (51). They found an apparently increased prevalence of active duodenal ulceration in cirrhosis caused by alcoholism (12.2%), chronic active hepatitis due to HBV (9.4%), and primary sclerosing cholangitis (8.5%).

The mechanism underlying the association between peptic ulcer disease and cirrhosis is unclear. There is conflicting data regarding serum gastrin levels and acid secretion in cirrhotic patients (52,53). Decreased hepatic metabolism of GI hormones and altered mucosal blood flow have been hypothesised as pathogenic factors. There are no reports on the prevalence of *H pylori* infection in cirrhotic patients. In addition, there has been no studies examining the subset of patients who develop ulcers, particularly in relation to

acid secretion. It is known that portacaval shunting increases the sensitivity to endogenous and exogenous gastrin but it is not clear how this increased sensitivity can lead to an ulcer diathesis (53).

Renal Failure and Transplantation

There are conflicting data regarding the prevalence of peptic ulcer during maintenance haemodialysis. Some studies suggest that the prevalence of DU is increased in patients prior to the commencement of haemodialysis. There is an increased risk of DU after renal transplantation. Acid secretion may be low, normal, or high in chronic renal failure on haemodialysis. During haemodialysis, basal acid hypersecretion has been increased in some series but not in others (42). Increases in acid secretion following transplantation have been reported but are controversial. Although hypergastrinaemia occurs in renal failure, an inverse relationship to acid secretion reflects underlying atrophic gastritis with no evidence for gastrin driven acid hypersecretion. There is no solid support for decreased renal metabolism of gastrin resulting in hypersecretion (42).

In renal transplantations, it appears that many ulcer cases are a form of stress ulceration. Peptic ulcer in uraemic patients is not linked to *H pylori* as the occurrence of antibodies with *H pylori* is similar in renal failure patients with or without peptic ulcer (54).

Other Disease Associations

Peptic ulcer disease is more common in patients admitted with venous thromboembolism, pulmonary embolism, coronary artery disease, patients with reduced exocrine pancreatic function, patients with renal calculi associated with multiple endocrine neoplasia (MEN I) syndrome, reflecting the presence of both gastrinoma and hypoparathyroidism (42).

2.2.5. Disorders Associated With A Decreased Incidence Of Duodenal Ulcer Disease

Patients with atrophic body gastritis (type A gastritis) have a decreased incidence of peptic ulcer. Auto-immune diseases associated with atrophic gastritis such as Addison's disease, auto-immune thyroid disease and hypoparathyroidism also have a low incidence of peptic ulcer (55).

There is also a negative association between gastric carcinoma and DU disease. This is probably due to the fact that most patients with gastric carcinoma have advanced atrophic gastritis with acid hyposecretion which reduces the risk of DU disease.

2.3. AETIOLOGY OF DUODENAL ULCER DISEASE

The pathogenesis of duodenal ulcer disease is multi-factorial. Common and uncommon causes are implicated. By far, the commonest forms of duodenal ulcer are associated with *H pylori* infection, non-steroidal anti-inflammatory drug usage and stress ulceration. Amongst the uncommon specific forms of DU disease are: gastrinoma, mastocytosis, basophilic leukaemias and antral G cell hyperfunction/hyperplasia. Other infections, in particular viral, such as herpes simplex virus Type I and CMV, have also been implicated. Vascular insufficiency caused by crack cocaine-associated perforations has been described. Radiation induced and chemotherapy induced duodenal ulcers are also reported in the literature.

Acid hypersecretion sets the stage for many cases of duodenal ulcers but it is naive to think that ulcers occur simply from the primary failure of regulatory mechanisms of acid secretion or mucosal defence. It is more likely that ulcers occur when the normal mechanisms controlling acid secretion and mucosal defence are disrupted by super-imposed factors such as *H pylori* infection, NSAID usage etc. Although NSAID induced and stress induced ulcers are common, they will not be specifically discussed in the subsequent sections as they are not directly related to the work presented in this thesis. The following sections review some of the abnormalities which have been documented in

duodenal ulcer disease and their relevance to the pathogenesis of duodenal ulcer disease is discussed.

2.3.1. Genetics Of DU Disease

Familial Aggregation of DU

Twenty-50% of DU patients have a positive family history for DU compared with 5-15% of non-ulcer subjects (49). First degree relatives of patients with DU have a three-fold increase in the prevalence of DU but not of GU. Concordance of DU is more common in monozygotic than dizygotic twins, suggesting a genetic element but incomplete concordance of ulcer in monozygotic twins indicated that non-genetic factors were also operative (49,56).

Genetic Markers in Peptic Ulcer

Several indirect genetic markers have been linked to peptic ulcer disease. Subjects with blood group O have a 30% increase in risk of DU compared to subjects with blood groups A, B or AB. There is a 50% increase in the risk of DU in subjects who do not secrete ABO blood group antigens into body fluids (the so-called non-secretors), such as saliva. Subjects with both blood group O and non-secretor status have a 150% increase in risk of developing DU (49,56). It is not clear how *H pylori* infection fits with these old associations. A

report by Mentis et al indicated that ABO status was related to ulcer independently of H pylori (57).

Familial Clustering of DU Disease

There have been reports suggesting that association between peptic ulcer disease and autosomal dominant inheritance of hyperpepsinogenaemia I. However, Mertz et al re-analysed sera from these cases and discovered that there was an 87% seropositivity for *H pylori* infection in subjects with a pepsinogen I level >100ng/ml. The seropositivity was only 35% in those with pepsinogen I level less than this value (58). Both pepsinogen I and antibodies to *H pylori* increased with age of the subjects in a fashion very similar to that observed in other studies of *H pylori* antibodies. It is likely therefore that the prevalence of peptic ulcer disease and hyperpepsinogenaemia I reflects familial clustering of *H pylori* infection rather than autosomal dominant inheritance of hyperpepsinogenaemia I.

Similarly, old data suggested that a subset of DU patients who are hypergastrinaemic have hyperfunction of their antral G cell population. However, since the discovery of *H pylori* and its resultant hypergastrinaemia, it is more likely that the association between hypergastrinaemia and peptic ulcer disease is caused by *H pylori*.

2.3.2. Psychologic Factors In Peptic Ulcer Disease

Psychodynamic factors have always been implicated in the pathogenesis of peptic ulcer disease. These factors have not been correlated with pathophysiologic parameters against the background of *H pylori* to define the subset of persons in whom these mechanisms constitute important, predisposing or exacerbating factors.

Psychologic Conflict and Personality Type

This subject is surrounded by controversy and few conclusions can be made from the published work. The classic theory defined the ulcer personality characterised by an exaggerated dependency / independency conflict. This view is now largely rejected by most investigators. It appears that duodenal ulcer patients frequently display personality disorders suggesting a psychodynamic element in the pathogenesis of their disease. The nature of the personality disorders is variable, reflecting the heterogeneity of the population examined as well as the methodology and definitions used by the investigators.

Role of Physical and Psychologic Stress

There is no doubt that peptic ulcer disease seems to be exacerbated by stressful life events. During the blitz of London in the autumn of 1941, an increased number of perforated peptic ulcers were reported (59). Twenty-five

per cent of prisoners of war developed DU during a follow-up period compared with an 11% occurrence in non-captured veterans (59). The person's interpretation and reaction to stress is probably more important than the intensity of external stress. It is important to distinguish between acute stressors, which are not excessive in DU patients, and chronic stressors such as high goal frustration, etc., which have been associated with the onset and relapse of ulcers (60).

The mechanism potentially linking psychologic factors to ulcers remain speculative. It is important to stress that DU occur only in patients with an adequate parietal cell mass to support an acid/peptic process. It is tempting to speculate that psychologic factors, via vagal mechanisms, may underlie an increase in basal and nocturnal acid outputs observed in some ulcer patients. Again it remains speculative whether chronic psychologic stress alters parietal cell mass. It is known that chronic vagal stimulation increases acid secretory mass in an animal model (61).

Psychodynamic factors may also alter mucus secretion, mucosal blood flow, and gastric motility and produce mucosal damage not clearly explained by an increase in acid secretion. Neural factors may play a role in the immune response and susceptibility to viral infection (62), providing a precedent for

psychodynamic factors possibly influencing gastric inflammatory response and resistance to *H pylori.*

2.3.3. Potential Risk Factors For DU Disease

A number of potential risk factors for DU disease have been implicated over the years. In the section below three of these factors are discussed and their contribution to DU disease is evaluated.

(A) Cigarette Smoking

There is sufficient evidence to link smoking to peptic ulcer disease: (1) Epidemiological studies indicate that smoking increases the risk for both DU and GU. This risk appears to be proportional to the amount smoked (63,64). However, in the light of the discovery of *H pylori*, the contribution of smoking to the pathogenesis of DU disease has been somewhat undermined. (2) Smoking impairs ulcer healing and promotes recurrences. (3) Smoking increases the risk of complications and the need for surgery (65). (4) Death rates from ulcer disease are greater in smokers.

Several mechanisms have been proposed for the effects of smoking on ulcer disease. These include: increased gastric emptying promoting duodenal acidification, increased basal and maximal acid secretion, decreased pancreatic bicarbonate secretion, altered gastroduodenal motility leading to reflux of duodenal contents, increased pepsinogen I secretion, decreased gastric mucosal prostaglandin production and altered blood flow (64,66-69). In general, these several effects of smoking are modest, variable, and

controversial; no single factor adequately explains the clinical impact of smoking on ulcer disease and there is not yet consensus on which factors contribute to pathogenesis.

(B) Alcohol

High ethanol concentrations damage the gastric mucosal barrier to hydrogen ion resulting in acute gastric erosions and haemorrhages (70). The acute effects do not appear to cause either gastritis or chronic peptic ulcer.

The effect of alcohol on gastric acid secretion is controversial and appears to reflect a biphasic dose response: concentrations between 1 and 4% produced stimulation of acid secretion while higher concentrations had no effect or inhibited secretion (71).

Wine and beer are strong stimuli of acid secretion in humans, producing 50-90% of maximal acid output. This stimulation of acid appears to be mediated to a large extent by gastrin (71,72).

(C) Diet

It is widely believed among lay people that dietary indiscretion is a cause of ulcers. While certain foods, beverages and spices cause dyspepsia, there are no convincing data that such foods cause, perpetuate or reactivate peptic ulcers.

Coffee is a strong stimulant of acid secretion and produces dyspepsia in many subjects. This is most probably due to enhanced oesophageal reflux (73). Caffeine does not seem to be the culprit as decaffeination does not reduce either effects of coffee (73). It is interesting to note that subjects with NUD, but not DU patients, experienced more dyspepsia with coffee than did normal controls (74). Attempts to link coffee consumption as a risk factor for ulcer disease have failed.

A variety of other caffeine- and noncaffeine- containing drinks (Coca-Cola, 7 UP, and tea) also potently stimulate acid secretion, producing more than 50% of maximal secretory response to pentagastrin (75).

2.3.4. Abnormalities of Acid and Pepsin Secretion in DU Patients

Since the time of Schwartz with his famous dictum "No Acid : No Ulcer", DU disease has been regarded as an acid/peptic disease. The arrival of *H pylori* infection on the scene has lead some to challenge this accepted wisdom and to propose a new dictum " No *H pylori* : No Ulcer". However, a number of well established abnormalities of acid and pepsin secretion are present in DU patients and it is important to try and account for these in the light of our new knowledge of *H pylori* infection.

Maximal Acid Output

The maximal acid secretory response of the stomach is an indirect measure of the parietal cell mass. DU patients have an increased maximal secretory response to stimulation with pentagastrin, gastrin, histamine, betazole and caffeine compared to normal subjects (76-80). It has been estimated that DU patients on average have a 1.5-2 times greater parietal cell mass than control subjects (78,81,82). It is important to note that a great degree of overlap exists between DU patients and healthy controls with only 20-50% of DU patients having elevated maximal acid outputs.

Basal, Nocturnal and Meal-Stimulated Acid Output

Basal acid output is increased in approximately one third of DU patients (76,79,83). Nocturnal acid output discriminates more effectively between DU patients and normal subjects (84-86) and is significantly higher in DU patients with little overlap with healthy controls. It is controversial whether meal-stimulated gastric acid secretion is increased in DU patients. The results are undermined by the difficulty of measuring acid output in the presence of food in the stomach. The acid secretory response to a meal appears to be prolonged in DU patients, remaining elevated compared to normals in the period 3 to 5 hours after a meal (79,83). Sustained acidification following a meal was also shown in studies which used continuos pH monitoring (87-88).

Possible Mechanisms for the Increased Secretory Capacity and Drive in DU Patients

The increased parietal cell mass characteristic of some DU patients may be the result of genetic factors or environmental factors or both. It is possible that some individuals are born with a larger parietal cell mass which is then subject to a variety of factors, some of which may be environmental, which act to attenuate this cell mass. However, the trophic factors which maintain the oxyntic mucosal mass in normal subjects or promote an increased secretory mass in some DU patients remain unknown. Gastrin is implicated as an important trophic factor for oxyntic mucosa stimulating oxyntic mucosal growth

(89,90) and increasing secretory mass in gastrinoma patients. However, as will be discussed in chapter 3, *H pylori* infection causes increased basal, meal- and bombesin- stimulated gastrin release in subjects with *H pylori* infection regardless of whether they are ulcer subjects or not. This finding renders studies of acid secretion and parietal cell mass in peptic ulcer uninterpretable unless *H pylori* status is simultaneously considered in both ulcer subjects and normal controls.

The vagal nerve also exerts trophic control over the oxyntic mucosa (91). Thirlby and Feldman (61), using a dog model, showed that vagal stimulation from sham feeding repeated over a six week period increased acid output from vagally innervated, but not denervated, gastric pouch. Histamine has not been reported to exert trophic effects on the oxyntic mucosa.

Thus, gastrin or vagal mechanisms or both are likely to be involved in the apparent increased secretory capacity of DU patients.

Regarding the increased secretory drive seen in DU patients, it is likely that a complex process involving increased stimulatory or diminished inhibitory activity of the endocrine, neural, paracrine or autocrine regulatory pathways might result in increased basal and interdigestive secretory drives. The nature

of these mechanisms and the abnormalities that result due to colonisation of the gastric mucosa by *H pylori* infection remain to be explored.

Pepsinogen and Pepsin

The human stomach produces two immunochemically distinct types of pepsinogen: pepsinogen I produced in the chief cells and mucus neck cells of the oxyntic mucosa and pepsinogen II which is present in the gastric body and antrum. Together they account for the acid protease activity of gastric juice.

Peptic activity is closely linked to gastric pH, with H^+ ions converting pepsinogen to the active protease pepsin. The pepsin is inactivated when the pH of gastric juice rises above 4.0. Acid plus pepsin is much more ulcerogenic than acid alone (92), a fact which is reflected in the label "peptic" which describes the majority of ulcers.

Serum pepsinogen I concentrations are elevated in 30-50% of DU patients. A high serum pepsinogen I level increases the risk of developing DU (92). Hyperpepsinogenaemia I may be a predictor of refractory or recurrent ulcer. A high pepsinogen II level increases the risk for GU (93).

It has recently been found that pepsinogen I and II levels are increased in the presence of *H pylori* infection (94), and eradication of the infection appears

to reduce serum pepsinogen I (95). What is also interesting is the finding that the apparent familial association of hyperpepsinogen I with peptic ulcer disease reflects familial clustering of *H pylori* infection (58). It is likely therefore that the abnormalities in the pepsins described in association with peptic ulcer disease may be induced by *H pylori* infection. To date, no studies have attempted to test the hypothesis that elevated pepsinogen I levels mark the subgroup of *H pylori* positive subjects with an ulcer diathesis compared with *H pylori* positive subjects without ulcers.

The work undertaken in this thesis has sought to explore some of the mechanisms involved in the pathogenesis of DU disease taking into account the presence of *H pylori* infection. In the following chapter I will review the effect of *H pylori* infection on gastric acid secretion which is likely to be the key factor in the pathogenesis of ulcer disease.

CHAPTER THREE

HELICOBACTER PYLORI COLONISATION AND ALTERATIONS IN GASTRIC ACID SECRETION

It is essential to realise that *H pylori* infection affects gastric physiology differently in the acute and chronic stages. Very little is known about the sequence of events following acute infection of *H pylori* and some evidence exists to suggest that the infection may turn off the production of acid for a short period. However, much work needs to be done in this field before we can make any generalisation. It is recognised, however, that the type of gastritis which is associated with ulceration both in the stomach and duodenum is the chronic form, the effects of which are discussed below.

3.1. *H PYLORI* AND GASTRIN

Numerous studies have shown that *H pylori* infection is associated with increased basal, meal stimulated and bombesin/gastrin releasing peptide stimulated gastrin concentrations which rapidly return to normal following eradication of the infection (96-101). The degree of hypergastrinaemia caused by *H pylori* is the same in DU patients and healthy volunteers (97). The elevated gastrin level associated with *H pylori* infection is due to an increase in the biologically active Gastrin-17 (G17) form of the hormone (102). G17 is mainly produced in the gastric antrum whereas G34 is mainly produced in the duodenum. The increase in the G17 form is consistent with *H pylori* predominantly affecting the antral mucosa. In addition, G17 is the main gastrin which increases in response to eating (102), and this is consistent with *H pylori* associated hypergastrinaemia being most marked postprandially.

3.2. MECHANISM OF *H PYLORI* ASSOCIATED HYPERGASTRINAEMIA

The mechanism by which *H pylori* infection causes increased antral gastrin release is unclear. Levi et al postulated that the ammonia produced by the high bacterium urease activity might raise antral surface pH and thereby prevent the physiological inhibition of gastrin release by intragastric acid (103). McColl et al in Glasgow have performed a number of studies in order to test this very plausible hypothesis. In the first study, *H pylori* ammonia production was increased *in vivo* by the intragastric infusion of urea to infected subjects, but found no change in the serum gastrin (104). Graham et al performed a similar study with the same result (100). Nujumi et al also assessed the effect of inhibiting *H pylori* urease activity *in vivo* by the oral administration of acetohydroxamic acid to infected subjects. Despite inhibiting bacterial urease activity by more than 80%, no change could be detected in serum gastrin (105). In a further study, the same authors completely suppressed *H pylori* urease activity by 36 hours of triple therapy but again could not demonstrate any fall in either basal or meal stimulated gastrin concentrations (106).

If *H pylori* were raising gastrin by its ammonia production blocking the inhibiting effect of intragastric acid, then *H pylori* associated hypergastrinaemia should be most marked at low intragastric pH, at least in the absence of acid.

However, Chittajallu et al found that *H pylori* infection raised gastrin by a similar percentage in the presence and absence of gastric acid (107). Likewise, Moss et al (108) and Karnes et al (109) found that *H pylori* infection was associated with a similar percentage increase in gastrin at pH 5.5 and pH 2.5.

Studies to date, therefore, do not support the hypergastrinaemia being secondary to bacterial ammonia interfering with the acid inhibitory control of gastrin release.

Another possible cause of *H pylori* induced hypergastrinaemia is that it is a compensatory response to the infection reducing gastric acid secretion. There is some evidence that acute *H pylori* infection may cause hypochlorhydria, and in some subjects this could persist and thus produce reflex hypergastrinaemia. Both McColl et al and Calam et al have studied the acid response to increasing doses of gastrin in DU patients before and following eradication of *H pylori* and have found no change in parietal cell sensitivity (110,111). This excludes the increased gastrin being secondary to the infection impairing parietal cell function in patients with DU. However, there are no data on the effects of chronic infection on parietal cell function in non-DU subjects.

The release of gastrin by the antral mucosa is under inhibitory control by somatostatin release from the antral D cells. The hypergastrinaemia could therefore be secondary to a relative deficiency of somatostatin. As somatostatin mainly exerts its effects locally in a paracrine fashion, it is necessary to look at the concentrations of the hormone in the mucosa rather than in the serum. Kaneko et al reported reduced immunoreactive/ somatostatin concentrations in gastric mucosal biopsies of patients with *H pylori* infection (112). However, a wide variety of upper gastrointestinal disorders were included in the study, and it is difficult to be sure whether the changes in somatostatin were related to the infection, to specific gastrointestinal disorders or to drug therapy. However, a significant correlation was noted between the severity of chronic inflammation and degree of depletion of somatostatin. In a study of 18 DU patients, Moss et al reported a rise in antral mucosal somatostatin/immunoreactive cell density at four weeks following eradication of *H pylori* infection (113). In a similar study in ten such patients they noted a rise in antral mucosal somatostatin mRNA at four weeks following eradication of the infection (113). Recently, Graham et al studied D cell numbers in volunteers with and without *H pylori* and in DU patients before and following eradication of the infection. There was a trend towards reduced D cell numbers in the infected, healthy volunteers, but this did not reach statistical significance. In addition, there was no difference in D cell numbers between patients and volunteers or between DU patients before and following

H pylori eradication (114). At present, therefore, the mechanism of *H pylori* induced hypergastrinaemia remains unclear.

3.3. EFFECT OF *H PYLORI* ASSOCIATED HYPERGASTRINAEMIA ON ACID SECRETION

Gastrin is generally accepted to be the major mediator of the gastric phase of acid secretion (115). The fact that *H pylori* increases biologically active gastrin, together with the finding in DU patients that this is not accompanied by any reduction in parietal cell responsiveness to gastrin, suggests that the infection should be causing significantly increased acid secretion. A number of studies have examined acid secretion by a variety of methods and these are discussed below.

3.3.1. Effect on Basal Acid Output

There are only a few adequately controlled prospective studies examining the effect of *H pylori* on basal acid output. There are only a few adequately controlled prospective studies examining the effect of *H pylori* on basal acid output. Montbriand et al studied basal acid output in ten patients with functional dyspepsia before and following suppression of *H pylori*. There was no statistically significant change, but 8 of the ten did show a fall,

suggesting that larger numbers might have produced a significant result (116). Levi et al did not observe any fall in basal acid output in ten DU patients examined after four weeks of triple therapy (117). However, in a more recent study the same group did observe a significant fall in a group of nine DU patients examined at four weeks following completion of eradication therapy (111).

The above studies indicated that *H pylori* might be increasing basal acid output, but that larger studies were required to clarify the question. The effect of *H pylori* and its eradication on basal acid output in health and disease is one of the aspects investigated in this thesis and will be discussed later.

3.3.2. Effect on Maximal Acid Output

There are few adequately controlled studies examining the effect of *H pylori* infection on maximal acid output. The only clear message is that eradication of *H pylori* infection does not cause any early reduction in maximal acid output in DU patients (116,117). There is a need for well controlled studies with adequate numbers of patients comparing DU patients and healthy volunteers with and without *H pylori*. In addition, studies are required of the longer term effects of eradicating *H pylori* on maximal acid output.

3.3.3. Effect on Intragastric Acidity and 24h pH

In 1988, McColl et al examined intragastric pH by means of *in situ* electrodes in twelve DU patients before and one month following eradication of the infection (118). There was no overall change in the median intragastric pH. However, the authors noted that there was a significantly greater rise in pH in response to the buffering effect of the meal following eradication of the infection. This was consistent with reduced acid secretion in response to the meals. However, when a smaller number of subjects were re-examined seven months later, the changes were no longer significant (96). Smith et al found no difference in median 24 hour intragastric acidity in a retrospective analysis comparing eight *H pylori* seropositive and eighty-seven seronegative young healthy volunteers (119). Eradication of the infection in the eight subjects did not produce any change in intragastric acidity when re-examined one month or six months later (99).

At present, therefore, the effect of *H pylori* on intragastric acidity remains unclear. One problem with intragastric pH is that it is a relatively insensitive method of assessing changes in gastric acid secretion. This is partly due to its poor reproducibility. Fimmel et al found that the day to day variation in median 24 hour pH, using *in situ* electrodes, was in the region of 1 pH unit (120). This variability can be explained by the fact that *in situ* electrodes can move within the gastric lumen and daytime pH varies by approximately 1 pH unit between

the antrum and body (121). In addition, alkaline reflux of duodenal contents or close contact between the electrode and mucosa can cause intermittent and marked rises in pH through the night (121). Day to day variations in the order of 1 pH unit are a major problem, as this represents a ten-fold variation in hydrogen ion concentration. Another weakness of intragastric pH or acidity studies is that they only assess hydrogen ion concentration and not the volume of gastric juice secreted, which is a major determinant of acid output. These problems associated with intragastric acidity measurements are exemplified by the fact that ranitidine 300mg, which markedly inhibits gastric acid secretion, may only increase median 24 hour intragastric pH in DU subjects from 1.1 to 1.4, i.e. an increase of only 0.3 pH units (122).

In view of the insensitivity of intragastric pH studies, the lack of consistent change following eradication of *H pylori* in the small number of subjects examined to date in no way excludes a significant change in acid secretion.

From the above discussion we can conclude that *H pylori* infection is significantly affecting the regulation of gastric acid secretion. Subjects with the infection have exaggerated basal and meal stimulated gastrin concentrations but the effect of this on acid secretion is still not clear. Methods available for

studying gastrin mediated acid secretion have yielded conflicting results and the effect of eradicating the infection on acid secretion remains controversial.

AIMS OF THE THESIS

The main aim of this thesis was to clarify the role of *H pylori* infection in the regulation of gastric acid secretion in man. The objectives were as follows:

(1) To devise a new approach to the study of gastrin mediated acid secretion through the use of the substance gastrin releasing peptide (GRP). The description and reproducibility of this test are presented in chapter four.

(2) To study basal and GRP stimulated gastrin and acid secretion in healthy volunteers with and without *H pylori* infection and in DU patients with the infection.

(3) To study the effect of eradicating *H pylori* infection on basal and GRP stimulated gastrin and acid secretion in healthy volunteers and DU patients.

(4) To study the mechanism of exaggerated acid secretion seen in DU patients and the role of *H pylori* infection in this.

(5) To study the effect of treatment with H_2 antagonists on basal and GRP stimulated gastrin and acid secretion in healthy volunteers.

(6) To study the effect of *H pylori* infection on basal and GRP stimulated gastrin and acid secretion in patients with non ulcer dyspepsia.

CHAPTER FOUR

THE GRP TEST: A NEW CLINICAL TEST OF GASTRIC ACID SECRETION : DESCRIPTION & REPRODUCIBILITY DATA

4.1. INTRODUCTION

The first project undertaken in my thesis was to devise a new clinical test to enable us to study gastrin mediated acid secretion without using a meal as the stimulus. Our knowledge of gastric physiology was helpful in that respect as we were aware of the central role played by a substance called gastrin releasing peptide (GRP) in the regulation of gastric acid secretion. GRP is present in nerves throughout the GI tract and in particularly high concentrations in the antral mucosa of the stomach (123,124). The neuropeptide plays a key role in the mediation of meal-stimulated acid secretion (125). Peptone in the stomach stimulates intramural neurones to release GRP which then stimulates the G cells in the antral mucosa to release gastrin which in turn stimulates the parietal cells to secrete acid. Acid secretion in response to GRP is thus a result of the combined functional response of the antral and oxyntic mucosae. GRP also activates inhibitory pathways involved in the regulation of acid secretion (126-128). GRP was therefore chosen as the stimulus to study gastrin mediated acid secretion.

The present study was undertaken to assess the reproducibility of this new test before using it to study the effect of *H pylori* infection on gastric acid secretion.

4.2. PATIENTS AND METHODS

Forteen subjects (9 males) were studied. Mean age was 35 years (range 24-53). Five had chronic duodenal ulcer disease with *H pylori* infection, 4 were healthy volunteers with *H pylori* infection, and 5 were healthy volunteers without *H pylori* infection. Duodenal ulcer patients were asked to stop antisecretory therapy at least four weeks prior to the secretory studies. None of the healthy volunteers was on any medication and none reported any major gastrointestinal symptoms. *H pylori* infection was confirmed in the DU patients by microscopic examination of antral biopsies, rapid urease test (CLO test) on antral biopsy and by the ^{14}C urea breath test as described below. In healthy volunteers *H pylori* status was determined by the ^{14}C urea breath test.

Each subject had their basal and GRP-stimulated acid secretion assessed under identical conditions on two separate days (day 1 and day 2). The investigator was blind to the result of the first test. The mean time interval between days 1 and 2 was 3.4 weeks (range 1-26). DU patients remained off antisecretory therapy until the completion of the reproducibility studies. Gastrin samples from the two study days for each subject were assayed in the same batch.

The ^{14}C urea breath test

This is a standard test in our laboratory and has been validated and used extensively. It involves giving a test dose of 0.4 MBq of ^{14}C urea in 0.3ml water in 25ml water.

The subject is fasted from 21:00hr the previous night. The subject is weighed with indoor clothes and shoes on and then asked to clean their teeth without swallowing any water and discarding all rinsings into running water in the basin. The subject is shown how to give a "Breath Sample" and this is taken as "Base". A test meal consisting of 200ml of Ensure is given followed by the test dose, then two rinsings of 25ml water. This is taken as time zero. The subject is then asked to re-clean their teeth as above and then give breath samples every 10 minutes till the end of the test. For most studies we use the short 30 minute breath test and take the 20 minute sample count as the important result. Counts above 40 are regarded as positive. Those below 20 are regarded as negative. Those which fall between 20 and 40 are treated as equivocal.

The GRP Test

All subjects reported at 09:00am following a 12 hour fast. A nasogastric tube (Anderson Inc., New York) was swallowed and its position in the dependent part of the stomach checked by the water recovery test. This involved aspirating the contents of the stomach until no contents could be recovered. Sixty ml of warm water were then infused into the stomach through the nasogastric tube over a period of 30 seconds. After one minute the water was aspirated and its volume checked. Recovery was judged satisfactory if more than 95% of the 60 ml were recovered. After emptying the stomach, intermittent suction was applied using an intermittent suction unit (Ohmeda, Columbia, U.S.A.) which applies suction for 20 seconds in each 32 second cycle. Three 15 min collections were obtained basally and at each of the following rates of I.V. infusion of GRP: 10 and 40 $pmol.kg^{-1}.h^{-1}$. Blood samples were collected every 15 minutes for gastrin determination and the plasma stored at -20 ^{0}C.

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

Basal acid output was calculated by taking the mean of all three samples prior to GRP infusion. Acid output for each GRP infusion rate was calculated by taking the mean of the second and third 15 min collections.

Gastrin was measured by radioimmunoassay using antiserum R98 which has a sensitivity of 5ng/l [129]. The basal gastrin value for each subject was determined by taking the mean of the three samples obtained prior to the start of GRP infusion. The gastrin value at each infusion rate of GRP was determined by taking the mean of the values at 30 and 45 minutes of each infusion.

Preparation of GRP

GRP was purchased from Cambridge Research Biochemicals (Cheshire, England) in 1mg aliquots of freeze-dried lyophilised powder. Subsequent preparation was undertaken by the Western Infirmary Pharmacy Department under sterile conditions. Each aliquot was made up into a 10ml stock solution by dissolving in sterile water and 0.1ml of 50% acetic acid solution was added to stabilise into solution. Vials containing 100μg of GRP in 1ml of solution were prepared and stored at -80 ^{0}C until the day of each study. For each study the content of each vial was further diluted in 0.9% NaCl solution. The peptide solution was filtered through a low protein binding bacterial filter (Gelman

Sciences, Northampton, England) before the final concentrations of the peptide were made up.

Statistics

Reproducibility of the GRP test was assessed by calculating estimates of the between day coefficient of variation for both gastrin and acid at basal, 10 and 40pmol.kg^{-1}.h^{-1} of GRP. The study was approved by the Western Infirmary Ethical Committee.

4.3. RESULTS

Reproducibility data is presented for basal gastrin and acid secretion and for gastrin and acid secretion at 10 and 40pmol.kg^{-1}.h^{-1} of GRP infusion.

Basal

The mean basal gastrin concentration was 49ng/l on day 1 (range: 22-115) and 46ng/l on day 2 (range: 12-125)(Fig.4.1.a). The mean basal acid output was 4.3mmol/h on day 1 (range: 0.5-13.2) and 4.4mmol/h on day 2 (range: 0.4-12))(Fig.4.1.b).

The coefficient of variation for gastrin was 29% and for acid 32%.

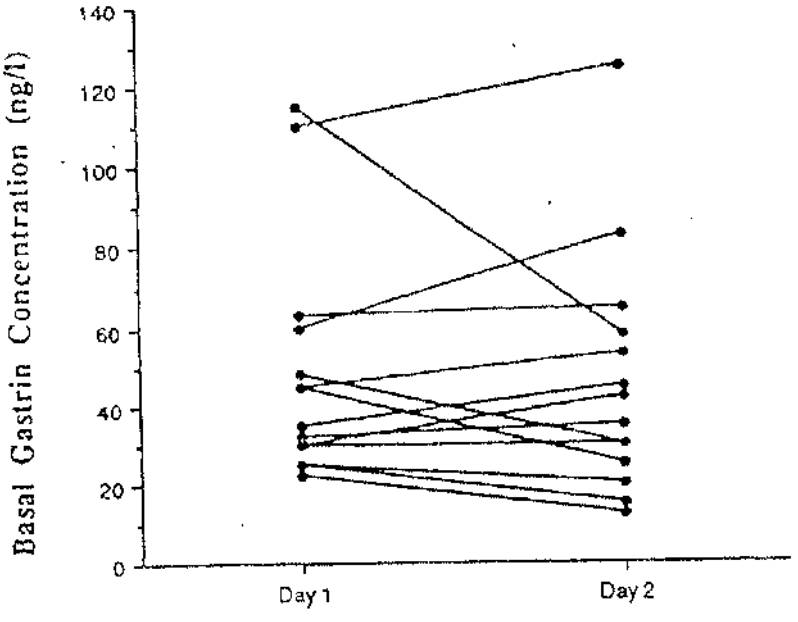


Fig. 4.1.a.

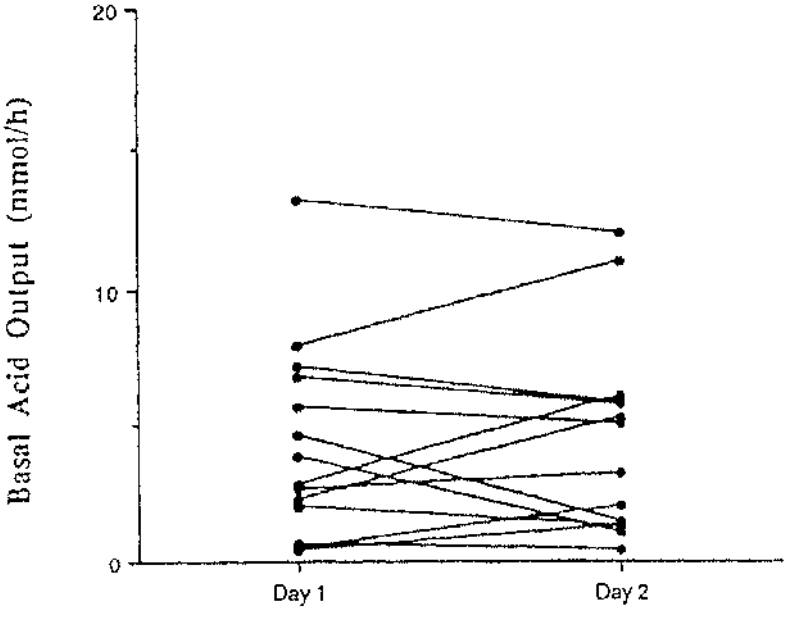


Fig. 4.1.b.

Fig. 4.1. Basal gastrin concentrations (a) and basal acid output (b) on the two study days.

GRP (10pmol.kg^{-1}.h^{-1})

The mean gastrin concentration at 10pmol.kg^{-1}.h^{-1} of GRP was 133ng/l on day 1 (range: 18-400) and 127ng/l on day 2 (range: 20-250)(Fig.4.2.a). The mean acid output at 10pmol.kg^{-1}.h^{-1} of GRP was 14.7mmol/h on day 1 (range1.5-51.9) and 14.8mmol/h on day 2 (range: 2.4-56.1)(Fig.4.2.b). The coefficient of variation for gastrin was 27% and for acid 11%.

GRP (40pmol.kg^{-1}.h^{-1})

The mean gastrin concentration at 40pmol.kg^{-1}.h^{-1} of GRP was 191ng/l on day 1 (range: 30-600) and 195ng/l on day 2 (range: 33-570)(Fig.4.3.a). The mean acid output at 40pmol.kg^{-1}.h^{-1} of GRP was 21.8mmol/h on day 1 (range: 1.0-58.8) and 22.0mmol/h on day 2 (range: 0.9-64)(Fig.4.3.b). The coefficient of variation for gastrin was 19% and for acid 6%.

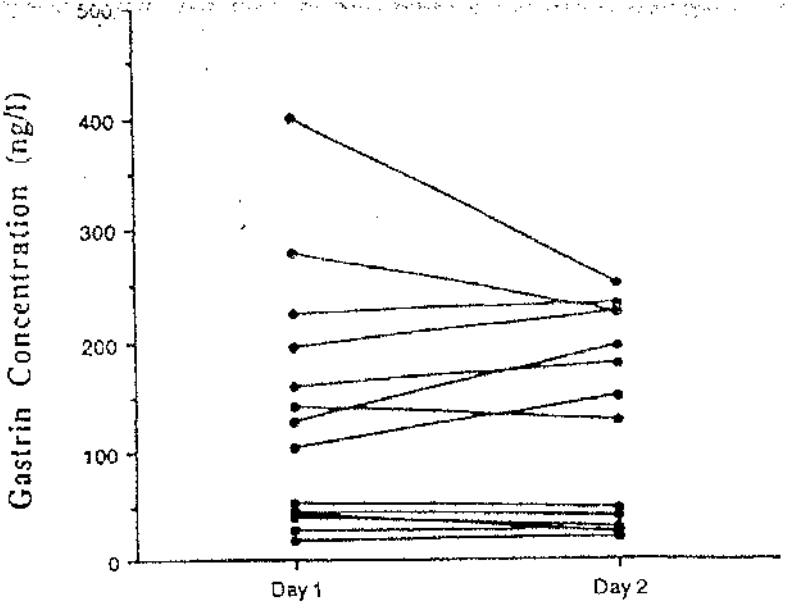


Fig. 4.2.a.

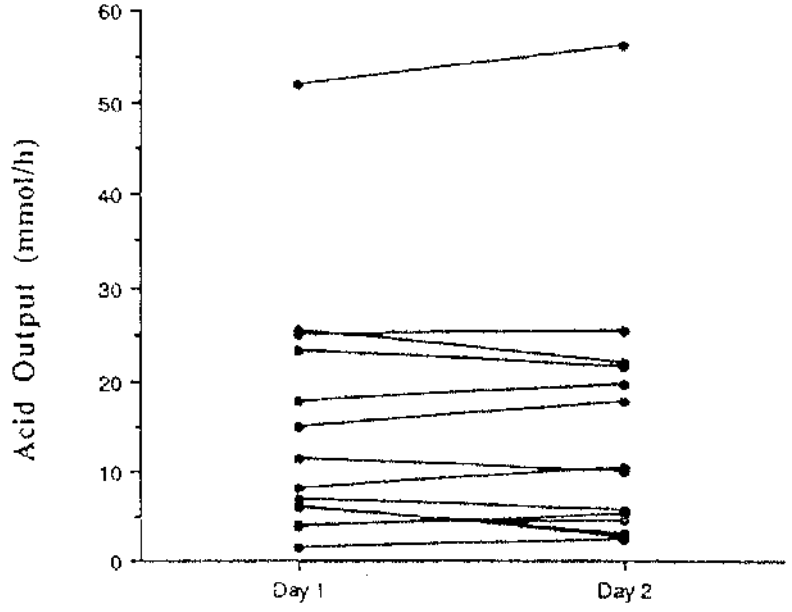


Fig. 4.2.b.

Fig. 4.2. Gastrin concentrations (a) and acid output (b) in response to 10pmol/kg/h of GRP on the two study days.

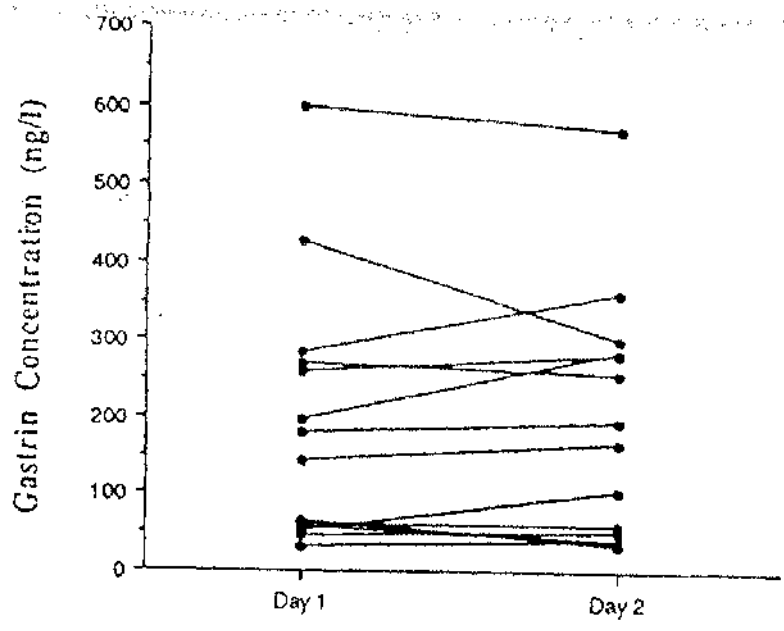


Fig. 4.3.a.

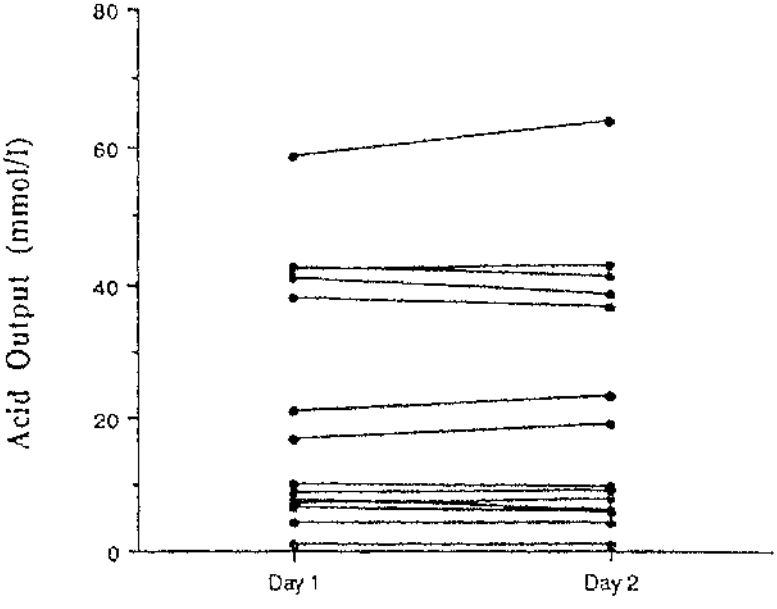


Fig. 4.3.b.

Fig. 4.3. Gastrin concentrations (a) and acid output (b) in response to 40pmol/kg/h of GRP on the two study days.

4.4. DISCUSSION

Gastrin releasing peptide (GRP) is a 27 amino acid peptide which has been detected in neurones throughout the gastrointestinal tract [123]. It acts exclusively as a neurotransmitter substance [124] whose main function in the stomach is mediation of gastrin release from antral G cells. The release of gastrin in response to food in the stomach has been shown to be mediated by GRP neurones [125]. In addition to stimulating gastrin release GRP is known to stimulate the release of other gastrointestinal regulatory peptides in man including cholecystokinin, gastric inhibitory peptide, vasoactive intestinal peptide, neurotensin, enteroglucagon and somatostatin [126-128], which exert an inhibitory control on gastrin release and acid secretion. GRP has thus emerged over the last few years as perhaps the most central neurotransmitter involved in the release of antral regulatory peptides.

The GRP test of acid secretion has several attractive qualities. The first is its ability to stimulate acid secretion through release of endogenous gastrin allowing the simultaneous assessment of the release of gastrin by the antral mucosa and the acid secretory response of the oxyntic mucosa to the endogenous gastrin. The second is that GRP also stimulates the release of gastric inhibitory peptides such as somatostatin and cholecystokinin and in this way simulates the response to eating. Thirdly, the GRP test allows the study of

gastrin-mediated acid secretion without the technical difficulties associated with the presence of food within the stomach.

The results of our reproducibility studies indicate that both arms of the test, i.e. the gastrin and acid secretion responses, are highly reproducible at both concentrations of GRP employed. We recommend that GRP should be used at the higher infusion rate of 40pmol.kg^{-1}.h^{-1}. At this rate the reproducibility of gastrin and acid secretion was 19% and 6% respectively. This was better than reproducibility at the lower infusion rate of 10pmol.kg^{-1}.h^{-1} (27% and 11% for gastrin and acid respectively). Another reason for using the 40pmol.kg^{-1}.h^{-1} infusion rate is that the gastrin levels achieved with this rate are similar to those achieved in response to eating a meal.

Reproducibility was not as high under basal conditions compared to the stimulated state and perhaps reflects varying degrees of reflux of alkaline duodenal contents into the stomach.

Experience to date (which includes the major part of the work presented in this thesis) indicates that GRP has no adverse side effects. We have administered it on 300 separate occasions to 150 individuals with no adverse reactions. In 25 individuals we have administered GRP in a dose up to 400pmol.kg^{-1}.h^{-1} (i.e. 10 times higher than the dose used in this study) without

any adverse reactions. Sixty subjects have received it on two separate occasions and 25 subjects on three separate occasions without evidence of allergic reactions. GRP has also been used by several other investigators and no adverse reactions reported [101,130,131]. Due to the interest raised by our use of GRP as a test of gastric secretory function, a commercially available preparation of GRP has recently entered the market manufactured by Clinalfa AG, Switzerland. Vials containing 50 μg of human GRP are sold ready for clinical use.

The GRP test is a simple, safe, and highly reproducible test of acid secretion. It is a powerful new tool for investigating the pathophysiology of upper gastrointestinal disorders.

In the following chapters we describe the use of GRP in investigating the effect of *H pylori* infection on the regulation of gastric acid secretion.

CHAPTER FIVE

THE EFFECT OF *HELICOBACTER PYLORI* INFECTION ON GASTRIC ACID SECRETION IN HEALTHY VOLUNTEERS AND DUODENAL ULCER PATIENTS

5.1. INTRODUCTION

It is now well established that *Helicobacter pylori* is the major acquired factor in the pathogenesis of duodenal ulcer disease. The infection is found in >95% of DU patients and numerous studies have demonstrated that eradicating it markedly lowers the ulcer relapse rate (37-40). The mechanism by which this infection which predominantly affects the gastric mucosa predisposes to ulceration of the duodenum has been the subject of much speculation .

Work carried out in our own unit in Glasgow as well as in other units has shown that both DU patients and healthy volunteers with *H pylori* have increased basal and meal stimulated gastrin concentrations which fall following eradication of the infection (95,96,98,99,132). This hypergastrinaemia is due to an increase in the biologically active G17 form of the hormone (102). Studies have also shown that the parietal cell sensitivity to gastrin is unaffected by *H pylori* (110,111) and, therefore, the increased hormone level is not simply a compensatory response to the infection impairing acid secretion.

Though gastrin is recognised as the major mediator of meal stimulated acid secretion (115), the effect of *H pylori*-associated hypergastrinaemia on acid secretion has remained unclear. As discussed in chapter 3 this is largely due

to the technical difficulty of reliably measuring acid secretion in response to a meal. In view of these problems we proceeded to develop the GRP test of acid secretion as described in chapter 4. Having assessed the reproducibility of this new tool and found it to be highly reproducible, we proceeded to study the effect of *H pylori* infection on acid secretion stimulated by the intravenous administration of GRP. This neuropeptide stimulates the G cells in the antral mucosa to release gastrin which in turn stimulates the parietal cells to secrete acid. This allows the simultaneous assessment of the combined functional response of the antrum and body of the stomach. GRP also stimulates the release of a variety of other hormones which exert an inhibitory control on acid secretion including cholecystokinin, gastric inhibitory peptide, vasoactive intestinal peptide, neurotensin, enteroglucagon and somatostatin (128,133). Acid secretion in response to GRP is therefore the product of the combined effects of these stimulatory and inhibitory control pathways and in this way resembles acid secretion in response to a meal.

In this study we examined basal and GRP stimulated acid secretion in healthy volunteers with and without *H pylori* infection and in DU patients with the infection. We also examined the effect of eradication of *H pylori* on basal and GRP stimulated acid secretion in healthy volunteers and DU patients and proceeded to examine the long-term effects of eradication of the infection on acid secretion in DU patients.

5.2. PATIENTS AND METHODS

Twenty five *H pylori* positive patients (17 males) with endoscopically confirmed chronic duodenal ulcer disease, 25 *H pylori* positive healthy volunteers (17 males), and 25 *H pylori* negative healthy volunteers (17 males) were studied. The three groups were matched for age and body weight. There were 20 smokers In the DU group and 6 in each of the other 2 groups. Duodenal ulcer patients were asked to stop any antisecretory therapy at least four weeks prior to the secretory studies. None of the healthy volunteers was on any medication and none reported major gastrointestinal symptoms. *H pylori* infection was confirmed in the DU patients by microscopic examination of antral biopsy, rapid urease test (CLO test) on antral biopsy and by ^{14}C urea breath test. In healthy volunteers, *H pylori* status was determined by the ^{14}C urea breath test.

Secretory studies

All subjects reported at 09:00h following a 12 hour fast. An orogastric tube (Anderson Inc., New York) was swallowed and its position in the dependent part of the stomach checked by the water recovery test. After emptying the stomach, intermittent suction was applied using an intermittent suction unit (Ohmeda, Columbia, U.S.A.) as described in chapter 4. Three 15 minute collections were obtained basally and at a GRP infusion rate of both 10 and 40 $pmol.kg^{-1}.h^{-1}$. Blood samples were collected every 15 min for gastrin determination and the plasma stored at -20 ^{0}C. The secretory studies were all performed with the investigator blind to the subjects' *H pylori* status.

The preparation of GRP is described in chapter 4. The volume and pH of each gastric juice collection were recorded and its hydrogen ion concentration measured by titration with 0.1M NaOH to pH 7 using an autotitrator (Radiometer ETS 822).

Basal acid output was calculated by taking the mean of all three 15 min samples prior to GRP infusion. Acid output for each GRP infusion rate was calculated by taking the mean of the second and third 15 min collections.

The measurement of gastrin is described in chapter 4.

Eradication of *H pylori*

Following the above secretory studies, 18 DU patients and 15 healthy volunteers with *H pylori* infection were treated with tripotassium dicitratobismuthate 120mg t.i.d., metronidazole 400mg t.i.d. and amoxycillin 500mg t.i.d. for 3 weeks. One month following completion of this therapy their ^{14}C urea breath test was repeated to determine *H pylori* status. Their secretory studies were also repeated at this point. Ten of the treated DU patients were also re-examined one year following triple therapy with repeat ^{14}C urea breath test and secretory studies. The ten DU patients re-examined at one year consisted of those whose acid output remained elevated at one month following eradication of *H pylori*.

Statistics

Statistical analysis of unpaired data was performed using the Mann-Whitney U test and of paired data using the Wilcoxon test. A 'p' value of <0.05 was taken as significant. Correlation of gastrin concentrations and acid output was assessed using linear regression analysis.

The study was approved by the Western Infirmary ethical committee.

5.3. RESULTS

The repeat ^{14}C urea breath test at one month after completion of the triple anti-*H pylori* therapy indicated eradication of the infection in 16 of the 18 DU patients and 14 of the 15 healthy volunteers. All ten DU patients who were re-examined at one year post-eradication therapy remained free of the infection.

Basal Gastrin

The basal gastrin concentration (ng/l) was similar in the *H pylori* positive healthy volunteers (median=45, range: 10-90) and *H pylori* positive DU patients (60, range: 22-175) and both were higher than the *H pylori* negative healthy volunteers (32, range: 15-50)($p<0.005$ for either) (fig.5.1.). One month following eradication of *H pylori* the median plasma gastrin concentration in the 16 DU patients fell from 50 (range: 22-150) pre-eradication to 40 (range: 12-70)($p<0.01$ versus pre-eradication) and in the 14 healthy volunteers from 48 (range: 10-90) to 32 (range: 7-110)($p<0.02$ versus pre-eradication). At one month post-eradication both values were not significantly different from the value in the *H pylori* negative healthy volunteers.

The median basal gastrin concentration in the 10 DU patients who were followed up for one year was 52ng/l (range: 22-150) pre-eradication and fell to

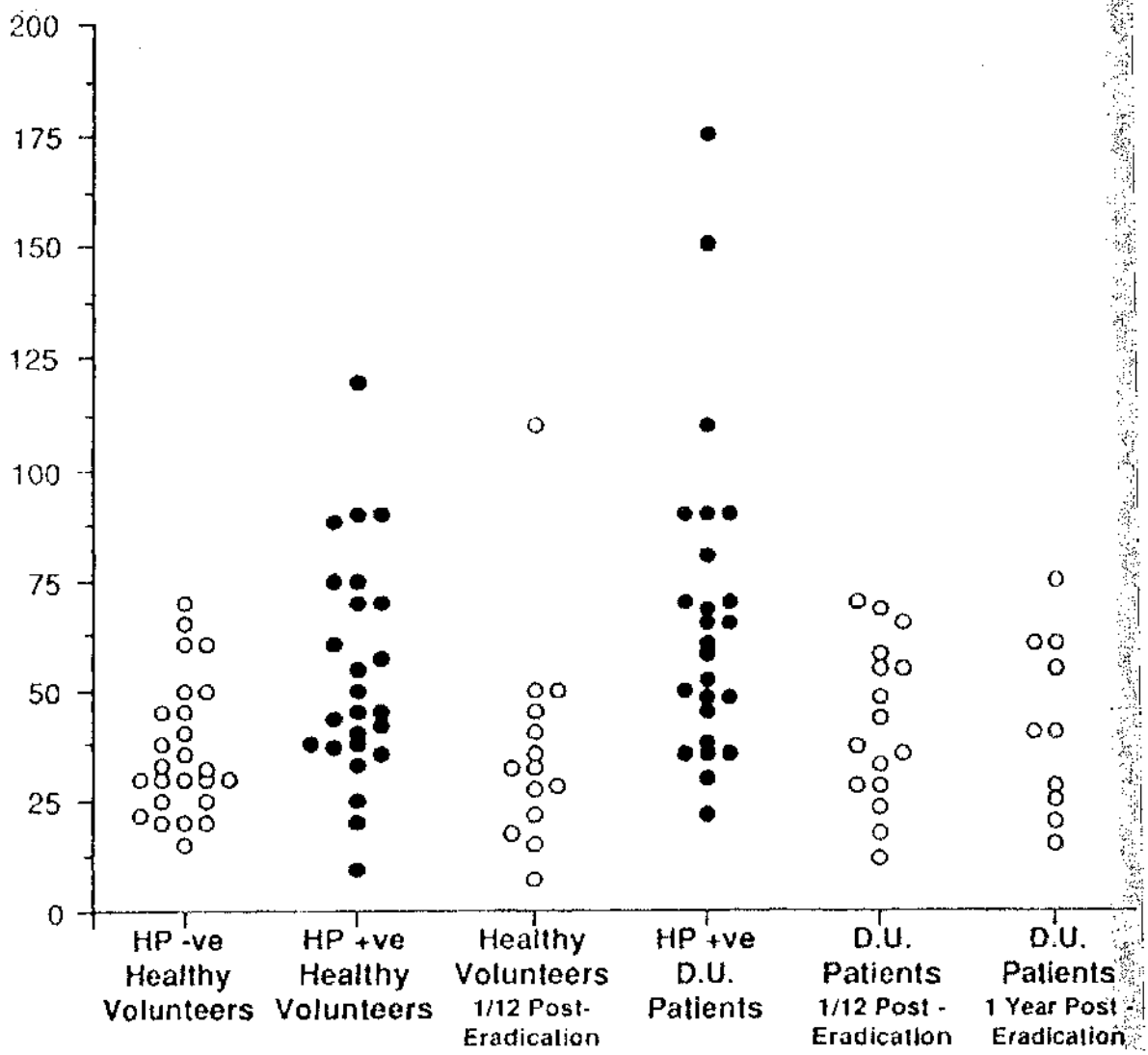


FIG. (5.1.)

Basal gastrin concentrations in healthy volunteers and DU patients of varying *H pylori* status (● indicates *H pylori* positive). Compared to the *H pylori* negative healthy volunteers, basal gastrin values are increased only in the *H pylori* positive healthy volunteers ($p<0.005$) and the *H pylori* positive DU patients ($p<0.005$).

36 (range: 12-68) at one month post-eradication ($p<0.01$) and was similar one year later (median = 40, range: 15-75)(fig.5.1.).

Basal Acid Secretion

The median basal acid output (mmol/h) was higher in the *H pylori* positive (2.9, range: 0.5-13.3) compared to negative (1.8, range: 0.5-7.9) healthy volunteers ($p<0.05$) (fig.5.2.). In addition, it was increased in the *H pylori* positive DU patients (6.6, range: 2.6-25.8) compared to both the *H pylori* negative healthy volunteers ($p<0.0001$) and *H pylori* positive healthy volunteers ($p< 0.001$). Eradication of *H pylori* lowered the median basal acid output in the 16 DU patients from 7.2mmol/h (range: 3.1-25.8) pre-eradication to 3.3 (range: 1.2-11.3) at one month post-eradication ($p<0.01$ versus pre-eradication), representing a median reduction of 50% (20% - 80%). However, at this time point post-eradication the basal acid output in the DU patients was still increased compared to the *H pylori* negative healthy volunteers ($p<0.01$). At one month following eradication of *H pylori* in the 14 healthy volunteers their median acid output was 1.8 (range: 0.2-13.1) which was not significantly different from their pre-eradication value (median = 2.0, range: 0.5-13.5)($p=0.8$). The basal acid outputs in the two DU patients and the one healthy volunteer in whom *H pylori* infection was not eradicated were similar before (3.7, 6.3, and 1.2) and following (4.8, 6.1 and 2.4 respectively) triple therapy.

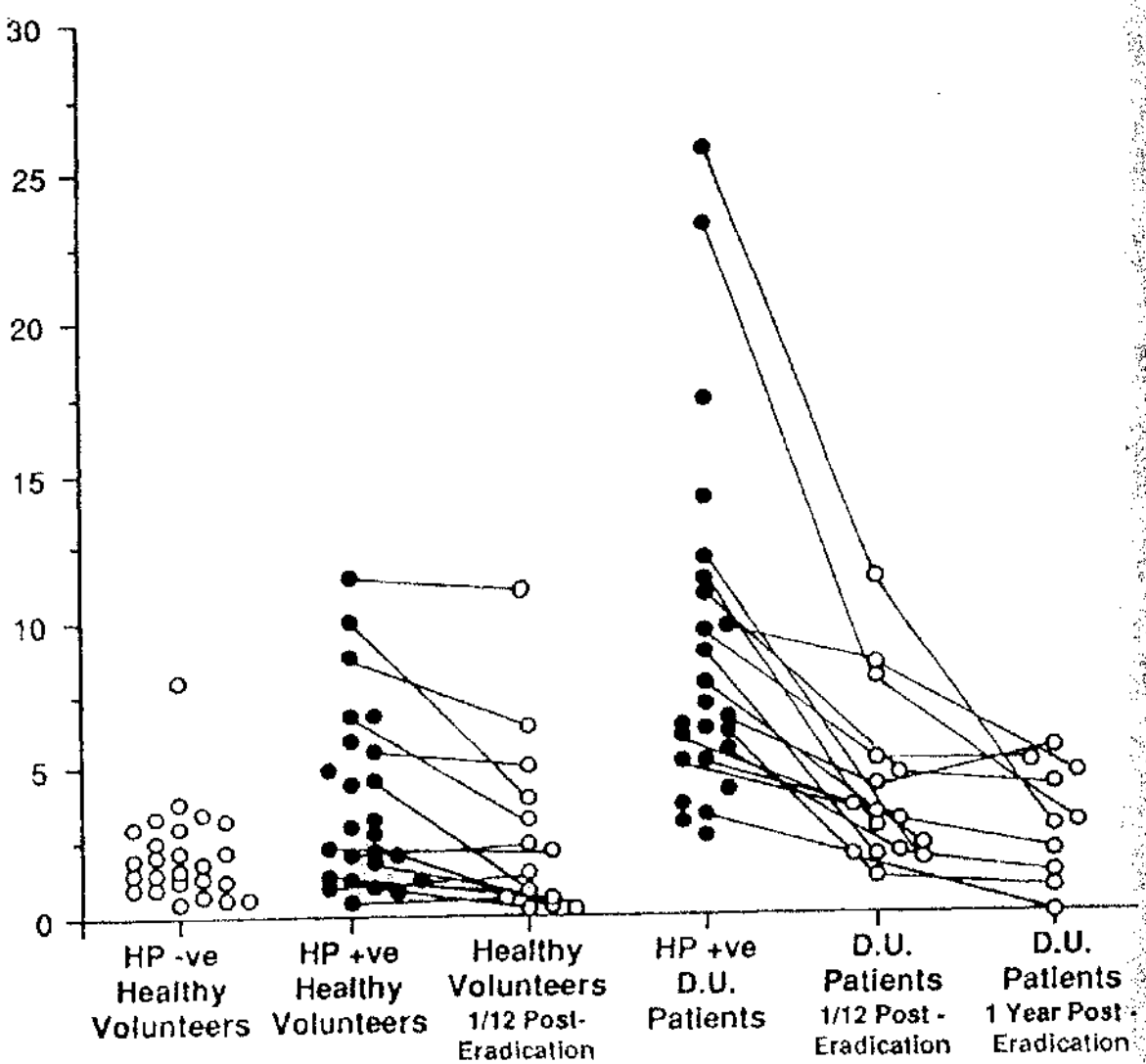


FIG. (5.2.)

Basal acid output in healthy volunteers and DU patients of varying *H pylori* status (● indicates *H pylori* positive). Compared to the *H pylori* negative healthy volunteers, basal acid output is increased only in the *H pylori* positive healthy volunteers ($p<0.05$), *H pylori* positive DU patients ($p<0.0001$), and DU patients one month post eradication ($p<0.01$).

The median basal acid output in the 10 DU patients who were followed up for one year was 8.1mmol/h (range: 3.1-25.8) pre-eradication and fell to 4.5mmol/h (range: 1.2-11.3)($p<0.01$ versus pre-eradication) at one month post-eradication and this fell further to 2.5mmol/h (range: 0.0-5.1) at one year post-eradication ($p< 0.04$ versus one month post-eradication) when it became similar to *H pylori* negative healthy volunteers (fig.5.2.).

Correlation Between basal gastrin and basal acid

In *H pylori* positive DU patients there was no correlation between basal gastrin and basal acid output ($r=0.27$, $p< 0.2$). Correlation between basal gastrin and basal acid output was stronger in the *H pylori* positive healthy volunteers but still failed to reach statistical significance ($r=0.37$, $p<0.07$).

Gastrin Response to GRP

At the GRP infusion rate of 40pmol.kg^{-1}.h^{-1} the median gastrin concentration (ng/l) was increased to a similar level in the *H pylori* positive healthy volunteers (238, range: 38-563) and *H pylori* positive DU patients (255, range: 90-600) and each was higher than that of the *H pylori* negative healthy volunteers (70, range: 28-157) ($p<0.002$ for each)(fig.5.3.). At one month following eradication of *H pylori* the median gastrin concentration in response to GRP 40pmol.kg^{-1}.h^{-1} in the 16 DU patients fell from 270 (range: 95-600)

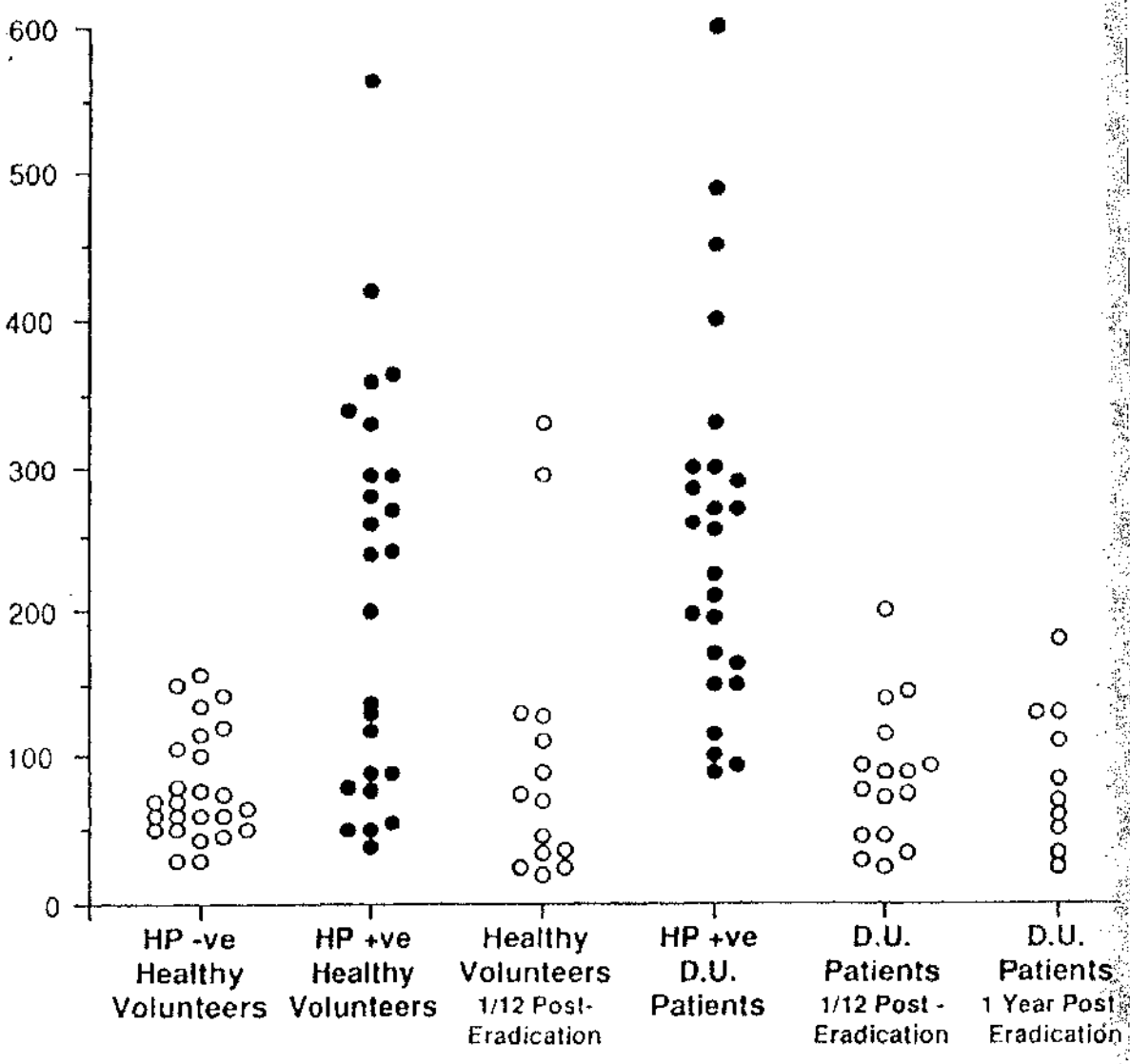


FIG. (5.3.)

Gastrin concentrations in response to stimulation with GRP (40pmol.kg^{-1}.h^{-1}) (● indicates *H pylori* positive). Compared to *H pylori* negative healthy volunteers gastrin concentrations are increased only in the *H pylori* positive healthy volunteers ($p<0.002$) and in *H pylori* positive DU patients ($p<0.002$).

pre-eradication to 84 (range: 23-200)($p<0.01$ versus pre-eradication). In the 14 healthy volunteers eradication of *H pylori* lowered the median gastrin concentration in response to GRP 40pmol.kg^{-1}.h^{-1} from 249 (range: 35-563) pre-eradication to 58 (range: 20-330) at one month post-eradication ($p<0.01$ versus pre-eradication). At one month post-eradication the values in both the DU patients and healthy volunteers were similar to those of the *H pylori* negative healthy volunteers (fig.5.3.).

The median gastrin concentration in response to 40pmol.kg^{-1}.h^{-1} GRP in the 10 DU patients who were followed up for one year was 278ng/l (range: 95-600) pre-eradication and fell to 90 (range: 30-200) at one month post-eradication and was similar one year later (78, range: 25-180)(fig.5.3.).

The gastrin concentration in the five groups of subjects at the GRP 10pmol.kg^{-1}.h^{-1} infusion rate showed the same pattern of response to that seen in response to GRP 40pmol/kg/h. We chose to present the results of the individual data points for the 40pmol.kg^{-1}.h^{-1} GRP rate as the gastrin levels stimulated by this are closer to those seen following a meal.

Acid Response to GRP

At GRP 40pmol.kg^{-1}.h^{-1} the median acid output (mmol/h) in the *H pylori* positive healthy volunteers (19.0, range: 1.0-38.3) was approximately 3 times

that of the *H pylori* negative healthy volunteers (6.3, range: 2.8-20.9)($p<0.001$)(fig.5.4.). At this infusion rate the median acid output in the *H pylori* positive DU patients was 39.1 (range: 17.9-64) which was approximately twice that of the *H pylori* positive healthy volunteers ($p<0.005$) and 6 times that of the *H pylori* negative healthy volunteers ($p<0.001$). In the 16 DU patients whose H pylori infection was eradicated their median acid output fell from 39.6 (range: 18.9-64) pre-eradication to 16.6mmol/h (range: 3.9-24)($p<0.01$) at one month post-eradication but was still higher than the H pylori negative healthy volunteers ($p<0.005$). In the 14 healthy volunteers who were eradicated of the infection the median acid output was 15.6mmol/h (range: 1.0-38.3) pre-eradication and this fell to 6.7 (range: 0.8-21.0) one month post-eradication. Acid secretion did not fall in the two DU patients and the one healthy volunteer in whom the infection was not eradicated being 17.9, 39 and 15.1 mmol/h before treatment and 28.4, 37.6 and 15.0 mmol/h respectively at one month following treatment.

The median acid output in response to 40pmol.kg^{-1}.h^{-1} GRP in the 10 DU patients who were followed up for one year was 39.6mmol/h (range: 21.5-57) pre-eradication and fell to 19.0mmol/h (range: 11.3-24)($p<0.01$ versus pre-eradication) at one month post-eradication and then fell further to 8.0mmol/h (range: 5.2-18.6)($p<0.01$ versus one month post-eradication) at one year post-eradication when it became similar to the values of the *H pylori* negative

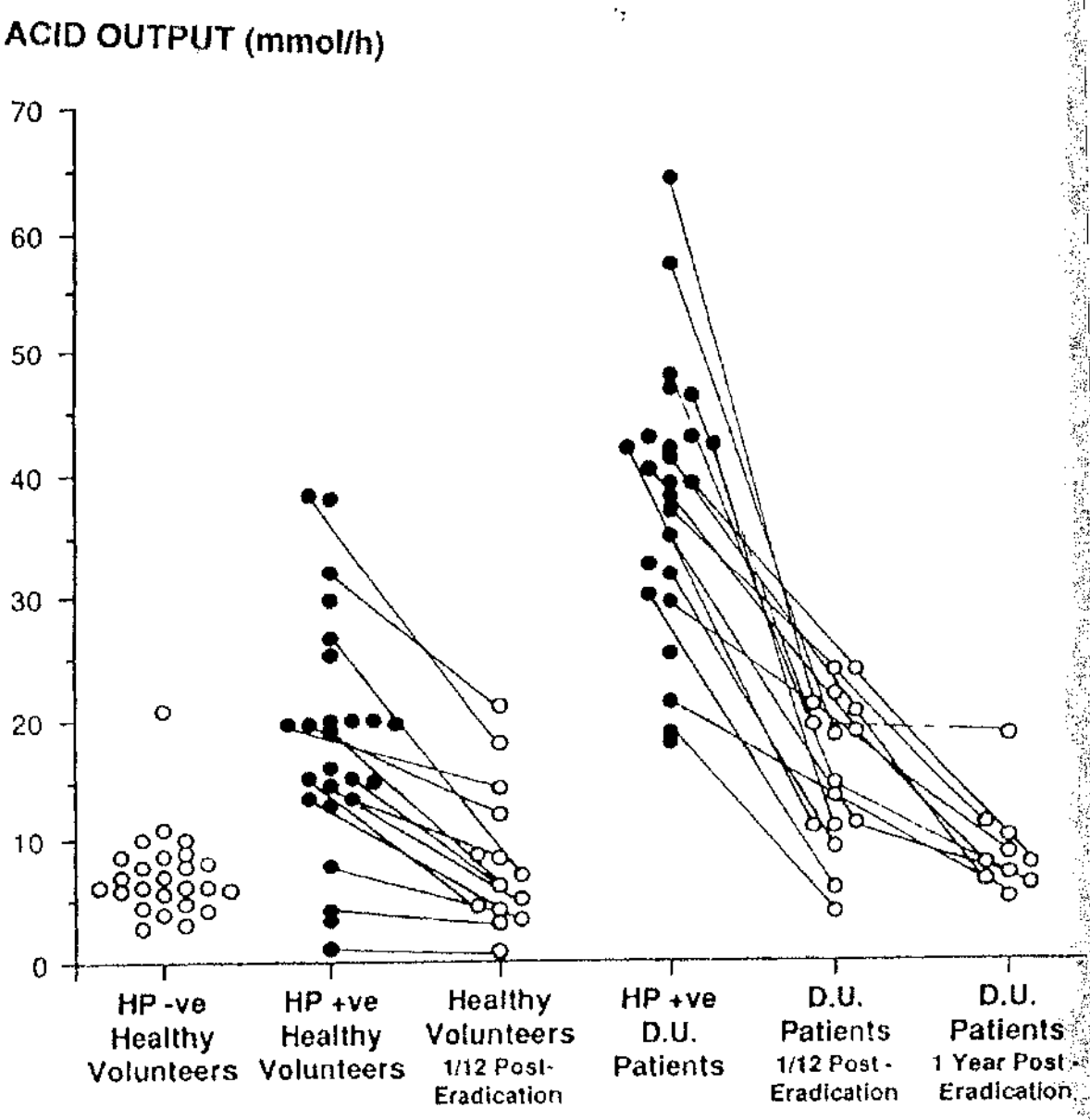


FIG. (5.4.)

Acid output in response to stimulation with GRP (40pmol.kg^{-1}.h^{-1})(● indicates *H pylori* positive). Compared to *H pylori* negative healthy volunteers, acid output is increased in *H pylori* positive healthy volunteers ($p<0.001$), *H pylori* positive DU patients ($p<0.001$) and DU patients one month post eradication $P<0.01$). The ten DU patients who were re-examined at one year post eradication were the ten who had the highest acid output at one month post eradication.

healthy volunteers (median = 6.3, range: 2.8-20.9)(p=0.1)(fig.5.4.). The GRP-stimulated acid output in this group of ten DU patients has fallen by a median of 78% (range: 60% - 87%) at one year post-eradication compared to pre-eradication values.

Correlation Between GRP Stimulated Gastrin and Acid

In *H pylori* positive DU patients there was no correlation between GRP stimulated gastrin concentration and acid output (r=0.12, p= 0.58). In *H pylori* positive healthy volunteers there was significant correlation between GRP stimulated gastrin concentration and acid output (r=0.50, p<0.01)(Fig.5.5.).

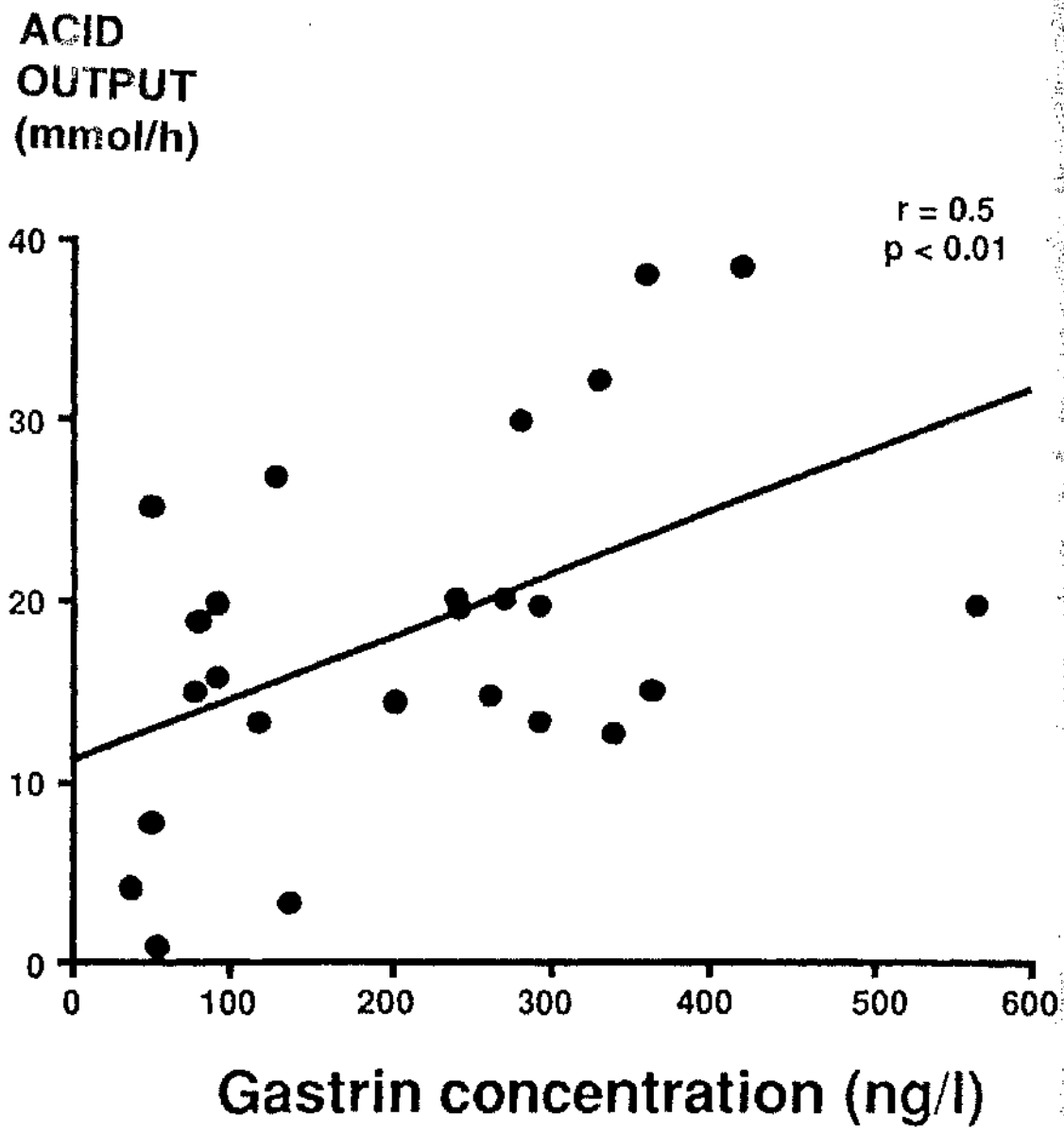


FIG. (5.5.)

Correlation between gastrin concentrations and acid output during stimulation with GRP (40pmol.kg^{-1}.h^{-1}) in the *H pylori* positive healthy volunteers.

5.4. DISCUSSION

H pylori infection is common in the general population and its prevalence increases with age (45). It has been recognised for some time that the infection causes chronic inflammation of the gastric mucosa (134). In addition, numerous studies have shown that the infection causes increased circulating gastrin concentrations (95,96,98,99,132). However, the effect of *H pylori* infection and the accompanying hypergastrinaemia on gastric acid secretion has been unclear.

The present studies demonstrate that *H pylori* infection in healthy volunteers is associated with an increase in both basal and stimulated acid output. Compared with true normal controls (i.e. *H pylori* negative healthy volunteers), infected healthy volunteers have a 2-fold increase in basal acid output and 3-fold increase in GRP stimulated acid output. The fact that the increased acid output resolved fully following eradication of *H pylori* indicates that the infection is the cause of the acid hypersecretion.

The increased acid secretion in the infected healthy volunteers appears to be mainly or entirely secondary to increased antral gastrin release. This is supported by several observations. The first is the fact that the increased basal and GRP stimulated acid outputs were both associated with increased gastrin

release. The second is the finding of a significant positive correlation between acid secretion and gastrin levels both basally and during GRP. The third is the fact that the acid hypersecretion fully resolved at the same time as resolution of the hypergastrinaemia, at one month post eradication.

The *H pylori* positive healthy volunteers also differed from the uninfected healthy volunteers in showing a much wider range of acid secretion. This was particularly marked during GRP stimulation when the acid output in 24 of the 25 *H pylori* negative healthy volunteers showed a 4-fold range (from 2.8-10.9) whereas the *H pylori* positive healthy volunteers were widely scattered between 1.0 and 38.3 representing a 38-fold range. This wide range in acid secretion in the *H pylori* positive healthy volunteers could be explained by the wide variation in the degree of hypergastrinaemia caused by the infection in different subjects.

The finding that *H pylori* infection markedly alters gastric secretory function in apparently healthy volunteers has important implications for gastrointestinal research. It means that much of the large literature on human gastric physiology and pathophysiology is flawed as a substantial proportion of those considered to be true normals will have had disturbed gastric function due to *H pylori* infection. It is important that all future studies of human gastric function include normal controls who are *H pylori* negative. As shown in our present

studies, true normal controls have a tight range of gastric secretory function which will facilitate future research.

In the DU patients, both basal and GRP stimulated acid outputs were increased 6-fold compared to *H pylori* negative healthy volunteers. The fact that acid secretion in DU patients is so markedly increased in comparison to the true physiological range strongly supports a role for acid in the pathophysiology of DU disease.

The basal and GRP stimulated acid outputs of the DU patients were both twice those of the *H pylori* positive healthy volunteers, despite the two groups having similar gastrin levels both basally and in response to GRP. This indicates that the DU patients are producing twice as much acid as the *H pylori* healthy volunteers for the same level of gastrin stimulation.

Eradicating *H pylori* infection in the DU patients reduced their basal and GRP stimulated gastrin levels to normal values within one month and this was associated with a 50% fall in basal acid output and a 68% fall in GRP stimulated output. However, at this time point the basal and GRP stimulated acid outputs were still twice normal (i.e. *H pylori* negative healthy volunteers values). This again demonstrated that the DU patients were producing twice as much acid secretion as the non-DU subjects with equivalent gastrin levels.

These findings indicate that the 6-fold increase in acid secretion in the DU patients is due to the combination of two factors: (1) Increased antral gastrin release and (2) An exaggerated acid response to gastrin stimulation. The first factor is the same as that noted in the *H pylori* positive healthy volunteers and resolves within one month of eradicating the infection. The second factor is a specific feature of the DU patients and it persisted at one month post eradication.

It is interesting to compare our findings using GRP with those of Hirschowitz et al using the closely related peptide bombesin which exerts similar biological effects (133). They studied the gastrin and acid response in DU patients and healthy controls prior to the recognition of *H pylori.* Acid output to bombesin was increased approximately 3 fold in the DU patients. The exaggerated acid response was again due to the combination of two defects: (1) increased antral gastrin release and (2) an increased acid response by the oxyntic mucosa to gastrin stimulation which was most apparent at higher bombesin infusion rates. The findings by Hirschowitz et al are thus very similar to our own findings. The fact that we have found a 6 fold exaggerated acid response compared to their 3 fold exaggerated response may be explained by the fact that we had a group of *H pylori* negative volunteers to define true normal whereas a proportion of the "normals" in the earlier study would have

had an exaggerated response due to unrecognised *H pylori* infection. Our own studies extend the work of Hirschowitz et al by showing that the defects in acid regulation are secondary to *H pylori* and fully resolve following its eradication.

In the present study we were able to examine whether this second factor present in the DU patients i.e. the exaggerated acid response to gastrin stimulation, represented a genetic factor predisposing to DU disease or was another reversible acquired factor. This was done by re-examining the ten DU patients whose acid output remained elevated at one month post-eradication of *H pylori*. Within one year their acid output fell by a further 50% and became equivalent to that of the *H pylori* negative healthy volunteers. Likewise, their basal acid output fell by a further 39% over the year and again became equivalent to that of the *H pylori* negative healthy volunteers. This further fall in acid secretion was not accompanied by any further fall in gastrin and therefore represented resolution of the second factor i.e. the increased acid response to gastrin stimulation.

Though the eventual resolution of the second defect in the DU patients indicates that it is not a genetic defect, its precise explanation is unclear. It may represent trophic effects of *H pylori*-induced hypergastrinaemia on the oxyntic mucosa. Even physiological concentrations of gastrin exert trophic effects on the oxyntic mucosa (135) and long-term exposure to the modestly

increased levels caused by *H pylori* could have significant effects. In addition, the further increase in gastrin associated with acid-inhibitory therapy could increase the trophic effects on the oxyntic mucosa. Such changes would take several months to resolve following restoration of normal gastrin levels, due to the long half-life of the parietal cell (136,137).

There has been considerable reluctance among clinicians to accept the pathogenic role of *H pylori* in DU disease despite several trials confirming markedly reduced ulcer relapse rates following eradication of the infection (37-40). Part of this reluctance can be explained by the conceptual difficulties in understanding how an infection of the stomach can predispose to ulceration of the duodenum. Our finding that basal and GRP stimulated acid outputs are increased 6-fold in duodenal ulcer disease and that the acid hypersecretion eventually fully resolves following eradication of *H pylori* provides a scientific explanation for the role of the infection in DU disease. When permanent resolution of the marked acid hypersecretion can be achieved by a single course of *H pylori* eradication therapy, there is little justification to continue to treat DU patients with repeated courses of expensive acid suppressive therapy.

There are also clinical implications from our finding that *H pylori* infection is accompanied by a 3-fold increase in acid secretion in the general population.

At present, acid inhibitory agents are prescribed to patients who have a wide variety of diseases other than duodenal ulceration in which reduction of acid secretion is thought to be of benefit. A high proportion of such patients will have *H pylori* infection and permanently lowering their acid secretion by eradicating the infection could be helpful in their long-term management.

One of the paradoxes associated with *H pylori* infection is the fact that it is on the one hand associated with DU disease which is a disorder of acid hypersecretion and, on the other hand, associated with gastric cancer (31-33) which is a disease of acid hyposecretion (138). This may be explained by our finding that *H pylori* infection is associated with a wide range of abnormalities of acid secretion. Indeed, when our *H pylori* positive healthy volunteers and DU patients are combined, the acid secretion associated with the infection ranges from below to many times above the true normal range. Our studies indicate that the top of the range is associated with DU disease and it is tempting to speculate that those at the bottom end may be the ones at risk of gastric cancer. The reason why *H pylori* infection produces different degrees of acid hypersecretion in different subjects is unclear.

In addition to considering the pathophysiological significance of *H pylori* induced disturbances in acid secretion in DU patients and in other diseases, one has to consider its importance in the 50% of the world's population who

carry the infection but have no apparent GI disease. In many of the developing countries, the prevalence of *H pylori* is almost 100% and it is acquired in early childhood (30,48). This high prevalence raises the possibility that the infection could be conferring some benefit in those regions. The major cause of death in such countries is enteric infections and gastric acid secretion is an important way in which the body defends itself from food borne infections (139). It is possible that the increased acid secretion induced by *H pylori* infection provides protection from more serious enteric infections in the developing world.

In conclusion, the fact that acid secretion is increased 6-fold in DU patients suggests that this is likely to be the key pathophysiological defect in DU disease. The finding that this increased acid secretion fully resolves following eradication of *H pylori* explains the role of the infection in DU disease. The finding that *H pylori* infection produces a 3-fold increase in acid secretion in the general population has major implications for other GI diseases.

CHAPTER SIX

INVESTIGATION OF THE MECHANISM OF EXAGGERATED ACID RESPONSE TO GRP IN DUODENAL ULCER PATIENTS

6.1. INTRODUCTION

In chapter 5 we observed that GRP stimulated acid output was increased 3-fold in *H pylori* positive healthy volunteers and 6-fold in *H pylori* positive DU patients, compared to *H pylori* negative healthy volunteers. In addition we found that the acid output in the DU patients fell by 68% at one month following *H pylori* eradication but was still twice as high as the *H pylori* negative healthy volunteers despite normalisation of their gastrin concentrations. This exaggerated acid response to GRP seen at one month post-eradication fully resolved when the subjects were re-examined one year later.

In the present study we have proceeded to investigate the mechanism of the exaggerated acid response to GRP seen in the DU patients. It is well established that DU patients have a larger parietal cell mass than normal subjects. It is likely that part of the exaggerated acid response observed in DU patients is due to their increased parietal cell mass. This increased parietal cell mass might be a result of the trophic effect of *H pylori*-induced hypergastrinaemia on the oxyntic mucosa. If this is the case then the increased parietal cell mass should resolve following eradication of *H pylori* and resolution of the hypergastrinaemia.

Another possible explanation for the exaggerated acid response to GRP in the DU patients is failure of inhibitory control processes. As discussed previously, acid output measured in response to stimulation with GRP is the end product of stimulatory pathways through the action of gastrin and inhibitory pathways through the action of CCK and other hormones. To assess whether the inhibitory limb of acid secretion is functional it is possible to compare the maximal acid response to endogenous gastrin released by GRP with the maximal acid response to exogenous gastrin. If the inhibitory limb is functional the former will be a fraction of the latter. If on the other hand the inhibitory limb is non functional the two acid responses will approximate. This approach was adopted in this study to examine the mechanism of the exaggerated acid response to GRP seen in the DU patients.

6.2. PATIENTS AND METHODS

Ten *H pylori* negative healthy volunteers (7 males), ten *H pylori* positive healthy volunteers (7 males), and ten *H pylori* positive DU patients (7 males) were studied. The three groups were matched for age and body weight. Duodenal ulcer patients were asked to stop any antisecretory therapy at least four weeks prior to the secretory studies. None of the healthy volunteers was on any medication and none reported major gastrointestinal symptoms. *H pylori* infection was confirmed in the DU patients by microscopic examination of antral biopsy, rapid urease test (CLO test- Delta West Pty Ltd, Australia) on antral biopsy and by ^{14}C urea breath test. In healthy volunteers, *H pylori* status was determined by the ^{14}C urea breath test. The ^{14}C urea breath test was performed as previously described in chapter 4.

Assessment of Maximal Acid Response to GRP Stimulated Gastrin and to Exogenous Gastrin-17

All subjects reported at 09:00h following a 12 hour fast. The secretory studies were performed in an identical fashion to that described in chapter 4.

Acid output was assessed basally and in response to GRP infused at the following rates: 10,40,100 and 200 $pmol.kg^{-1}.h^{-1}$ each for 45 minutes. All but 4 of these subjects also had their maximum acid response to Gastrin-17

assessed on a separate day. Following a 45 minute basal collection, Gastrin-17 was administered intravenously at a rate of 2000 $ng.kg^{-1}.h^{-1}$ for 60 minutes. Gastric juice was collected in 4x15 minute aliquots.

Maximal acid response to pentagastrin was determined in a further 11 DU patients who did not have any assessment of their GRP response.

GRP infusions were prepared as described in chapter 4. Pentagastrin was purchased from ICI (Cheshire, England). Gastrin-17 was purchased from Cambridge Research Biochemicals (Cheshire, England).

The volume and pH of each gastric juice collection were recorded and its hydrogen ion concentration measured by titration with 0.1M NaOH to pH 7 using an autotitrator (Radiometer ETS 822).

Basal acid output was calculated by taking the mean of all three 15 min samples prior to GRP or pentagastrin infusion. Acid output for each GRP infusion rate was calculated by taking the mean of the second and third 15 min collections. Maximal acid output to exogenous gastrin stimulation was calculated by taking the mean of all four 15 minute collections during the infusion.

Gastrin was measured by radioimmunoassay as described in chapter 4.

Eradication of *H pylori*

Following the above secretory studies, all 21 DU patients were treated with tripotassium dicitratobismuthate 120mg t.i.d., metronidazole 400mg t.i.d. and amoxycillin 500mg t.i.d. for 3 weeks. One month following completion of this therapy their ^{14}C urea breath test was repeated to determine *H pylori* status.

At one year post treatment GRP stimulated acid output was re-assessed in the ten DU patients. Pre-treatment, seven of these had had the prolonged GRP assessment as well as their maximal acid response to Gastrin-17 assessed and therefore they had these same tests again at this point. At this time point, however, the 200pmol.kg^{-1}.h^{-1} infusion rate of GRP was omitted as it had become apparent that the maximal response occurred at lower doses. The 11 additional DU patients who had only had a pentagastrin test pre-treatment each had this repeated at one year post-treatment. Each patient having secretory tests at one year post-treatment also had a repeat ^{14}C urea breath test at this time to exclude re-infection.

Analysis

V_{max} was estimated by fitting the data to a Langmuir model in which V_{max} was the primary parameter of interest. This was appropriate in 33 out of the 40

cases (82.5%). In the remaining 7 cases the acid level increased and then decreased with increasing doses of GRP and the maximal acid output was therefore estimated by inspection.

Statistics

Statistical analysis of unpaired data was performed using the Mann-Whitney U test and of paired data using the Wilcoxon test. A 'p' value of <0.05 was taken as significant.

The study was approved by the Western Infirmary ethical committee.

6.3. RESULTS

The repeat ^{14}C urea breath test at one month after completion of the triple anti-*H pylori* therapy indicated eradication of the infection in all DU patients. All 21 DU patients who were re-examined at one year post-eradication therapy remained free of the infection.

Maximal Acid Output to Exogenous Gastrin in DU Patients Before and One Year Following Eradication of *H pylori*

The median maximal acid output to exogenous gastrin in the 20 *H pylori* positive DU patients pre-treatment was 42mmol/h (range: 21-64). This was unchanged in the 18 patients re-examined at one year post-eradication, being 38.5mmol/h (range: 22-63)(Fig.6.1.).

Maximal Acid Output to GRP

The median maximal acid output (mmol/h) to GRP was higher in the *H pylori* positive (20, range 5.5-35.9) versus negative healthy volunteers (6.4, range 3.6-8.6) ($p<0.005$)(Figs.6.2.,6.3.). The median value in the *H pylori* positive DU patients (39.2, range 24.6-54.7) was higher than both the *H pylori* negative healthy volunteers ($p<0.0002$) and *H pylori* positive healthy volunteers ($p<0.001$). At one year post-eradication of *H pylori* the median value in the DU patients had fallen to 7.9 (range: 4.1-22.6)($p<0.006$ versus pre-

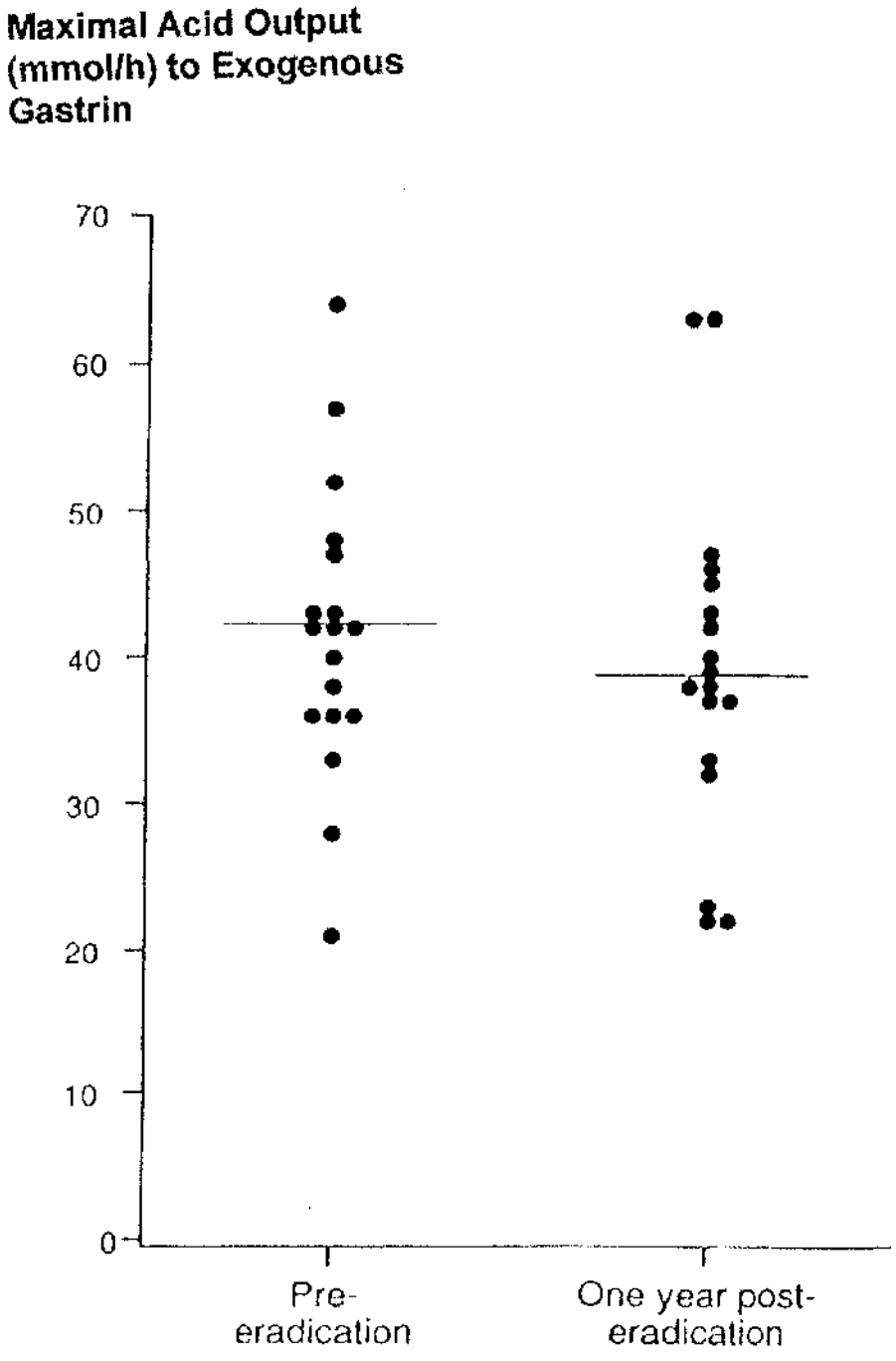


FIG. (6.1.)

Maximal acid output to exogenous gastrin in the 18 DU patients examined before and one year following eradication of *H pylori*. Eleven of the patients were examined following pentagastrin and seven following Gastrin-17. The median values are indicated by horizontal lines.

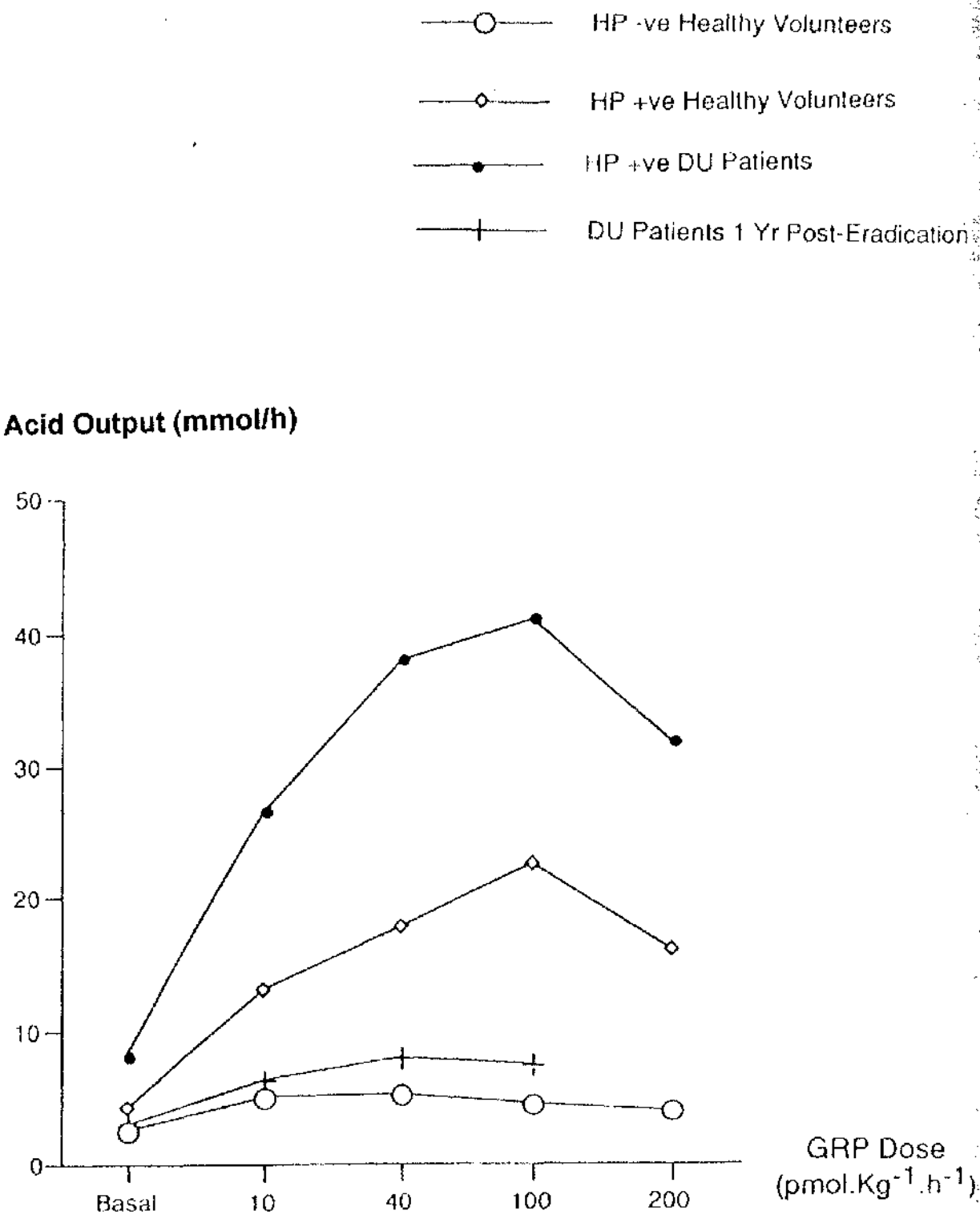


FIG. (6.2.)

Acid output in response to increasing doses of GRP in *H pylori* negative healthy volunteers (n= 10), *H pylori* positive healthy volunteers (n= 10), and DU patients (n= 10) before and one year following eradication of *H pylori*. The values are medians.

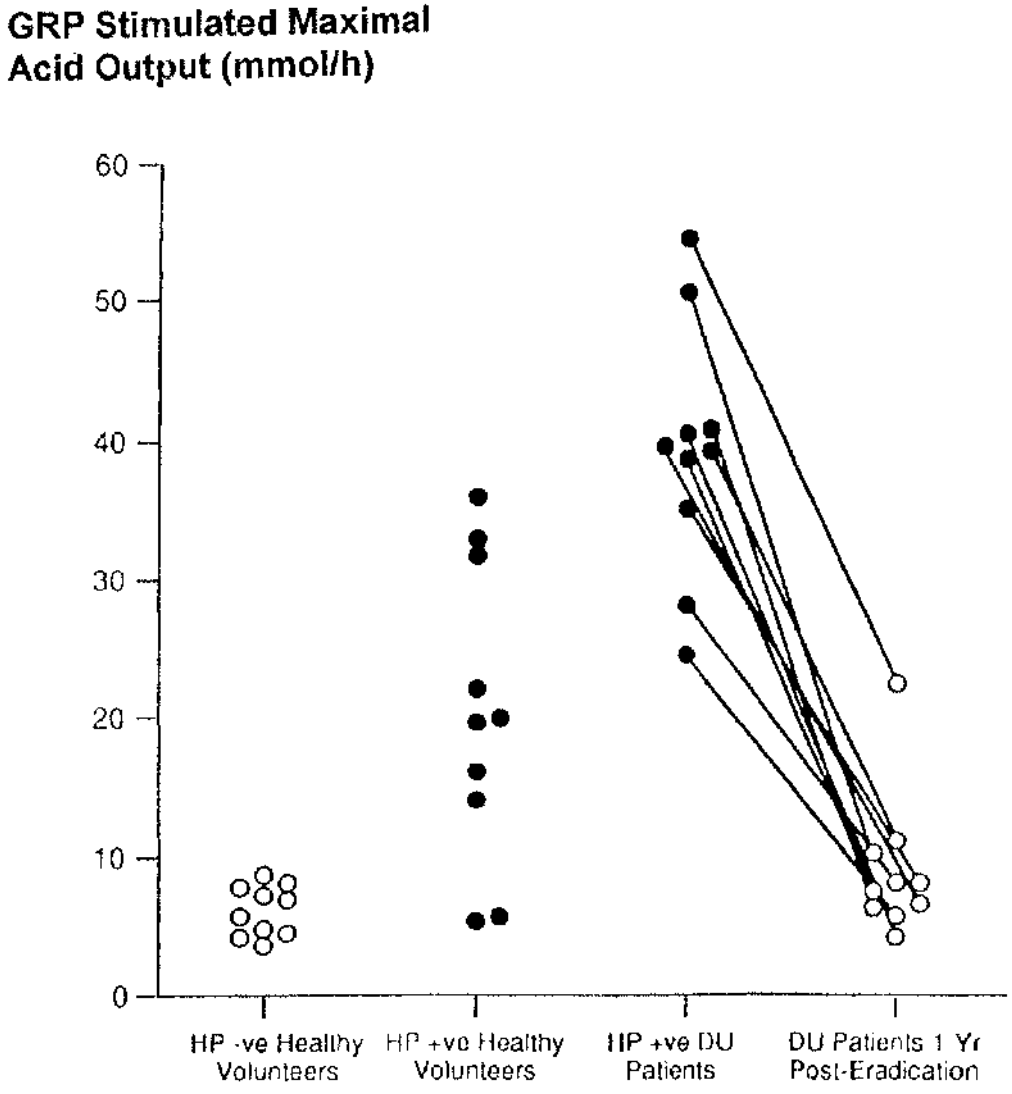


FIG. (6.3.)

Calculated maximal acid output to GRP stimulation in the various groups of subjects studied (● indicates *H pylori* positive). The *H pylori* positive healthy volunteers are higher than the *H pylori* negative volunteers ($p<0.005$). The *H pylori* positive DU patients are higher than all other groups ($p<0.001$) but became similar to the *H pylori* negative healthy volunteers at one year post eradication.

eradication) which was equivalent to that of the *H pylori* negative healthy volunteers. The maximal acid response to GRP stimulation in the infected DU patients and healthy volunteers occurred at approximately 100pmol.kg^{-1}.h^{-1}, compared with 40pmol.kg^{-1}.h^{-1} in the *H pylori* negative healthy volunteers or DU patients post-eradication. (Fig.6.2.).

Maximal Acid Output to GRP Versus Exogenous Gastrin-17

In the subjects described immediately above the median maximal acid output to exogenous Gastrin-17 was similar in the *H pylori* negative (25.6, range 18-51.6) and positive (30.8, range 22.1-40.1) healthy volunteers (fig.6.4.). The median value in the DU patients was 43.0 (range: 36-58.5) which was higher than that of the other two groups ($p<0.01$). The value in the DU patients was unchanged at one year following eradication of *H pylori* (median = 38; range: 32-63).

In the *H pylori* negative healthy volunteers the median value for the maximal acid response to GRP as a proportion of maximal acid output to exogenous Gastrin-17 was 27% (range: 9%-38%)(Fig.6.5.). The corresponding value was higher in the *H pylori* positive healthy volunteers at 70% (range: 25%-91%)($p<0.005$). The value in the DU patients was 83% (range: 50%-97%) which was higher than the *H pylori* negative healthy volunteers ($p<0.001$) but not significantly different from the *H pylori* positive healthy volunteers. At one

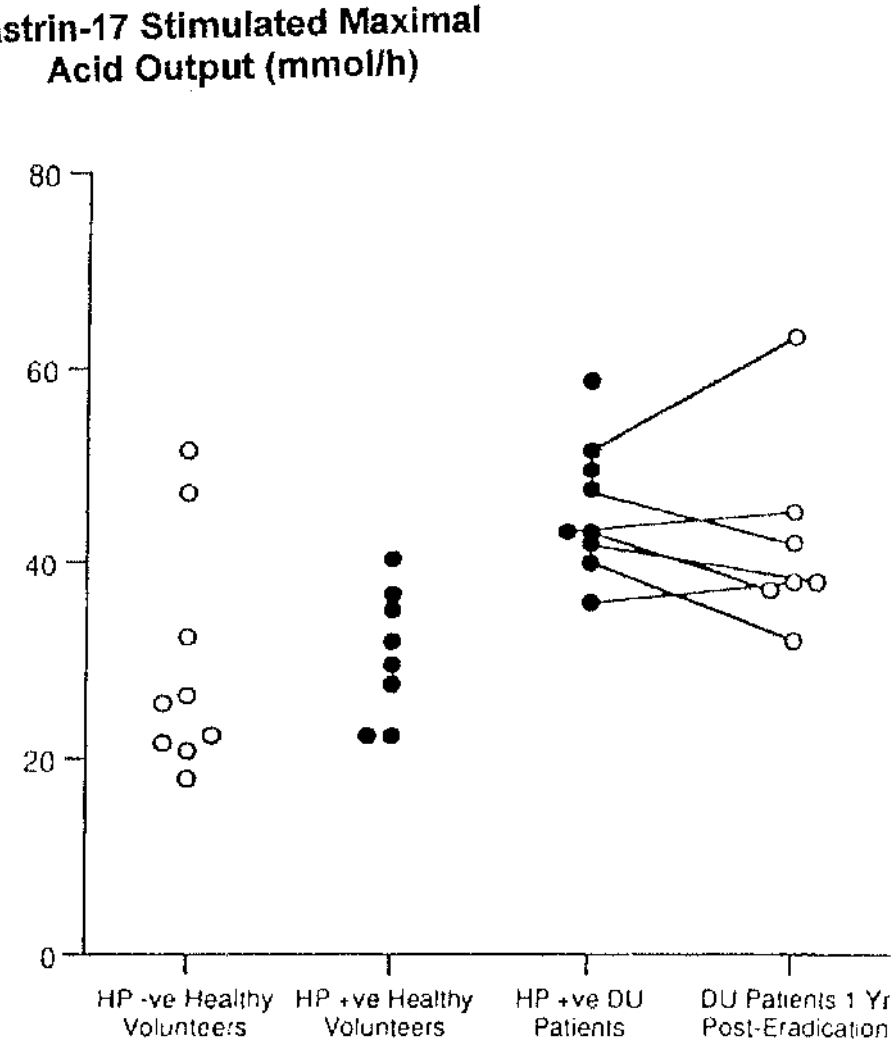


FIG. (6.4.)

Maximal acid output to Gastrin-17 in the subjects from the various groups who also had their maximal response to GRP assessed (● indicates *H pylori* positive). The DU patients examined before and one year post *H pylori* eradication are identified by continuous lines. The *H pylori* positive DU patients were higher than the healthy volunteers with and without *H pylori* ($p<0.01$ for both) and were unchanged at one year post eradication.

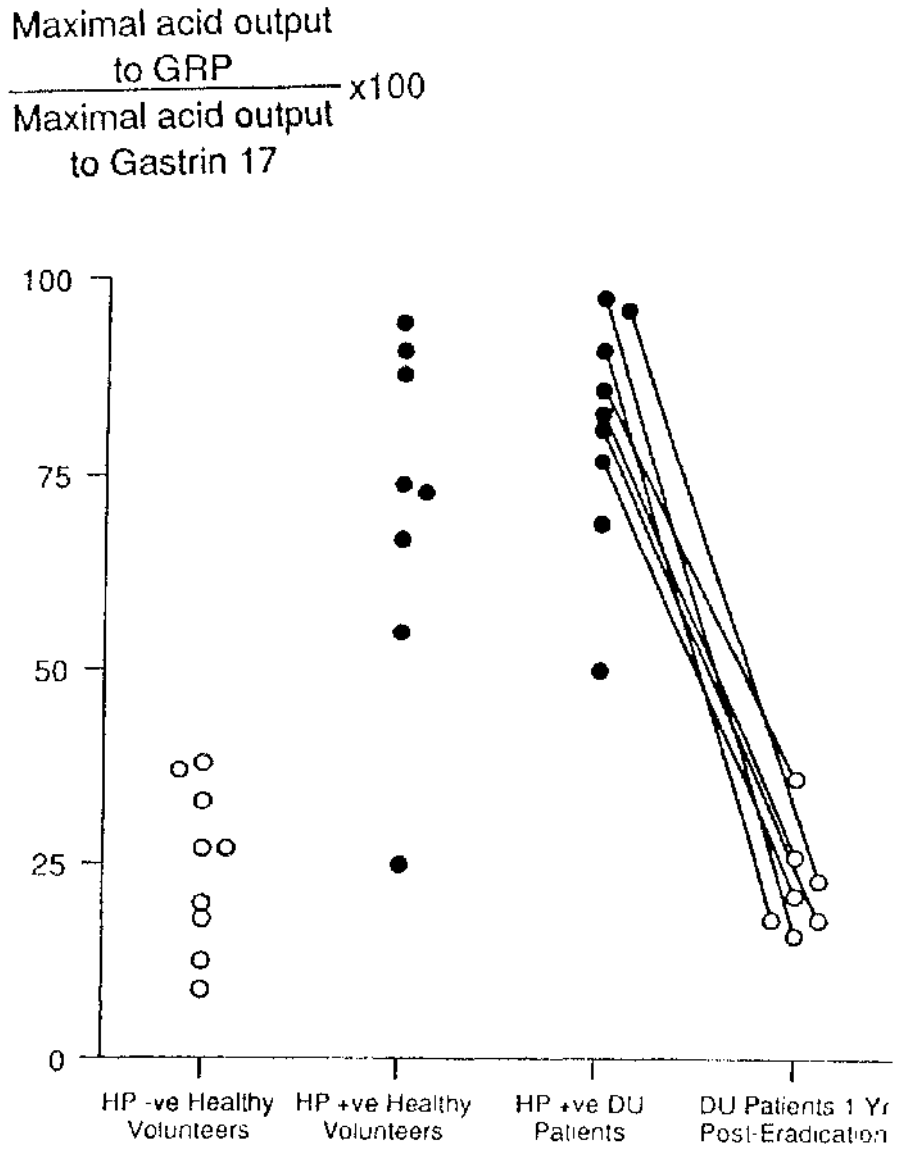


FIG. (6.5.)

Maximal acid output to GRP as a percentage of maximal acid output to Gastrin-17 in *H pylori* negative healthy volunteers, *H pylori* positive healthy volunteers, and DU patients before and following eradication of *H pylori* (joined by continuous lines)(● indicates *H pylori* positive). The values are similar in the *H pylori* positive healthy volunteers and DU patients and both higher than the *H pylori* negative healthy volunteers or DU patients at one year post eradication.

year post-eradication the value in the DU patients had fallen to 21% (range: 16%-36%)($p<0.001$ versus pre-eradication) which was similar to the value in the *H pylori* negative healthy volunteers.

6.4. DISCUSSION

In the present study we examined the effect of eradicating *H pylori* on the parietal cell mass in the DU patients. In some patients this was assessed using supramaximal doses of Gastrin-17 and in others using pentagastrin. These two methods have been shown to be equivalent in assessing maximal acid response to exogenous gastrin (140). DU patients are known to have a large parietal cell mass with their maximal acid response to pentagastrin being 1.5 to 2 times higher than that of healthy controls (77). It is possible that this increased parietal cell mass is due to long-term trophic effects of *H pylori* induced hypergastrinaemia. However, we found no reduction in maximal acid response to exogenous gastrin at one year post-eradication. This is consistent with other studies which found no change at earlier time points following eradication therapy (98,111,116), and also with the one previous study which re-examined patients at one year post eradication (141). Following curative resection of gastrinomas, the increased parietal cell mass caused by the trophic effect of the hypergastrinaemia resolves within 3-6 months (137) and therefore one would expect to see resolution within the 12 month period assessed in our study. However, this does not completely exclude a role for *H pylori* induced hypergastrinaemia in contributing to the increased parietal cell mass in DU patients. It is now recognised that the infection is usually acquired

at an early age (142) and it is possible that exposure to hypergastrinaemia at early stages of development may have irreversible consequences.

In the present studies we were able to investigate the relationship between the maximal response to GRP and the maximal response to exogenous gastrin in the various groups studied. In *H pylori* positive DU patients the maximal acid response to GRP stimulated gastrin was 83% of that to exogenous gastrin. This is consistent with 3 previous studies with bombesin in DU patients which reported the maximal response to that peptide to be between 80% and 100% of that to exogenous gastrin (133,143,144). In the *H pylori* positive healthy volunteers the maximal acid response to GRP was 70% of that to exogenous gastrin which was not significantly different from the DU patients. However the value in the *H pylori* negative healthy volunteers was markedly lower at 27%. Two previous studies have examined the maximal acid response to bombesin in healthy volunteers and both found it to be approximately 50% of that achieved with exogenous gastrin (133,145). The value of 50% in these previous studies in healthy volunteers is equivalent to our value when we combine our *H pylori* positive and negative healthy volunteers and is consistent with a proportion of those previously studied having *H pylori* infection. In the present study we were able to extend the previous studies by demonstrating that the reason for the maximal acid response to GRP/bombesin approximating to that of exogenous gastrin was

related to *H pylori* in that the abnormality fully resolved following eradication of the infection.

The fact that *H pylori* infection causes the maximal acid response to GRP to approach the maximal acid secretory response to exogenous gastrin may provide an insight into the pathophysiological basis of the hypersecretory response. GRP stimulates the G cells to release gastrin and this in turn stimulates the parietal cells, directly or via histamine release from ECL cells, to secrete acid (130,143). However GRP also activates neuroendocrine pathways which exert inhibitory control on gastric secretion. It does this by stimulating the release of a range of neuropeptides (including CCK, secretin, gastric inhibitory peptide, vasoactive intestinal peptide, neurotensin and enteroglucagon) (128,133,146) which inhibit the acid response both at the level of gastrin release and acid response to gastrin stimulation (147-153). The activation of the CCK mediated inhibitory control by GRP/bombesin is demonstrated by the fact that the administration of the CCK A receptor antagonist to healthy human volunteers increases their gastrin and acid response to GRP/bombesin by 50% (154). The inhibitory neuropeptides which are released in response to GRP stimulation are thought to exert their inhibitory influence via stimulating the release of somatostatin from D cells which acts in a paracrine fashion on the G cells and parietal and/or ECL cells (155-163). However, in the case of secretin the inhibitory response is thought

to be partly mediated via prostaglandin release (164). In some animals GRP also exerts inhibitory control directly on the stomach as well as via the central nervous system, but the importance of these pathways in man has not been defined (165,166). The administration of GRP therefore simultaneously activates these stimulatory and inhibitory control pathways of acid secretion and a likely explanation for the exaggerated response to GRP stimulation induced by *H pylori* is impairment of the inhibitory control. Strong evidence in favour of this is the finding that increasing doses of GRP allowed one to attain an acid response almost equivalent to the maximal secretory capacity in *H pylori* positive individuals whereas only 27% of the maximal response could be achieved in uninfected controls. *H pylori* infection has been shown to induce a reduction in immunoreactive somatostatin concentrations and somatostatin mRNA expression in the gastric mucosa (112,113) and this is consistent with the inhibitory control being disrupted at the level of somatostatin.

Our own unit in Glasgow and others have previously looked at the effect of *H pylori* on the inhibition of peptone stimulated gastrin and acid induced by intragastric acid (107,108,167). These studies found no defect in this control except for the one prolonged study in which impairment was seen in the second and third hour of intragastric acid and peptone infusion (167). A normal response to inhibitory control exerted by intragastric acid does not preclude impaired inhibitory control mediated by the various intestinal hormones

stimulated by GRP. Acid in the stomach can inhibit gastrin release by directly blocking the stimulatory effects of amino acids and amines on antral G cells (168,169). In contrast the inhibitory control exerted by the intestinal inhibitory hormones is indirect being mediated via somatostatin release.

In the studies presented in chapter (5) we found that GRP stimulated acid output in the *H pylori* positive DU patients was twice that of the *H pylori* positive healthy volunteers. This could not be explained by the degree of hypergastrinaemia as it was similar in both groups. The increased acid response to gastrin in the *H pylori* positive DU patients, however, may be explained by their increased parietal cell mass. The fact that GRP stimulated acid output in the DU patients returned to normal at one year following eradication of *H pylori* despite no resolution of the increased parietal cell mass may be explained by the return of the normal inhibitory control both at the level of gastrin release and of acid response to gastrin. The fact that the exaggerated gastrin response resolved before the exaggerated acid response to gastrin may be explained by differences in the mediation of the inhibitory control in the antral and oxyntic mucosae (153).

It could be argued that the more exaggerated acid response in the DU patients compared with the *H pylori* positive healthy volunteers, was related to rebound hypersecretion following the withdrawal of their acid inhibitory therapy. This is indeed a valid point and one which will have to be addressed. The studies presented in chapter (7) will attempt to answer this question.

We showed in chapter (5) that the increased basal acid output which is characteristic of DU patients (83) also fully resolved following eradication of *H pylori* infection. Moss and Calam have also recently reported a 65% fall in basal acid output following eradication of the infection in their DU patients (111). The cause of the increased basal acid output in the DU patients is unclear but is likely to be due to *H pylori* impairing the tonic inhibitory control of the large parietal cell mass characteristic of the DU patients (77).

Our findings of the effect of *H pylori* on the control of gastric secretion are likely to be relevant to the mechanism by which *H pylori* predisposes to duodenal ulceration. As already discussed the exaggerated response to GRP caused by *H pylori* is likely to be due to failure of inhibitory control exerted by neuropeptides mainly released from the duodenum and intestine. This inhibitory control is likely to serve as a means by which the duodenum and intestine can protect itself from excess exposure to acid delivered from the stomach. Failure of this protective feedback control of acid secretion by the

duodenum is therefore a likely explanation for the mechanism by which *H pylori* predisposes to DU.

In 1977, prior to the recognition of *H pylori,* Malagelada et al performed elaborate studies of meal stimulated acid secretion in DU patients and healthy volunteers (170). These studies differed from most previous or subsequent studies of meal-stimulated acid secretion by the fact that they did not artificially elevate intragastric pH and thus interfere with the physiological response to the meal. They found that the ulcer patients had inappropriately prolonged acid response and increased rate of acid delivery into the duodenum following the meal. The gastrin response was similar in the two groups. They concluded that this was most likely due to failure of the duodenal regulatory mechanisms that simultaneously control acid secretion and acid emptying. Their findings are consistent with our present finding of impaired inhibitory control of the acid response to gastrin in the DU patients which is fully reversed following eradication of *H pylori*

Our findings may also explain why *H pylori* leads to duodenal ulceration in only a proportion of those carrying the infection. Those who develop duodenal ulceration are known to have a greater parietal cell mass and maximal acid secretory capacity (77). The *H pylori*-induced loss of inhibitory control in a subject with a large gastric acid secretory capacity is likely to be more injurious

to the duodenum than the loss of control in a subject with a normal or small secretory capacity. The development of the clinical disease may therefore be explained largely by the combination of the acquired factor i.e. *H pylori*-induced loss of inhibitory control plus the genetic factor i.e. large parietal cell mass.

CHAPTER SEVEN

THE EFFECT OF RANITIDINE TREATMENT ON BASAL AND GRP STIMULATED GASTRIN AND ACID SECRETION IN HEALTHY VOLUNTEERS

7.1. INTRODUCTION

H_2 receptor antagonists are valuable agents for the healing of duodenal and gastric ulcers and for relieving the symptoms associated with these conditions. However, a proportion of patients treated with these acid suppressive drugs experience a rapid resurgence of dyspeptic symptoms on discontinuing therapy. The recurrence of dyspeptic symptoms may simply be due to the restitution of previous levels of acid secretion. However, another possible explanation of the early and marked resurgence of dyspeptic symptoms is that it is related to rebound acid hypersecretion.

We have demonstrated in chapter five that *H pylori* positive DU patients have a six fold increase in their GRP stimulated acid secretion compared to *H pylori* negative healthy volunteers. We proceeded to examine the mechanism of this exaggerated acid response to GRP in chapter six and our findings are consistent with *H pylori* disrupting the inhibitory pathways controlling acid secretion. The DU patients differed from the healthy volunteers in one very important aspect in that DU patients had been exposed to acid inhibitory therapy. The effect of this form of therapy on the regulatory pathways controlling acid secretion remain largely unknown. Although all our DU patients were studied at least four weeks following discontinuation of acid suppressive therapy, we were concerned that at least part of this exaggerated

acid response to GRP may in fact be due to the phenomenon of rebound acid hypersecretion.

Most of the early studies which examined acid secretion following withdrawal of H_2 antagonists measured acid output in response to supraphysiological doses of histamine or pentagastrin and found no evidence of rebound hypersecretion (171-175). However, several more recent studies have used other methods of determining acid secretion and have reported the presence of significant rebound hypersecretion. In 1986, Frislid et al found that gastric acid secretion in response to a simulated meal in healthy volunteers was increased by more than 30% when examined 60 hours after completing a four week course of ranitidine 150mg b.d. (176). In 1989, Fullarton et al studied nocturnal acid output before and two days following a four week course of nizatidine 300mg nocte in patients with healed duodenal ulcers (177). Before commencing treatment the median acid output was 39.4mmol/10h and this increased to 74.1mmol/10h after treatment, representing a median increase of 77%. In 1991 Nwokolo et al reported an increase in nocturnal intragastric acidity one day after completing a twenty-eight day courses of either ranitidine, cimetidine or nizatidine in healthy volunteers (178). They also observed increased day-time intragastric acidity following the ranitidine therapy. In 1992, Kummer et al studied nocturnal acid output following a six week course of ranitidine 300mg nocte in patients with

active duodenal ulcers (179). On the third day after stopping treatment, 10 hour nocturnal acid output was 86.2 mmol compared with 54.7 mmol before treatment, representing a mean increase of 58%. In a similar study Fullarton et al observed a 44% increase in nocturnal acid output following ranitidine (180). These recent studies indicate rebound acid hypersecretion is evident when studied by tests which do not over-ride the normal regulatory controls of acid secretion as occurs when measuring maximal acid output.

As we have demonstrated in the previous chapters, GRP has proved to be a useful stimulus of gastric secretory function. In the present study we employed the GRP test to study the phenomenon of rebound acid hypersecretion following a two month course of a commonly prescribed H_2 antagonist (ranitidine). In view of the fact that *H pylori* alters the regulation of gastric acid secretion we have studied subjects with and without the infection.

7.2. SUBJECTS AND METHODS

Ten *H pylori* negative (8 males) and ten *H pylori* positive (8 males) healthy volunteers were studied. All subjects were matched for age and body weight (mean age for *H pylori* negative subjects = 29, range: 18-50; mean age for *H pylori* positive subjects = 30, range: 18-56). None had ever been exposed to acid inhibitory therapy and none had consulted their doctor with chronic dyspeptic symptoms. *H pylori* status was confirmed in each subject within 2 weeks of entry to the study by the ^{14}C urea breath test.

Secretory Studies

All subjects had their basal and GRP stimulated gastrin and acid output measured within 2 days prior to starting ranitidine therapy. For this the subjects reported at 09:00h following a 12 hour fast. A nasogastric tube was swallowed and secretory studies were performed as described in chapter four. Three 15 minute collections were obtained basally. An intravenous infusion of GRP was then started at a rate of 40pmol.kg^{-1}.h^{-1} and continued for 45 minutes during which a further three 15 minute collections of gastric juice were obtained. Blood samples were collected every 15 minutes for gastrin determination and the plasma stored at -20 ^{0}C.

Following their pre-treatment secretory tests the subjects were then given a pack containing 60 ranitidine (Zantac) tablets 300mg each. They were instructed to take one 300mg tablet at 22:00h each night for the following 60 days. Compliance with the treatment was ensured by regular reminder phone calls and by pill counting at the end of the study.

The secretory studies described above were repeated in an identical fashion sixty hours and ten days after the last ranitidine dose. In three patients the secretory studies were performed again 17 days after stopping ranitidine.

Basal acid output was calculated by taking the mean of all three 15 minute samples prior to GRP infusion. Acid output in response to GRP infusion was calculated by taking the mean of the second and third 15 minute collections.

Gastrin was measured by radioimmunoassay using antiserum R98 which has a sensitivity of 5ng/l (102). It binds G17 and G34 with equimolar potency. The basal gastrin value for each subject was determined by taking the mean of the three samples obtained prior to GRP infusion. The gastrin value in response to GRP infusion was determined by taking the mean of the two values at 30 and 45 minutes of the infusion.

Statistics

Statistical analysis was performed for matched pairs using the Wilcoxon test with significance taken at the 5% level.

The study was approved by the Western Infirmary ethical committee and all subjects gave written consent for the study.

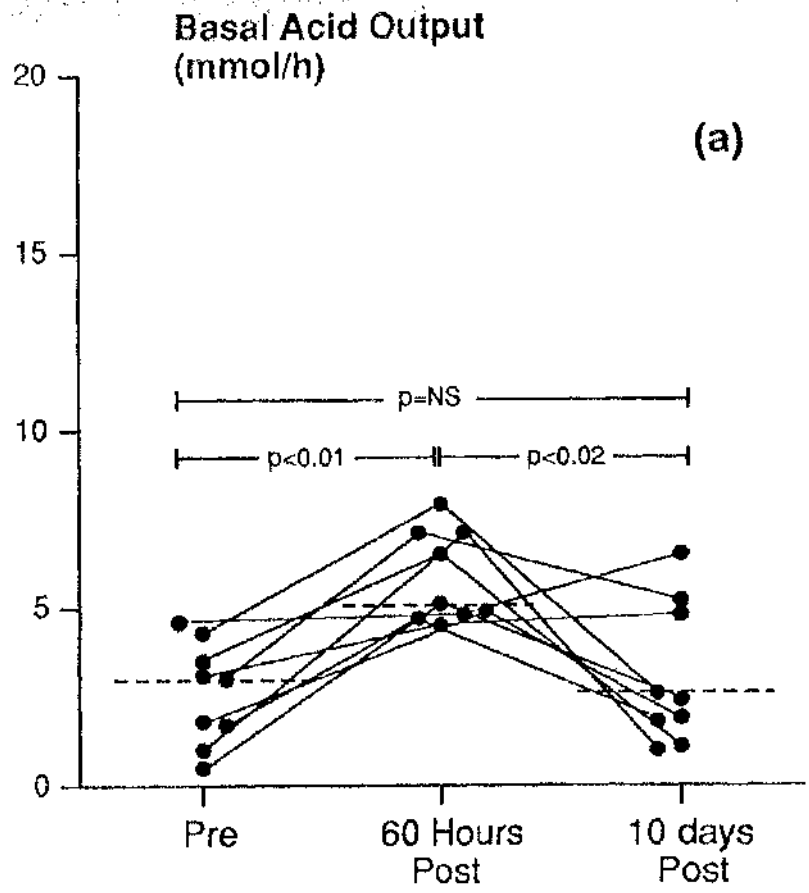
7.3. RESULTS

Compliance

Eighteen out of 20 subjects (9 in each group) completed the study with 100% compliance with the ranitidine treatment. The remaining 2 subjects (both male) were excluded from the post-treatment studies on the basis of poor compliance with treatment as assessed by pill counts at the end of the study.

Basal acid output and gastrin concentrations

The median basal acid output of the 18 subjects pre-treatment was 3.3 mmol/h (range: 0.5-11.5). Sixty hours post-treatment it increased to 6.9 mmol/h (range: 4.7-17.8) representing a median increase of 109% compared to pre-treatment values ($p<0.01$). The basal acid output returned to pre-treatment values by day 10 (median 4.8; range: 1.8-14.9)(Fig. 7.1.a&b). This increase in basal acid output was not accompanied by any change in the median plasma



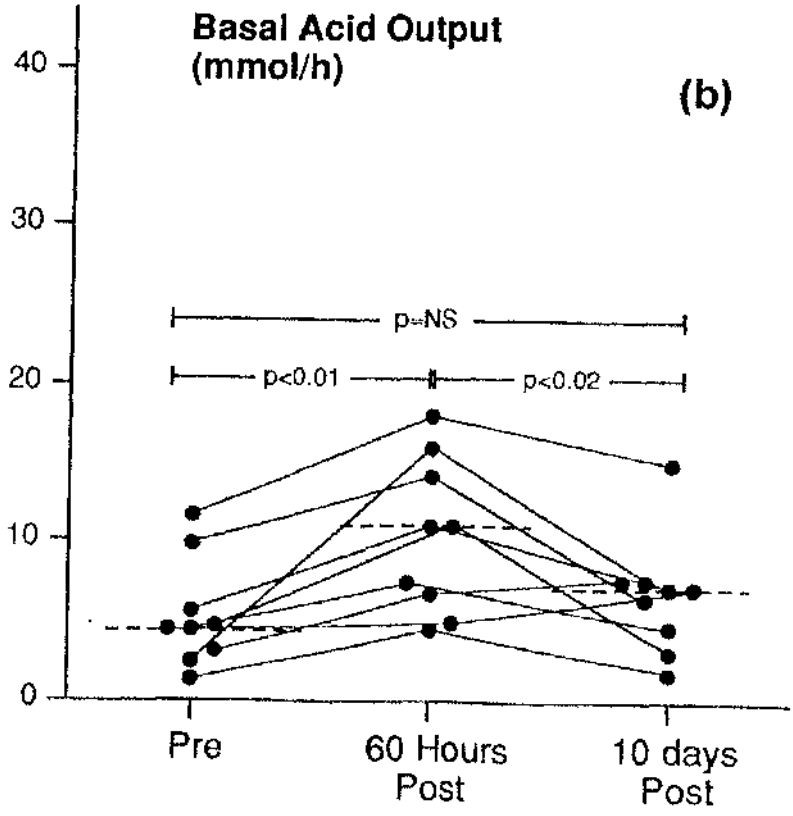


FIG. 7.1.

Basal acid output (mmol/h) in *H pylori* negative subjects (a) and *H pylori* positive subjects (b), pre, 60 hours post and 10 days post ranitidine treatment.

gastrin concentrations: pre-treatment = 33 ng/l, range: 15-105; 60 hours post-treatment = 43 ng/l, range: 10-195; 10 days post-treatment = 43 ng/l, range: 10-180 (Fig. 7.2.a&b).

Analysis of the subjects according to *H pylori* status showed that the 9 positive subjects had higher basal gastrin concentrations (median = 35, range: 30-105) and higher basal acid output (median = 4.5, range: 1.2-11.5) pre-treatment than the uninfected subjects (median gastrin = 20, range: 15-65; median basal acid output = 3.0, range: 0.5-4.6) ($p<0.05$ for both). Both groups were similar with respect to the degree of rebound in their basal acid output (median = 137%, range: 4%-840% for the negative subjects and median = 96% range: 7%-587% for the positive subjects). Neither group showed significant rebound in basal gastrin concentrations.

GRP stimulated acid output and gastrin concentrations

The median GRP stimulated acid output of the 18 subjects pre-treatment was 10.0 mmol/h (range: 4.6-29.8). Sixty hours post-treatment it increased to 15.6 mmol/h (range: 11.7-36.6) representing a median increase of 68% compared to pre-treatment values ($p<0.01$). The median GRP stimulated acid output fell to 12.4 mmol/h (range: 5.1-40.3) by day 10 but was still significantly higher than pre-treatment value ($p<0.05$)(Fig. 7.3. a&b). However, the difference represented only 9% and was due to 4 subjects whose acid outputs

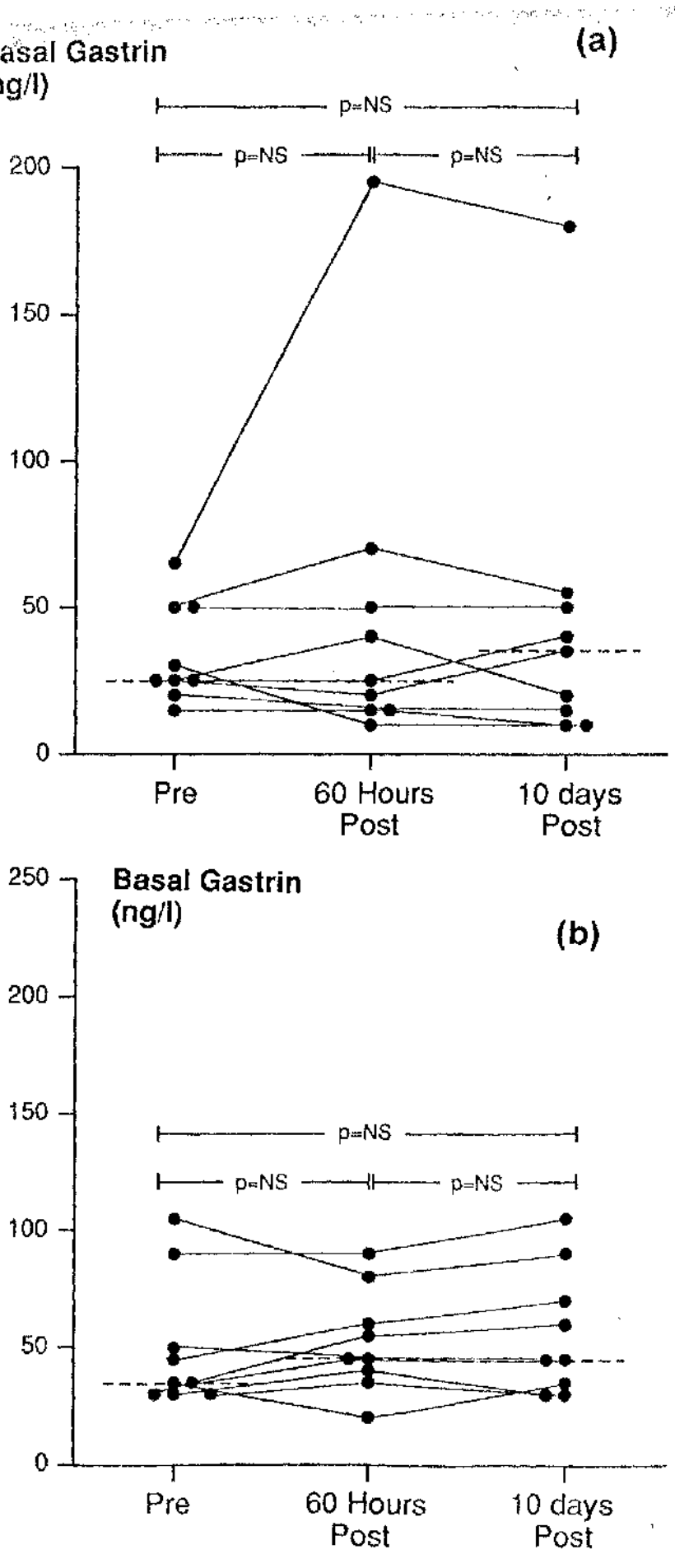
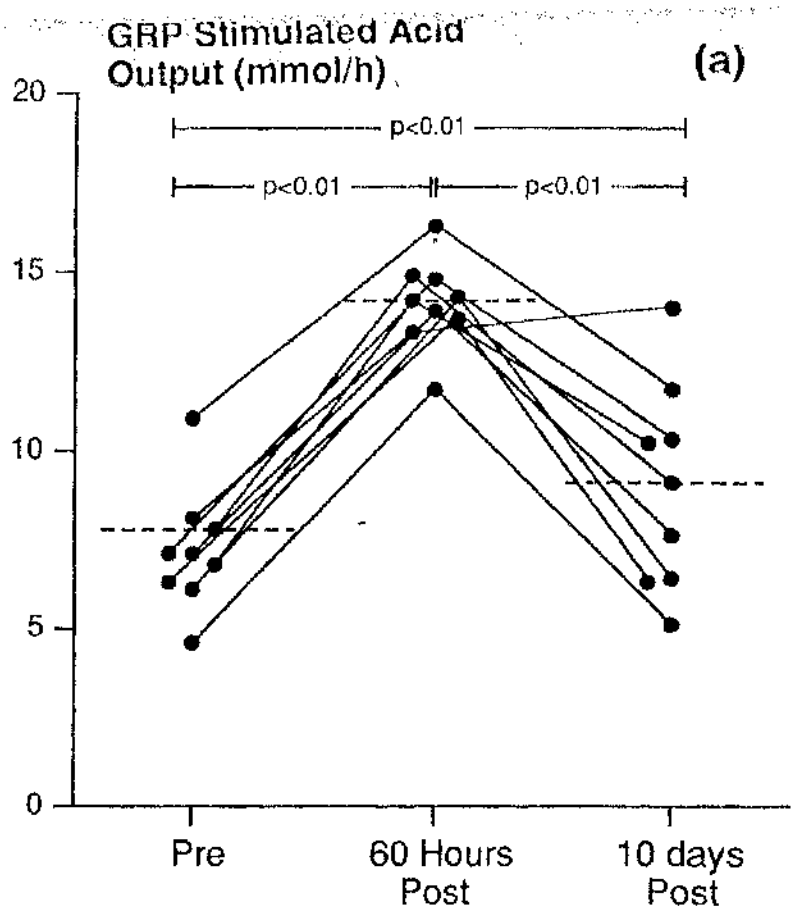


FIG. 7.2.

Basal gastrin concentrations (ng/l) in *H pylori* negative subjects (a) and *H pylori* positive subjects (b), pre, 60 hours post and 10 days post ranitidine treatment.



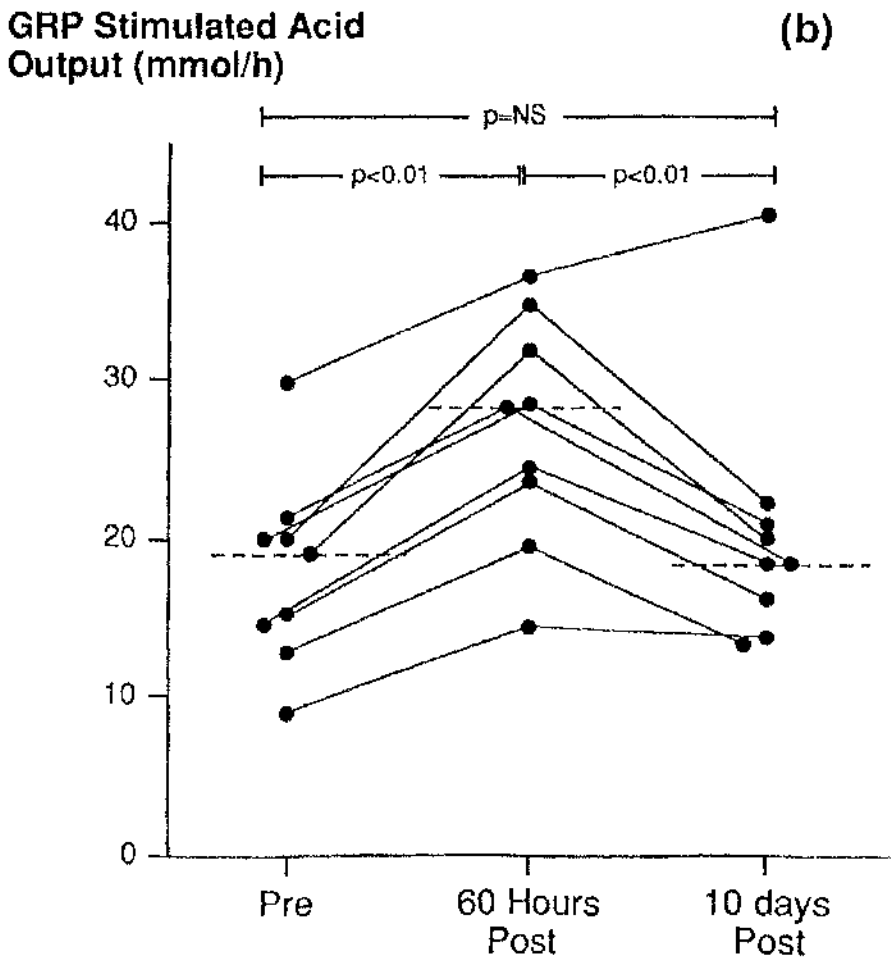
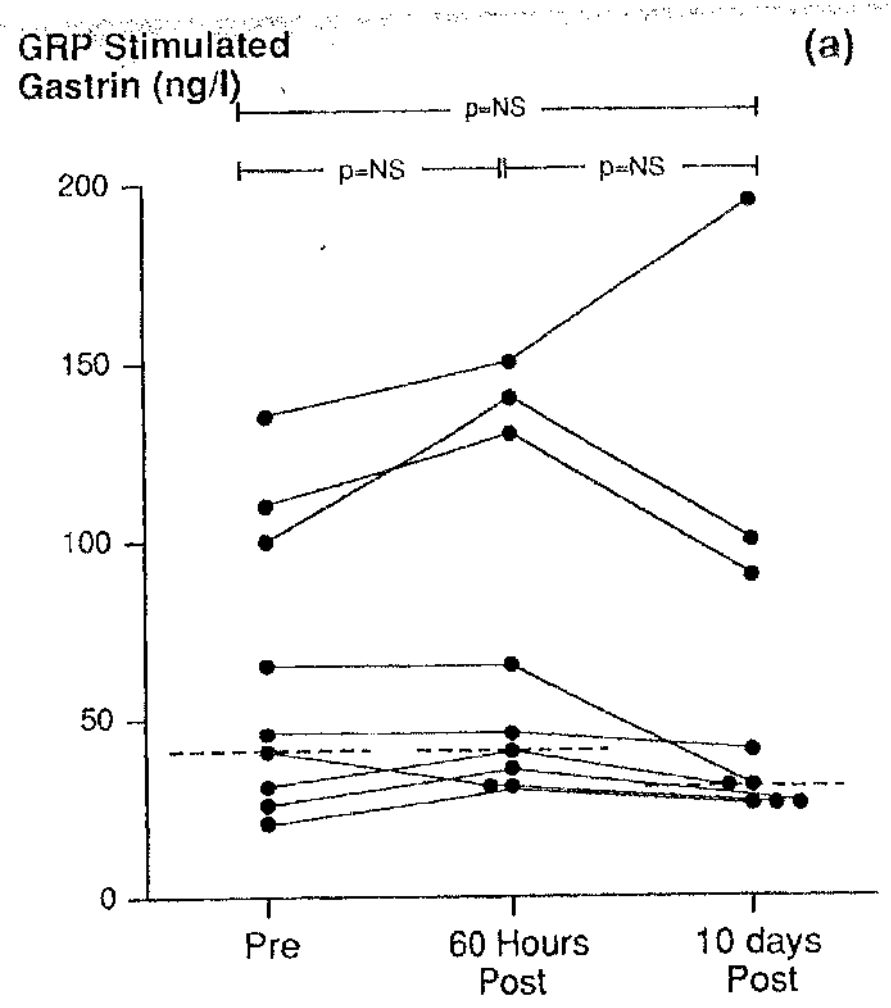


FIG. 7.3.

GRP stimulated acid output (mmol/h) in *H pylori* negative subjects (a) and *H pylori* positive subjects (b), pre, 60 hours post and 10 days post ranitidine treatment.

remained relatively high 10 days post treatment (6.8, 8.1, 7.1 and 29.8 pre-treatment compared to 10.2, 14, 10.3 and 40.3 respectively 10 days post-treatment). These 4 subjects were re-examined one week later (i.e. 17 days post-treatment) and their GRP stimulated acid outputs fell to 6.5, 7.9, 7.6 and 30.3 respectively which was equivalent to their pre-treatment value. The increase in GRP stimulated acid output was not accompanied by any significant change in the GRP stimulated plasma gastrin concentrations: pre-treatment = 83ng/l, range: 20-250; 60 hours post-treatment = 100, range: 30-225; 10 days post-treatment = 70, range: 25-250,(Fig. 7.4.a&b).

Analysis of the subjects according to *H pylori* status indicated that the 9 positive subjects had higher GRP stimulated gastrin concentrations (median = 95, range: 55-250) and acid output (median = 19.0, range: 9.0-29.8) pre-treatment than the uninfected subjects (median gastrin = 45, range 20-135; median acid output = 7.8, range: 4.6-10.9)($p<0.01$ for both). The *H pylori* negative subjects showed a higher percentage of rebound in their GRP stimulated acid output than the positive subjects (median = 108%, range: 50%-154% vs median = 56%, range: 23%- 74% respectively)($p<0.01$). Neither group showed significant rebound in GRP stimulated gastrin concentrations.



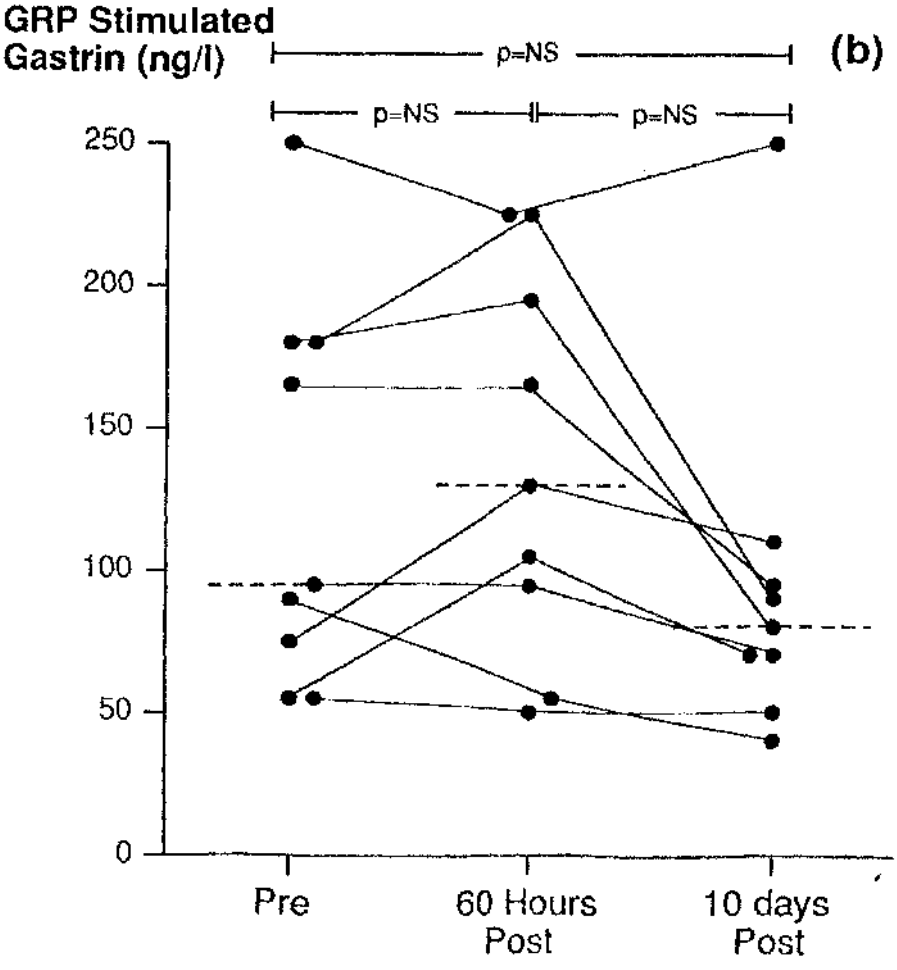


FIG.7.4

GRP stimulated gastrin concentrations (ng/l) in *H pylori* negative subjects (a) and *H pylori* positive subjects (b), pre, 60 hours post and 10 days post ranitidine treatment.

7.4. DISCUSSION

The present studies demonstrate that a standard two month course of ranitidine therapy is associated with marked rebound of both basal and GRP stimulated acid secretion in subjects with and without *H pylori* infection. In the group as a whole, basal acid output increased by 109% and GRP stimulated acid output by 68% at sixty hours after stopping ranitidine therapy. This rebound acid hypersecretion largely resolved by day 10 post-treatment.

The finding of rebound basal acid hypersecretion is consistent with the three previous studies of nocturnal acid output reporting mean increases ranging from 44% to 77% following withdrawal of H_2 antagonist therapy (177,179,180). Rebound acid output has not been studied previously following GRP stimulation but our results are in keeping with the report by Frislid et al who observed rebound hypersecretion following a sham-meal (176). There is therefore now strong evidence that H_2 antagonists produce rebound hypersecretion of both basal and stimulated acid output.

In the present study, we included subjects with and without *H pylori* infection. This was done as the infection has been shown to alter the normal regulation of gastrin release and acid secretion and could therefore modify rebound acid hypersecretion. *H pylori* positive healthy volunteers have

increased basal, meal-stimulated and GRP-stimulated serum gastrin concentrations compared with uninfected subjects (97,119). In addition, infected volunteers have a 1.5-2 fold increase in basal acid output and 2-3 fold increase in GRP stimulated acid output compared to controls. These disturbances in gastrin and acid secretion fully resolve following eradication of the organism. The *H pylori* positive healthy volunteers included in the present study showed this previously reported increase in basal and GRP stimulated gastrin and acid output.

The percentage increase in basal acid output following discontinuation of ranitidine therapy was similar in *H pylori* positive and negative healthy volunteers. However, the percentage increase in GRP stimulated acid output was higher in the *H pylori* negative volunteers than in those with the infection. This may be explained by the fact that GRP stimulation in *H pylori* positive healthy volunteers produces an acid output which is equivalent to approximately 70% of their maximal acid output. Consequently, those with the infection only have the capacity to increase GRP stimulated acid secretion by approximately 50% which approximates to the rise noted in the present study. In contrast, GRP stimulation in subjects without *H pylori* produces an acid response of only about 25% of their maximal acid secreting capacity and they therefore have a greater reserve in which to increase their acid output.

The mechanism of the rebound acid hypersecretion following H_2 antagonist therapy is unclear. It cannot be explained by an increase in the parietal cell mass as numerous previous studies have shown no change in maximal acid output to stimulation with pentagastrin or histamine following treatment with H_2 antagonists (171-175). In addition, it does not appear to be directly mediated by increased gastrin release as neither the basal or GRP stimulated acid rebound were associated with any rebound increase in gastrin levels. In keeping with our findings, previous studies of rebound hypersecretion have also found no evidence of rebound hypergastrinaemia (172,173,177,178,180). In addition, the rebound hypersecretion cannot be explained by increased parietal cell sensitivity to gastrin as studies by Aadland and Berstad (1974) found this unaltered in DU patients or healthy volunteers when examined 60 hours and 84 hours after completing a 6 week course of cimetidine (175).

There is some evidence that rebound acid hypersecretion following H_2 antagonist therapy may be explained by an increased sensitivity to histamine. Aadland and Berstad examined acid secretion in response to submaximal and supramaximal doses of histamine in healthy volunteers before and following four weeks treatment with cimetidine (175). They found that the acid response to the low dose of histamine increased significantly due to an increased parietal cell sensitivity to the amine. Jones et al studied acid secretion in patients with healed duodenal ulcers before and after three months treatment

with ranitidine 150mg nocte (181). Following withdrawal of treatment , they found an increased acid response to stimulation with the H_2 agonist, impromidine, and also an increased acid inhibitory response to the H_2 antagonist, ranitidine. This was again interpreted as showing an enhanced sensitivity of the H_2 receptor possibly due to an increase in the number of H_2 receptors i.e. up-regulation.

Other possible explanations for the rebound hypersecretion also need to be considered. Acid secretion basally and in response to a meal or GRP depends upon the interaction of both stimulatory and inhibitory controls. The rebound hypersecretion observed by each of these methods could therefore be explained by down-regulation of the inhibitory controls especially those which are activated by acid in the stomach or duodenum. The inhibitory control of acid secretion by the oxyntic mucosa is partly mediated via the release of somatostatin from fundic mucosal D cells and acid suppression by omeprazole reduces somatostatin mRNA in the fundic mucosa of animals (182).

The present studies clearly demonstrate substantial rebound acid hypersecretion following discontinuation of H_2 antagonist therapy. The clinical significance of this phenomenon with respect to rapid resurgence of symptoms remains to be determined. It has also been shown that the rebound in acid secretion largely resolves within two weeks of discontinuing therapy in healthy

volunteers. This suggests that the four week interval off treatment after which our DU patients were studied in the previous chapters was probably adequate to avoid any false increase in their acid outputs caused by rebound. This however does not exclude the possibility that long-term acid inhibition, which most DU patients are under, may in fact be contributing to their ulcer diathesis by damaging the inhibitory control pathways in a similar fashion to *H pylori* infection.

CHAPTER EIGHT

THE EFFECT OF *H PYLORI* INFECTION ON GRP STIMULATED GASTRIN AND ACID SECRETION IN NUD PATIENTS

8.1. INTRODUCTION

Dyspepsia is a very common disorder with a prevalence of 30-40% in the general population of the UK (183,184). The disorder accounts for 10-20% of GP consultations (185) and 30% of hospital gastroenterology referrals (186). In over 50% of dyspeptic patients upper GI investigations are non-definitive and these patients fall into the category of non-ulcer dyspepsia (NUD)(187,188). Consequently, NUD is the commonest diagnosis in patients presenting with dyspepsia.

The recent recognition of the pathogenic role of *H pylori* infection in duodenal ulcer (DU) disease is transforming the management of that condition and removing the need for long-term treatment with expensive acid inhibitory therapy. The cause of NUD remains unknown but there is considerable interest in its relationship to DU disease and in the possible role that *H pylori* infection may have in the condition.

Our own results presented in chapter five have shown that *H pylori* positive DU patients have a 6-fold increase in acid secretion in response to stimulation by GRP, when compared with *H pylori* negative healthy volunteers. GRP stimulated acid secretion is also increased in healthy volunteers with the infection but their median output is only 3 times that of *H pylori* negative

healthy volunteers. The very marked increase in acid secretion in DU patients is likely to represent a key pathophysiological defect underlying their DU disease. We also showed that the hypersecretion of acid in both *H pylori* positive DU patients and healthy volunteers is entirely due to the infection as it fully resolves following eradication of the organism.

This recognition of disturbed gastric secretory function caused by *H pylori* and associated with DU disease may be helpful in investigating the pathogenesis of NUD. In particular the assessment of GRP stimulated acid secretion may provide a means of identifying NUD subjects with a similar disturbance of gastric function as DU patients and which could be reversed by eradication of their *H pylori* infection.

The aim of the present study was to determine whether the underlying disturbance of acid secretion characteristic of DU disease is also present in a proportion of *H pylori* positive NUD patients.

8.2. PATIENTS AND METHODS

Twenty five *H pylori* positive NUD patients (16 males) were studied. Their median age was 35 years (range: 18-59). The patients were recruited consecutively from our GI outpatient clinic and all fulfilled the following three criteria: (1) a six month or longer history of dyspepsia. This consisted of upper abdominal or retrosternal pain, discomfort, heartburn, nausea, vomiting, or other symptom considered referable to the proximal alimentary tract, and unrelated to exercise. (2) No macroscopic abnormality of the upper GI tract demonstrable despite at least two upper GI investigations including one endoscopy. (3) Evidence of *H pylori* infection as confirmed by microscopic examination of antral biopsies, rapid urease test (CLO test) on antral biopsy and ^{14}C urea breath test.

The above NUD patients were compared to three groups of subjects: 25 *H pylori* negative healthy volunteers (17 males), 25 *H pylori* positive healthy volunteers (17 males), and 25 *H pylori* positive DU patients (17 males). The DU patients had all been confirmed to have an active ulcer by endoscopic examination within the previous 12 months. All groups were matched for age and body weight.

NUD and DU patients were asked to stop any antisecretory therapy at least four weeks prior to the secretory studies. None of the healthy volunteers was on any medication and none had consulted the medical profession on account of dyspepsia. *H pylori* infection in the DU patients was confirmed as for the NUD patients. In the healthy volunteers, *H pylori* status was determined by the ^{14}C urea breath test.

Secretory Studies

All subjects reported at 09:00h following a 12 hour fast. The GRP test was performed in an identical fashion to that described in chapter four. Three 15 minute collections were obtained basally and at each of the following rates of I.V. infusion of GRP: 10 and 40pmol.kg^{-1}.h^{-1}. Gastrin and acid output were measured as described previously.

Statistics

Statistical analysis was performed using the Mann-Whitney U test. A 'p' value of <0.05 was taken as significant.

In the NUD patients linear regression analysis was performed to look for correlation between acid output and age and duration of dyspeptic symptoms.

The study was approved by the Western Infirmary ethical committee.

8.3. RESULTS

Basal Gastrin

The basal gastrin concentration (ng/l) was similar in the *H pylori* positive healthy volunteers (median = 45, range: 10-90), *H pylori* positive NUD patients (60, range: 25-270), and *H pylori* positive DU patients (60, range: 22-175) and all were higher than the *H pylori* negative healthy volunteers (32, range: 15-50) ($p< 0.005$ for all three)(Fig.8.1.).

Gastrin Response to GRP

At the GRP infusion rate of 40pmol.kg^{-1}.h^{-1} the median gastrin concentration (ng/l) was increased to a similar level in the *H pylori* positive healthy volunteers (238, range: 38-563), *H pylori* positive NUD patients (225, range: 55-700), and *H pylori* positive DU patients (255, range: 90-600) and each was higher than that of the *H pylori* negative healthy volunteers (70, range: 28-157)($p< 0.002$ for each)(Fig.8.3.).

The gastrin response in the four groups of subjects at the GRP infusion rate of 10pmol.kg^{-1}.h^{-1} showed the same pattern of response to that seen in response to GRP 40pmol.kg^{-1}.h^{-1}. We chose to present the results of the individual data points for the 40pmol.kg^{-1}.h^{-1} GRP rate as the gastrin levels stimulated by this are closer to those seen following a meal.

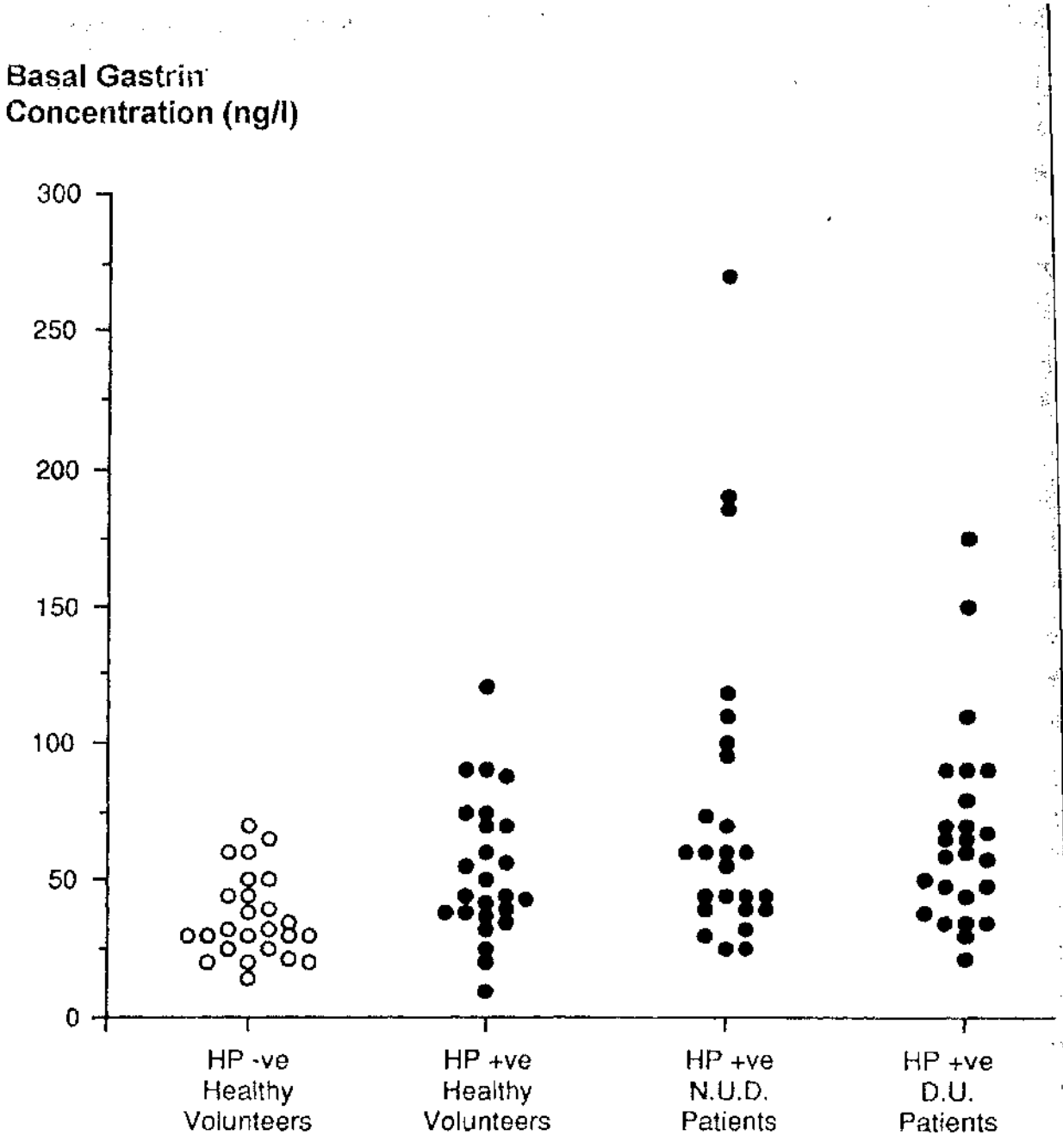


FIG. (8.1.)

Basal gastrin concentrations in the four groups of subjects studied. The *H pylori* positive NUD patients were higher than the *H pylori* negative healthy volunteers ($p<0.002$) and similar to the *H pylori* positive healthy volunteers and DU patients.

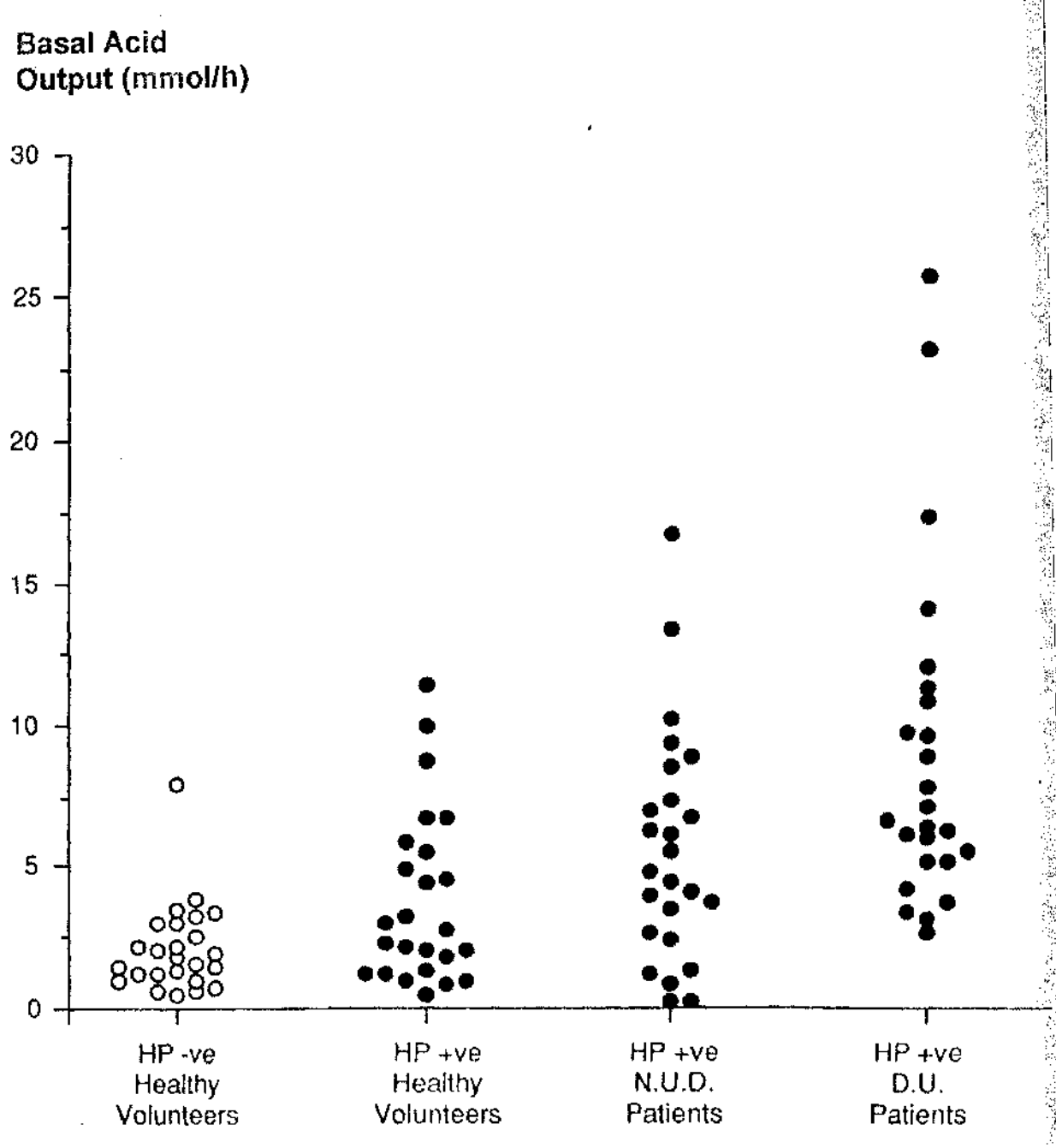


Fig. (8.2.)

Basal acid output in the four groups of subjects studied. The *H pylori* positive NUD patients were higher than the *H pylori* negative healthy volunteers ($p<0.01$) and lower than the DU patients ($p<0.03$), but not different from the *H pylori* positive healthy volunteers.

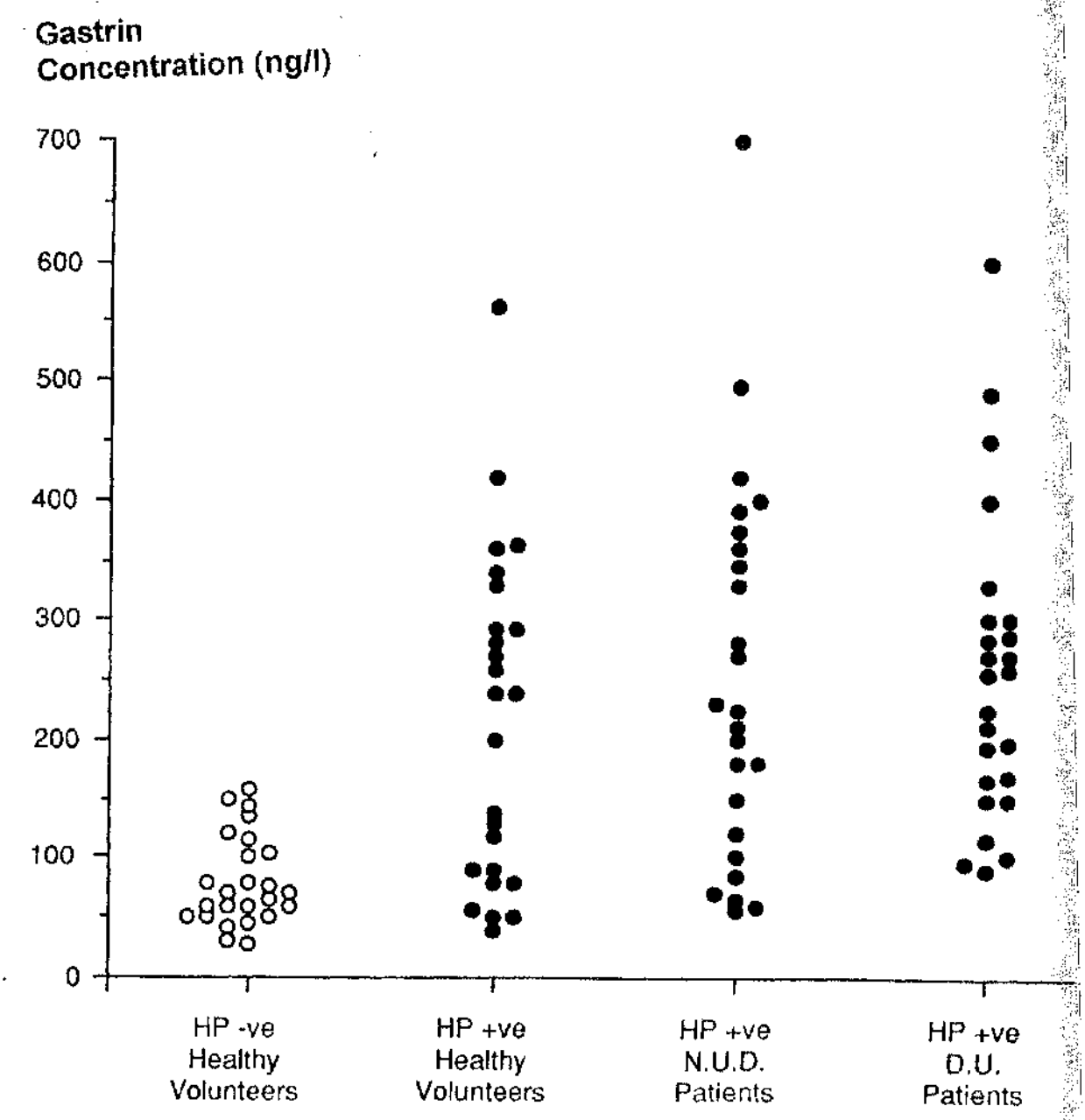


Fig. (8.3.)

Serum gastrin concentrations in response to stimulation with gastrin releasing peptide (40pmol.kg^{-1}.h^{-1}) in the four groups studied. The *H pylori* positive NUD patients were higher than the *H pylori* negative healthy volunteers ($p<0.002$) and similar to the *H pylori* positive healthy volunteers and DU patients.

Basal Acid Secretion

The median basal acid output (mmol/h) was higher in the *H pylori* positive healthy volunteers (2.9, range: 0.5-13.3)($p<0.05$) and *H pylori* positive NUD patients (4.8, range: 0.3-16.7)($p<0.01$) compared to the *H pylori* negative healthy volunteers (1.8, range: 0.5-7.9). However, there was no statistically significant difference between the basal acid output in the *H pylori* positive healthy volunteers and NUD patients ($p=0.2$). The median basal acid output in the *H pylori* positive DU patients was 6.6mmol/h (range: 2.6-25.8) and was significantly higher than that of the *H pylori* positive NUD patients ($p<0.03$), *H pylori* positive healthy volunteers ($p<0.001$), and *H pylori* negative healthy volunteers ($p<0.0001$)(Fig.8.2.).

Acid Response to GRP

At GRP 40pmol.kg^{-1}.h^{-1} the median acid output (mmol/h) in the *H pylori* positive healthy volunteers (19.0, range: 1.0-38.3) was approximately 3 times that of the *H pylori* negative healthy volunteers (6.3, range: 2.8-20.9)($p<0.001$). At this infusion rate the median acid output in the *H pylori* positive NUD patients was 29.6 (range: 5.2-46.5) which was approximately 5 times that of the *H pylori* negative healthy volunteers ($p<0.0001$) and 1.5 times that of the *H pylori* positive healthy volunteers ($p<0.001$). The median acid output in the *H pylori* positive DU patients was 39.1 (range: 17.9-64) which was approximately 6 times that of the *H pylori* negative healthy volunteers ($p<0.0001$) and twice

that of the *H pylori* positive healthy volunteers ($p<0.005$). Acid output was significantly higher in the *H pylori* positive DU patients compared to the *H pylori* positive NUD patients ($p<0.001$)(Fig.8.4.).

Acid outputs in response to 10 $pmol.kg^{-1}.h^{-1}$ of GRP followed the same pattern in the four groups as those obtained in response to 40 $pmol.kg^{-1}.h^{-1}$.

In the NUD patients linear regression analysis showed that there was no correlation between acid output and either age ($r=0.2$, $p=0.87$), or duration of dyspeptic symptoms ($r=0.1$, $p=0.9$).

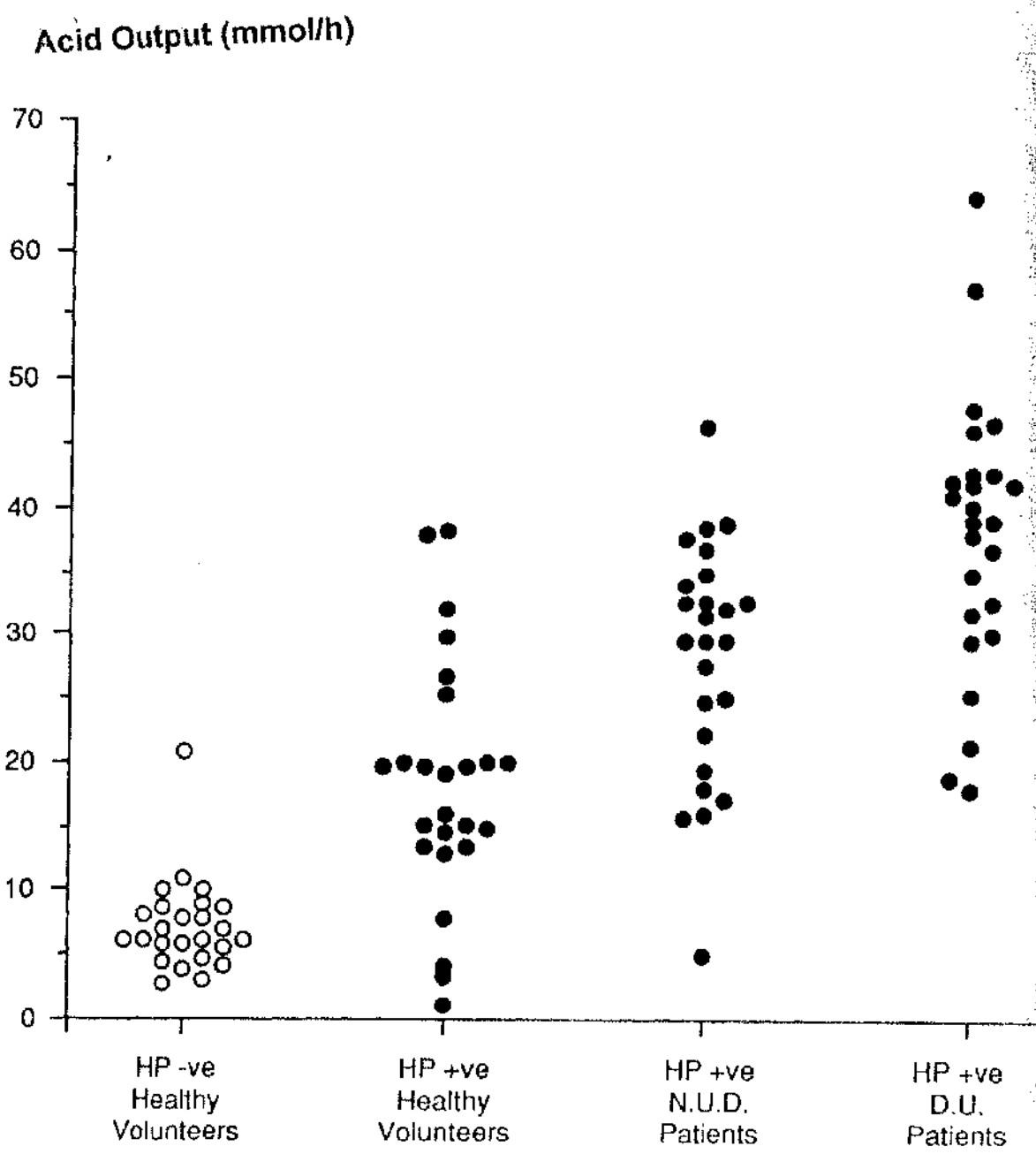


Fig. (8.4.)

Acid output in response to stimulation with gastrin releasing peptide (40pmol.kg^{-1}.h^{-1}). The acid output in the *H pylori* positive NUD patients was higher than the *H pylori* negative healthy volunteers ($p<0.0001$) and *H pylori* positive healthy volunteers ($p<0.001$), and lower than the DU patients (0.001).

8.4. DISCUSSION

H pylori is now established as the major acquired factor in the pathogenesis of DU disease. The mechanism by which it predisposes to DU disease is likely to be related at least in part to its effects on gastric acid secretion. *H pylori* increases basal, meal stimulated and GRP stimulated gastrin concentrations and the magnitude of the hypergastrinaemia is similar in DU patients and healthy volunteers with the infection (96-101,132). The hypergastrinaemia is accompanied by a 3 fold increase in GRP stimulated acid output in infected healthy volunteers and a more marked 6 fold increase in DU patients. DU patients with *H pylori* thus resemble infected healthy volunteers in having a similarly exaggerated gastrin response but differ from them in producing twice as much acid for equivalent gastrin levels basally and during GRP stimulation. In the present study we have studied gastric function in *H pylori* positive NUD patients to see whether a subgroup have a similar disturbance to DU patients.

Both basal and GRP stimulated gastrin concentrations were increased to a similar extent in the NUD patients compared with *H pylori* positive healthy volunteers and DU patients. This confirms previous findings that the magnitude of basal and stimulated hypergastrinaemia is similar in the presence of *H pylori*

infection regardless of whether the subject is healthy or has dyspeptic disease (97).

The basal acid output was significantly increased in the *H pylori* positive NUD patients compared to the *H pylori* negative healthy volunteers, but was also significantly less than in the DU patients. There was no statistically significant difference in basal acid output between the infected healthy volunteers and NUD patients, though there was a trend in favour of it being higher in the latter.

GRP stimulated acid output in the NUD patients was 5 times higher than the *H pylori* negative healthy volunteers but significantly lower than the *H pylori* positive DU patients. The GRP stimulated acid output in the NUD patients was also 60% higher than that of the *H pylori* positive healthy volunteers. The median GRP stimulated acid output in the NUD patients thus fell half way between that of the *H pylori* positive healthy volunteers and the *H pylori* positive DU patients. This could be explained by approximately half the NUD patients having GRP stimulated acid secretion similar to the DU patients and half having acid secretion similar to the *H pylori* positive healthy volunteers. Alternatively, it could be due to NUD patients having GRP stimulated acid output which falls between that of DU patients and infected healthy volunteers.

The former explanation is the more likely as previous studies indicate that NUD is a heterogeneous condition (189).

The mechanism of the increased GRP stimulated acid secretion in the NUD patients was similar to that in the DU patients. The DU patients had a similar gastrin response to the infected healthy volunteers and thus their higher acid response was due to an increased acid response to gastrin during GRP stimulation. Likewise the NUD patients had the same gastrin response to GRP as the infected healthy volunteers and thus their higher acid output was again due to an increased acid response to gastrin during GRP stimulation.

The finding that a subgroup of *H pylori* positive NUD patients have a similar disturbance of gastric function to DU patients may be relevant to the underlying cause of their dyspeptic disease. It is well recognised that symptoms in DU patients correlate poorly with the presence of actual ulceration (190-194) and the pain is thus not simply due to the effect of excess acid on an active ulcer. The precise mechanism of pain in DU disease is unknown but is likely to be due to the interaction of excess acid secretion and inflammation of the gastroduodenal mucosa (195). This mechanism could also explain the pain in the NUD patients who have an exaggerated GRP stimulated acid response similar to the DU patients.

If a subgroup of *H pylori* positive NUD patients have a similar basis to their dyspeptic symptoms as DU patients then eradication of the infection is likely to benefit them in a similar way to DU patients. Such treatment would both lower their GRP stimulated acid secretion and resolve the inflammation of their mucosa. Previous studies of the value of eradicating *H pylori* in patients with NUD have produced conflicting results and this is consistent with the infection playing a role in only a subgroup of NUD patients (196-198). The challenge now is to find a means of identifying the subgroup of patients with NUD whose symptoms are secondary to their *H pylori* infection and who are therefore most likely to benefit from its eradication. Assessment of GRP stimulated acid secretion might provide a means of identifying such patients.

CHAPTER NINE

SUMMARY AND CONCLUSIONS

H pylori infection is the commonest chronic bacterial infection world-wide. It has been associated with a variety of upper gastrointestinal diseases including gastritis, duodenal and gastric ulcer disease, non ulcer dyspepsia and gastric carcinoma. The mechanism by which this common infection predisposes to these diseases remains poorly understood. The work carried out in this thesis has sought to explore the effect of the infection on gastric secretory function in man.

The first objective of the thesis was to develop a new approach to the study of gastrin mediated acid secretion without using food as the stimulus, thus avoiding all the technical difficulties and inaccuracies associated with this. This was achieved by using the substance gastrin releasing peptide (GRP). Acid secretion measured in response to intravenous infusion of GRP is the end product of the stimulatory and inhibitory mechanisms controlling acid secretion and reflects the combined functional response of the antrum and body of the stomach. It simulates the response to eating and represents a more physiological test of gastrin mediated acid secretion than most of the currently available tests of acid secretion. The reproducibility of the GRP test was assessed and was found to be highly reproducible for both gastrin and acid secretion. We recommend the use of the GRP infusion rate of 40 $pmol.kg^{-1}.h^{-1}$ as this was found to be the most reproducible for both gastrin and acid secretion. In addition the gastrin levels achieved in response to

stimulation by this dose are comparable to those achieved in response to stimulation by a test meal.

Using this new and highly reproducible tool we proceeded to study acid secretion in a variety of subjects with and without *H pylori* infection. We have shown that GRP stimulated acid secretion is increased six fold in DU patients with the infection compared to *H pylori* negative healthy volunteers (true normals). This exaggerated acid response is likely to represent a key pathophysiological defect which underlies DU disease. We also showed that eradication of *H pylori* infection leads to complete normalisation of basal and GRP stimulated gastrin and acid secretion in DU patients. The gastrin abnormality resolves within one month of eradication and the acid abnormality takes upto one year to fully resolve. These novel findings have shed considerable light on the pathophysiology of DU disease and the role of *H pylori* infection in it.

We have also shown for the first time that GRP stimulated acid secretion is increased two to three fold in *H pylori* positive healthy volunteers compared to true normals. This redefinition of true normality has allowed the clear separation of the duodenal ulcer diathesis without any overlap with normals. Since half the world's population is colonised with *H pylori* infection, these findings will have to be taken into account when designing future research

projects on gastric function. The finding that *H pylori* infection produces a two to three fold increase in acid secretion in the general population which resolves following eradication of the infection may also be of relevance to other dyspeptic disorders such as reflux disease.

We proceeded to investigate the mechanism of the exaggerated acid response to GRP in DU patients and presented evidence compatible with this being due to impaired inhibitory control of acid secretion. This was shown by demonstrating that true normals achieve a small proportion of their maximal acid secretory capacity when stimulated with GRP compared to stimulation with exogenous gastrin and this is consistent with GRP activating an intact inhibitory pathway capable of attenuating the acid response to GRP. In contrast, DU patients achieve almost all their maximal acid secretory capacity in response to stimulation with GRP compared to stimulation with exogenous gastrin consistent with the inhibitory pathway being defective and incapable of attenuating the acid response to GRP.

The finding that eradication of *H pylori* infection did not alter the maximal acid secretory capacity of DU patients suggests that *H pylori* does not play a part in determining this and that other factors such as the genetic makeup of the individual is probably involved. As such one might speculate that the individuals who are predisposed to developing DU disease are those who are

born with a large parietal cell mass and who subsequently acquire *H pylori* infection. This then damages their ability to attenuate and control the amount of acid secreted by the stomach in response to appropriate stimuli with a marked increase in the amount of acid delivered to the unprotected duodenal mucosa. Future research should now focus on the nature of the defect in the inhibitory control mechanisms caused by *H pylori* infection. This will be of relevance to the understanding of DU disease as well as other upper GI diseases which have been linked to *H pylori* infection.

We proceeded to examine the effect of the most commonly prescribed medication for dyspepsia, i.e. ranitidine, on acid secretion in healthy volunteers with and without *H pylori* infection. This was done to investigate whether treatment with H_2 antagonists leads to rebound acid hypersecretion following withdrawal of therapy which could contribute to the exaggerated acid response seen in DU patients. We showed that a two month course of ranitidine leads to a doubling of basal acid output and a 68% increase in GRP stimulated acid output two days after withdrawing treatment. This rebound acid hypersecretion is not associated with any significant change in gastrin concentrations and fully resolves within ten days of stopping ranitidine. All our DU patients were studied at least four weeks after stopping acid inhibitory therapy. However, DU patients are usually on this medication for much longer periods and the long-term effects of chronic suppression of acid secretion

remain unknown. It is still possible that part of the exaggerated acid response seen in DU patients is due to rebound acid hypersecretion following acid suppressive therapy. This suggests albeit controversially that these drugs may be accentuating the DU diathesis and are contributing to the secretory abnormalities seen in DU patients. Our findings of significant rebound in both basal and GRP stimulated acid secretion offer an explanation for the common clinical problem of rapid resurgence of dyspeptic symptoms following discontinuation of acid suppressive therapy. Future work will now focus on the clinical aspects of this phenomenon by assessing in a double blind placebo controlled fashion the presence and severity of dyspeptic symptoms following discontinuation of acid suppressive therapy in healthy volunteers. We will also proceed to investigate the mechanism of the rebound phenomenon by examining the effect of acid suppressive therapy on parietal cell sensitivity and on the acid inhibitory control mechanisms.

Finally, we used the GRP test to study acid secretion in patients with non ulcer dyspepsia (NUD) and *H pylori* infection. We showed that NUD patients display a wide spectrum of acid secretion ranging from low to very high. A significant proportion of NUD patients showed an exaggerated acid response to GRP similar to DU patients i.e. they displayed the DU diathesis. This raises the exciting possibility that this group of NUD patients could be cured by eradication of their *H pylori* infection. This hypothesis needs to be tested in a

large double blind placebo controlled trial which will examine the value of eradication of the infection in NUD patients. We have designed such a trial funded by the Medical Research Council and work has already started in our unit on this large project. Over the next three years new guidelines for the management of NUD patients will hopefully become available.

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ABSTRACTS

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