HELICOBACTER PYLORI AND PEPTIC ULCER DISEASE, WITH SPECIAL REFERENCE TO THE WESTERN CAPE

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Louw JA, Zak J, Lucke W, Le Roux E, Jaskiewicz K, Winter T, Lastovica A and Marks IN. Triple therapy with sucralfate is as effective as triple therapy containing bismuth in eradicating *Helicobacter pylori* and reducing duodenal ulcer relapse rates. Scand J Gastroenterol 1992;27 Suppl 191:28-31.

Louw JA, Girdwood AH, Jaskiewicz K, Zak J, Trey G, Lucke W, Truter H and Kotze TJvW. *Helicobacter pylori* prevalence in non - ulcer dyspepsia: ethnic and socioeconomic differences. S Afr Med J 1993;83(3):169-171.

Louw JA, Falck V, van Rensburg C, Zak J, Adams G and Marks IN. Distribution of *Helicobacter pylori* colonization and associated gastric inflammatory changes: differences between patients with duodenal and gastric ulcers. J Clin Path 1993;46:754-756.

Louw JA, Lucke W, Jaskiewicz K, Lastovica AJ, Winter TA and Marks IN. *Helicobacter pylori* eradication in the African setting, with special reference to re-infection and duodenal ulcer recurrence. Gut 1995;36:544-547.

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Louw JA, Girdwood AH, Jaskiewicz K, Zak J, Callanan MD and Marks IN. When should the Rapid Urease Test be interpreted - 1 minute, 10 minutes, 1 hour or 24 hours? S Afr Med J 1991;80:47.

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Louw JA, Jaskiewicz K, van Rensburg C, Lucke W, O'Keefe S and Marks IN. The proton pump inhibitors (PPIs) omeprazole, lansoprazole and pantoprazole have a similar effect on gastric *H. pylori* distribution. Am J Gastroent 1994;89(8):1379.

ABSTRACT

Helicobacter pylori and peptic ulcer disease, with special reference to the Western Cape.

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This study investigates various aspects of the role of *Helicobacter pylori* in peptic ulcer disease, with particular reference to the Western Cape. The following aspects are addressed:

1. Gastric involvement by the organism in duodenal and gastric ulcer disease: This was investigated by evaluation of multiple biopsies taken from the antrum and body of peptic ulcer subjects, and by the characterisation of the gastritic process and *H. pylori* colonization densities in these. The data showed that while the infection is predominantly antral in duodenal ulcer disease, it is found with equal frequency in the body of gastric and duodenal ulcer subjects. The density of infection, and the magnitude of the inflammatory response in the body were, however, less pronounced in duodenal ulcer subjects. This is compatible with current theories regarding the differences in the gastritic process and acid secretion found in duodenal and gastric ulcer subjects.

2. Duodenal involvement by the organism in peptic ulcer disease: This was investigated by histological evaluation of duodenal biopsies taken from tissue immediately adjacent to duodenal ulcers. These samples were investigated for the presence of gastric metaplasia and *H. pylori* colonization. The findings confirmed a high incidence of gastric metaplasia and *H. pylori* infection in relation to the duodenal ulceration. While this finding is at variance with that of workers from other Third World countries, it is in keeping with findings from developed countries and is compatible with current theories implicating the organism in the development of duodenal ulcer disease.

3. The effect of *H. pylori* infection on gastric secretion of acid and pepsin, factors strongly implicated in the pathogenesis of peptic ulcer disease: This was studied by investigating the effect of healing and the immediate and long-term effect of *H. pylori* eradication on the basal and pentagastrin stimulated secretion of acid and pepsin. We were unable to identify any consistent effect of eradication on acid and pepsin secretion. This is similar to the findings of other workers, and adds appreciably to the limited data on long term follow-up available in the literature. We conclude that the postulated changes in gastric secretion following *H. pylori* eradication are unlikely to be mediated by changes in parietal cell mass, as has been suggested, and have drawn attention to possible shortcomings in the traditional methodology used to investigate gastric acid secretory function (as opposed to acid secretory capacity).

4. The prevalence of *H. pylori* infection was determined in 169 dyspeptic subjects representative of the patients seen in the Western Cape Teaching Hospitals. A high

prevalence of the infection was confirmed, while a striking ethnic difference in prevalence was identified, which is probably attributable to the large differences in socioeconomic development inherent in South African communities.

5. The effect of *H. pylori* eradication on the natural history of duodenal ulcer disease was assessed by follow-up of "eradicated", and similarly treated "non-eradicated" subjects, over a period of two years. This confirmed that eradication of the organism is associated with dramatically improved duodenal ulcer relapse rates and, importantly, that re-infection by *H. pylori* is rare even in our population with a high prevalence of the infection.

6. Finally, a number of treatment strategies were investigated in an attempt to formulate a safe, acceptable and effective treatment strategy for *H. pylori*. The study indicated that conventional "Triple Therapy" is acceptable in our population, due in part to the relatively low incidence of metronidazole resistance encountered in this population, but that "Triple Therapy" using low dose tetracycline is not. We investigated the effect of acid suppression on the organism, both as a monotherapy strategy and when acid suppression is used in combination therapy. This led to the conclusion that the use of the proton pump inhibitor omeprazole is ineffective in eradicating the organism when used as monotherapy, while omeprazole and amoxicillin dual therapy is extremely well tolerated by patients and eradicates the organism in approximately two thirds of subjects.

This study supports the hypothesis that *H. pylori* is an important factor in the pathogenesis of peptic ulcer disease and confirms that the infection is extremely prevalent in our community. Despite the high prevalence, the data indicates that a peptic ulcer management strategy, which includes *H. pylori* eradication, is likely to be successful in the Western Cape.

DECLARATION

I, Jacob Albertus Louw, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part thereof has been, is being, or is to be submitted for another degree to this or any other University.

Signed:

Dated:

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CHAPTER 1

INTRODUCTION

Until recently, acid-pepsin secretion has dominated our thinking on the etiology and management of peptic ulcer disease. Schwarz's time-honoured dictum of "no acid - no ulcer" led to the 20th century vogue of using a variety of increasingly potent acid inhibitory agents in the management of peptic ulcer disease. The rationale for this approach was supported by the efficacy of such therapies in healing ulcers. The almost inevitable ulcer relapse following successful short-term healing, however, highlighted differences between the healing of the ulcer and the cure of the disease. The cause of the disease remained elusive.

The acid and pepsin model appeared too simplistic in terms of ulcer etiology, and attention was focussed on other factors considered to be "protective" (i.e. protecting the gastroduodenal mucosa against acid and pepsin aggression). Thus, peptic ulcer disease was seen as the result of an imbalance between these "protective" and aggressive factors. Once again, this model was supported by the efficacy of the so-called mucosal protective agents in healing ulcers, without affecting acid secretion. Ulcer relapse after healing with the mucosal protective agents, although perhaps less frequent than that following healing with acid inhibitory agents, continued to pose a problem.

The problem of ulcer relapse prompted the use of effective (and costly) medical maintenance therapy, which reduced the liability of ulcers to relapse. This in turn impacted on surgical practice, elective peptic ulcer

surgery for "intractable" disease becoming a less attractive option for the management of uncomplicated peptic ulcer disease.

Over the past decade perceptions regarding the etiology and management of peptic ulcer disease have changed dramatically. This followed on the rediscovery of *Helicobacter pylori* in the early 1980's and the finding that successful treatment of the infection in peptic ulcer sufferers (referred to as the conventional term "eradication" in this thesis) dramatically reduced the liability for ulcers to relapse. This finding forced the gastroenterological community to question existing views on the pathogenesis of peptic ulcer disease and, in particular, raised the possibility that peptic ulcer disease may be an infectious disease. Opposition to the perceived role of *H. pylori* in ulcerogenesis has, however, been stubborn.

There are several reasons why clinicians have been reluctant to accept an etiological role for *H. pylori* in peptic ulcer disease in the face of evidence that eradication of the organism drastically modifies the natural history of the disease. These include an inherent reluctance of clinicians to jettison the time-honoured understanding of the pathogenesis of peptic ulcer disease, the efficacy and safety of conventional therapies in healing peptic ulceration, and an apparent lack of a plausible and cohesive pathophysiological role for the organism in the disease. In addition, evolving and rapidly changing concepts with regard to ulcer pathogenesis and eradication strategies tested the credulity of most people, let alone sceptics. The reluctance to accept *H. pylori* as an important factor in the etiology of peptic ulcer disease is further complicated in the African setting by the belief that the diseases associated with *H. pylori* infection,

with the exception of gastritis, are uncommon in the socioeconomically disadvantaged and highly infected population of Africa.

H. pylori related research has dominated clinical gastroenterological research over the past decade. Most of this research has originated in developed, First World countries. There has been some indication that findings generated in developed countries may not necessarily be applicable to the situation in socioeconomically underdeveloped Third World countries. The epidemiology of the infection appears to be different, socioeconomically disadvantaged communities having a much higher prevalence than their affluent counterparts. The implications of this for eradication strategies have not been adequately studied. The selection of antibiotics used for eradication may also be influenced by antibiotic sensitivities peculiar to socioeconomically disadvantaged communities.

The objective of this thesis is to investigate the role of *H. pylori* infection in peptic ulcer disease with special reference to the largely socioeconomically disadvantaged population we serve. A comprehensive review of the literature is presented to establish the historical perspective of the subject, and a number of clinical studies will be presented to:

1. Investigate the characteristics of the gastric involvement of *H. pylori* in peptic ulcer disease.

2. Study the phenomenon of duodenal gastric metaplasia and *H. pylori* infection in duodenal ulcer subjects.

3. Investigate the role of *H. pylori* in determining the abnormalities of gastric secretion associated with specifically duodenal ulcer disease.

4. Assess the prevalence of the infection locally and attempt to identify factors predisposing to the infection.

5. Determine the efficacy of eradication as a management strategy in our "high prevalence" community, as determined over a 24 month period, with specific reference to re-infection rates following eradication.

6. Investigate the efficacy and tolerability of eradication strategies.

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION:

Peptic ulcer disease is a major health problem since it is a significant cause of human suffering and a considerable consumer of health care resources. Despite intense research interest in the pathogenesis of peptic ulcer disease, standard texts stated as recently as 1985 that "we know nothing about the causes of the development and persistence of ulcers, why ulcers heal or relapse, or why and how patients with ulcers may or may not have pain, bleeding or perforation" (Boyd, 1985). Notwithstanding the lack of a basic understanding of the pathogenesis of peptic ulcer disease, a vast volume of information has accumulated with regard to the epidemiology of peptic ulceration, possible etiological factors and the natural history of peptic ulcer disease.

2.2 EPIDEMIOLOGY OF PEPTIC ULCERATION:

While it is estimated that Western populations may have a lifetime prevalence for peptic ulcer disease of up to 10% (Langman 1979), historical epidemiological data with regard to peptic ulcer disease is difficult to interpret. This difficulty arises mainly from the lack of suitable diagnostic modalities in early studies, which made both detection of peptic ulceration and the differentiation of peptic ulcers into gastric or duodenal ulceration difficult. Symptomatic studies lack sensitivity and specificity and cannot reliably predict the site of ulceration (Dunn 1962, Edwards 1968, Feldman 1980). As endoscopically controlled trials have become the reference standard for peptic ulcer studies, it has become

clear that asymptomatic ulcers occur commonly and may persist for months (Boyd 1984a, Boyd 1984b). It is likely, therefore, that historical prevalence data underestimates the true prevalence of peptic ulceration.

Although estimations of absolute prevalence rates may be suspect, epidemiological observations based on "objective" evidence such as perforation rates and mortality statistics have indicated an important trend over the past two centuries. Several parameters have indicated a remarkable change in the disease profile with regard to ulcer incidence and age and sex prevalence over this period. Peptic ulceration appears to have been rare in the nineteenth century. Based on perforation rates, it apears that peptic ulceration in the nineteenth century was predominantly gastric, occurring in young women (<30 years) of low social class (Jennings 1940). Duodenal ulcers appear to have been very uncommon until the second quarter of the twentieth century, at which time the incidence increased, reaching a peak incidence, as estimated by complication rates, in the 1950's (Susser 1962). This increased incidence earned peptic ulceration, and especially duodenal ulceration, the label of "disease of civilisation". Jennings' warning two decades earlier that "too much attention has been paid to the constitutional factor in peptic ulcer and not enough to environmental causes" appears to have gone largely unheeded (Jennings 1940).

A steady decline in the mortality from peptic ulceration became apparent following the end of the Second World War. Significantly, this decrease in peptic ulcer mortality affected age groups unequally, declining in young men and women, while rising in subjects older than 65 years (Susser 1962). Susser hypothesised that this phenomenon could best be explained by a cohort phenomenon, implying that each generation carried

its own particular risk of developing peptic ulcers in adult life. In analysing available data, Susser identified a number of trends that would support the cohort theory. These included: (i) an increase in the average age of death associated with peptic ulceration; (ii) an increase in the average age at which peptic ulcer perforations occurred and (iii) a reversal in the social class grading associated with peptic ulceration, social class mortality showing age shifts indicating that specific cohorts of patients carried their own risk for peptic ulceration. The latter was suggested by analysis of serial censuses, which indicated that each generation carried forward its own particular risk of peptic ulceration. This observation was re-inforced by the reversal in the "typical" socioeconomic profile associated with peptic ulceration. Whereas the disease was one of affluence early in the twentieth century, it became clear by 1951 that the disease was affecting predominantly the lower socioeconomic classes in the younger cohorts (Susser 1962).

It should be noted that the decrease in incidence of peptic ulceration is not a universal finding. The limited epidemiological data available from Africa, specifically Southern Africa, suggests that the incidence of peptic ulceration is increasing in the African population. While seemingly high and low prevalence areas for peptic ulceration exist in Africa, there is some evidence that there is a rising incidence in urban and adjacent areas, even in low prevalence areas (Tovey 1975). In South Africa, data from Durban suggests that there is an increase in the incidence of peptic ulceration in the African population, admission rates for duodenal ulceration increasing some 12-fold in the period 1950-1976 (Robbs 1979). Data from the metropolitan area of Johannesburg also supports the hypothesis that urbanisation is associated with an increase in the incidence of peptic ulceration in the African population (Segal 1978).

There is no ready explanation for the observed changes in the incidence rates, age-and-sex profiles for peptic ulcer disease. It has been postulated that this change could reflect the effect of the stresses of an early phase of urbanisation (Susser 1962). It seems plausible, however, that we are in fact observing a "cohort effect" (Sonnenberg 1984), which may in turn reflect changes in exposure to environmental factors. In recent times *Helicobacter pylori* has emerged as the major environmental factor implicated in the pathogenesis of peptic ulcer disease.

2.3 PEPTIC ULCER DISEASE: READILY HEALED, BUT NEVER CURED?

Perceptions regarding the effective management of peptic ulcer disease have changed dramatically over the past few years. Healing of the ulcer crater is no longer a problem and at the present time the real challenge of management is the prevention of the almost inevitable ulcer relapse, which occurs within 12 months of healing in approximately 80% of successfully treated patients (Hirschowitz 1983). The pathogenetic model of acid-pepsin aggression versus mucosal resistance (Soll 1990) has provided a rationale for the use of either acid inhibitory or mucosal protective therapy for ulcer healing; a number of approaches and agents are available.

2.3.1 SHORT TERM HEALING:

The agents used in ulcer healing can be categorized as either manipulators of the acid/pepsin milieu (inhibitors of acid secretion), antacids or as drugs that enhance the mucosa's capacity to resist acid/pepsin aggression (mucosal protective agents).

2.3.1.1 Acid neutralizing agents:

Antacids are widely used in patients with dyspepsia. Their once unchallenged role in peptic ulcer therapy, however, has been seriously undermined by the impact of the newer anti-secretory agents. Although there is little doubt that antacids are capable of healing duodenal ulcers, the optimum dose has not been defined. Peterson et al showed that a large dosage antacid regimen with an acid neutralizing capacity (ANC) of 1008 mmol/day (15 ml of antacid = 20 - 40 mmol) was superior to placebo in healing duodenal ulcer (Peterson 1977). The role of antacids in peptic ulcer healing was recently reviewed by Marks et al. This review noted that antacid dosages with an ANC of about 120-160 mmol/day may be effective in ulcer healing, but that an ANC of less than 100 mmol/day is ineffective. The antacid formulation (liquid or tablet) does not appear to affect efficacy (Marks 1992). Available evidence also suggests that gastric ulcer healing rates are comparable to those achieved with the H_2-receptor antagonists (cimetidine and ranitidine) when treatment is continued for 6 - 12 weeks (Marks 1992).

2.3.1.2 Inhibitors of acid secretion:

These drugs act on the various processes involved in the control of acid secretion. The muscarinic blockers, the sophisticated anticholinergics, act by blocking the cholinergic receptors of the stomach. The H_2-receptor antagonists act by blocking the parietal H_2-receptor (Feldman 1990). These drugs, developed by Sir James Black and co-workers and first registered for clinical use in England in 1976 (Molinder 1994) were, until recently, the most potent acid inhibiting agents. The most recent additions to the list of anti-secretory agents are the "proton pump

inhibitors" (PPIs). These drugs, which inhibit the final step of acid secretion, produce a greater and longer lasting inhibition of acid secretion than the H_2-receptor antagonists when used in conventional doses.

The gastric H^+, K^+-ATPase (proton pump) is one of the phosphorylating ATPases. The pump is a protein heterodimer, composed of two (alpha and beta) subunits. The 100kDa alpha subunit comprises approximately 1035 amino acids (Maeda 1990), and is seen as the functional or catalytic subunit (Hirschowitz 1995). The beta subunit is smaller, comprising approximately 290 amino acid residues, and is probably required for stabilization and transposition of the alpha subunit (Reuben 1990).

In its inactive state the pump is found in the cytoplasm of the parietal cell. Following activation, the pump is translocated from the cytoplasm to the membrane of the secretory canaliculus, and following phosphorylation by ATP, undergoes a conformational change which enables it to transport H^+ from the cytoplasm, through the canalicular membrane and to release it through its extracytoplasmic domain (Wallmark 1980). This movement is electrically balanced by the transport of K^+ into the cell.

The PPIs in clinical use at present are substituted benzimidazoles and are long-acting inhibitors of acid secretion, as they bind the proton pump irreversibly by covalent binding (Helander 1993). While the three available drugs - omeprazole, lansoprazole and pantoprazole - differ in the number and location of binding sites with the pump, they are all acid dependant prodrugs, the parent drugs being inactive. These are all weak bases which are concentrated in the acidic conditions within the

secretory canaliculus of the parietal cell where they are protonated and converted to their active, sulfenamide, form. The reactive sulfenamides then bind covalently to cysteine residues of the exposed (activated) pump, thereby irreversibly blocking acid secretion (Maton 1991). As the final step of acid secretion is blocked by these agents, they inhibit secretion of acid from the parietal cell, irrespective of the hormonal/neural stimulus, and are therefore the most potent inhibitors of acid secretion.

Since ulcer healing rates have been shown to be directly related to the degree of acid suppression over a 24 hour period (Jones 1987), it is not surprising that the PPIs are claimed to allow more rapid and effective treatment of acid-related diseases of the digestive tract than has been possible with the H_2-receptor antagonists (Walan 1989).

2.3.1.3 Mucosal protective agents:

These agents heal ulcers without affecting acid secretion. Examples include sucralfate, a basic aluminium salt of sucrose octasulphate, and the colloidal bismuth agents. Their mode of action is not completely understood, but they appear to coat the ulcer base. These drugs are of particular interest in that initial ulcer healing with these agents has been shown to delay duodenal ulcer relapse significantly when compared to healing by the H_2-receptor antagonists (Lee 1985, Lam 1987). The reason for this is not clear, but an improved quality of healing of the ulcer scar (Moshal 1979, Tovey 1989), a lack of changes in H_2-receptor regulation and sensitivity, found with H_2-receptor antagonist therapy (Marks 1989, Jones 1988, Fullarton 1989) have been invoked to explain this finding. It is of particular interest to note that both these agents have

been shown to have anti-*H. pylori* effect (Gorbach 1990, Hui 1989); this may be a further important factor responsible for the delay in ulcer relapse following therapy with these agents.

Misoprostol, the prostaglandin analogue in clinical use, is included in this group although there is evidence that the drug's acute healing effect may be linked to its ability to suppress acid secretion (Debas 1989).

2.3.1.4 Ulcer healing rates:

Most of the currently available drugs have excellent healing rates. The proton pump inhibitors are clearly more effective than the other agents in short term healing and show two week duodenal ulcer healing rates as high as 70 - 80% (Marks 1991, Ekstrom 1992), a rate traditionally considered the four week H_2-receptor antagonist healing rate. Non-healing at four weeks occurs in less than 5% of duodenal ulcers with PPI treatment, while gastric ulcer healing rates lag behind, and longer treatment periods are generally needed.

2.3.2 LONG TERM MANAGEMENT OF PEPTIC ULCER DISEASE:

2.3.2.1 Requirements of a long term management strategy for peptic ulcer disease:

The minimum requirements of any long term management strategy have to be: **firstly**, the reduction or abolition of acute exacerbations and **secondly**, the prevention of potentially serious complications, such as perforation and bleeding, which are estimated to occur at a rate of approximately 3% per annum, almost doubling to 5% in patients who have previously suffered complications (Elashoff 1983). Therapy should,

of course, be safe, and preferably affordable. Various strategies have been developed to manage this tendency for ulcers to relapse. The delaying effect of the mucosal protective agents specifically on duodenal ulcer relapse rates has already been mentioned. The use of maintenance therapy, either "on demand", repeated courses or the more satisfactory continuous maintenance therapy, has proved successful in managing symptomatic ulcer relapse.

2.3.2.2 Medical maintenance therapy for peptic ulcer disease:

Medical maintenance therapy has traditionally been considered as "on demand", i.e the patient uses treatment when symptomatic, repeated courses of treatment with documented recurrences, or long term medical maintenance. Controlled data with regard to the first two approaches is scarce, although there is some evidence that these two approaches are equally effective in managing symptomatic recurrences, but that acute ulcer healing is significantly more commonly achieved with full course therapy (Thorat 1990). While clearly an attractive cost saving option, there is no data comparing "on demand" therapy to long term, low dose, medical maintenance therapy, an approach clearly shown to prevent duodenal ulcer relapse over periods of up to nine years, while protecting the patients against the complications of bleeding and perforation (Penston 1993a). As complications can occur with asymptomatic ulceration, which occurs commonly in peptic ulcer disease, it seems unlikely that "on demand" therapy will be a reasonable approach in patients who need medical maintenance, if prevention of ulcer complications is a goal of maintenance therapy.

Fry, in the pre - H_2-receptor antagonist era, produced peptic ulcer follow-up data to suggest that peptic ulcer disease "burns itself out" over a period of 10-15 years. This offered the hope that the morbidity of peptic ulcer disease could be improved if patients could be managed with maintenance until they were over the active period of the disease. Fry claimed that, in a study of 212 patients, peptic ulcer symptoms diminished after 5-10 years (Fry 1964). Fry's study was flawed, however, in that the sample size diminished as the follow-up period progressed. At 5 years 198 (93%) of the sample of 212 patients were evaluated, at 10 years this had shrunk to 182 (88%) and at 15 years a mere 51 (24%) of the original 212 were evaluable. Furthermore, Fry excluded 28 subjects (16%) of the original sample who underwent elective surgery for severe symptoms, making it difficult to interpret the reduction in severely impaired functional gradings demonstrated during his follow-up. While claiming to support Fry's findings, the study by Greibe et al - also in the pre-H_2-receptor antagonist era - in fact showed that, 13 years after diagnosis, 41% of patients remained symptomatic, while 22% had undergone surgery for the disease (Greibe 1977). In practice, maintenance studies have, as a rule indicated that, following cessation of maintenance therapy, ulcers recur. This has been shown for symptomatic relapse following short term maintenance (12 months) (Gudmand-Hoyer 1978) as well as following an average of 7.5 years of maintenance therapy with acid suppression (Penston 1993b). While some workers have suggested that maintenance therapy with acid suppression modifies the natural history of duodenal ulcer disease, with reduced relapse incidences following cessation of therapy (Holtman 1993, Walan 1985, Susi 1991), the bulk of evidence supports the view that duodenal ulcer disease is a protracted disease, which continues to affect patients

for many years and that the natural history is unchanged by acute or maintenance therapy strategies.

2.3.2.3 Surgery for peptic ulcer disease?

Until recently, acid reducing surgery has been the only management strategy capable of preventing ulcer relapse. Although not perfect, medical therapy with the associated benefits of improved healing efficacy, safety, and the ability to maintain ulcer patients free of clinical relapse and complications, albeit at huge cost, has impacted severely on elective peptic ulcer surgery. While surgery had already shown a decreasing tendency, probably related to the decline in peptic ulcer disease referred to in 2.2 above, the introduction of the H_2-receptor antagonists was associated with a marked reduction in surgery for peptic ulceration (Bloom 1991). This is not surprising. The ease with which healing is achieved has removed the "ulcer resistant to medical therapy" from the list of indications for elective surgery, while the policy of a "pill a day keeps the surgeons at bay" (Wormsley 1985) has made surgery for "intractable ulceration" an exercise in cost benefit evaluation. It remains to be seen whether the surgeon's fairly recent acquisition of minimally invasive (i.e. laparoscopic) surgical skills will have any significant effect on this mindset.

It is apparent that neither surgical nor medical management strategies are ideal for the management of peptic ulcer disease. The recent "rediscovery" of *H. pylori* and the shift in emphasis from acid and pepsin aggression to *H. pylori* infection as an important factor in the etiology of peptic ulcer disease offers, for the first time, the prospect of a "medical cure" for peptic ulcer disease.

2.4 *HELICOBACTER PYLORI*: A NEW, UNIQUELY GASTRIC, PATHOGEN:

2.4.1 Isolation of a "new" gastric organism:

The "discovery" of *H. pylori* occurred in the early 1980's, thanks to the close cooperation between Barry Marshall, a registrar in Internal Medicine undergoing training in Gastroenterology, and Robin Warren, a histopathologist, at the Royal Perth Hospital, Western Australia (Marshall 1988, Goodwin 1993). Warren alerted Marshall to the presence of spiral bacteria in gastric biopsy specimens stained with a silver staining method. While most of a list of 25 patients in whom Warren had identified the organism had symptoms, no clear clinical syndrome could be linked to gastric infection with the organism. The link with gastritis was presumptively made when a patient was fortuitously treated with tetracycline: although poorly characterised, the patient reported an improvement in his symptoms; more important, however, was the finding that the patient's gastritis had also resolved (Marshall 1988).

The time had come to inform the world of the "discovery" of the gastric organisms. Apparently, clinician and histopathologist could not agree on the wording of their first communication (Goodwin 1993), and the world was informed of their findings in two separate letters to The Lancet in 1983. In his letter, Warren describes the characteristics of gastritis associated with the organism (Warren 1983), while Marshall emphasises the uniqueness of the new bacterium and, with amazing vision, already speculates at this early stage that the organism may play an important role in gastritis and its associated diseases, "i.e. peptic ulcer and gastric cancer" (Marshall 1983).

In the words of Goodwin "until a microbe is cultured and characterised, histopathological observation of the new organism remains tantalisingly incomplete" (Goodwin 1986); the culture of the organism was finally accomplished in Perth on the 14th of April 1982 (Marshall 1984b). This was accomplished with a considerable deal of good fortune. Since the organism was originally thought to be a *Campylobacter*, culture plates were discarded after 48 hours of incubation. Only after the plates were left incubating over the annual Easter break, which lasted five days, were the characteristic transparent colonies seen (Goodwin 1993). The process of allocating an identity to this new organism now began.

Review of the literature reveals that Warren and Marshall were not the first to describe the presence of spiral organisms in human stomachs. The first report of gastric spiral organisms in humans was made, in 1906, by Krienitz who used Giemsa staining and found spiral organisms in the gastric contents of a subject with carcinoma of the lesser curvature (Krienitz 1906). Gastric spiral organisms were again reported toward the middle of the century, but were soon forgotten, for a number of reasons. In 1938, Doenges reported on the presence of gastric spirochaetes in post mortem samples in 43% of 242 stomachs, but could not come to any conclusions, because of the quality of the biopsies (Doenges 1938). In 1940, Freedberg and Barron noted, using a silver staining method, the presence of spirochaetes in 13 of 35 human stomachs resected for peptic ulceration or carcinoma. Interestingly, they noted that the organisms were "rarely found in the mucosa of gastric tissue resected for duodenal ulcer without attendant gastric ulcerations". They also concluded that these organisms represented an opportunistic, non-pathologic colonization of diseased mucosa (Freedberg 1940). This

impression was strongly re-inforced by the work of Palmer, who could not identify "spirochaetes in human gastric suction biopsies" in a large sample of 1180 biopsies from 1000 adult subjects - using standard hematoxylin and eosin staining techniques and not silver stains - and concluded that the previously observed spirochaetes were merely oral flora, colonizing abnormal/diseased gastric mucosa (Palmer 1954). It should be noted that most of the suction biopsies came from the fundal region.

With the benefit of hindsight, the most significant observation with regard to gastric pathogens was probably made by Steer and Colin-Jones in the mid 1970's (Steer 1975). The availability of endoscopic techniques allowed these workers to study large numbers of antral gastric biopsies. They observed Gram negative, spiral organisms under the mucus layer in 80% of their gastric ulcer patients, but none on normal mucosa. Inappropriate culture conditions yielded only *Pseudomonas aeruginosa*. While the organism was, therefore, erroneously assumed to be *Pseudomonas aeruginosa* (cultured pseudomonas are not spiral) the true importance of their observation went unappreciated. Since the bacteria were associated with gastritis and leukocytes appeared to migrate through the epithelium in response to the organism, their observations suggested that these organisms were not innocent commensals as had been suggested before.

This was not, however, the last time that gastric microbacteria were noted. In 1979, Fung et al demonstrated curved bacteria by electronmicroscopy in gastric sections of patients with the endoscopic diagnosis of "gastritis". Once again there is no description of tissue invasion, the bacteria "adhering to the luminal surface of the mucosal

cells" (Fung 1979). This observation was not interpreted at all. At about the time that Warren and Marshall published their observations in The Lancet, a retrospective publication by Rollason et al was accepted for publication in the Journal of Clinical Pathology. These workers found spiral organisms in 42% of 310 consecutive endoscopic gastric biopsy specimens and found that the organisms were associated with gastritis (Rollason 1984).

The significance of the presence of urease activity in the stomach also appears not to have been appreciated and interest fluctuated in much the same way as it did for gastric bacteria. Urease activity was described in the stomachs of animals in 1924 (Luck 1924). It was considered that this activity arose from the gastric mucosal cells and that it served to protect the gastric mucosa against acid. It was noted in 1926 that the vomitus of uremic patients contained large amounts of ammonia (Bliss 1926) and concluded that this represented an escape mechanism whereby gastric mucosal urease could convert excess urea to ammonia, which could be excreted as vomitus. By studying gastrectomy specimens, Fitzgerald and Murphy noted an association between gastric urease and peptic ulceration in 1950; the source of the urease activity remained unknown and it was postulated that the urease activity protected the gastric mucosa from acid by buffering hydrogen ions with ammonia (Fitzgerald 1950). Using a different approach, Freisinger linked the presence of increased titers of antibodies to urease to gastric ulceration (Freisinger 1963).

Two items of information, generated a decade apart, suggested that the urease activity was bacterial in origin. Lieber and Le Fevré noted that the gastric urease activity disappeared after tetracycline administration,

thereby indicating a bacterial origin (Lieber 1959). Almost ten years later, in 1968, Delluva and co-workers added further support to the hypothesis that the gastric urease activity was bacterial in origin when they observed that germ free animals never exhibited urease activity in the stomach (Delluva 1968).

It is clear, therefore, that the major contribution of Warren and Marshall in 1983 was not the "re-discovery" of a gastric spiral organism. Their true contribution was the linking of *H. pylori* to a clinicopathological entity - gastritis - and their enthusiasm to link the organism to other gastroduodenal diseases (Warren 1983, Marshall 1983).

2.4.2 Microbiology and taxonomy:

H pylori is the index species of a new bacteriological genus, *Helicobacter*, a name first published in 1989 (Goodwin1989). *H. pylori* is a Gram negative, microaerophilic, curved or spiral bacterium, which posseses up to six polar flagellae, which are sheathed (Buck 1990). The organism was initially thought to belong to the *Campylobacter* genus when cultured in 1984, although the authors noted at this early stage that certain morphological features suggested that this was not a typical *Campylobacter* (Marshall 1984a). Initial concern regarding the classification focused on the flagellar morphology, organisms of the *Helicobacter* genus having sheathed flagellae, whereas organisms of the *Campylobacter* genus do not. Despite these morphological inconsistencies and based on light microscopical similarities and the guanine plus cytosine ratio of their DNA (35.8 - 37.1 mol%), the name *Campylobacter pyloridis* was proposed for the new organism (Marshall 1984b) and subsequently validated in 1985 by the International Union of

Microbiological Societies (IUMS 1985). Two years later, however, it was pointed out that the specific epithet "*pyloridis*" was grammatically incorrect (Hartmann 1987) and the name was changed to *C. pylori*. (Marshall 1987).

It soon became clear, however, that the grammatical mistake was not the only one to have been made in the naming of this unique organism. Reference has already been made to the morphological differences with other organisms of the *Campylobacter* genus; during the latter part of the 1980's it became clear that there were more fundamental morphological, biochemical and genomic reasons for considering a re-allocation of genus.

Morphologically, the organism passes a superficial test for *Campylobacter*. It is a curved, rod shaped, Gram negative organism, which is motile and grows on medium similar to that of *Campylobacter* species. Ultrastructural analysis, however, reveals a number of differences between this new organism and *Campylobacters*. Reference has been made to the presence of 4-6 sheathed flagellae, whereas *Campylobacters* have only one, unsheathed polar flagella at one or both ends of the cell (Marshall 1984a, Goodwin 1985). It was also shown that *H. pylori* possessed several other unique ultrastructural features. These were: (i) the ends of the bacterium do not taper; (ii) no terminal concavity is present at the location of the flagellae; (iii) flagellar discs contain no radial structures; (iv) the organism possesses structures internal to the plasma membrane; (v) the cell is smooth and (vi) flagellar sheaths are in continuity with the unit membrane of the cell wall (Owen 1989, Goodwin 1985, Jones 1985, Goodwin 1986).

Analysis of the cellular components of *H. pylori* also indicated that this was a new genus. The cellular fatty acid content of the organism, a reliable taxonomic tool independent of the growth conditions and culture medium of the organism (Jantzen 1985), was found to differ significantly from that of the *Campylobacter* genus, both qualitatively and quantitatively (Goodwin 1985). The lack of the respiratory quinone, thermoplasmaquinone-6 in *H. pylori* (Goodwin 1985), as well as the unique SDS-PAGE protein electrophoretic patterns of the organism (Pearson 1984, Megraud 1985) also suggested that this organism was in fact not a *Campylobacter*.

Molecular techniques finally delivered conclusive evidence that the organism which had been described by Marshall and Warren, and eventually cultured in 1984 from human gastric mucosa, was not a *Campylobacter*. Analysis of the 16S ribosomal RNA sequence of the organism showed that it was more closely related to *Wolinella succinogenes* than to other *Campylobacter* species, but noted significant differences with the Wollinellas so as to justify a separate genus (Paster 1988, Owen 1989).

Thus, in October 1989, the *Helicobacter* genus was officially introduced and *Helicobacter pylori* was accepted as the correct name for *Campylobacter pylori* (Goodwin 1989), *Helicobacter mustelae* also being included in this new genus. The name "*Helicobacter*" describes two of the key morphological characteristics of the genus: helical *in vivo*, but often rodlike *in vitro*. Since 1989, the new genus seems to have justified its creation and by 1992 it contained nine distinct species (Editorial, The Lancet 1992). Three years later, in 1995, the genus has expanded even

more and now, following the recent addition of *Helicobacter bilis* (Fox 1995), contains 12 species already formally named. These are: *H. pylori, H. mustelae, H. muridarum, H. felis, H. nemestrinae, H. acinonyx, H. cinaedi, H. fennelliae, H. rappini, H. hepaticus, H. pametensis, H. canis,* and *H. bilis.* In addition, the genus will probably be expanded eventually by the inclusion of a number of species with a strong homology to the *Helicobacter* genus. It is now accepted that the *Helicobacters* are capable of producing disease in the gastrointestinal tract and associated viscera in a number of host species.

2.4.3 *Helicobacter pylori*: adapted to colonize gastric mucosa specifically:

It has become evident that *H. pylori* is by no means an incidental colonizer of human gastric mucosa. The organism is, in fact, unable to colonize mucosa other than gastric and is unique in its ability to survive in the hostile gastric environment, thought in the past to harbour oral flora only transiently, except in patients with achlorhydria. It was believed that the acid in the stomach would destroy most bacteria (Gianella 1972). *H. pylori* appears to be equipped to colonize gastric mucosa specifically and, following successful colonization, is adapted to survive in the hostile gastric environment.

There is ample histological evidence that *H. pylori* is found only in gastric epithelium, even if located outside the stomach - i.e. gastric metaplastic tissue in the duodenum as well as Barrett's metaplasia of the oesophagus (Loffeld 1992, Wyatt 1990), Meckel's diverticula (DeCothi 1989) and ectopic gastric mucosa in the rectum (Pambianco 1988). Goodwin was the first to suggest that *H. pylori* specifically binds gastric epithelium, but

not the altered, intestinal metaplastic tissue found in the stomach (Goodwin 1986). The organism is consistently found within and beneath the gastric mucus (Marshall 1983) and only rarely penetrates the gastric epithelium (Hazell 1986). Initial observations suggested that the organisms congregated at intercellular junctions, and it was postulated that the organism migrated to these junctions because nutrients may be more readily available (Hazell 1986). Subsequently, workers have refuted the claim that the organism is most commonly seen at or near intercellular junctions and speculated that the observations made with regard to the preferred location of the organism in the past, may have been spurious, but confirmed the organism's predilection for the surface of gastric epithelial cells (Thomsen 1990). Ultrastructural studies have since indicated that the *H. pylori*/epithelial interaction is analogous to that of enteropathogenic *E. coli*, which form specific adhesion sites with host epithelia. This similarity was first referred to by Goodwin et al (Goodwin 1986) and further evidence for this was supplied by Hessey and co-workers in 1990. Ultrastructural analysis of biopsies taken from 83 subjects with dyspepsia revealed that the organism interacted with the gastric epithelium in one of three ways (Hessey 1990). The majority of adhesion sites were "abutting" with an unchanged epithelial contour, while interactions which were associated with changes in epithelial contour (adhesion pedestals and indentation sites) were found less commonly.

Further evidence for the cell-lineage specificity of *H. pylori* , as well as the specific preference of binding sites, has been gathered from the study of the interaction between the organism's adhesins and gastric receptor molecules. Based on hemagglutination studies, Evans suggested that *H. pylori* possessed a fibrillar based, sialic acid-bearing "colonization

factor" (Evans 1988). Later work by Lingwood and Slomiany suggested that *H. pylori* may interact with specific sulfate containing glycerolipid receptors in the gastric mucosa (Lingwood 1989, Slomiany 1989). Further clinical relevance was given to these observations by the work of Boren et al. Their work indicated that the Lewis^b blood group antigen may mediate the attachment of *H. pylori* to gastric mucosal cells (Boren 1993). They also suggest that these receptors may be less readily available in blood group A and B subjects, an observation which, if confirmed, may explain the greater risk for developing gastric ulceration attributed to people expressing the blood group O.

2.4.4 *Helicobacter pylori*: adapted to survive in the gastric environment:

The human stomach is an extremely hostile environment for bacteria. Gastric acid is germicidal at a pH of less than 4 and represents an important barrier to pathogens (Giannella 1972). While a host of bacteria can be demonstrated in the human stomach following a meal, these disappear and few bacteria are detected after a pH of 3 has been attained. Studies in normochlorhydric, "normal" subjects, suggests that permanent colonization of the stomach by conventional bacteria is rare, being found only in southern India (Drasar 1989). There are a number of reasons why *H. pylori* can successfully colonize and survive in this hostile environment.

Motility is important for the survival of the organism in the stomach and is provided by the two to six polar, sheathed flagellae. This allows the organism to be motile in a viscous environment (Hazell 1986), thus allowing it to progress from the gastric lumen, through the mucus to its final attachment on the gastric epithelium.

Bacterial adhesion, discussed in 2.4.3, is an important determinant for the successful colonization of the gastric mucosa by *H. pylori*. Once in this position, the organism is protected from the acid peptic environment in the stomach, as the pH at the epithelial surface approaches neutral.

***H. pylori* urease** appears to be essential for the survival of the organism in an acid environment, and it has been suggested that the presence of this enzyme, with both acid and alkaline pH optima (Taylor 1988), is essential for gastric colonization, as the presence of an enzyme with an acid pH optimum is restricted to organisms which colonize the stomach; these organisms have been shown to have a survival advantage in an acid environment, when compared to "non-gastric" *Helicobacters*. (Ferrero 1991). This advantage may be important in the initial colonization phase of *H. pylori*, when it is exposed to the acid environment of the gastric lumen. The fact that hypochlorhydria enhances colonization efficacy (Marshall 1985, Morris 1987), suggests that protection against acid exposure would be beneficial to *H. pylori* survival, at least in the early, colonization, phase.

It has been suggested that the urease enzyme would protect the organism against acid degradation by establishing an alkaline microenvironment surrounding the organism. In this regard it should be noted that the enzyme has an extremely high affinity for urea substrate, and that the intact cell wall does not offer a barrier to urea substrate (Mobley 1988); the enzyme activity is, in fact, present both within and on the surface of the organism (Tytgat 1992). This enzyme would, therefore, be capable of hydrolysing environmental urea substrate both in an acid and an alkaline environment, producing ammonia and

bicarbonate. In an acid environment, this alkaline microenvironment would protect the organism against hydrogen ions, by neutralisation. Marshall initially offered some proof that urea hydrolysis does occur in the stomachs of patients infected by *H. pylori* (Marshall 1986), an observation which has been exploited to diagnose current gastric *H. pylori* colonization by means of the urea breath test (Graham 1987) and "CLOtest" (Marshall 1987b). Marshall has, furthermore, shown that urea protects the organism from the bactericidal effect of acid (Marshall 1990).

Despite the evidence cited above, the precise role of the urease enzyme is still the subject of controversy. There is some evidence that this acid-protective effect of the urease enzyme may not be its principal function, and that it may be essential for the mobilisation of urea nitrogen for use by the organism, as is the case for a large number of eu- and prokaryotic organisms (Mobley 1989). The interpretation of its function is further complicated by the fact that its peak activity is at an alkaline pH (Mobley 1988) and that there is some indication that the organism does not survive in the presence of urea, unless the environment is acidic (Clyne 1994). This is difficult to reconcile with the organism's survival on the gastric epithelium and below the mucus layer.

2.4.5 *Helicobacter pylori*: a pathogen:

The initial histopathological description of *H.pylori* in gastric mucosa, as well as the eventual culture of the organism, although important milestones in the "*Helicobacter pylori* story", went little further than the observations of gastric organisms made in the past (2.4.1). While the original observations had already made the morphological link with

gastritis (Warren 1983), the true challenge was to prove that the organism was not merely an opportunistic colonizer of diseased gastric mucosa, a view which had been held by some (Varis 1988). It soon became evident, however, that the "commensal" theory was extremely unlikely. Observations in patients with idiopathic, type A gastritis associated with pernicious anemia, indicated that the organism was found far less frequently than in control populations(Flejou 1989, Fong 1991), while the organism was not identified at all in patients with eosinophilic gastritis, Crohn's gastritis or Menetrier's disease (Ormand 1991). The post-operative stomach with bile reflux also appears not to predispose to *H. pylori* colonization, but rather to protect against it (Offerhaus 1989).

Final proof that the organism is a pathogen, causing gastritis, comes from two ingestion studies. In both, ingestion of the organism led to gastritis, which was self-limiting in one subject (Marshall 1985), but required therapy in the second (Morris 1987, Morris 1991). Analysis of the clinical syndrome, with raised gastric pH, was strongly reminiscent of so-called epidemic gastritis with achlorhydria (Ramsey 1979), an association apparently confirmed by the observations of Graham et al in a patient infected during an acid secretory study, with histological control permitting proof of infection by *H. pylori* (Graham 1988a).

Koch's postulates have, therefore, been fulfilled for *H. pylori* as a causative agent for gastritis. (i) There is a high association of the infection with gastritis, (ii) the organism has been consistently isolated in samples from gastritic subjects, (iii) inoculation has caused gastritis in human volunteers and (iv) the organism has been identified in these cases of gastritis following ingestion (Marshall 1985). It is now

appreciated that the organism is the major cause of type B or "hypersecretory"/diffuse antral gastritis (Correa 1980), the type of gastritis associated with duodenal ulcer disease. The question arises whether *Helicobacter pylori* can be further implicated in the etiology of peptic ulcer disease?

2.5 AETIOPATHOLOGICAL FACTORS ASSOCIATED WITH PEPTIC ULCER DISEASE:

While lacking a clear etiological factor, the disease has been increasingly seen to represent an imbalance between "aggressive" (acid, pepsin) and "defensive" factors (mucus and bicarbonate secretion, mucosal blood flow, cellular regeneration) - a concept which has found support in the success of the therapeutic approach to peptic ulcer disease. A host of pathophysiologic changes has been observed in patients with peptic ulcer disease. These changes, although not "causative" by themselves, have given insight into the process of peptic ulceration. Any etiological agent has to be assessed for its ability to elicit these changes.

2.5.1 PHYSIOLOGICAL CHANGES ASSOCIATED WITH PEPTIC ULCER DISEASE:

2.5.1.1 Acid and pepsin secretion and peptic ulcers:

As far back as the turn of the century Schwarz stated that peptic ulceration was "a product of self-digestion; it results from an excess of autopeptic power of gastric juice over the defensive power of gastric (and intestinal) mucosa" (Schwarz 1910). Although acid should probably be viewed as the main aggressive factor in the pathogenesis of peptic ulceration, this is probably because of its relationship to pepsin, as

without acid the peptic activity of gastric juice will be lost; gastric juice (acid) without peptic activity has little digestive power (Samloff 1989).

A number of abnormalities in acid secretion have been noted in peptic ulceration. These are not uniformly found in patients with ulceration and often overlap with findings in normal subjects. Furthermore the findings of different studies have been contradictory.

Parietal cell mass has been shown by Card and Marks to be larger in duodenal ulcer subjects than in non-ulcer controls (Card 1960); functionally this is reflected in the elevated maximal acid output in response to secretagogue stimulation found in 30-50% of duodenal, but not gastric ulcer subjects (Lam 1980). Important subgroups of patients have been identified in these hypersecretors: based on Grossman's study in 1963 it has been suggested that patients with a younger age of onset of the disease are more likely to be hypersecretors (Grossman 1963), while Lam has suggested that patients with a positive family history of duodenal ulceration will be more likely to hypersecrete acid (Grossman 1963, Lam 1983). Abnormalities in secretory patterns have also been noted in groups of patients with ulceration. Most important of these observed changes is the elevation in basal acid secretion found in approximately 30% of duodenal ulcer subjects, which is reflected in an increase in nocturnal acid secretion in some, but not all, duodenal ulcer patients (Lam 1984).

While changes in parietal cell mass, basal and maximal acid secretion have been noted in groups of patients with duodenal ulceration, there is also some indication that the control of acid secretion may be affected in patients with duodenal ulceration. The cephalic phase of the control of

acid secretion, as evaluated by modified sham feeding, food teasing or insulin hypoglycaemia, is abnormal in a significant proportion of ulcer subjects. While giving highly variable results, approximately 50% of patients appear to have an abnormally high output of acid in response to cephalic stimulation (Lam 1984). Walsh et al have demonstrated an apparent lack of feedback inhibition of acid secretion in duodenal ulcer subjects, associated with, and possibly caused by, an abnormal gastrin response to meals (Walsh 1975). The sensitivity of parietal cells to exogenous stimuli has also been shown to be abnormal in a subset of patients, duodenal ulcer patients requiring less exogenous gastrin than normal subjects to achieve a given level of acid secretion (Lam 1984, Isenberg 1975); others have not been able to confirm these findings (Roxburgh 1994).

In summary, no single abnormality in gastric acid secretion has been identified as a key factor in ulcerogenesis; the major clinical relevance of gastric acid secretion in ulcerogenesis is seen mainly in the importance and efficacy of acid suppressive therapy in ulcer healing.

Gastrin has also been extensively investigated, as it has been perceived as the endogenous stimulator of gastric acid secretion. The most consistent finding has been an abnormal increase in circulating gastrin following food intake (Blair 1987). This increase appears not to be associated with an increase in antral G-cell numbers, thus suggesting an abnormality in G-cell function (Creutzfeldt 1976). A deficiency of somatostatin, the paracrine inhibitor of gastrin release, which may explain the abnormal gastrin secretion patterns, has been observed by some (Chayvialle 1978), but the deficiency does not appear to be present in all duodenal ulcer subjects, Polak et al demonstrating the deficiency

only in subjects with duodenal ulceration and G-cell hyperplasia (Polak 1978).

The importance of pepsin in the development of peptic ulceration has been previously mentioned. The enzyme was further implicated in the pathogenesis of duodenal ulceration by the observation that serum levels of pepsinogen I, the systemically secreted component of pepsin, were increased in 60% of patients with duodenal ulceration (Samloff 1975), and by the observation that families with a prominent history of duodenal ulceration have elevated levels of serum pepsinogen I (Rotter 1979). This was considered a genetic marker for duodenal ulcer disease. Others observed an increased tendency for duodenal ulcers to relapse in the presence of elevated serum pepsinogen I levels (Sumii 1989). Samloff associated an increased serum level of pepsinogen I (130 μg/l or more) with an increased risk of duodenal ulceration (Samloff 1986), further presumptive proof that the "idiopathic" elevation of pepsinogen had pathogenic significance, either independently or as a marker for acid hypersecretion (Samloff 1989).

There is evidence, however, that many of the observations made with regard to pepsinogen may have been misinterpreted; duodenal ulceration is invariably associated with gastritis, predominantly antral but, as will be indicated later in this thesis, also involving the body of the stomach to some degree (Chapter 3). As gastritis is an acknowledged cause of increased pepsinogen levels (Samloff 1989), it is possible that the observed changes in serum pepsinogen levels merely reflected the gastric mucosal disease associated with duodenal ulceration.

The relationship between acid secretion, pepsin secretion, gastrin and *Helicobacter pylori* will be further explored in Chapter 5 of this thesis.

2.5.1.2 Changes in gastric motility and duodenal pH in ulceration:

If gastric acid and pepsin are important in the pathogenesis of duodenal ulcer disease, either their secretion or their delivery to the duodenum may be abnormal, leading to increased levels at the target site. While the study of duodenal pH is fraught with methodological difficulties, some workers have found evidence that duodenal hyperacidity occurs in duodenal ulceration, while others have not (Lam 1984, Eriksen 1988).

The duodenum has pH sensitive receptors which attempt to maintain the pH in the duodenum at approximately 6 by regulating the amount of acid delivered to it. This may be achieved by slowing gastric emptying, or by inhibition of acid secretion. As duodenal acid neutralization (by mucosal and pancreatic bicarbonate) appears to be normal in duodenal ulcer subjects (Lam 1984), it is not surprising that increased gastric emptying and abnormal feedback control of acid secretion have been noted in duodenal ulcer subjects. The abnormalities in gastric emptying have, however, not been uniformly found, although the majority of studies appear to indicate that gastric emptying is accelerated. The methodology as well as the study subjects used in these studies have not, however, been uniform (Malagelada 1980).

Gastroduodenal motility has been poorly studied in relation to *H. pylori* status. The limited information to date does not indicate that *H. pylori* infection is associated with an increased gastric empying rate. One study showed a normal gastric emptying rate in infected patients (Prakash

1987) and another indicated delayed gastric emptying (Wegener 1988), while a study of interdigestive motor function did not identify a difference between *H. pylori* infected and non-infected dyspeptic individuals (Pieramico 1993).

2.5.1.3 Gastroduodenal inflammation - The role of *Helicobacter pylori*:

Marshall makes the point that gastritis as a clinical entity has always been neglected in clinical gastroenterology (Marshall 1988). This is particularly true of the English speaking world. Peptic ulcer disease has, however, been associated with gastritis for many years. Gastritis occurs commonly in gastric ulcer disease, and is almost invariably present in duodenal ulcer disease (Wyatt 1987, Greenlaw 1980), where it was thought to be usually mild in nature and to affect exclusively the gastric antrum (Schrager 1967). The gastritis associated with duodenal ulceration was so typical as to be considered a specific gastritic entity by some workers (Correa 1988). Others have indicated that the gastritis associated with duodenal ulceration may be more widespread (Penston 1990). It is to Marshall's credit that he immediately made the connection between gastritis caused by *H. pylori* and peptic ulceration. This association between *H. pylori*-induced gastritis and peptic ulcer disease has now been well documented, and gastritis is found in most gastric ulcer subjects and almost all duodenal ulcer subjects (Rauws 1989; see Chapter 3 of this thesis for a more detailed discussion).

The relationship between duodenitis and duodenal ulcer disease has always been the subject of controversy. In recent years it has been demonstrated that gastric metaplasia commonly occurs in the duodenal

bulb. Kreuning et al found evidence of gastric metaplasia in 64% of 50 "healthy" individuals (Kreuning 1978), while Shousha found gastric metaplasia in the duodenum of 45 of 60 patients with dyspepsia (Shousha 1983).

Gastric metaplasia of the duodenum was first described by James in a preliminary communication to the second World Congress of Gastroenterology. Examining operative biopsies taken from the first part of the duodenum, he noted that patches of gastric epithelium were commonly found in the duodenum of patients with duodenal ulceration, but not gastric ulceration or carcinoma (James 1964). These findings were, notably, more striking in subjects with acid hypersecretory states, suggesting that the phenomenon was related to acid hypersecretion. This postulate was tested by Rhodes using a cat model and intramuscular injections of histamine to stimulate acid secretion (Rhodes 1964). He found that gastric epithelium in the duodenum developed in cats subjected to acid hypersecretion, and postulated that the gastric epithelium originated in the necks of Brunner's glands.

Steer was the first to document the presence of *H. pylori* in the duodenum of patients with duodenal ulceration (Steer 1984). Working independently of Warren and Marshall, and apparently unaware of their findings in gastric epithelium, he described an "S-shaped bacillus" on gastric metaplastic tissue in eight of eleven patients with duodenal ulceration.

These findings were given clinical importance by the findings of Carrick et al (Carrick 1989). These workers studied antral and duodenal pinch biopsies from 137 patients: 46 with active duodenal ulceration, 44 with

healed ulcers and 47 normal subjects. Their data indicated that duodenal gastric metaplasia was a risk factor for the development of duodenal ulceration - risk ratio 6.2 - which was increased dramatically by the presence of *H. pylori* in the duodenum - risk ratio 51.

2.5.2 ENVIRONMENTAL ULCEROGENS:

As discussed in paragraph 2.2, the epidemiology of peptic ulcer disease, which has shown quite marked changes in the past century, suggests that environmental factors may be involved in the pathogenesis of the disease. A number of so-called environmental ulcerogens have been identified.

2.5.2.1 Ulcerogenic drugs:

Salicylates and nonsteroidal anti-inflammatory drugs: It was first noted in 1938 that aspirin induced acute gastric damage (Douthwaite 1938). Since that time, analgesic drugs have been increasingly incriminated in the pathogenesis of chronic peptic ulceration, a fact made more important because of the widespread use of these drugs. While there is general acceptance that aspirin and non steroidal anti-inflammatory drugs (NSAIDs) cause peptic ulceration, the best, prospective data supporting this view comes from a fairly recent study by Kurata et al, undertaken prospectively in 34 198 Seventh Day Adventist Church members (Kurata 1992). A number of questions remain unanswered. Specifically, the risk for developing duodenal ulceration, although not quantified, does not appear to be increased (but complications from duodenal ulceration are) by NSAID use. In contrast, gastric ulceration is found more commonly with NSAID use, suggesting these drugs cause gastric ulceration and exacerbate the risk of complications with duodenal ulceration (Talley

1992). Most series identify NSAID use with an increased risk for the serious complications of peptic ulcer disease (Fries 1989).

It is also not clear why only a minority of NSAID users develop ulceration, the point prevalence being of the order of 20% at any given time, many of them being asymptomatic (Talley 1992). Attempts have been made to identify factors associated with an increased risk of ulceration. These include: the age of the patient, older age representing a higher risk; a high dosage of NSAID; multiple NSAID use; a past history of peptic ulcer disease; the use of corticosteroids and severe arthritis-related disability (Hollander 1992).

While the precise pathogenetic mechanism of NSAID-associated ulceration remains unclear, it is likely that the bulk of the NSAID effect is mediated by disruption of the mucosal barrier (Soll 1990). This effect may be mediated by both local and systemic mechanisms, since the administration of rectal NSAIDs and prodrugs does not protect the user against the gastropathy (Soll 1991). While the local irritant effect of the drugs could be mediated by their solubility at acid pH and their protonation status in the stomach (protonated agents causing more local damage), the mediation of the systemic gastropathic effect remains speculative. It is believed, however, that these drugs probably disrupt the mucosal barrier by affecting the synthesis of prostaglandins, important paracrine mediators of mucosal integrity (Hollander 1992). This concept derives support from the therapeutic success of prostaglandin analogues in preventing NSAID associated gastropathy (Graham 1993a).

It is of interest to note that one study, at least, has suggested that there is no synergism between NSAIDs and the major environmental ulcerogen,

H. pylori, in the development of gastropathy. In this study, gastropathy appeared to be more frequent in patients not infected by *H. pylori* than in those in whom the infection was found. This finding was interpreted as being supportive of an earlier observation that resistance to salicylate induced injury of the gastric mucosa correlated with histological evidence of what is now designated "chronic active gastritis" (i.e. neutrophil invasion of the mucosa), which is now considered a hallmark of *H. pylori* infection (Graham 1991). The relationship between NSAIDs and *H. pylori* is still not completely understood, however, as a second study has suggested that *H. pylori* infection is, in fact, a risk factor for the development of NSAID gastropathy (Heresbach 1992). The results of suitably designed interventional studies should clarify the issue in the future.

Corticosteroids: The role of corticosteroid use in predisposing to peptic ulceration was for long unclear and controversial. The meta analysis by Messer et al, which included 59 studies, concluded that both peptic ulceration and the complications thereof, were significantly more common in patients using corticosteroids (Messer 1982). Chronic use of oral corticosteroids, high daily dosage and corticosteroid use in patients with a past history of peptic ulceration appear to be risk factors for the development of peptic ulceration, although the mechanism remains obscure.

2.5.2.2 Personal habits:

Smoking: The association between smoking and peptic ulceration is contentious. Evidence supporting a role for smoking comes from three areas. **Epidemiologically**, there is some evidence that smoking can be

associated with peptic ulceration, which is found more commonly in smokers than non-smokers (Friedman 1974). The early studies were, however, not endoscopically controlled and in one of the few endoscopically controlled studies reported to date, no difference could be found between the incidence of ulceration in smokers and non-smokers (Wursch 1977). Even if a degree of association between peptic ulceration and smoking is identified, causality will be difficult to prove as smoking may be only a confounding variable for other factors, such as socioeconomic status, and by inference, *H. pylori* gastritis. Observations on the **pathophysiologic effect** of smoking on gastric physiology, thought to be important in the etiology of peptic ulceration, have also given conflicting results. Observations made with regard to the effect of smoking on acid secretion, gastric emptying and pancreatic bicarbonate secretion have been conflicting (Boyd 1985) and cannot be invoked as an argument in favour of an association between smoking and duodenal ulceration. Much has been made of the results of **clinical studies** indicating that smoking both delays ulcer healing and contributes to the extremely high incidence of relapse associated with ulceration (Korman 1981, Katschinski 1991, Sontag 1984). These studies may, however, be flawed, as ulcers are not stratified for size in the"speed of healing" assessments. Sontag et al's follow-up study showed a clear advantage of non-smokers over smokers - a relapse rate of 21% (non-smokers) vs 72% (smokers). Despite the aforegoing, the etiologic role of smoking in duodenal ulceration remains unproven, as it may merely represent a marker for a character trait or etiologic agent. This possibility is further emphasised by the interesting findings of Borody et al. These workers demonstrated that the smoking habit did not influence the recurrence rate in ulcer subjects in whom *H. pylori* had been been eradicated (Borody 1992).

Alcohol consumption: There is insufficient data to link the consumption of alcohol to the etiology of ulceration (Friedman 1974, Boyd 1985).

2.5.2.3 Infectious agents as ulcerogens:

Herpes simplex virus type I (HSV-I) was the first infectious agent to be suggested as a possible ulcerogen, based on the similarity of susceptible age groups, the periodicity and the tendency to recur, at the same site, during periods of "stress" (Neumann 1967). The isolation of the organism from the vagus ganglion (Warren 1978) implied that the opportunity exists for the organism to get to the upper gastrointestinal tract. Vestergaard and Rune showed that antibody levels to HSV-I were higher in duodenal ulcer subjects than in controls, but that the prevalence of the antibodies to HSV-I was no different between the two groups (Vestergaard 1980). The finding that cimetidine is useful in the treatment of herpesvirus infection can be interpreted as support for the somewhat tenuous hypothesis implicating herpesvirus infections in the pathogenesis of peptic ulceration (van der Spuy 1980).

Helicobacter pylori: It is clear, therefore, that a number of factors are associated with duodenal ulcer disease. However, whenever a population of patients with ulcers is investigated for the presence of one of these potentially important factors, one finds that, while as a population, patients with duodenal ulcer disease differ (statistically) significantly from those without duodenal ulceration, considerable overlap exists. Thus none of the pathophysiological phenomena discussed above can be considered as being a universal and essential feature of chronically recurrent duodenal ulcer disease. The exception is the presence of antral

gastritis and gastric *H. pylori* infection. The role of *H. pylori* in peptic ulcer disease will be explored further in the following section.

2.5.3 *HELICOBACTER PYLORI* AND ULCEROGENESIS:

2.5.3.1 Evidence suggesting a role for *Helicobacter pylori* in peptic ulcer disease:

In his first letter to The Lancet, Marshall already linked *H. pylori* to peptic ulcer disease (Marshall 1983). He did this through his astute observation which linked the presence of type B (antral predominant) gastritis to peptic ulcer (duodenal ulcer) disease. Acceptance of a role for the organism in peptic ulceration was, however, not readily achieved. The gastroenterological world was divided during most of the 1980's; in one camp were outspoken critics of the *H. pylori* theory, claiming that *H. pylori* gastritis was a "normal" phenomenon (Peterson 1990) and advising physicians not to rush in with antimicrobial therapy for peptic ulcer disease (Petersen 1989). In 1988, Graham et al concluded from their data generated in post HSV-patients that, "while *C. pylori* infection may contribute, it is not the cause of duodenal ulcer" (Graham 1988b). Graham mustered additional arguments to lend further support to the point of view that *H. pylori* "is not the cause of peptic ulcer disease", concluding that attempts at identifying and treating the infection were still within the realm of research activities (Graham 1988c).

Recognition of *H. pylori* as a cause of ulceration was promoted by the excellent report of the working party assembled for the 1990 World Congresses of Gastroenterology in Sydney, Australia (Tytgat 1990). This report attempted to reconcile the differences between the "Schwarzians" and "Pylorites" by accepting that peptic ulceration was a multifactorial

disease, while asserting that *H. pylori* was "undoubtedly the major factor in the multifactorial disease, that is peptic ulceration". This meeting also appears to have given direction to research in the following couple of years, by stating that "our priority is not to ignore the other contributing factors (in the pathogenesis of peptic ulcer disease) but rather to identify how they interact with the organism".

Such has been the success of this approach, that apparent sceptics have been able to state that "we now recognize three major causes of peptic ulcer disease: *Helicobacter pylori* infection, the use of nonsteroidal anti-inflammatory drugs and pathological hypersecretory states such as Zollinger-Ellison syndrome" (Graham 1993b). The bridging of the "credibility gap" (McKinlay 1990) has been based on the evidence especially of interventional studies, and has clearly included some notable converts to the *H. pylori* cause!

More recently the recognition of *H. pylori* as a cause of peptic ulcer disease appears to have been formalised by the National Institutes of Health Consensus Statement (NIH 1994). While being guarded about the etiological association between the organism and peptic ulceration, the role of *H. pylori* is acknowledged *de facto* by the very strong recommendation that all ulcer patients infected with *H. pylori* be treated for the infection.

In trying to muster the evidence in favour of an etiological role for *H. pylori* in peptic ulcer disease, it has immediately to be conceded that Koch's postulates will never be fulfilled for peptic ulceration, as they have been for gastritis (Marshall 1985). These postulates state that the agent 1) must be found only in patients with the disease, 2) must be

obtained from the diseased subject and grown outside the body, 3) these cultured agents must, once purified, cause the same disease in a susceptible animal when introduced and 4) should be found in the diseased areas produced by such introduction (Marshall 1985). While Koch's criteria represent a landmark in the evolution of evidence-based medicine, they are obviously inappropriate for a number of infectious states where carriers exist, as well as for a number of chronic, or "slow" infections. Koch's hypothesis also cannot satisfactorily identify an association between a single factor and a disease of multifactorial etiology. Fortunately, epidemiologists have provided criteria by which causality of an association between an agent and a disease process can be assessed (Hill 1965). The evidence linking *H. pylori* to the etiology of peptic ulcer disease in terms of Hill's criteria will now be discussed.

2.5.3.1.1 *H. pylori* and peptic ulceration: strength of association:

H. pylori is consistently found in association with duodenal ulceration (>90% of cases), and is commonly found in association with gastric ulcer disease. Supporting data is summarised in table 2.1. Only reports which assessed gastric *H. pylori* status are included.

Table 2.1: *H. pylori* in peptic ulcer disease:

Reference	Gastric ulcer n (% HP+)	Duodenal ulcer n (% HP+)
Marshall 1984a:	22 (77)	13 (100)
McNulty 1984:	8 (63)	20 (95)
Langenberg 1984:	3 (66)	9 (100)
Burnett 1984:	19 (53)	7 (57)
Lambert 1985:	23 (65)	61 (95)*
von Wulffen 1986:	18 (72)	54 (83)
Pettross 1986:	28 (54)	25 (60)
Fiocca 1987:	30 (90)	34 (88)
Niemelä 1987:	33 (58)	NR
Humphreys 1988:	12 (92)	32 (93)
Graham 1988d:	20 (80)	85 (90)
Rauws 1988:	27 (96)	36 (100)
Hui 1991a:	NR	144 (98)
Saita 1993:	NR	20 (80)
Louw 1993 (included in this thesis):	40 (88)	44 (98)

* Duodenal ulcer/duodenitis"
NR Not reported

While the organism, and associated gastritis, are commonly found in subjects without peptic ulceration (see chapter 6), the association with peptic ulcer disease is further emphasised by the fact that the organism is extremely rare in populations known not to develop peptic ulceration, evidence for such infection being found in only 2 of 274 Australian Aborigines (Dwyer 1988). The apparent lower infection rates associated with gastric ulceration should also be interpreted with care, as NSAIDs may be responsible for *H. pylori* negative gastric ulceration.

Duodenal ulceration does occur in the absence of *H. pylori* infection, but is extremely rare. McColl et al could identify only 12 *H. pylori* negative duodenal ulcer subjects over a 5 year period (McColl 1993). Of these,

only six could be considered "idiopathic", as four were related to regular NSAID intake, one to Crohn's disease of the duodenum and one to Zollinger-Ellison syndrome. McColl could identify three abnormalities of gastric physiology associated with the cases of idiopathic, *H. pylori* negative, duodenal ulceration: 1) Hypergastrinaemia; 2) Increased basal and peak acid output and 3) Rapid gastric emptying of liquids and solids, this being the only factor that clearly distinguished the *H. pylori* negative idiopathic ulcer patients from *H. pylori* positive controls.

2.5.3.1.2 *Helicobacter pylori*: temporal relationship with the development of peptic ulceration:

Reference has already been made to the data indicating that *H. pylori* is not a contaminant of diseased mucosa, but rather the principal cause of gastritis (2.4.5). Some insight into the temporal relationship between the organism and duodenal ulcer can be gained from the work of Sipponen et al who have shown that the gastritis associated with *H. pylori* is a strong risk factor for the development of duodenal ulceration (Sipponen 1990a). In this follow-up study of 454 consecutive patients with known gastric histology, 321 subjects with gastritis could be identified. Eighteen (6%) of these subjects developed duodenal, 5 pyloric, 7 antral and 4 gastric body ulcers. This contrasts strongly with the single patient who developed an ulcer in the cohort of 133 subjects without gastritis at entry. The 10 year cumulative probability for developing ulceration in gastritic subjects can thus be estimated at 10.6% (95% Confidence Interval[CI] = 7.2 - 14%) while the value was 0.8% (95% CI = 0 - 2.2%) for the non-gastritic group. This study indicates that gastritis, associated with *H. pylori*, precedes ulceration and is a risk factor for its development.

2.5.3.1.3 *Helicobacter pylori*: Biological gradient in peptic ulcer disease:

There is limited data to support the hypothesis that an increased degree of *H. pylori* infection is associated with an increased risk of duodenal ulceration (Alam 1992, Khulusi 1994). A similar claim has been made in the case of gastric ulceration (Leung 1992). One must agree with the views of Genta that the science of quantifying gastric colonization density by *H. pylori* is, unfortunately, not exact (Genta 1992).

Precise quantification techniques will have to be devoloped before the question of biological gradient for *H. pylori* in peptic ulcer disease will be settled.

2.5.3.1.4 *Helicobacter pylori* and peptic ulcer disease: plausibility of the association:

One of the principal problems with the plausibility of the *H. pylori*/duodenal ulcer disease association was the difficulty in reconciling a gastric infection with a duodenal disease. This conceptual difficulty has to a large extent been overcome by the finding that the organism can bind gastric metaplastic tissue in the duodenum (c.f. paragraphs 2.4.3 and 2.5.1.3 and chapter 4). Once in the duodenum, the organism has the ability to induce an inflammatory response as it has been shown both to secrete a chemotactic factor for monocytes and neutrophils (Craig 1992, Nielsen 1992) and to activate neutrophils, probably by secretion of N-formyl-methionyl-leucyl-phenyalanine (Mooney 1991). Infection with the organism also appears to be associated with the principal mediators of the inflammatory response - tumor necrosis factor alpha and Interleukin-6

(Crabtree 1991a) and there is some indication that the organism may increase the local production of Paf-acetheter (previously "platelet activating factor"), a suggested cause of gastroduodenal ulceration (Denizot 1990).

Once in the duodenum, the organism has several potential mechanisms by which it can damage the mucosa, independently from the host's inflammatory response. There is some evidence that the organism has the potential to inhibit the gastric laminin and integrin interaction, thereby potentially compromising the mucosal integrity (Piotrowski 1991). There are conflicting views on the pathogenic potential of the urease enzyme to mediate mucosal damage by way of ammonia production. Using a rat model, Tsuji et al have demonstrated the capacity for ammonia to injure gastric mucosa, and have elucidated the mechanism by which damage can be caused - an inhibition of cellular respiration and energy metabolism (Tsuji 1992), but there is no proof that this mechanism is applicable in the human subject (El Nujumi 1992). The organism also possesses a number of enzymes with the potential capacity to affect the gastroduodenal mucus layer. Although there is no proof that these enzymes are responsible, Sarosiek et al have shown that the mucus gel layer is thinner in *H. pylori* infected individuals than in controls and have speculated that this may be as a result of the action of microbial lipase, protease and phospholipase A_2 action (Sarosiek 1991). While there is experimental evidence that this breakdown of gastric mucus is not mediated by an *H. pylori* derived extracellular protease (Sidebotham 1991), the degrading of mucus is not questioned. This effect on the gastroduodenal gel, as well as the damage to the epithelial integrity at the site of infection, appears to be compatible with the "leaking roof"

hypothesis for peptic ulceration put forward by Goodwin (Goodwin 1988).

The organism also produces "toxins", at least one of which is able to induce cellular vacuolisation in epithelial cell line cultures. This "vacuolating cytotoxin" (now termed VacA) was first identified in broth culture filtrates (Leunk 1988), and cytotoxin producing strains have since been shown to be more commonly present in subjects with peptic ulceration (Figura 1989). The cytotoxin has been characterised by Cover et al, whose findings indicated that the vacuolating activity is contained in 128 and 82 kilodalton (kDa) proteins, 128 kDa protein containing strains being more prevalent in *H. pylori* infected individuals with peptic ulcer disease (Cover 1990). Crabtree et al were also able to demonstrate serologically, by examining the gastric IgA response, the presence of a 120 kDa protein associated with the organism. Recognition of the 120 kDa protein was strongly associated with peptic ulcer disease, 25 of 57 subjects recognising the protein having peptic ulceration, while none of the 19 patients who did not recognize the protein had peptic ulceration (Crabtree 1991b). In a related development, a gene, the Cag A (cytotoxin associated gene A), has been cloned and sequenced (Tummuru 1993, Covacci 1993). The Cag A gene product is a hydrophilic, surface-exposed protein of 128 kDa which appears to be essential for the expression of the cytotoxin activity (and can therefore serve as a marker for it), but which does not mediate the cytotoxic activity directly.

The recent description of a mouse model and the study of *H. pylori* pathogenicity in this model has shed some light onto the importance of CagA and VacA. In what represents an important advance in the ability of workers to study *H. pylori* in an animal model, Marchetti and co-

workers have succeeded in infecting mice with viable *H. pylori*. Their findings indicate that CagA and VacA positive strains (now called "type I" strains) are associated with gastric pathology resembling human disease (loss of gastric architecture, epithelial erosions and ulceration and inflammatory cell infiltration of the lamina propria). Infection with VacA and CagA negative strains ("type II" strains), on the other hand, was associated with a mild inflammatory infiltration (Marchetti 1995).

The evidence for specific toxins and strain differences in *H. pylori*, which may influence the pathogenicity of the organism, may explain why not all infected subjects develop peptic ulceration. These findings further enhance the plausibility of an association between *H. pylori* and peptic ulcer disease, as they provide a basis to explain why not all subjects infected with the organism go on to develop ulceration.

The stated aim of the *H. pylori* working party at the 1990 World Congresses of Gastroenterology was "not to ignore the other contributing factors (to the development of peptic ulceration) but rather to identify how they interact with the organism and initiate the ulcerative process" (Tytgat 1990). One of the key observations in this regard has been the finding of an exaggerated meal-stimulated gastrin release in duodenal ulcer patients infected by *H. pylori*, and its ablation after therapy for *H. pylori* (Graham 1990). This observation, by implication, links infection by *H. pylori* to abnormalities of acid secretion and has led to the "gastrin link hypothesis" for *H. pylori* and duodenal ulceration (Levi 1989). This hypothesis links antral *H. pylori* colonization to inapproriate hypergastrinaemia and, resulting from that, inappropriately increased acid secretion. This theme will be discussed further in chapter 5 of this thesis.

2.5.3.1.5 *Helicobacter pylori* and peptic ulceration: the effect of therapeutic interventions:

The most convincing evidence in favour of an etiological role for *H. pylori* in peptic ulcer disease comes from studying the effect of successful treatment of the organism on both the healing of ulcers and on the ulcer relapse rates in successfully treated patients.

Although largely ignored at the time, the first indication that antimicrobial therapy has a role in the management of peptic ulcer disease was already published in 1969, in Spanish (cited by Diaz, 1986). Metronidazole, given inadvertently to peptic ulcer sufferers apparently had a beneficial effect on the outcome of treatment, an observation later confirmed (Diaz 1986). It is the ability of successful *H. pylori* treatment to influence the natural history of peptic ulceration that has indicated that the organism may have etiological importance in the disease. As can be seen from table 2.2, ridding the host of *H. pylori* has a marked beneficial effect on the relapse rates of duodenal ulceration, in socioeconomically advanced, First World countries.

Table 2.2: The effect of *H. pylori* "eradication" on the natural history of duodenal ulceration: a sample of studies published as peer reviewed papers, with a minimum 12 month follow-up and allowing comparison between *H. pylori* positive and negative subjects:

Author:	N	Follow-up (months)	HP Positive Relapse (%)	HP Negative Relapse (%)
Coghlan 1987:	39	12	19/24 (79)	4/15 (27%)
Marshall 1988b:	68	12	37/44 (84)	5/24 (21)
Rauws 1990:	38	12	17/21 (81)	0/17 (0)
Hentschell 1993:	99	12	40/53 (75)	1/46 (2)
Forbes 1994:	63	84	11/26 (42)*	8/37 (22)*
			9/26 (35)**	3/37 (8)**
Louw 1995 and this thesis:		12	14/21 (67)##	4/27 (15)#
		24	15/21 (71)##	5/27 (18)#

* Clinical relapse
** Endoscopically or radiologically confirmed relapse.
\# 1 = re-infected, 1 = gastrinoma.
3 of 21 patients lost to follow-up

The total number of patients subjected to *H. pylori* eradication can be increased many-fold by the inclusion of abstracts and letters - the trend is clear and sustained, however.

Other than the obvious beneficial effect of *H. pylori* eradication on the natural history of duodenal ulcer disease, the data in table 2 identifies two further interesting findings. Firstly, it is clear that the earlier studies had a higher recurrence rate than those done later. The explanation for this is probably that the original investigators thought that their patients were free of the organism when they were not. This problem with the ability of current diagnostic methodology to identify low bacterial loads (discussed further in chapter 8) led in 1990 to the definition of **eradication** as the absence of the organism at least four weeks following

cessation of anti *H. pylori* therapy, while the inability to demonstrate the organism immediately following therapy was considered **clearance**, which did not imply successful treatment (Tytgat 1990).

The second factor identified in table 2 is the role of clinical relapse in assessing the success of an eradication strategy. Although the phenomenon of "clinical relapse", i.e. ulcer-like dyspepsia occurring in patients following eradication, is still poorly recorded, the retrospective data of Forbes et al suggests that this may be an important factor. One of the major benefits of a successful eradication strategy will be the cost saving incurred by the reduced need for maintenance therapy or repeated courses of therapy for recurrent duodenal ulceration. The effect of the occurrence of eradicated, dyspeptic, ulcer free patients on diagnostic and therapeutic strategies and hence cost structures still needs to be adequately investigated.

Much less data has been generated with regard to the effect of *H. pylori* eradication on the natural history of gastric ulcer disease, but the limited data available suggests that *H. pylori* eradication has a similar beneficial effect on gastric ulcer disease, with lower relapse rates noted in the studies published to date (Table 2.3). The major difficulty with gastric ulcer disease, however, has been the difficulty of positively excluding NSAID use in the patients studied.

Table 2.3: The effect of *H. pylori* eradication on the natural history of gastric ulcer disease. Summary of studies with at least 12 months of follow-up data available:

Author:	N	Follow-up (months)	HP Positive Relapse (%)	HP Negative Relapse (%)
Seppälä 1993:	159	12	63/133(47)	0/26(0)
Bayerdörffer 1993:	102	12	(51)	(3)
Labenz 1994:	50	12	10/18(56)	1/32(3)

A role for *H. pylori* in peptic ulcer disease is further suggested by the observation, initially made by Graham, that eradication of the organism is associated with improved ulcer healing rates (Graham 1991b). This observation has been further strengthened by the finding that both gastric and duodenal ulcers heal with antibacterial "triple therapy" (Hosking 1994, Sung 1995).

The effect of therapeutic intervention on the healing and subsequent relapse of peptic ulcer disease therefore strongly favours a role for *H. pylori* in the pathogenesis of peptic ulcer disease.

2.5.3.1.6 *Helicobacter pylori* and peptic ulceration: coherence of theories linking infection with ulceration:

Any causal association between *H. pylori* infection and peptic ulcer disease should be reconcilable with the known changes in the demographics of peptic ulcer disease referred to in 2.2, i.e. the declining incidence in Western populations, the apparent increase brought on by

urbanisation, the change in the age spectrum as well as the socioeconomic classes principally involved in the disease.

The epidemiologic characteristics of *H. pylori* infection as they are now understood and which will be discussed in greater detail in chapter 6, are entirely compatible with the observed changes in the prevalence of gastroduodenal ulceration.

One of the essential features of the epidemiology of *H. pylori* in developed countries is its increasing prevalence with age (Dooley 1989, Graham 1991c, Sitas 1991). The infection is also more prevalent in subjects from a socioeconomically disadvantaged background (Graham 1991c), which probably represents a surrogate marker for parameters such as large family size, crowded living conditions and, probably, suboptimal sanitation, factors which have been identified as risk factors for the development of the infection in childhood (Mendall 1992). Available evidence suggests that the increase in the *H. pylori* infection rate seen with age in Western population samples does not represent accrual of the infection during the subject's lifespan. Infection appears to occur predominantly in the childhood years, poor socioeconomic status during childhood being a risk factor for infection in adult subjects (Malaty 1994). Cullen et al could also demonstrate by serial serological assessment in 141 subjects over a 21 year period that seroconversion is uncommon during adulthood (Cullen 1993).

These findings with regard to the epidemiology of the infection in developed countries are consistent with a so-called cohort effect - that is, the apparent age-dependent variation in the prevalence of *H. pylori* reflects the changes in acquisition of the infection during childhood. The

risk for acquisition of the infection, being dependent on socioeconomic factors, may also be influenced by early urbanisation. This cohort effect with regard to the acquisition of *H. pylori* infection is similar to that described with peptic ulcer disease (Sonnenberg 1984) and offers a plausible alternative explanation to Susser's "stress of early urbanisation" for the changes observed in the epidemiology of peptic ulcer disease around the middle of this century (Susser 1962), while reminding one of Jennings' view that too little attention has been focussed on environmental factors in the search for possible causes of peptic ulceration (Jennings 1940).

2.5.3.1.7 and 8 *Helicobacter pylori* and peptic ulceration: experimental evidence linking the organism to the disease and an animal analogy:

It is not possible, at present, to satisfy these criteria set by Hill. If, however, the findings of Marchetti et al, referred to in paragraph 2.5.3.1.4, are confirmed and extended, these criteria may also eventually be met (Marchetti 1995).

In summary, while the bulk of the evidence linking *H. pylori* to ulcerogenesis is inferential, and a causal role cannot be unequivocally proven for the organism in what has to be viewed as a disease of multifactorial etiology, *H. pylori* goes a long way in satisfying Hill's criteria (Hill 1965). The association of the infection with ulceration is strong and consistent. Factors have been identified which could explain why infection is not invariably associated with peptic ulceration (strain variation, presence of gastric metaplasia in the duodenal cap). Available evidence indicates that the infection precedes the development of ulceration, and there is some evidence in favour of a biological gradient,

although proof of this is still tenuous. There is no doubt that in Western society eradication favourably influences the outcome of duodenal ulceration not associated with NSAID use or primary acid hypersecretory states (Zollinger - Ellison syndrome). The same is probably true for gastric ulceration, although the number of subjects reported on at the time of writing is still relatively few. In addition, a strong, perhaps causal, association between *H. pylori* and peptic ulceration is entirely compatible with the known epidemiogy of the disease.

The discovery of *H. pylori*, and the rapid accumulation of data causally linking it to peptic ulceration, has changed the perception that peptic ulcer disease is caused by primary abnormalities of gastric physiology. As a result, peptic ulcer disease is increasingly seen as an infectious disease.

2.6 *HELICOBACTER PYLORI* IN THE AFRICAN SETTING:

The data with regard to *H. pylori* infection in Africa appears to differ from that generated from outside Africa in several respects; the findings "out of Africa" underline the complicated, multifactorial nature of the diseases associated with *H. pylori* infection.

2.6.1 *H. pylori* in Africa North of the Limpopo:

Two epidemiological findings typify the nature of *H. pylori* infection in Africa: the infection appears to be acquired at an early age and the infection rate is extremely high.

Most studies from the African continent have indicated that the infection is acquired at a young age. Holcombe, studying a random sample of 268 subjects in northeastern Nigeria concluded that the majority of the

population (82%) had seroconverted by the age of 10 years (Holcombe 1992), while overall 85% of the population studied had serological evidence of *H. pylori* infection, a finding confirmed by serological findings from populations in Algeria and the Ivory Coast (Megraud 1989), where 80-90% of the sera tested were positive for *H. pylori* antibodies.

These serological findings have been confirmed in endoscopic studies. Wyatt et al studied 39 dyspeptic patients by endoscopy in Ghana, West Africa (Wyatt 1987b) and found that 38 of these had gastritis, all associated with *H. pylori*. In addition, 14 (37%) of these patients had endoscopically proven duodenal ulceration. Glupczynski et al studied a population of 324 dyspeptic patients in Zaire and found evidence for *H. pylori* infection in 88% of subjects. They found that 38 (12%) had duodenal ulceration and 23 (7%) gastric carcinoma and, furthermore, that nine of the ten teenagers in the sample were already infected (Glupczynski 1991).

Overall, it appears that 70-97% of dyspeptic patients are infected by *H. pylori,* while approximately 80% of asymptomatic subjects studied also have evidence of the infection (Holcombe 1992b). This high prevalence and early infection is, of course, entirely compatible with the observations regarding the epidemiology of the infection made in Western society i.e. the desperate socioeconomic deprivation, poor and crowded living conditions and poor access to water and sanitation would be expected to lead to early infection and an extremely high prevalence of the infection.

The conceptual difficulty lies, however, in the belief that the diseases commonly associated with *H. pylori* infection in socioeconomically

advanced populations, with the exception of gastritis, are thought to be uncommon in the highly infected African subjects.

This appears to be the case with peptic ulceration, where the so-called high and low prevalence areas for peptic ulceration (high = Nile/Congo watershed and coastal West Africa, low = northern savannah of West Africa) do not appear to be paralleled by differences in *H. pylori* prevalence. In addition, the overall prevalence of ulceration in the highly infected African population is still less that that for their less commonly infected counterparts from the First World (Holcombe 1992b). This observation may be erroneous, however, and based on poor epidemiological data and pick-up rates of gastrointestinal pathology in Africa. This is suggested by the preliminary report of Willemin et al, who found, endoscopically, gastric carcinoma in 2 (4%) and peptic ulceration in 14 (32%) of 44 patients with dyspepsia (Willemin 1993). These findings are also supported by those of Wyatt et al, who reported the presence of frank duodenal ulceration in 14 (37%) of 38 patients with *H. pylori* associated gastritis (Wyatt 1987). Although less dramatic, Glupczynsky's data also suggests that peptic ulceration may not be as rare as believed (Glupczynski 1991). The "African Enigma" may, therefore, not be an enigma, but merely another example of under reporting due to the poor medical infrastructure on the continent.

Graham has proposed an explanation for the apparent lack of an increased prevalence of duodenal ulceration in the presence of an extremely high prevalence of the infection in Africa (Graham 1992). The proposal is that patients from poor socioeconomic backgrounds are infected early; rather than developing antral predominant gastritis with hypergastrinaemia and, possibly, acid hypersecretion with secondary

gastric metaplasia in the duodenum, these subjects go on to develop pangastritis with hypochlorhydria. Although plausible, this theory ignores the evidence in favour of a cohort theory for acquisition of the infection, also found in Western society, as well as the apparent low incidence of gastric ulceration and gastric carcinoma, two other gastric disease-states associated with *H. pylori* infection. It is clear that the "African Enigma" is not readily rationalised, and that basic epidemiological data with regard to the disease states associated with *H. pylori* is lacking in the African continent.

If epidemiological data from Africa is in fact correct, the limited *H. pylori* prevalence data from Africa may be interpreted as supporting the hypothesis that mere infection by the organism is unlikely to be a sufficient factor for the development of disease - genetic, environmental and organism (strain) differences may be important in determining the development of disease.

A second important aspect of the infection in Africa has been the observations with regard to antibiotic sensitivity - this is further described in chapter 8.

2.6.2 *Helicobacter pylori* in South Africa:

With the exception of the work reported in this thesis, the study of *H. pylori* in South Africa has been limited to epidemiological observations, with no data relating to the factors influencing the prevalence of the infection in South African population groups, disease associations, the clinical and physiological effects at eradication as well as different eradication strategies.

The first observations with regard to *H. pylori* prevalence were made in 1985, in patients from the Gauteng Province (Crewe-Brown 1985). These workers detected the organism in 58 (82%) of 71 patients with duodenal ulceration, 9 (90%) of 10 patients with gastric ulceration, while the organism was detected in 46 (59%) of 78 control subjects.

Wright et al were the first to report on the prevalence of the infection in dyspeptic subjects in the Cape Town area (Wright 1987). They reported the infection in gastric biopsy samples in 73% of 49 patients with varied gastroduodenal pathology (gastric ulceration = 24, duodenal ulceration = 6, gastritis only = 12 and 7 with normal mucosa). In the same year prevalence figures for Durban were published in abstract form, and published in paper form the next year (Miller 1988). This group studied 224 dyspeptic patients with varied gastroduodenal pathology and reported an overall prevalence of the infection of 82%. The same group published their serologically determined prevalence rates for a random sample of patients in Kwazulu-Natal, confirming that the prevalence of the infection was in fact high, with 22 of 38 (58%) of the subjects in the 5 - 10 year age group already infected (Sathar 1991).

Jaskiewicz et al reported the prevalence of the infection in an unselected sample of 178 patients from a population of dyspeptic subjects considered to be at high risk for gastric carcinoma (Jaskiewicz 1989). They reported an overall incidence of 90% in patients with gastroduodenal ulceration, with a prevalence of 72% in "non ulcer dyspeptics". The high prevalence of the infection in the African population was confirmed in a study from Johannesburg by Dawes et al who reported an overall prevalence of 81% in a study sample of 90

patients (Dawes 1991), while the Bloemfontein group reported a prevalence rate of approximately 81% in black subjects selected for the presence of gastritis, finding that only 53% of 17 white gastritic subjects were positive for the organism (Grundling 1992).

In summary, the data generated in South Africa has been largely limited to observations with regard to the prevalence of the infection. We have set out to investigate the factors influencing the prevalence of the infection, to investigate factors which may be important in the pathogenesis of peptic ulcer disease, to examine the effect of eradication on the natural history of peptic ulcer disease in our population with a high prevalence of the infection and, finally, to determine an effective therapeutic approach in the population we serve.

CHAPTER 3

DIFFERENCES IN GASTRIC *HELICOBACTER PYLORI* COLONIZATION PATTERNS AND ASSOCIATED GASTRIC INFLAMMATORY CHANGES IN DUODENAL AND GASTRIC ULCER DISEASE

3.1 INTRODUCTION:

Gastric *Helicobacter pylori* infection is strongly associated with duodenal ulcer disease and is also commonly found in association with gastric ulceration (Marshall 1984a, Rauws 1988). It is, however, an extremely prevalent infection and has been found in up to 88% of patients studied in the African setting, making it probably the most common bacterial infection on this continent (Megraud 1989, Wyatt 1987b, Glupczynski 1991, Holcombe 1992).

H. pylori is uniquely adapted to survive in the hostile gastric environment, and is in fact unable to colonize mucosa other than gastric. This unique affinity for gastric mucosa has been demonstrated to be the result of a specific receptor based mechanism (Lingwood 1989), with the fibrillar hemagglutinin component of the organism acting as a "colonization factor" (Evans 1988). Historically, gastritis has commonly been found in gastric and duodenal ulceration, the patterns of gastritic involvement differing. Correa has described the gastritis associated with duodenal ulceration as being of the antral predominant or "hypersecretory" pattern (Correa 1988). This corresponds to the reported distribution of the organism in the stomach in patients with duodenal or pyloric ulcer disease - the organism being found mainly in the antrum with sparing of the body/fundal mucosa (Levi 1989). It has been postulated that the latter allows for the characteristic hypergastrinaemia and acid

hypersecretion of duodenal and pyloric ulcer disease. Gastric ulceration, on the other hand, occurs against the background of a more widespread gastritic process (Gear 1971, Tatsuta 1986), which may reflect a more widespread colonization by *H. pylori*, or a difference in the immunological response of the gastric mucosa to the organism in these conditions. This may be reconciled with the lower levels of acid secretion found in gastric ulcer disease.

3.2 AIM OF STUDY:

The high infection rate in the community we serve (Louw 1993, van Wyk 1993) coupled with the absence of known antrum-specific colonization factors, raised the question as to whether the gastric distribution of the organism differs in gastric and duodenal ulceration. This study was carried out to determine the gastric distribution of *H. pylori* in patients with duodenal and gastric ulceration and, in addition, to examine the gastric mucosal inflammatory response to the organism in these disease states.

3.3 PATIENTS AND METHODS:

3.3.1 Patients:

Patients with newly diagnosed, uncomplicated gastric and duodenal ulceration were recruited from the Gastroenterology outpatient services of the Groote Schuur and Tygerberg Hospitals. All patients gave informed consent for participation in the study which was approved by the Ethics and Research Committee of the University of Cape Town.

3.3.2 Endoscopy:

All patients were subjected to a routine upper gastrointestinal endoscopy, using Xylocaine local anaesthetic with or without sedation with midazolam 2.5mg IV. The ulcer site was defined as duodenal or gastric; because of the difficulty in classification, pyloric channel ulcers were excluded from the study. Two endoscopic pinch biopsies each were taken from the antrum (<5cm from the pylorus) and a further two biopsies from the posterior wall of the gastric body. Samples were preserved in formalin, and evaluated by a single pathologist, who had no knowledge of the ulcer diagnosis.

3.3.3 *H. pylori* status:

H. pylori status was determined histologically, in sections stained by the Giemsa method. The density of colonization was scored semi-quantitatively as:

0 = organisms not found after thorough search using Giemsa stained material.

1 = organisms found only after thorough search of more than one high power field of Giemsa stained material.

2 = organisms found in every high power field, present only in crypts.

3 = organisms abundant, in crypts and mucus layer.

3.3.4 Gastritis:

Gastritis was classified according to the guidelines of the Sydney classification system (Misiewicz 1990), the scoring limited to categories

0 (none), 1 (mild), 2 (moderate) and 3 (severe). Only samples incorporating muscularis mucosae (ie. full thickness mucosa) were evaluated for atrophy. Gastritis was assessed as being either "predominantly antral" or "pangastritis" according to the presence of chronic inflammatory changes found in the samples from body and antral mucosa.

3.3.5 Statistical analysis:

Statistical analysis of categorical data was by means of the Fisher's exact test and the Mantel-Haenszel Chi-square test, and continuous variables were evaluated by means of Student's t - test, both by using the EPISTAT statistical package. A p value of < 0.05 was considered significant.

3.4 RESULTS:

The results are summarised in Tables 3.1, - 3.4 as well as figure 3.1

3.4.1 Patient characteristics:

Adequate antral and fundic biopsies were obtained from 40 of 43 gastric and from all 44 duodenal ulcer patients. The gastric ulcer patients were significantly older than the duodenal ulcer group, mean (SD) age 52(13) years for gastric ulcers and 42(15) years for duodenal ulcers, $p < 0.01$].

3.4.2 *H. pylori* prevalence:

The overall incidence of *H. pylori* infection was 88% (35/40) in gastric ulcer patients and 98% (43/44) in duodenal ulcer patients. This difference in incidence was not significant.

Figure 3.1: Graphic representation of the observed gastric distribution of *H. pylori*:

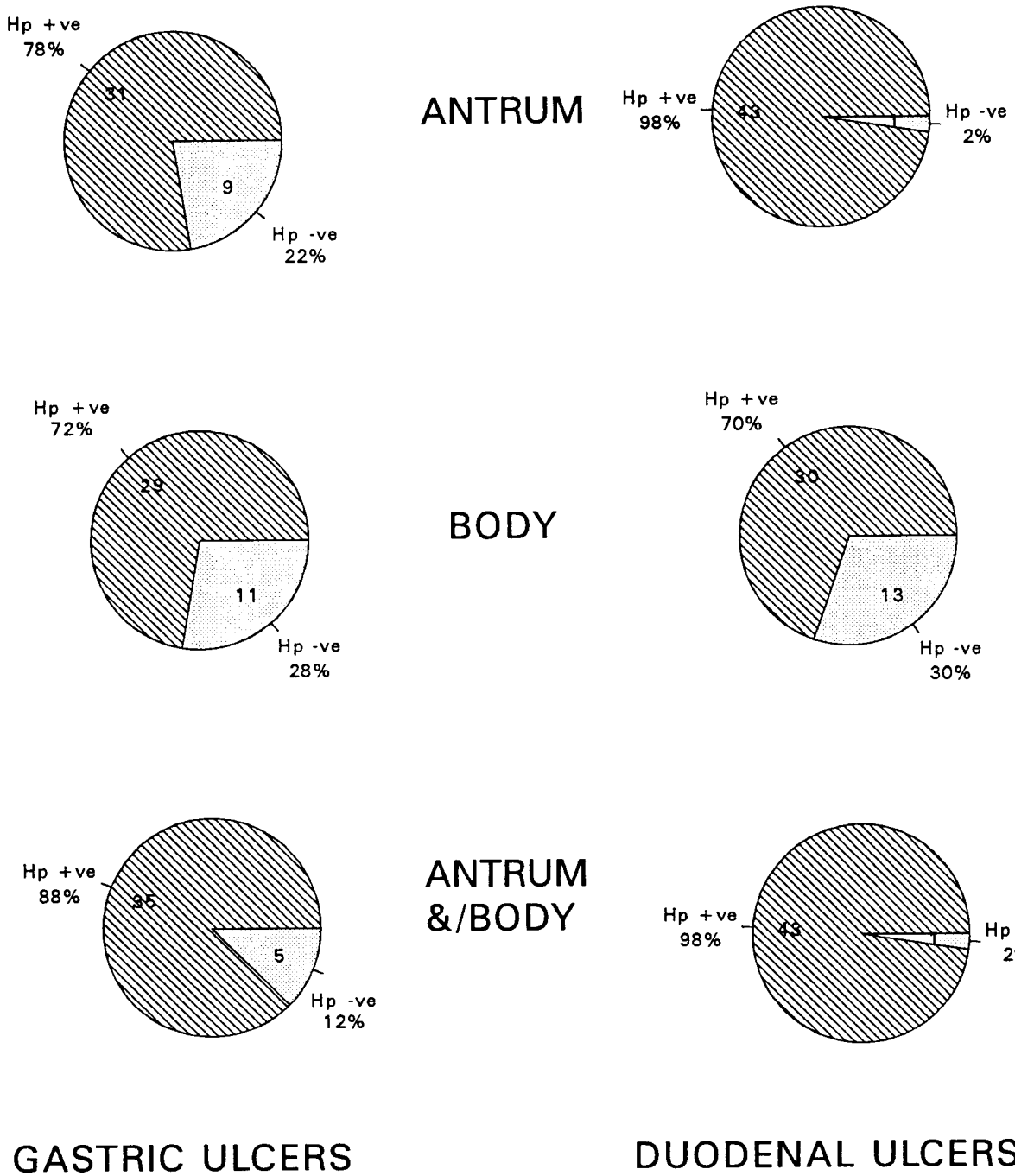


Table 3.1: Characteristics of *H. pylori* activity in biopsy samples:

	Gastric ulcers	Duodenal ulcers	p
Patients:	40	44	
Age: mean ±SD:	52 ±13	42 ±15	<0.01*
***H. pylori* distribution:**	+/-	+/-	
Antrum:	31/9	43/1	<0.01**
Body:	29/11	30/13	NS**
Antrum and/or body:	35/5	43/1	NS**
Density of colonization:			
Antrum: 0 or 1:	12	6	
2 or 3:	28	38	NS#
Body: 0 or 1:	22	36	
2 or 3:	18	7	<0.01#

NS Not significant
* Student's t-test
** Fisher's exact test
\# Chi-square

3.4.3 Pattern of distribution of *H. pylori:*

H. pylori was found more frequently in the antrum of duodenal ulcer than of gastric ulcer patients - 43/44 vs 31/40, $p < 0.01$. The pattern of *H. pylori* colonization was different in the two groups of patients. Antral colonization was found more frequently in duodenal ulcer patients than was body colonization - 43/44 positive in the antrum, 30/43 positive in the body, $p < 0.001$. No difference was found in the distribution of the organism in the gastric ulcer group - 31/40 (antrum) vs 29/40 (body).

Although the pattern of distribution differed, the incidence of body colonization did not differ in duodenal ulcer and gastric ulcer patients - 30 of 43 duodenal ulcer patients were colonized in the body of the stomach, compared to 29 of 40 gastric ulcer patients. There was, however, a significant difference in the density of body colonization, with the gastric ulcer group having significantly more subjects in the 2 and 3

density categories (18 of the 29 in the gastric ulcer group, vs 7 of the 30 in the duodenal ulcer group - $p < 0.01$).

3.4.4 **Pattern of distribution of gastritic changes:**

As summarised in Table 3.2, and given in detail in tables 3.3 and 3.4, differences were noted in the gastritic characteristics of the two ulcer groups. Active gastritis (i.e. neutrophil activity) was found more commonly in the body of gastric ulcer subjects, and atrophy was also more likely to be found in this setting (8 of 39 vs 0 of 43, $p < 0.01$). The incidence of antral intestinal metaplasia also differed markedly between the groups - 15 of the 40 patients in the gastric ulcer group showed evidence of antral intestinal metaplasia, vs 3 of the 44 patients in the duodenal ulcer group - $p < 0.001$. When patients were stratified into three arbitrarily chosen age groups (15-40 years, 40^{+}-60 years and > 60 years), and the data analysed by the Mantel-Haenszel Chi-square test controlling for age, the differences in prevalence of both antral intestinal metaplasia ($p < 0.01$) and body atrophy ($p < 0.05$) remained significant, although the significance levels were clearly lower, and only marginal in the case of body atrophy.

Table 3.2: Characteristics of gastric morphology:

	Gastric ulcers +/-	Duodenal ulcers +/-	p
Neutrophil activity:			
Antrum:	36/4	40/4	N/S*
Body:	11/29	4/40	<0.05*
Mucosal atrophy:			
Antrum:	31/2	28/5	NS*
Body:	8/31	0/43	<0.01
Intestinal metaplasia:			
Antrum:	15/15	3/41	<0.001
Body:	3/37	0/44	NS*

NS Not significant
* Fisher's exact test.

Table 3.3: Categorisation of histological characteristics, antrum:

	Duodenal ulcer				Gastric ulcer			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Chronic inflammation:	0	3	33	8	0	2	30	8
Neutrophil activity:	4	28	11	1	4	14	21	1
H. pylori density:	1	5	20	18	9	3	16	12
Mucosal atrophy:	5	11	16	1	2	7	21	3
Intestinal metaplasia:	41	1	2	0	25	5	4	6

Table 3.4: Categorisation of histological characteristics, body:

	Duodenal ulcer				Gastric ulcer			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Chronic inflammation:	25	18	1	0	11	19	9	1
Neutrophil activity:	40	2	2	0	29	6	5	0
H. pylori density:	13	23	7	0	11	11	14	4
Mucosal atrophy:	43	0	0	0	31	2	5	1
Intestinal metaplasia:	44	0	0	0	37	2	0	1

The gastritic process tended to be predominantly antral in duodenal ulceration, while in gastric ulceration a pangastritic process was more common. The gastritis was deemed to be predominantly antral in 39 of

the duodenal ulcer subjects, vs 23 of the gastric ulcer subjects, and pangastritic in 5 of the former and 17 of the latter - $p < 0.01$.

3.5 DISCUSSION:

Our findings confirm that in the population we serve in the Western Cape, gastric *H. pylori* is strongly associated with peptic ulcer disease, only 1 (2%) of 44 subjects with duodenal ulceration having no evidence of the organism. The infection was also very common in the gastric ulcer patients, being present in the antrum or body in 35 (88%) of patients in this sample.

This study confirms that the gastritis in duodenal ulcer disease is predominantly antral in nature, while it is more widespread in gastric ulceration. The finding that gastric ulcer disease is more commonly associated with antral intestinal metaplasia, which may affect the likelihood of demonstrating the organism in the antrum, but which may in turn reflect the outcome of chronic *H. pylori* associated gastritis (Craanen 1992), is consistent with the findings of earlier studies (Schrager 1967, Gear 1971), but appears to be at variance with a recent study (Sobala 1992), where this difference was not found when the data was analysed controlling for age. It should be noted that in the study of Sobala et al there was a trend for an increased incidence of intestinal metaplasia, which was lost when the data was analysed after being controlled for age. While there was a clear reduction in the significance level when our findings were stratified for age, significance was not lost.

Our findings with regard to the distribution of gastritic changes in the different forms of peptic ulceration are also consistent with the theory

that the topography of gastritis may have aetiological significance in peptic ulcer disease, duodenal ulceration being associated with the predominantly antral form of gastritis, with relative sparing of the parietal cell mass, while gastric ulceration tends to develop in the presence of a more generalised gastritis, which may affect the oxyntic region and hence, the acid secretory capacity of the stomach (Ball 1961, Sipponen1990b, Tatsuta 1986). This finds aetiopathological significance in the observation that while duodenal ulcer disease may be associated with increased acid secretion, this is unlikely in gastric ulceration (Petersen 1975, Collen 1993).

The similar incidence of *H. pylori* in the body of the stomach in the duodenal and gastric ulcer disease groups may be construed as being at variance with the established view that patients with duodenal ulcer disease have fundal "sparing" of *H. pylori* colonization (Queiroz 1988, Levi 1989). However, the finding that both fundal colonization density and morphologic parameters (neutrophil "activity" and mucosal atrophy) in the body were significantly lower in the duodenal than in the gastric ulcer group was clearly in keeping with the concept of relative fundal sparing in duodenal ulcer disease.

These fundal inflammatory morphologic changes were associated with a significantly higher colonization density in the gastric ulcer group. In this regard, the findings of the present study are in keeping with earlier reports suggesting a correlation between the magnitude of the inflammatory response and the density/depth of *H. pylori* colonization (Bayerdörffer 1992, Neri 1992, Alam 1992), rather than the presence of *H. pylori per sè.* Although the ability of different strains to produce varying inflammatory responses by different cytotoxin profiles or varying

efficiency in activating the inflammatory response cannot be excluded, Figura et al could not identify specific putative virulence characteristics of *H. pylori* (urease levels, motility and degree of adhesion) which could predict the severity of gastritis in infected subjects (Figura 1992). While gastritis appears to be the common expression of gastric *H. pylori* infection, Fox et al have also identified a high prevalence *H. pylori* strains capable of producing a vacuolating cytotoxin in a population with a high prevalence of atrophic gastritis (Fox 1992). Strain variation may not, however, be an important factor in determining the severity of gastritis.

A number of factors may be important in determining the difference in the colonization density gradient in duodenal and gastric ulcer disease. Firstly, it is possible that this merely reflects the duration or chronicity of the infection. This appears to find some support in the finding of a significantly older mean age, as well as the presence of markers for chronic gastritis (ie atrophy and intestinal metaplasia) in the gastric ulcer group. The relationship of age to the chronicity of gastritis is not simple, however, and the duration of the gastritis may be better assessed by the chronicity of symptoms, an approach used by earlier studies (Gear 1971). Available evidence, generated in studies on the natural history of duodenal ulcer associated type B gastritis, suggests that the inflammatory changes, with occasional exceptions, do not progress to include the body/fundus (Kekki 1984, Tatsuta 1986). The gastritis in patients with gastric ulcer or gastritis without peptic ulcer disease is, however, progressive (Kekki 1984, Maaroos 1985, Villako 1990). It thus seems unlikely, based on current data, that *H. pylori* infection spreads proximally up the body of the stomach from the antrum in duodenal ulcer subjects. However, suitably designed longitudinal studies should address this important question, as the controversial phenomenon of duodenal

ulcer "burnout", suggested by Fry (Fry 1964), may be explained by proximal spread of the gastritic process, with resultant hypochlorhydria. Current observations of the non-spreading nature of the antral gastritis in duodenal ulcer disease offer little support for Fry's hypothesis of duodenal ulcer "burnout".

Secondly, as gastric colonization by *H. pylori* appears to be mediated by specific *H. pylori* colonization factors for gastric glycerolipid receptors (Lingwood 1989), it is conceivable that there may be strain variations in the affinity of the organism's colonization factors for the gastric glycerolipid receptors. This may be one of the factors that will explain the apparent strain variation associated with peptic ulcer disease (Crabtree 1991b). On the other hand, it is possible that the distribution of the mucosal glycerolipid receptor distribution may differ in subjects, giving rise to host related differences in colonization patterns, resulting in or associated with, different gastroduodenal diseases. There is currently no evidence that this may explain the "host factor" thought to be implicated in the selection of patients who will develop peptic ulcer disease.

Finally, patients with duodenal ulcer disease may be resistant to colonization of the oxyntic mucosa by *H. pylori*, by virtue of the increased acid secretion found in this group of patients, which in turn may protect the oxyntic mucosa from bacterial colonization (Graham 1989).

In summary, this study confirms the established finding with regard to the distribution of gastritis in duodenal and gastric ulcer disease. It shows, however, that in the population studied, the frequency of

colonization of the gastric body by *H. pylori* is equally common in gastric and duodenal ulcer disease, but that the density of colonization and the inflammatory changes associated with the infection are less marked in duodenal ulcer subjects. The differences in the expression of the inflammatory response appear to be linked to differences in colonization density. Our findings further emphasize the multifactorial nature of the pathogenesis of peptic ulcer disease, as host or environmental factors may be invoked to explain the differences in colonization patterns and inflammatory responses observed.

CHAPTER 4

THE INCIDENCE OF DUODENAL GASTRIC METAPLASIA AND *HELICOBACTER PYLORI* COLONIZATION IN DUODENAL ULCER SUBJECTS FROM A DEVELOPING COUNTRY

4.1 INTRODUCTION:

One of the difficulties of implicating *H. pylori* in the pathogenesis of duodenal ulcer disease has been the conceptual difficulty in implicating what appears to be a predominantly gastric infection in the pathogenesis of duodenal ulceration. While the "gastrin link" hypothesis (Levi 1989), discussed in Paragraph 2.5.3.1.4 and Chapter 5 of this thesis, attempted to make the link between gastric (antral) infection and duodenal ulcer disease by means of the postulated effect of the organism on gastrin and acid secretion, the organism is capable of inducing a local inflammatory reaction, thereby at least having the potential to cause localised mucosal damage (Paragraph 2.5.3.1.4). The inability of *H. pylori* to bind to mucosa other than gastric has been alluded to (Para 2.4.3). For *H. pylori*, therefore, to be directly involved in duodenal inflammation the organism would have to have gastric mucosa to which it can bind.

The presence of (apparent) gastric tissue in the duodenum of subjects was reported for the first time by James in 1963 and published in article form in 1994, although he alludes to a number of earlier workers who may have described the phenomenon in humans, but whose work did not allow for firm conclusions to be drawn (James 1964). James speculated on the origin of the "duodenal gastric tissue", concluding that, based on the findings in Zollinger Ellison syndrome, the gastric tissue in the duodenum probably represented a protective response to acid exposure.

This speculation was supported by the experimental study of Rhodes, who subjected cats to histamine injections over a period of up to four weeks and compared the duodenal morphology to that of uninjected controls (Rhodes 1964). Patches of gastric epithelium were noted in ten of the 16 injected cats and in only one of the seven control animals.

Steer was probably the first to identify *H. pylori* in gastric metaplastic tissue in duodenal ulcer subjects (Steer 1984), although, much like his earlier report of gastric bacteria in peptic ulceration (Steer 1975), a connection was not made with existing data at the time. Seemingly unaware of the work and suspicions of Marshall and Warren, published the previous year (Marshall 1983, Warren 1983), Steer published his findings in 11 patients with duodenal ulceration, documenting duodenal bacteria, associated with gastric metaplasia, in four of the 11. At the same time, he reported that he could not identify bacteria in three of the 11 patients in whom gastric metaplasia could be identified.

Gastric metaplasia has been identified as a risk factor for duodenal ulceration (Carrick 1989). In their study of 137 subjects (46 with active duodenal ulceration, 44 with healed ulcers and 47 "normal" subjects), Carrick et al estimated that, while the presence of antral infection by *H. pylori* was associated with a risk ratio of 7.6, the presence of gastric metaplasia in the duodenum carried a risk ratio of 6.2 for the development of duodenal ulceration. The presence of duodenal infection with *H. pylori* was associated with a much higher estimated chance for the development of duodenal ulceration, however, the risk ratio being calculated as 51. The compounding effect of *H. pylori* infection on the risk ratio for duodenal ulceration is not unexpected, as gastric metaplasia does not appear to be uncommon in the absence of duodenal ulceration.

Carrick et al identified gastric metaplasia in 15 (32%) of 47 normal subjects, but could only identify *H. pylori* in one (2% of total) normal subjects. In the pre-*H. pylori* era, Kreuning et al reported gastric metaplasia in 32 (64%) of 50 patients without clinical or endoscopic evidence of gastroduodenal disease (Kreuning 1978), while Shousha et al found metaplastic tissue in 30 (86%) of 35 dyspeptic subjects without peptic ulceration (Shousha 1983).

The prevalence of gastric metaplasia and *H. pylori* in duodenal ulceration has been studied in a number of developed countries (Table 4.1).

Table 4.1: Reported incidence of gastric metaplasia and duodenal *H. pylori* colonization in duodenal ulcer subjects from developed countries.

Author:	Sample	Gastric metaplasia + n (% of sample)	*H. pylori* + n (%of sample)
Shousha 1983:	8	8 (100)	NR
Steer 1984:	11	7 (64)	4 (36)
Johnston 1986:	53	N/R	45 (85)
Johnston 1988:		N/R	85 (96)
Carrick 1989:	46	42 (91)	38 (83)
Taha 1993:	25	16 (64)	N/R
Noach 1993:	61*	55 (90)	N/R
Satoh 1993:	75	54 (72)	25 (33)

N/R Not Reported.
* Estimate of *H. pylori* prevalence cannot be made as group includes eradicated subjects.

While most of these observations are based on studies evaluating the gastric status of *H. pylori*, Amarapurkar et al have claimed that the incidence of gastric metaplasia and *H. pylori* infection in the duodenum is lower in developing than in developed countries (Amarapurkar 1993). These workers took multiple duodenal biopsies from 46 subjects with active duodenal ulceration. They detected gastric metaplasia in only

13.3% of these and found *H. pylori* in 8.3% of the 46 subjects - detection rates much lower than those reported from most Western studies.

4.2 AIM OF STUDY:

This descriptive study was undertaken to determine the prevalence of gastric metaplasia and duodenal *H. pylori* infection in subjects with active duodenal ulceration, in our Third World population.

4.3 PATIENTS AND METHODS:

4.3.1 Patients:

Seventy four patients with active duodenal ulceration, recruited as part of two duodenal ulcer studies, were investigated. These patients gave fully informed, written consent, and the studies were approved by the Ethics and Research Committee of the University of Cape Town.

4.3.2 Endoscopy:

All patients were endoscoped using standard Olympus fibreoptic gastroscopes of the P and X series. Patients received Xylocaine local anaesthetic spray for pharyngeal anaesthesia and received midazolam, 2.5mg IV as sedation, unless contraindicated.

4.3.3 Biopsies:

Each patient had biopsies taken from the antrum and duodenum, as close as possible to, and within 5mm of, the duodenal ulcer. Two gastric biopsies were taken from the antrum, within 5cm of the pylorus. The

duodenal biopsy was taken as close to the ulcer margin as possible, while attempting to avoid the ulcer slough.

Gastric and duodenal biopsies were placed in 10% neutral formalin before being further processed for histolopathological examinations. All biopsies were stained with haematoxylin and eosin and, in cases where *H. pylori* could not be readily identified, additional sections were stained by the modified Giemsa technique. All histopathological sections were evaluated, or reviewed, by the same pathologist.

4.4 RESULTS:

The results are summarised in table 4.2.

Table 4.2: Summary of antral and duodenal *H. pylori* and gastric metaplasia status:

	Males n (% of total)	Females n (% of total)	Total
Patients entered:	57 (77)	17 (23)	74
Patients evaluated:	50 (82)	11 (18)	61
Antrum, *H. pylori* positive:	49 (82)	11 (18)	60
Duodenum, gastric metaplasia positive:	38 (88)	5 (12)	43
Duodenum, *H. pylori* positive:	30 (88)	4 (12)	34
Duodenum, gastric metaplasia and *H. pylori* positive:	30 (88)	4 (12)	34

Dual biopsies were attempted in 74 patients. Fifty seven of these were males and 17 females, with a mean age of 38.6 years (SD 11 years.). Of these 74 patients, the results from 13 are not included because of the poor quality of the duodenal biopsies. All but three of the study subjects were from the socioeconomically disadvantaged, non-white community

(see Chapter 6). Fifty of the evaluable patients were male and 11 female, while the age (±SD) of the group was 38.3 (±11.4) years.

H. pylori was detected histologically in the antrum in all but one of the 61 patients reported here. In this patient, gastric metaplasia was not present in the duodenum nor could *H. pylori* be identified in the duodenum.

H. pylori could be identified in the duodenum in 34 (56%) of the 61 subjects. Duodenal gastric metaplasia could be identified in 43 (70%) of the 61 subjects. Thirty four (79%) of the subjects with evidence of gastric metaplasia in the duodenum were positive for duodenal *H. pylori* as well.

The patients with gastric metaplasia in the duodenum tended to be younger than those without. Those with detectable metaplasia were 36 (±10) years, while those without detectable metaplasia were 43 (±13) years. This difference in age distribution was not, however, significant ($p > 0.05$, Student's t-test).

While female subjects made up 18% of the evaluated sample group, they represented only 12 % of the metaplasia positive group and 33% of the gastric metaplasia negative group. This apparent difference in gender distribution did not achieve statistical significance ($p > 0.05$, Fisher's exact test).

4.5 DISCUSSION:

Our findings, both with regard to the presence of gastric metaplasia and *H. pylori* in the duodenum of duodenal ulcer subjects in this sample of patients, representing predominantly the socioeconomically disadvantaged segment of our community, are in keeping with those reported from Western/developed communities, and differ markedly from the findings of Amarapurkar et al, discussed earlier (Amarapurkar 1993). We may indeed have underestimated the prevalence of both gastric metaplasia and *H. pylori* infection. The fact that the organism was found in ±80% of subjects in whom gastric metaplasia was detected suggests that the infection is a frequent concomitant of duodenal ulcer, but that "biopsy error" may be responsible for an underestimation of the true prevalence. This finds some support in the work of Wyatt et al, who estimated that a single duodenal biopsy would detect only 63% of the actual incidences of gastric metaplasia found by multiple biopsies (Wyatt 1990). Marshall, on the other hand, has claimed a pick-up rate of 92% for gastric metaplasia, with a single biopsy, taken from the ulcer margin (Marshall 1988).

Preliminary reports from two other developing countries are similar to our own with regard to the presence of metaplasia in duodenal ulcer disease. Zhuo et al found duodenal gastric metaplasia in 53 (82%) and duodenal *H. pylori* in 44 (69%) of 64 Chinese duodenal ulcer patients from the Guangzhou Province (Zhuo 1994). The experience from Algeria is similar, with gastric metaplasia being detected in 44 (73%) of 60 patients with duodenal ulceration (Biad 1994).

The finding that duodenal gastric metaplasia occurs commonly in duodenal ulcer subjects, and that the duodenum is commonly infected by *H. pylori* in these patients, is compatible with the theory that gastric colonization by the organism may predispose to duodenal ulceration, the gastric *H. pylori* spreading to the duodenum in subjects with gastric metaplasia, thereby providing a local agent capable of weakening the duodenal mucosal resistance. This concept, described by Goodwin as the "leaking roof" theory of ulcerogenesis for duodenal ulcer disease, suggests that duodenal colonization of gastric metaplastic tissue by *H. pylori* leads to weakening of the mucosal barrier, by virtue of the documented direct toxicity of the organism, as well as by its ability to induce an inflammatory response at the site of infection (Discussed in paragraph 2.5.3.1.4). This weakening of the mucosal barrier, it is argued, allows the inherent aggressive agents, pepsin and gastric acid, to damage the mucosa (Goodwin 1988). This theory is compatible with clinical experience, in that acid (and thus also pepsin secretion) suppression, as well as therapy that enhances mucosal protection, can be expected to heal the duodenal ulcer, while failure to eradicate the organism would subject the diseased mucosa to acid/peptic attack once therapy is withdrawn, leading to ulcer relapse.

The relationship between duodenal gastric metaplasia and gastric *H. pylori* may of course be more complex. If the "gastrin link" hypothesis (Levi 1989) is accepted (discussed in more detail in Chapter 5), *H. pylori* may be able to influence its own progression to the duodenum. As discussed, it is generally accepted that gastric metaplastic tissue in the duodenum represents a protective response to noxious stimuli in the duodenum, principally acid. If the "gastrin link" hypothesis is correct,

antral *H. pylori* infection will be associated with increased acid secretion in subjects, the duodenum will be exposed to an increased acid load, gastric metaplasia will develop, allowing the gastric organism to migrate to the duodenum, there weakening the mucosal barrier and leading to ulceration. On the basis of genomic studies, there can be little doubt that the same strain of *H. pylori* is usually responsible for the infection in both the stomach and duodenum of patients. Tee et al, using ribotyping, have shown in a sample of five patients that the same strain could be identified in different regions of the stomach, and in the duodenum (Tee 1992), while Prewett et al showed chromosomal DNA homology in organisms from gastric and duodenal biopsy sites in 13 of 15 patients (Prewett 1992).

There is some indication that gastric metaplasia of the duodenum is a reversible condition. This has been suggested by the finding that the prevalence of gastric metaplasia in the duodenum is lower following highly selective vagotomy than before surgery (Wyatt 1987). There is evidence, furthermore, that the incidence of gastric metaplasia is lower in patients treated with continuous acid suppression, when compared to those treated with intermittent acid suppression (Tucci 1990). If the "gastrin link" hypothesis is correct, eradication of the organism from the stomach may (eventually) be associated with a reduction in the extent of duodenal gastric metaplasia. The relationship between gastric metaplasia in the duodenum and *H. pylori* infection of the stomach and duodenum has been well investigated in only one study. Noach et al reported on their findings on the extent of gastric metaplasia following the eradication of *H. pylori* (Noach 1993). These investigators failed to identify a significant reduction in the extent of gastric metaplasia in the duodenum in 43 patients studied at least a year following eradication. It should be

noted, however, that a strong trend towards a reduction in the extent of gastric metaplasia was found, but this failed to reach significance ($p = 0.08$). Given the possibility of sampling error, as well as the lack of a specific time-frame in which the regression of gastric metaplasia can occur, the question of the effect of eradication on the regression of gastric metaplasia, and by inference gastric acid secretion, remains unanswered. This is further emphasised by the observation of Tucci et al, who demonstrated that long-term (5 years), continuous acid suppression significantly reduced the incidence of duodenal gastric metaplasia (Tucci 1990). It is clear that longer term post eradication follow-up studies are needed to elucidate the role of *H. pylori* in the development of duodenal gastric metaplasia.

In summary, the data shows that the prevalence of gastric metaplasia in duodenal ulcer subjects from our predominantly socioeconomically disadvantaged community in Western Cape is similar to that reported from First World or socioeconomically developed countries. Our findings are compatible with theories implicating *H. pylori* in the pathogenesis of duodenal ulcer disease, the organism being almost universally present in the stomach of duodenal ulcer subjects and, despite the potential for sampling error because of the single duodenal biopsy, being found in relation to duodenal ulceration in the majority of subjects.

CHAPTER 5

THE EFFECT OF ULCER HEALING AND *HELICOBACTER PYLORI* ERADICATION ON GASTRIC PHYSIOLOGY

5.1 INTRODUCTION:

"*Helicobacter pylori* clearly causes peptic ulcer, but how it does so is less clear" - this (under)statement by Calam, made in 1993, is still relevant (Calam 1993). The organism has, however, been causatively linked to many of the abnormalities in gastric physiology associated with peptic ulcer disease (discussed in paragraph 2.5.1.1 of this thesis). Interest has focussed especially on the role of the organism in the changes observed with regard to gastric acid secretion, pepsin and pepsinogen secretion as well as the effect of infection with the organism on the regulators of gastric physiology: gastrin, histamine and somatostatin.

Abnormalities in the secretion of gastrin, a regulator of gastric acid secretion, were the first to be linked to infection with *H. pylori.* Although Brady et al stated in 1988 that they were "unable to identify any consistent relationship between *C. pylori* and acid secretion and serum gastrin" (Brady 1988), this was merely the first of a number of conflicting observations with regard to gastric physiology that have been made. In the following year, Levi et al reported that they had identified an association between antral *H. pylori* infection, an increased basal and stimulated gastrin level and peak acid output in 25 patients, when compared to 6 patients without antral *H. pylori* colonization (Levi 1989). They proposed that antral colonization with *H. pylori* increased gastrin release and speculated that the chronic gastrin drive would increase acid secretion, by both direct stimulation and by its trophic effect on the parietal cell mass. Although not considered at the time, this mechanism

would also allow for increased pepsinogen secretion, an effect which is also gastrin and acid mediated (Basson 1988). The "gastrin link" hypothesis was born.

Observations on the relationship between *H. pylori* and increased gastrin secretion were soon extended, and its implications questioned. McColl et al demonstrated that the levels of basal and meal stimulated gastrin decreased in nine (non ulcerated) duodenal ulcer subjects following eradication (McColl 1989). They noted, however, that overall intragastric acidity, as measured by intraluminal pH meter, as well as nocturnal acid output, considered important in the pathogenesis of duodenal ulcer disease, did not decrease in these patients. It was observed, however, that daytime acid secretion may have been altered by eradication, as there appeared to be a reduced acid output in response to meals, an observation of some importance when the complex control of meal-stimulated gastric acid secretion is considered. These workers reported on their experience after a seven month follow-up period, and concluded that the decrease in gastrin was not associated with an early or late reduction in gastric acidity (McColl 1990). A similar observation, with an apparent inconsistency in the relationship between acid secretion and gastrin status, was made by Smith et al. These workers studied the 24-hour gastrin profile of 95 healthy subjects undergoing 24-hour intragastric pH-monitoring (Smith 1990), and found an obvious hypergastrinaemia in the eight subjects infected with *H. pylori*, but once again could not link this to increased 24-hour gastric acidity - they in fact demonstrated a "low, but normal" 24-hour intragastric acidity, a finding similar to that of Peterson et al (Peterson 1993). Graham et al also studied the effect of *H. pylori* eradication on the gastrin response to meal stimulation, the most common gastrin abnormality in duodenal ulcer disease (Graham 1990). They documented a reversal of the exaggerated

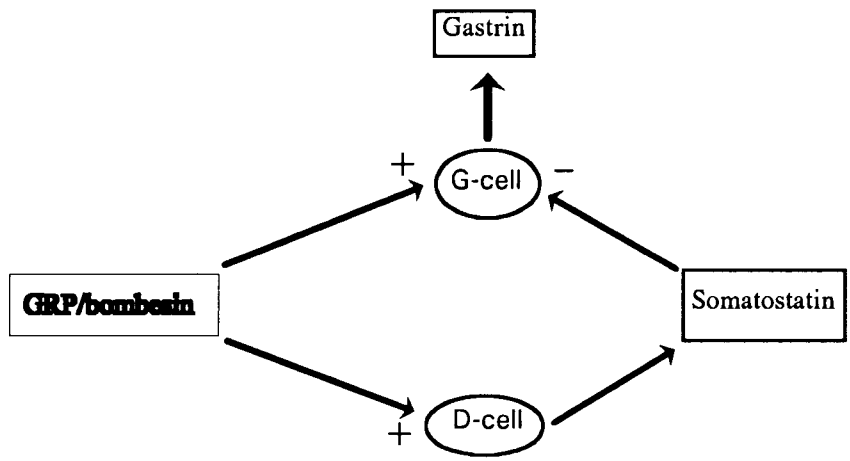
meal-stimulated gastrin release in subjects following the eradication of *H. pylori*. A further apparent contradiction of the gastrin link hypothesis was noted by Chittajallu et al, who found that the degree of hypergastrinaemia associated with *H. pylori* infection was the same in duodenal ulcer subjects and asymptomatic volunteers (Chittajallu 1992), although they found a decrease in serum gastrin concentrations following *H. pylori* eradication in duodenal ulcer subjects (Chittajallu 1992c).

Despite isolated reports to the contrary (Testino 1993), the association between *H. pylori* infection and abnormal gastrin secretion is now widely accepted. Further observations have identified both morphological and functional abnormalities which may be responsible for the abnormal gastrin response. Sankey et al have indicated that immunostaining of antral gastrin cells is quantitatively increased in *H. pylori* gastritis, an apparent contradiction of earlier findings in duodenal ulcer subjects (Sankey 1990, Creutzfeldt 1976). Other workers have provided plausible evidence for an abnormality of the control of gastrin release rather than an increase in G cell number as the underlying abnormality associated with *H. pylori* gastritis. Hirschowitz et al have shown that duodenal ulcer subjects have an exaggerated gastrin release in response to bombesin stimulation when compared to controls (Hirschowitz 1985). Subsequent studies have indicated that this effect of bombesin (or gastrin releasing peptide, GRP) was reversed by the eradication of *H. pylori* (Graham 1991d, Beardshall 1992).

GRP/bombesin and somatostatin have an interesting functional relationship in the stomach. The somatostatin, or D-cells, lie in close proximity to the gastrin cells in the antrum where somatostatin exhibits an inhibitory paracrine influence on gastrin release (Schubert 1991). GRP/bombesin stimulates both gastrin and somatostatin release from the

antrum, the nett effect on gastrin release thereby reflecting the interaction between the stimulatory effect of GRP/bombesin and the inhibitory effect of somatostatin, released from the D-cell by GRP/bombesin, both on the antral G-cell. The abnormal response of gastrin to GRP/bombesin does not, therefore, neccessarily reflect G-cell hyperfunction. The "hyperresponsiveness" may merely reflect the breakdown of inhibitory - somatostatin - control, an hypothesis which would be compatible with observations made regarding the paucity of D-cells in subjects with duodenal ulceration (Polak 1978).

Figure 5.1: Relationship between GRP/bombesin, somatostatin and gastrin secretion:



The relationship between *H. pylori* gastritis and gastric somatostatin has been investigated and found to be compatible with the hypothesis that infection by the organism in some way decreases the levels of

somatostatin in the stomach. Kaneko et al have demonstrated a reduction in immunoreactive-somatostatin in the gastric mucosa and juice of subjects infected by *H. pylori* (Kaneko 1992), a finding confirmed by Sumii et al in the antrum of infected subjects (Sumii 1993). Somatostatin measurements may be problematic, however, as the peptide is of a paracrine nature in the stomach, being destroyed locally. Moss et al therefore used molecular techniques to demonstrate a rise in antral somatostatin messenger RNA, as well as the number of immunoreactive D-cells, following eradication (Moss 1992). Le et al also reported an increase in antral D-cell numbers following eradication (Le 1993), but Graham et al could not show this (Graham 1993c). It is worth noting that most of these observations relate to the antrum, the principal site of gastritis in duodenal ulcer disease. We have shown, however, that the body of the stomach is also commonly colonized, albeit mildly, by *H. pylori* in duodenal ulcer disease (Chapter 3).

It would appear, therefore, that most of the abnormalities observed with regard to gastrin secretion in *H. pylori* infected individuals, whether ulcerated or not, can be explained by mechanisms related to *H. pylori* infection. The question remains: what does this mean in functional terms - is the hypergastrinaemia associated with abnormalities of acid secretion, or does it represent a peculiarity with no functional/pathophysiologic importance? While the effect of acute *H. pylori* gastritis is clearly a suppression of acid secretion (Graham 1988a), the effect of the chronic infection on gastric secretion has not been elucidated. Many workers have attempted to demonstrate that the observed abnormalities in gastrin homeostasis in *H. pylori* infected subjects translate into real abnormalities of acid secretion. As can be seen from table 5.1, standard methodology has largely failed to do this.

Table 5.1: The effect of *H. pylori* on gastric acid secretion as assessed by exogenous gastrin stimulation or intragastric pH-monitoring.

Study	n	Model	Method	Findings
Brady 1988:	36	DU/GU & NUD	Pentagastrin	No relationship between HP and acid secretion.
Levi 1989#:	25&6	DU	Pentagastrin	Increase PAO;HP status by CLO only
McColl 1989*:	9	DU,pre & post erad	24Hr pH & nocturnal asp	Overall intragastric acidity and nocturnal acid output unchanged; ? reduced postprandial secretion.
Montbriand 1989:	10	NUD, pre & post erad	Pentagastrin	No consistent/significant change in acid output following eradication.
Fullarton 1991*:	8	DU, pre & post erad	Pentagastrin & meal stimulation	No difference in basal, BAO or MAO following eradication, or 12 months later.
Wagner 1992:	70	DU 21 Gastritis 35 Normal 14	24 Hr pH	No significant difference in intragastric acidity between HP positive and negative subjects
Bechi 1992:	37	NUD	24 Hr pH	No difference HP+ & HP-
Chittajallu 1992b*:	8	DU, pre & post erad	Pentagastrin, PCS by 50% MAO dose	No significant change in PCS
Moss 1993#:	9	DU, pre & post erad	G17 dose response studies	Reduction in Basal, but not stimulated, secretion following eradication. PCS unchanged.
Peterson 1993:	136 & 52	Healthy:- 63 HP+, 73 HP- DU: 52	BAO, PAO (pentagastrin, histamine, G17), meal stimulated	DU subjects: increased basal & PAO when compared to normal; lower BAO in HP+ normals. "Hypergastrinaemia related to HP, BAO and PAO not"
Chiba 1993:	22	DU - 9 Healthy - 13	Step dose pentagastrin	Trends for reduction in BAO, MAO, Total Acid Output and PCS, but no significant differences in DU subjects following healing & eradication.
Haruma 1994:	55	Healthy - 29 HP+, 26 HP-	Tetragastrin BAO, MAO	BAO similar in both groups, MAO slightly higher in HP+, but not significantly so.

*, # = Same center
HP = *H. pylori*
BAO = Basal acid output
M/PAO = Maximal/Peak acid output
Healthy = healthy volunteers
DU = duodenal ulcer
NUD = Non ulcer dyspeptics
PCS = Parietal cell sensitivity
Contr = Controls
G17 = Human gastrin heptadecapeptide

Thus, despite marked abnormalities in gastrin release attributed to *H. pylori*, standard clinical methodology has not identified a consistent abnormality of basal or stimulated acid secretion associated with the raised gastrin levels associated with *H. pylori* gastritis in a wide spectrum of study subjects. These studies may have one shortcoming, however. All but one have evaluated eradicated patients shortly (4 weeks) after eradication. However, in the one study performed 12 months following eradication on eight patients, no change in acid secretion was observed (Fullarton 1991). This suggests that the postulated effect of *H. pylori* on gastric acid secretion is unlikely to be mediated by gastrin-dependant changes in parietal cell mass.

While the relationship between *H. pylori*, gastrin and gastric acid secretion has been extensively studied, gastric pepsin secretion has not. It has been demonstrated that gastric *H. pylori* infection is associated with an increase in serum pepsinogen levels, thought to reflect increased gastric secretion of pepsin, and that this hyperpepsinogenaemia is reversed following eradication (Chittajallu 1992c, Oderda 1990). Two studies have also shown that *H. pylori*, or its components, can stimulate pepsinogen release from rabbit gastric glands (Cave 1991) and guinea pig gastric mucosa mounted in Ussing chambers (Young 1992). Data on intragastric pepsin in relation to *H. pylori* status is extremely limited, however. Yahav et al have studied the effect of eradication therapy on mucosal activated pepsinogen activity and found that it was lower in infected subjects and rose when evaluated three months following eradication therapy (Yahav 1992). This is a surprising finding, as pepsin is an important ulcerogen and serum pepsinogen levels have been noted to fall following eradication of *H. pylori*.

Given the theoretical shortcomings of the "acute" acid secretory studies and the paucity of data with regard to the effect of *H. pylori* eradication on gastric pepsin status and the perceived importance of these two "aggressive factors", we have attempted to investigate the relationship between gastric *H. pylori* and gastric secretion of acid and pepsin. This was done by studying the effect of *H. pylori* eradication on acid and pepsin secretion, both in the short term (one month following eradication) and longer term (12 months following documented eradication).

5.2 PATIENTS AND METHODS:

5.2.1 Patients:

Adult (18-65 years) patients with active duodenal ulcer disease were eligible for study. Patients had to be healthy, except for the presence of duodenal ulceration, while women of childbearing potential and patients with complicated ulceration were excluded from study. Patients were required to give informed, written consent for participation in the study, which was approved by the Ethics and Research Committee of the University of Cape Town. Patients were free to withdraw at any stage during the study.

5.2.2 Endoscopy schedule:

Patients were endoscoped at entry to the study, immediately following cessation of healing therapy, 72 hours after documented healing (the day before acid studies were performed), 4 weeks following cessation of eradication therapy and 12 months following documented eradication. Except for the initial endoscopy, whenever possible all endoscopies were performed by the same endoscopist.

Endoscopy was performed with fibreoptic equipment (Olympus XQ20 or PQ20 series), using midazolam 2.5mg IV as sedation (unless contraindications to its use existed or the patients requested that it not be administered). Standard cleaning and sterilization of equipment with 2% glutaraldehyde was used.

5.2.3 Therapy:

Patients were randomised to receive an H_2-receptor antagonist (ranitidine 300mg nocte) or sucralfate 2g bd. Initial ulcer healing therapy was for a period of six weeks, but a further period of four weeks was allowed if the ulcer was not healed after six weeks (healing = complete epithelization). Patients not healed after 10 weeks of therapy were considered not eligible for study.

Following documented ulcer healing, all patients were placed on "Triple Therapy". This consisted of a colloidal bismuth preparation - colloidal bismuth subcitrate - 120mg qid, metronidazole 400mg tds and tetracycline (or amoxicillin), 250mg (patients weighing <75kg) or 500mg (patients weighing > 75kg) qid. "Triple Therapy" was administered for a period of two weeks.

5.2.4 *H. pylori* status:

H. pylori status was determined at entry to study, following healing, a minimum of four weeks after cessation of therapy, as well as six and 12 months following documented eradication.

H. pylori status was determined from antral biopsies, taken within 5 cm of the pylorus. Biopsies were examined by rapid urease test (one biopsy)

according to the method of Arvind et al (Arvind 1988), histological assessment of biopsies stained by the modified Giemsa method (2 biopsies), and culture (one biopsy). Samples for histology were immediately placed in 10% neutralised formalin before further processing, while samples for culture were immediately placed and transported in a jar under microaerophilic conditions (Oxoid Gas Generating BR38, Basingstoke, Hampshire, UK). The specimen was cultured on tryptose blood agar (CM 233; Oxoid Ltd) containing lysed horse blood (10% volume/volume) at 37°C under microaerophilic conditions [12% CO_2, 88% air (6% O_2), 95% humidity] for a minimum of 7 days before being considered negative. *H. pylori* was positively identified by colony morphology and urease reaction (Christensen's urea slope).

Patients were considered *H. pylori* positive if the organism was detected with any of these tests.

5.2.5 Gastric secretory studies:

Acid secretory studies were performed (i). at entry to study, prior to commencement of therapy, (ii). 96 hours after documented ulcer healing, (iii). four to five weeks following cessation of "Triple Therapy" and (iv) 12 months later in those subjects in whom the organism had been eradicated.

Acid studies were performed as follows: Following an overnight fast, a nasogastric tube was passed and its position in the dependant part of the stomach confirmed by fluoroscopy. Saliva was aspirated by intermittent suction throughout the collection period and discarded. A basal collection period of one hour was followed by sequential intravenous administration

of pentagastrin at a dosage of of 0.1μg/kg ("low dose") and 6μg/kg ("high dose"), each for 60 minutes. Gastric juice was aspirated by continuous suction and samples for analysis collected for 10 minute periods.

Hydrogen ion concentration was determined by titration to pH 7 using 0.2M NaOH (Radiometer, Copenhagen). The acid output, in mmol/h, was calculated for the basal and stimulated periods by using the three highest consecutive 10-min acid outputs of each collection. The parietal cell sensitivity (PCS) was calculated as the ratio of low dose to high dose acid output, expressed as a percentage.

At the time of aspiration, samples were collected for assay of gastric juice pepsin concentration. After addition of glycerol (1ml/10ml juice) these samples were stored at -20^{0}C until analysed. Gastric juice pepsin determination was after the method of Basson et al (Basson 1988b), as follows: For each assay one aliquot of ^{125}I-labelled albumin and 0.25g of albumin were added to 25ml of a 0.031M HCl/0.05M KCl buffer, pH 1.8. To each 100 μl sample of gastric juice (or of diluted standard), 500μl (approx 100,000d.p.m) of the acid-albumin solution was added. All remaining pepsinogen in each sample was, thus, by activation, converted to pepsin. The samples were incubated for 40 minutes at 37^{0}C. One ml of 5% w/v trichloroacetic acid was added to each tube to precipitate the undigested albumin and the precipitate separated by centrifugation at 3000rpm for 35 minutes at 4^{0}C. The digested fragments of ^{125}I-albumin remaining in the supernatant were quantified by automated gamma scintigraphy. Each assay was standardised using purified porcine pepsin (Sigma Chemical Co, St Louis, Missouri), of known activity, to construct a standard curve, corresponding to pepsin concentrations of 10 - 10000 U/ml.

One unit of peptic activity was defined conventionally as the amount needed to produce a change in absorbance at 280nm of 0.001/minute at pH 2.0 at 37°C, measured as trichloroacetic acid-soluble products, using hemoglobin as substrate. Results are expressed as pepsin concentration (U/ml) and output (U $x10^3$/hour).

5.2.6 Statistical analysis:

Because of the sample size, a normal distribution of data points was not assumed and non-parametric methods were used in analysis. Analysis of the total data set was by means of the Wilcoxon signed rank (paired data points) and rank sum (unpaired data points) methods. In addition, a separate analysis of the available paired data points was done.

Statistical analysis was done using the Epistat software package.

5.3 RESULTS

Data from nine patients was available for analysis. Of these, seven are males and the mean age of the group (±SD) was 36 (±9.7) years at entry. Five patients received ranitidine as healing therapy and four were healed with sucralfate. Six patients were healed following six weeks of therapy and three after 10 weeks. *H. pylori* was eradicated in nine patients following "Triple Therapy". Eradication was questioned in one patient, as the culture was contaminated by fungal overgrowth; the 12 month follow-up data confirmed, however, that the organism was eradicated. One patient was re-infected by *H. pylori* at some stage following the six month endoscopy visit. As she was unequivocally eradicated of the organism up to the six month endoscopy, her initial gastric secretion studies are included; the 12 month studies have not

been analysed. The 12 month follow-up data from two other patients is not available.

5.3.1 Acid Secretion:

5.3.1.1 Basal acid output:

Data on basal acid output (BAO) was available in nine patients at entry, following healing and eradication and in six at the 12 month follow-up.

Table 5.2: Changes in BAO (mmol/h) during the follow-up period:

Healing Therapy	Entry	Healed	Eradicated	12 Months
Ranitidine	11.0	2.8	2.6	2.6
Ranitidine	5.8	3.2	2.4	1.6
Sucralfate	8.0	2.8	1.4	11.4
Sucralfate	2.4	0.2	2.0	4.6
Sucralfate	6.4	1.0	10.2	10.2
Ranitidine	5.6	3.6	1.2	N/A
Ranitidine	8.6	11.6	7.8	11.6
Sucralfate	13.4	2.8	5.6	N/A
Ranitidine	6.8	12.6	9.8	N/A
n:	9	9	9	6
mean:	7.6	4.5	4.8	7.0
median:	6.8	2.8	2.6	7.4
S.D.:	3.2	4.4	3.6	4.6

The BAO clearly tended to fall following healing and eradication, the BAO being lower than the entry values at these points in seven of the nine subjects. This reduction in BAO when compared to entry values did not, however, achieve statistical significance (Wilcoxon signed rank test). The BAO at 12 months was no different to the entry values (Wilcoxon rank sum test). When analysis is restricted to the six patients in whom 12 month follow-up data is available, this trend is similar: no significant reduction in BAO could be identified over the 12-month follow-up period

when compared to entry (entry median = 7.2, 12-month median 7.4, p = not significant (N/S).

5.3.1.2 Low dose pentagastrin stimulation:

Data on acid secretion in response to low dose pentagastrin stimulation was available in nine patients at entry, following healing and eradication and in six at the 12 month follow-up.

Table 5.3 Gastric acid secretory response (mmol/h) to low dose pentagastrin stimulation:

Healing Therapy	Entry	Healed	Eradicated	12 Months
Ranitidine	40.0	30.8	35.2	18.4
Ranitidine	31.0	25.6	18.2	23.4
Sucralfate	60.6	25.2	35.6	35.4
Sucralfate	11.4	13.6	19.0	17.7
Sucralfate	25.4	11.8	25.6	27.8
Ranitidine	15.4	21.2	32.0	N/A
Ranitidine	25.4	25.6	26.8	30.8
Sucralfate	20.8	19.2	29.8	N/A
Ranitidine	21.7	16.0	22.1	N/A
n:	9	9	9	6
Mean:	28.0	21.0	27.1	25.6
Median:	25.4	21.2	26.8	25.6
S.D.:	14.8	6.4	6.5	7.0

Once again, no statistically significant effect of eradication could be demonstrated in these subjects either in the short term (four weeks following eradication therapy) or in the long term (12 months following eradication). Following eradication, the acid secretion remained essentially unchanged in two subjects, increased in four and decreased in three. Twelve months following therapy, acid secretion, as compared to the entry values, was decreased in three subjects and increased in the remaining three. Analysis of the data for the six subjects with complete

data collection yielded similar results (entry median = 28.2, 12 month median = 25.6, p = N/S).

5.3.1.3 High dose pentagastrin stimulation:

Data, summarised in table 5.4, was available in nine patients at entry, after healing and following eradication and in six patients at the 12 month follow-up visit.

Table 5.4: Gastric acid secretory response (mmol/h) to high dose pentagastrin stimulation:

Healing Therapy	Entry	Healed	Eradicated	12 Months
Ranitidine	62.2	69.2	60.8	46.4
Ranitidine	60.4	62.4	35.4	31.0
Sucralfate	83.6	82.8	84.6	78.0
Sucralfate	37.4	34.4	32.8	28.4
Sucralfate	46.4	34.6	38.0	49.4
Ranitidine	44.2	37.4	38.8	N/A
Ranitidine	59.6	48.2	40.6	55.4
Sucralfate	51.8	40.0	54.2	N/A
Ranitidine	96.8	78.8	70.8	N/A
n:	9	9	9	6
Mean:	60.3	54.2	50.7	48.1
Median:	59.6	48.2	40.6	47.9
S.D.:	19.1	19.4	18.1	18.1

There was a clear trend for the acid secretory response to fall, when compared to entry, at the healing visit (secretion lower in seven, increased in two) and the post-eradication visit (lower in seven, increased in two). This trend achieved statistical significance in the post eradication study ($p<0.03$, Wilcoxon signed rank test). Significance was lost at the 12 month follow-up, although acid secretion was still lower than at entry in four of the six subjects available for analysis. Analysis of the data for the six subjects with a complete 12 month data set identified a similar

reduction in acid output following eradication, significance once again being lost at the 12 month follow-up visit (entry median = 60.0, post eradication median = 39.3, 12 month median = 47.9; p = 0.03, entry vs post-eradication, p = 0.06, entry vs 12 months).

5.3.1.4 Parietal cell sensitivity:

Changes in parietal cell sensitivity, summarised in table 5.5, could be assessed in nine patients following healing and eradication and in six 12 months following eradication.

Table 5.5: Parietal cell sensitivity (%) following healing and eradication:

Healing Therapy	Entry	Healed	Eradicated	12 Months
Ranitidine	64.3	44.5	57.9	40.0
Ranitidine	51.3	41.0	51.4	75.4
Sucralfate	72.5	30.4	42.1	45.0
Sucralfate	30.5	39.5	57.9	61.9
Sucralfate	54.7	34.1	67.4	56
Ranitidine	34.8	57.0	82.0	N/A
Ranitidine	42.6	53.1	66.0	55.6
Sucralfate	40.2	48.0	54.9	N/A
Ranitidine	44.8	41.0	62.4	N/A
n:	9	9	9	6
Mean:	48.4	43.2	60.25	55.7
Median:	44	41	57.9	55
S.D.:	13.7	8.5	11.2	12.6

No significant differences in PCS could be demonstrated during the follow-up period, following eradication. Although there was a tendency for the PCS to increase during the follow-up period, this did not achieve significance (p = 0.06, entry vs 12-month follow-up visit).

5.3.2 Gastric secretion of pepsinogen:

5.3.2.1 Basal gastric pepsin concentration and output:

Data on basal pepsin concentration and output, summarised in table 5.6, could be evaluated in nine patients at entry, seven at the healing stage, nine at the eradication phase and six at the 12-month follow-up.

Table 5.6: Basal gastric juice pepsin concentration and output:

Heal Rx	Entry		Healed		Eradicated		12 Months	
	Conc	*Out*	Conc	*Out*	Conc	*Out*	Conc	*Out*
Ran	2240	*197*	2950	*109*	3020	*127*	1445	*55*
Ran	1910	*164*	2089	*192*	2089	*129*	724	*35*
Sucr	2000	*192*	8320	*815*	4786	*172*	2660	*330*
Sucr	1413	*79*	N/A	*N/A*	1480	*104*	2138	*124*
Sucr	708	*115*	N/A	*N/A*	4467	*679*	1020	*153*
Ran	1290	*79*	1050	*67*	668	*20*	N/A	*N/A*
Ran	660	*88*	1150	*163*	1334	*99*	832	*98*
Sucr	1349	*183*	1349	*103*	1170	*84*	N/A	*N/A*
Ran	1350	*144*	1900	*340*	1260	*151*	N/A	*N/A*
n	9	*9*	7	*7*	9	*9*	6	*6*
Mean	1435	*138*	2686	*256*	2252	*174*	1469	*132*
Median	1350	*144*	1900	*163*	1480	*127*	1232	*111*
S.D.	542.9	*48.9*	2569	*262*	1501	*194.3*	778.3	*106*

Conc Pepsin concentration = U /ml
Out Pepsin output, U x10^3/h

Both pepsin concentration and output did not change significantly during the study period, although both exhibited a trend to rise on healing and fall to entry levels at the 12 month follow-up visit. Analysis limited to the subjects with continuous (complete) data sets confirmed this finding.

5.3.2.2 Gastric pepsinogen secretion in response to low dose pentagastrin stimulation:

Pepsinogen secretion in response to low dose pentagastrin stimulation could be determined in nine patients at entry, following healing and following eradication, and in six 12 months after eradication. The data is summarised in table 5.7.

Table 5.7: Gastric juice pepsin concentration and output in response to low dose pentagastrin stimulation:

Heal Rx	Entry		Healed		Eradicated		12 Months	
	Conc	*Out*	Conc	*Out*	Conc	*Out*	Conc	*Out*
Ran	2510	*783*	3695	*916*	4460	*776*	1349	*189*
Ran	1700	*435*	2630	*536*	2455	*378*	1479	*287*
Sucr	3981	*1330*	4790	*1044*	4678	*945*	1190	*257*
Sucr	1556	*209*	4571	*622*	1290	*266*	1687	*247*
Sucr	1496	*383*	2818	*496*	3715	*914*	1120	*202*
Ran	1170	*140*	1050	*180*	891	*171*	N/A	*N/A*
Ran	890	*203*	1290	*279*	1072	*187*	1811	*304*
Sucr	1412	*234*	1698	*282*	1290	*209*	N/A	*N/A*
Ran	1500	*504*	1910	*458*	1510	*438*	N/A	*N/A*
n	9	*9*	9	*9*	9	*9*	6	*6*
Mean	1801	*469*	2716	*534*	2373	*476*	1439	*247*
Median	1500	*383*	2630	*496*	1510	*378*	1414	*252*
S.D.	927	*379*	1379	*290*	1518	*317*	273	*45*

Conc Pepsin concentration = U /ml
Out Pepsin output, U $x10^3$/h

Interpretation of the data is hampered by the tremendous scatter of data points. The same trend seen in the basal data, i.e. a tendency for the pepsin concentration to rise on healing, only to fall again following eradication, is present. This tendency for the pepsin concentration achieves statistical significance for the healing visit ($p < 0.05$, Wilcoxon signed rank test, healing visit compared to entry). The decrease following

eradication does not achieve significance when compared to the entry data, but the 12 month concentration and output are both significantly lower than the healing concentration and output respectively ($p < 0.05$, Wilcoxon rank sum test). The trend for a reduction in pepsin output and concentration is confirmed when analysis is limited to the six subjects with complete data sets for the four evaluation points, but statistical significance is lost (concentration and output, healing vs 12 month $p = 0.06$).

5.3.2.3 Gastric pepsinogen secretion in response to high dose pentagastrin stimulation:

Gastric pepsinogen secretion in response to high dose pentagastrin stimulation could be assessed in nine patients at entry, eight following healing, nine following eradication and six at 12 months following eradication.

Table 5.8 Gastric juice pepsin concentration and output in response to high dose pentagastrin stimulation:

Heal Rx	Entry		Healed		Eradicated		12 Months	
	Conc	*Out*	Conc	*Out*	Conc	*Out*	Conc	*Out*
Ran	2950	*1115*	3980	*1819*	4780	*1692*	1496	*497*
Ran	1860	*662*	N/A	*N/A*	2371	*560*	1188	*264*
Sucr	3350	*1628*	4270	*2135*	3631	*1932*	1320	*652*
Sucr	1679	*430*	3162	*835*	870	*204*	1259	*247*
Sucr	1374	*429*	3388	*901*	3981	*1186*	920	*269*
Ran	740	*182*	690	*178*	692	*145*	N/A	*N/A*
Ran	1120	*441*	1580	*518*	1122	*314*	1585	*561*
Sucr	1096	*346*	1148	*303*	1050	*309*	N/A	*N/A*
Ran	1122	*570*	1350	*686*	1200	*468*	N/A	*N/A*
n	9	*9*	8	*8*	9	*9*	6	*6*
Mean	1699	*644*	2446	*921*	2188	*756*	1294	*415*
Median	1374	*441*	2371	*760*	1200	*468*	1289	*383*
S.D.	892	*451*	1404	*700*	1558	*674*	236	*176*

Conc Pepsin concentration = U /ml
Out Pepsin output, U $x10^3$/h

This data set is once again characterised by marked scatter of the data points. The tendency for the pepsinogen concentration to rise on healing and to fall following eradication, seen in the basal and low dose assessments is once again present, but does not achieve statistical significance, both within the complete data set and analysis of the five subjects with data at all study points.

Figure 5.2: Graphic representation of changes in gastric acid secretion following healing and eradication.

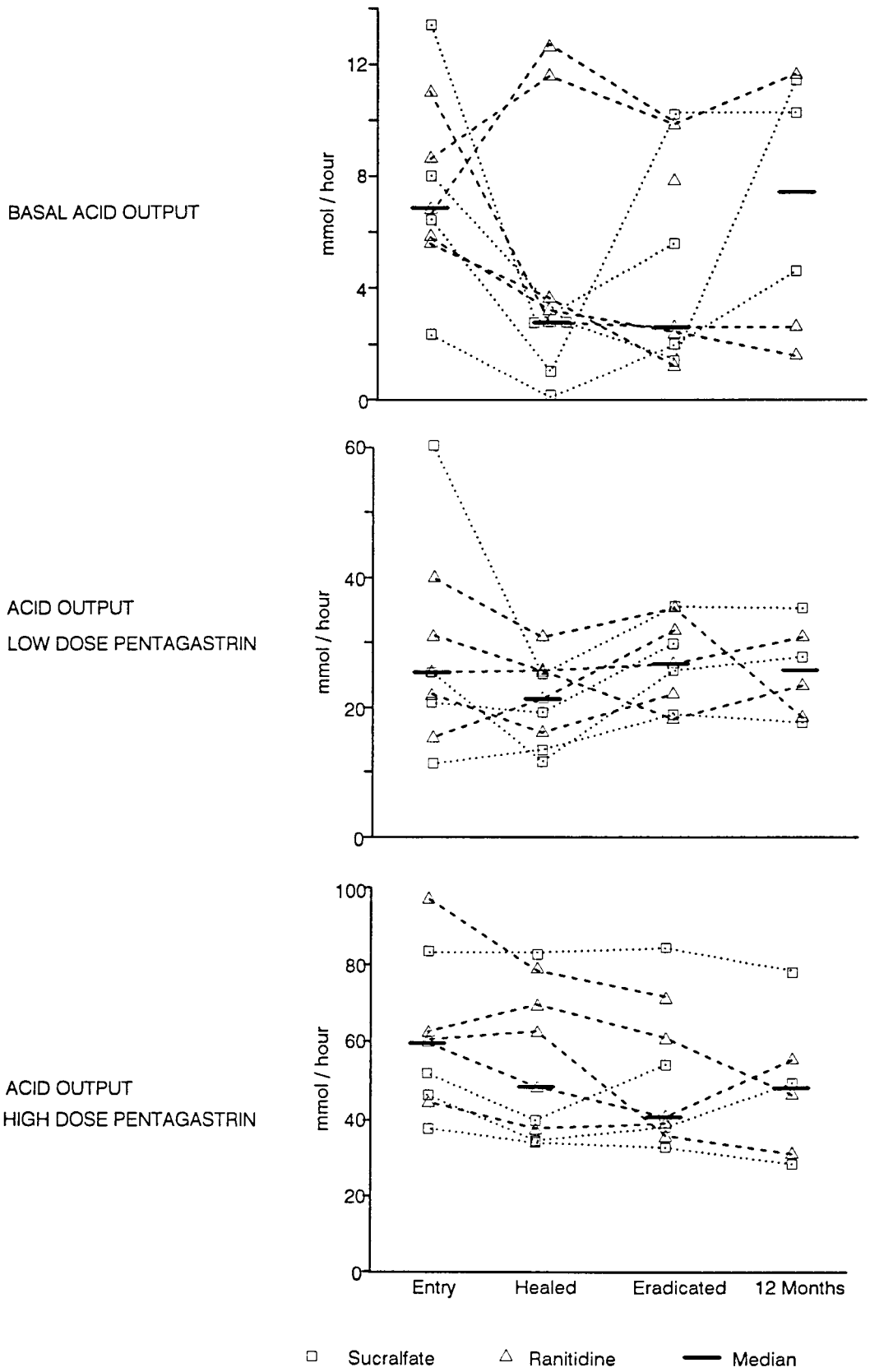
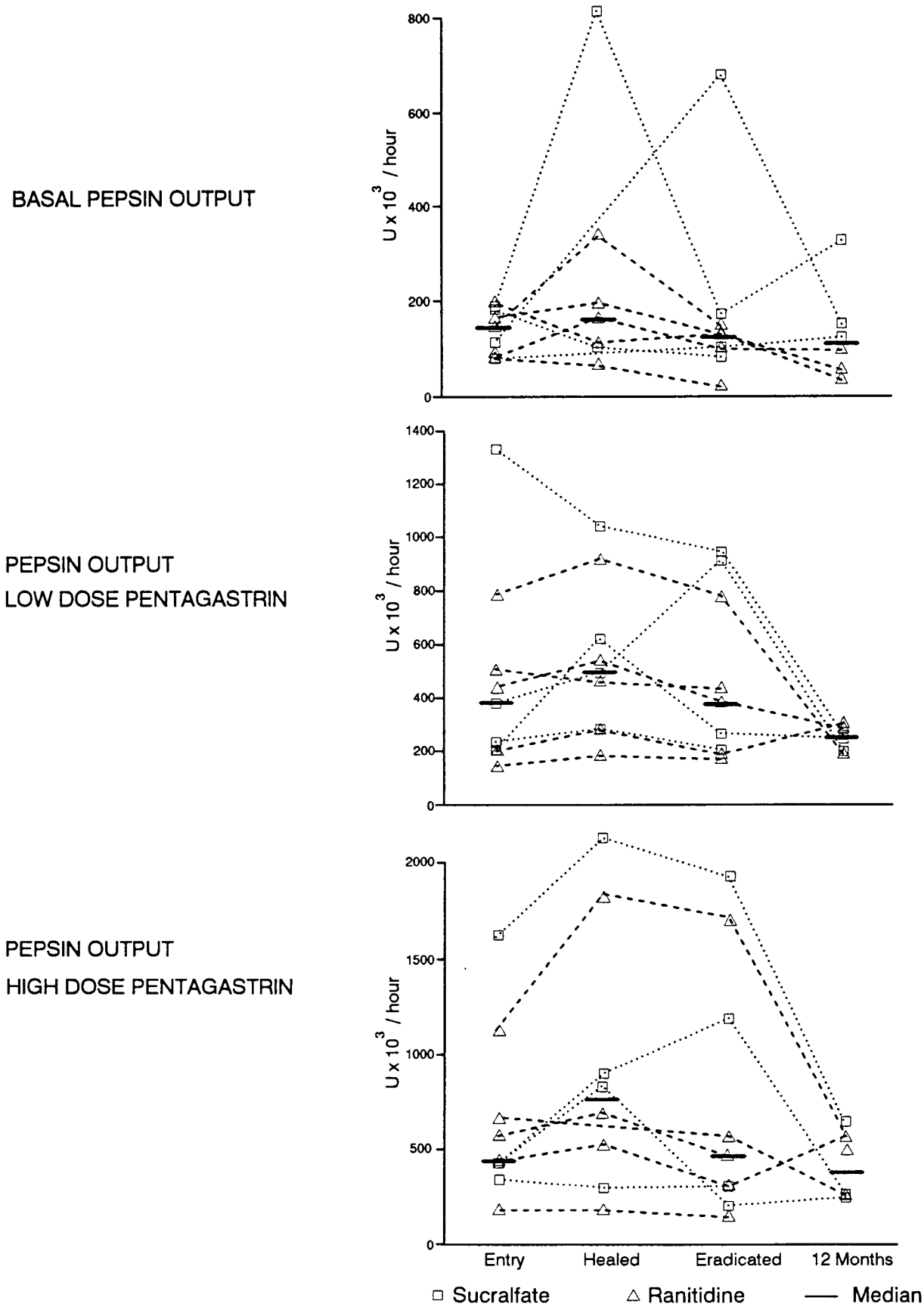


Figure 5.3: Graphic representation of changes in gastric pepsin output following healing and eradication.



5.4 DISCUSSION

In view of the wide scatter of the data, interpretation of the findings has to be cautious. Despite this limitation, certain trends with regard to gastric acid and pepsin secretion following eradication of *H. pylori* could be identified. The acid response to low-dose pentagastrin stimulation remained essentially unchanged. In contrast, a quite marked and, in this data set, significant, reduction in acid secretion following high dose pentagastrin stimulation occurred following eradication, a trend which persisted, but was not statistically significant, after 12 months. Parietal cell sensitivity to exogenous gastrin stimulation remained unchanged during the follow-up period. A sustained effect of eradication on any of the parameters of gastric acid secretion evaluated in these nine subjects, six of whom were followed up over the period of 12 months was thus not observed.

The findings are in general agreement with those of a number of investigators who have used standard, exogenous gastrin stimuli to investigate the effect of eradication (Table 5.1). The inability of the present study to demonstrate significant reductions in gastric acid secretion following eradication is, however, in sharp contrast to the findings of the Glasgow group who used Gastrin Releasing Peptide (GRP), the endogenous stimulant for gastrin release, to investigate the effect of eradication on gastric acid secretion. The Glasgow group have found that eradication of *H. pylori* is associated with marked decreases in acid secretion in healthy volunteers (El-Omar 1993), and duodenal ulcer subjects (El-Omar 1993b), and that the "abnormalities" in acid secretion associated with duodenal ulceration are reversed and normalised one year following eradication (El-Omar 1993c). It is noteworthy, however, that these results of El-Omar et al appear to be in conflict with earlier

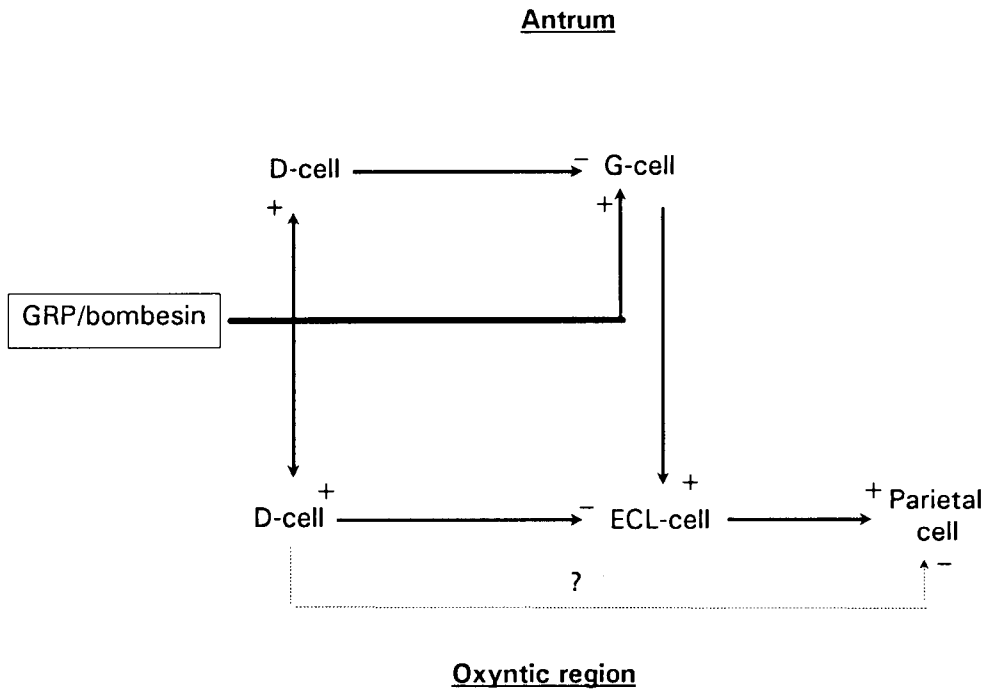
data from the same group, in which gastric acid secretion, as assessed by exogenous gastrin administration, remained unchanged following eradication (Fullarton 1991), a finding similar to that reported in this thesis.

These apparent differences with regard to the effect of eradication on gastric acid secretion may not, however, be irreconcilable; different stimuli have been used and it is clear that these agents stimulate acid secretion in different ways. The recognition of this difference in stimulation characteristics pre-dates the "*H. pylori* era" in peptic ulcer disease and was well demonstrated by Hirschowitz et al (Hirschowitz 1985). These workers identified a fundamental difference in acid secretory response patterns following stimulation with gastrin-17 and bombesin (or GRP), which mediates acid secretion by release of endogenous gastrin-17. The exaggerated release of gastrin following bombesin administration in duodenal ulcer subjects found in their study has already been mentioned. In addition, the data showed that high doses of GRP/bombesin were associated with a decreased acid secretory response in duodenal ulcer patients and controls. A higher GRP/bombesin dose was needed in duodenal ulcer patients before this decreased acid secretory response was noted, with inhibition occurring at a higher secretory plateau level than in non-duodenal ulcer subjects. This was interpreted as implying that an inhibitory control mechanism, which limited the acid secretory response, was stimulated by GRP/bombesin, but not by exogenous gastrin-17. Given the dosage differential between duodenal ulcer subjects and normals, it was postulated that subjects with duodenal ulceration were less sensitive to (or lacking in) this inhibitory mechanism.

It is possible that this observation, i.e of a dual stimulatory/suppressive effect of GRP/bombesin on gastric acid secretion, can explain the inability to demonstrate consistent changes in acid secretion following eradication when using exogenous gastrin stimulation. While Hirschowitz et al were only able to speculate on the nature of the suppressive mechanism activated by GRP/bombesin use, there is now evidence that the regulatory peptide, somatostatin, mediates the inhibition of gastrin secretion from antral G-cells while it also inhibits acid secretion (Wolfe 1988, Schubert 1990, Schubert 1991). As previously mentioned, it has been well demonstrated that GRP/bombesin stimulates antral G and D cells, thereby both stimulating (directly) and inhibiting gastrin secretion (paracrine action of somatostatin released from D-cells). In the oxyntic gland, GRP/bombesin stimulates the D cells, releasing somatostatin, which in turn inhibits parietal acid secretion in a paracrine fashion, either by direct inhibition of acid secretion by the parietal cell or by suppression of histamine release by the enterochromaffin-like (ECL) cell (Prinz 1993).

The observed differences in the results obtained with the two secretagogues - GRP/bombesisn and exogenous gastrin - may, in other words, reflect basic differences in their mode of action. On the one hand, GRP/bombesin appears to be more suited to the study of the global, physiological, **control** of acid secretion, while exogenous gastrin, which stimulates the parietal cell directly, is probably best suited to assess gastric secretory **capacity**.

Figure 5.4: Postulated interaction between GRP/bombesin, gastrin, histamine and acid secretion:



It is possible, therefore, that the assumption that *H. pylori* infection affects acid secretion directly is fallacious. The GRP/bombesin data suggests that *H. pylori* infection affects acid secretion indirectly, by reducing the inhibitory capacity of somatostatin on both gastrin release and endogenously stimulated acid secretion. This would explain the inability of standard, exogenous gastrin-based methodologies to identify changes in acid secretion following eradication, since the inherent capacity of the parietal cell to secrete acid as well as the parietal cell sensitivity to gastrin remain unaffected by eradication. Despite the temptation to link the reduction in gastrin concentration following eradication with a decrease in parietal cell mass, there is at present no hard evidence to suggest that *H. pylori* eradication is associated with a decrease in parietal cell mass.

Changes in the somatostatin "brake" on acid secretion would, on the other hand, be associated with the abnormalities in GRP stimulated acid secretion found by El-Omar et al and referred to above. The data indicating that *H. pylori* infection of the stomach is associated with a reduction in D-cells, immunoreactive somatostatin and evidence of a reduced synthesis of somatostatin, which is reversed by eradication of the organism, were mentioned in paragraph 5.1. GRP stimulation of an infected subject would therefore be associated with "unopposed" gastrin release and parietal cell secretion, leading to exaggerated acid secretion in the infected subject. Following eradication, and restitution of the D-cell complement and somatostatin content, GRP would co-stimulate gastrin and somatostatin release in the antrum, thus providing an explanation for the observed reduction in stimulated gastrin secretion following eradication. Co-stimulation of the D- and parietal cells in the oxyntic glands would be reflected by a lower acid secretory response in the eradicated subject. This effect would be essentially independent of changes in parietal cell mass, although any reduction in the latter would accentuate these findings.

In considering the effect of healing on acid secretion an interesting trend with regard to basal, low dose and high dose stimulated acid secretion was noted. Following healing with sucralfate, acid secretion tended to decrease, while consideration of the post-eradication data shows a tendency for the post-sucralfate acid secretion to increase again. The relevance of this observation lies firstly in the support it gives to earlier reports that ulcer healing with sucralfate is associated with a reduction in basal (Marks 1989, Johnston 1990, Moss 1992b), nocturnal (Kummer 1992) and stimulated acid secretion (Marks 1989, Johnston 1990b). Furthermore, the tendency of the acid secretion of patients in the present

study to increase again following eradication suggests that this effect is mediated by *H. pylori* independent mechanisms. This interpretation is at variance with that of Banerjee et al, from the Glasgow group, who have concluded that the reduction of gastric acid secretion following healing by sucralfate is mediated by suppression, but not eradication, of *H. pylori* (Banerjee 1994). While in agreement with the finding that sucralfate suppresses antral colonization by *H. pylori* (Hui 1989), we have previously demonstrated that this decrease in antral colonization is associated with a tendency for colonization to increase in the body of the stomach in patients treated with sucralfate. In addition, the overall gastric urease activity as assessed by urea breath test was unchanged following sucralfate therapy (Winter 1993). The finding that the overall gastric colonization following sucralfate therapy is not affected, and that our current data indicate a tendency for acid secretion to increase following eradication (when compared to post healing secretion), suggests that *H. pylori* suppression is unlikely to be responsible for the reduction in acid secretion following sucralfate healing. This is in accordance with the findings of Moss et al (Moss 1992b).

The results with regard to gastric pepsin secretion following eradication have two main elements: (i) a tendency for gastric pepsin levels to increase following healing (ii) a non-insignificant trend for reduction in both the output and concentration 12 months following eradication, when compared to entry values. This tendency reaches significance in the low and high dose stimulated samples when post-healing values are compared to the 12-month follow-up values.

There is no obvious explanation why gastric pepsin secretion should increase following the healing of duodenal ulceration, with either sucralfate or ranitidine therapy, but it would appear that this finding is

"real" and not artefactual. Support for this is found in earlier studies. Sandvik et al investigated the gastric acid and pepsin secretion in response to modified sham feeding (Sandvik 1985) and found that patients with inactive duodenal ulcer disease had pepsin outputs which were significantly greater than those of controls and those with active duodenal ulceration. This was primarily because of an altered secretory pattern, characterised by increased secretion during the late period of collection. This has not been previously noted with pentagastrin stimulation, however. It should be noted that our findings with regard to the effect of healing on pepsin secretion differ from those published by earlier workers. Achord found that, following duodenal ulcer healing, there was a significant reduction in both basal and stimulated (peak) pepsin output (as well as acid output) when compared to the values obtained for active ulceration (Achord 1981). The design of that study is, however, questionable as it appears that while the total sample consisted of 23 control subjects and 71 duodenal ulcer patients, only 11 subjects were studied prospectively (from crater to healing), and of these only eight had acid studies performed while an active ulcer crater was present. In this sub group, which resembles ours more closely, Achord could not demonstrate a significant reduction in gastric secretion "early" (within three months) or "late" (12 months) following healing.

We have considered the possibility that the ulcer healing agents may have led to a "rebound" secretion of pepsin in a manner similar to that described for acid secretion following H_2-receptor antagonist therapy (Fullarton 1989). There is, however, no support in the literature for a chief cell rebound hypersecretion following healing with ranitidine (Frislid 1986) or cimetidine (Aadland 1979), although it has to be conceded that the design of these two studies may not have been optimal for the detection of "rebound". Frislid studied normal subjects, while Aadland did

not specify the exact timing of the post-healing studies. Chief cell sensitivity also appears to be unaffected by H_2-receptor antagonist treatment (Aadland 1979b). There is no reason to suspect that the mucosal protective agents (sucralfate and bismuth) are associated with rebound pepsin hypersecretion. On the contrary, Baron et al showed a decrease in gastric pepsinogen secretion following bismuth therapy (Baron 1986).

The data generated over the 12 month period also does not support the hypothesis that gastric pepsin secretion is reduced following *H. pylori* eradication, basal 12 month concentration and output values being similar to entry values, while stimulated 12 month values were lower, but not significantly so, than entry values. Although the data set is limited by its size and the scatter of the data, this suggests that *H. pylori* is not directly involved in stimulating gastric pepsin secretion. This appears to be at variance with the findings in serum, where eradication is usually associated with a reduction in serum pepsinogen values (Oderda 1990, Chittajallu 1992) as well as *in vitro* data indicating that *H. pylori* or its components directly stimulate pepsin secretion (Cave 1991, Young 1992).

Data on the effect of eradication on the gastric pepsin concentration is scarce. There is some indication, however, that our results are in fact not artefactual. Yahav et al have reported on the peptic activity in 13 subjects, as estimated before and after *H. pylori* eradication therapy (Yahav 1992). These workers found that the total peptic activity increased three months following eradication, and argued that (non-stimulated) gastric peptic activity is in fact diminished during active infection.

Available evidence suggests that there is a lack of association between the three pepsin compartments following eradication. **Serum** pepsinogen levels tend to decrease following eradication, while our data indicates that basal **luminal** pepsin secretion remains unchanged. Yahav et al have, on the other hand, demonstrated that **mucosal** levels of pepsin are increased following eradication (Yahav 1992). These findings suggest that *H. pylori* or *H. pylori*-related factors (e.g. inflammatory response) are unlikely to directly stimulate pepsin synthesis, as mucosal levels are higher in the "healthy" mucosa following eradication. This has to be interpreted as reflecting an increase in zymogen stores in the restituted mucosa. An alternative explanation would be that eradication removes an *H. pylori* associated stimulus to pepsin secretion, as found *in vitro* (Young 1992), again unlikely in view of the finding that luminal pepsin concentrations are unaffected by eradication. Improvement in mucosal integrity following eradication, with a consequent decrease in mucosal leakage of pepsinogen, offers perhaps the best explanation for the decrease in serum pepsinogen levels. Serum levels of pepsinogen I and II have been shown be influenced by gastric mucosal histology, and by the histological changes following eradication, (Samloff 1982, Wagner 1994). The effect of eradication, and the gastric morphological changes associated with it, on luminal pepsin concentration have not previuosly been investigated.

As is the case with stimulated acid secretion, the inability to demonstrate a significant reduction (within the constraints of the data set) in stimulated gastric pepsin secretion has to be interpreted with care. Once again, the appropriateness of pentagastrin as a stimulus needs to be

evaluated. As with acid secretion, Hirschowitz et al have identified a defective inhibition of pepsin secretion following GRP/bombesin stimulation in duodenal ulcer subjects. In addition, the GRP/bombesin stimulated secretion characteristics were clearly different to the curves obtained with exogenous gastrin-17 (Hirschowitz 1985). The appropriateness of exogenous gastrin as a stimulus for evaluating the effect of eradication on pepsin secretion has, therefore, to be questioned, essentially for the same reason as it has to be questioned in the case of acid secretion - that is the inability of pentagastrin stimulation to simulate physiological stimulation, with the interaction between stimulatory and inhibitory control mechanisms. Somatostatin again is pivotal in this argument. Pentagastrin is an established calcium mediated pepsin secretagogue (Basson 1988) and will therefore directly stimulate the chief cell to secrete pepsin. Several factors are, however, involved in modulating chief cell pepsin secretion (Hirschowitz 1991). Somatostatin, shown to be reduced in *H. pylori* gastritis, has been demonstrated *in vitro* to block both cyclic-AMP and calcium mediated pepsin secretion (Basson 1988). As is the case with acid secretion, this may be the "inhibitor" postulated by Hirschowitz et al. By using pentagastrin as secretory stimulus, we would not be able to quantitate the effect of the restitution of somatostatin levels following eradication on pepsin secretion.

In view of the limitations of the data set, caution must prevail in the interpretation of these results. However, the complexity of the control mechanisms involved in both acid and pepsin synthesis and secretion is such that the delicate balance of stimulatory and inhibitory control might not be truly reflected by the use of pentagastrin, which may obscure or even override the physiological changes which may be associated with

H. pylori eradication. GRP/bombesin may therefore be the more appropriate stimulus to assess the functional changes associated with eradication, whereas exogenous gastrin stimulation can only reflect parietal cell mass and sensitivity. More studies are needed to unravel the complex relationship between *H. pylori* and gastric acid and pepsin secretion.

CHAPTER 6

PREVALENCE AND FACTORS INFLUENCING THE PREVALENCE OF *HELICOBACTER PYLORI* IN AN ETHNICALLY DIVERSE SAMPLE, WITHOUT GASTRODUODENAL PATHOLOGY, FROM THE WESTERN CAPE

6.1 INTRODUCTION:

Although many questions remain concerning the epidemiology of *H. pylori* in Western developed countries, the characteristics of the prevalence have been well characterised. The following generalised statements are applicable with regard to the characteristics of *H. pylori* prevalence in Western countries (Marshall 1994)

1. *H. pylori* infection is uncommon in young subjects, affecting about 20% of persons below the age of 40, but being found in approximately 50% of subjects older than 60 years.

2. *H. pylori* infection is uncommon among children.

3. Low socioeconomic status is a positive predictor of *H. pylori* infection.

The data for developing countries appears to be totally different, with available studies indicating that the prevalence of the infection is much higher than in developed countries, with a high prevalence of the infection in young subjects (Megraud 1989, Glupczynski 1991, Wyatt 1987, Holcombe 1992).

Prevalence data for *H. pylori* has been recorded by a number of workers in South Africa. In a study from the Gauteng province, Crewe-Brown et al reported on the prevalence of the organism in gastric ulcer subjects (90%), duodenal ulcer subjects (82%) and "control subjects" (59%) (Crewe-Brown 1985). Wright et al reported on the prevalence of the organism in 51 subjects with varied gastroduodenal pathology (73% positive) (Wright 1987), and the Durban group reported a prevalence of 82% in their sample of 224 subjects, once again with varied pathology (Miller 1988). Jaskiewicz et al, also based in the Western Cape, reported on the prevalence in "subjects at risk for gastric carcinoma", finding a 90% prevalence in subjects with peptic ulceration and 72% in patients with dyspepsia but without evidence of ulceration (Jaskiewicz 1989). Dawes et al confirmed the high prevalence of the infection in the Gauteng province, reporting an overall prevalence of 81% in a sample of 90 patients with varied gastroduodenal pathology presenting to a specific clinic in Johannesburg (Dawes 1991).

It is apparent, therefore, that while an attempt has been made to document the prevalence of *H. pylori* infection in South African communities, including the Western Cape, earlier studies have included patients with varying pathologies who are known, in Western studies at least, to have different risks for infection. In addition, no South African study has attempted to study factors which are considered important in determining the prevalence of the infection. These factors include race (Graham 1991c, Kang 1990), socioeconomic status (Graham 1991c, Sitas 1991) and close personal contact as found in institutionalised subjects (Berkowicz 1987).

6.2 AIM OF THE STUDY:

The aim of this cross-sectional study was, therefore, to determine the prevalence of *H. pylori* and to investigate the influence of a number of factors on the prevalence of infection by the organism in a clinically defined group of subjects. The latter was drawn from the population we serve; the demographic profile was considered representative of patients in the Western Cape area.

6.3 PATIENTS AND METHODS:

6.3.1 Patients:

All dyspeptic patients, presenting to the Gastrointestinal Clinic, Groote Schuur Hospital and a Cape Town based gastroenterological practice, were eligible for the study. The sample reflects the referral pattern of the clinic and practice and was not constructed as a population survey. Patients with peptic ulcer disease, gastric carcinoma and endoscopically detectable oesophagitis were excluded. All patients gave informed consent, and the study was approved by the Ethics and Research Committee of the University of Cape Town.

6.3.2 *H. pylori* status:

H. pylori status and the presence of gastritis were determined by histological assessment of adequate antral biopsies, taken within 5cm of the pylorus and stained by the Giemsa method. All histological assessments were carried out by one pathologist, experienced in the technique.

6.3.3 Data Collection:

Each patient was interviewed by his/her endoscopist, and the following data collected:

6.3.3.1 Demographic information:

This included age, racial group and gender.

6.3.3.2 Socioeconomic class and occupation:

Patients were classified according to their own, or the family breadwinner's status. Classification was according to the British Registrar General's guidelines (HMSO 1980). However, because a large section of the South African population falls mainly in the Registrar General's classes IV and V, we used a modification of this system, in which classes I, II and III are combined and called MRC Class I. Class IV becomes MRC class II and Class V becomes MRC class III. Pensioners were classified according to their previous occupation and unemployed patients were classified as MRC III.

6.3.3.3 Educational level:

Educational level was determined as the number of successful years spent in school, tertiary education and in-service training (in the case of apprenticeships and occupations requiring in-service training).

6.3.3.4 Subjects sharing accommodation:

The total number of people sharing accommodation with the patient was determined. This was determined as the number per house or shelter.

6.3.4 Statistical analysis:

Categorical data was analysed by the Chi - square test and continuous data by Student's t-test using the SAS, BMDP and EPISTAT statistical packages. Discrete Multivariate Analysis (DMA), applying the principles of a logistic regression model, was used to control for the following factors: race, gender, social class and number of persons sharing accommodation. A p value of less than 0.05 was regarded as significant.

6.4 RESULTS:

Adequate antral biopsy specimens were obtained from 169 of 179 patients studied - 47 White, 109 Coloured and 13 Black. Because of their small number and the compatibility of their demographic characteristics, the black patients were included in the non-white grouping for analysis.

The results are summarised in Tables 6.1 and 6.2.

6.4.1 Overall prevalence:

One hundred and six (63%) of the patients were infected by *H. pylori*.

Table 6.1: Summary of data, infected versus non-infected subjects:

	H. pylori positive n (% of total)	*H. pylori* negative n (% of total)	p
Total sample:	106 (63)	63 (27)	
Male:	55 (64)	31 (36)	
Female:	51 (61)	32 (39)	NS
Non-white:	87 (71)	35 (29)	
White:	19 (40)	28 (60)	<0.001*
Socioeconomic classification:			
MRC I:	43(53)	38 (47)	
MRC II:	27 (75)	09 (25)	<0.05**
MRC III:	36 (69)	16 (31)	
Age: mean ±SD:	44 ±13	53 ±18	<0.01#
$Education:			
10 years or less:	77 (73)	33 (52)	
>10 years:	29 (27)	30 (48)	<0.01*
$Persons sharing accommodation:			
3 or less:	47 (44)	37 (59)	
>3:	59 (56)	26 (41)	NS

* Chi-square # Student's t-test ** Bartholomew's test for order
NS not significant $ Intragroup % shown

6.4.2 *H. pylori* and gender:

No difference in the prevalence of the infection was found between genders, 64% of the male patients being infected compared to 61% of the female patients.

6.4.3 *H. pylori* and age:

In total, the infected individuals were younger than those not infected, the mean(SD) age being 44(13) years in the *H. pylori* infected group, and

53(18) years in the *H. pylori* negative group ($p < 0.01$, Student's t - test). This difference reflects the sample composition, with the mean(SD) age in the white group being 56(16) years, whereas it was 43(14) in the non-white group ($p < 0.001$, Student's t - test). Within the infected group, significantly more non-white patients were infected before 45 years of age when compared to the white group (59% vs 33%. $p < 0.05$, Chi - square test).

6.4.4 *H. pylori* and race:

A marked racial difference in prevalence was found, the non-white group having a prevalence of 71% and the white group 40% ($p < 0.001$, Chi - square test). When applying the multivariate model, controlling for the factors noted above, race remained a predictive factor for *H. pylori* infection.

6.4.5 *H. pylori* and socioeconomic class:

A significant difference in prevalence was found between the social classes, with an apparent inverse relationship between socioeconomic classification and *H. pylori* infection ($p < 0.05$, Bartholomew's test for order). However, with this particular data set and in the South African context in general, it is difficult to separate the influence of racial grouping and socioeconomic status and we found that this apparent significant difference in prevalence among the three social groupings could be ascribed to the racial distribution in these groups. Using the DMA model, racial grouping and social class were so strongly associated that social class could not be implicated as an independent predictor of *H. pylori* infection.

6.4.6 *H. pylori* and education:

There were significant differences in the education levels of the infected and non - infected groups. In the infected group, 77 (73%) of the patients had less than 10 years of education (the equivalent of a standard eight or Junior Certificate level), compared to only 33 (52%) in the non - infected group ($p = 0.01$, Chi - square test). Once again, this reflects the sample composition and the marked difference in education level between the two racial groupings (Table 6.2).

6.4.7 *H. pylori* and accommodation density:

The non - infected subjects appeared to have less persons sharing accommodation than the infected group. Thirty seven (59%) of the non - infected group had 3 or less persons sharing accommodation, compared to 47 (44%) of the infected group. However, this difference did not reach significance ($p = 0.09$, Chi - square test) and again reflects the marked differences noted between racial groupings. When investigated further in the DMA model, controlling for race, gender and and social class, no significant differences could be shown between infected and non-infected subjects.

Figure 6.1 summarises the main differences in demographic charactersistics of the *H. pylori* negative and positive groups, while Table 6.2 highlights some of the key differences between the two race groups and clearly demonstrates the marked differences with regard to age, level of education, the number of persons sharing accommodation and their distribution in the socioeconomic groups.

Figure 6.1: Demographic profiles of *H. pylori* negative and positive groups.

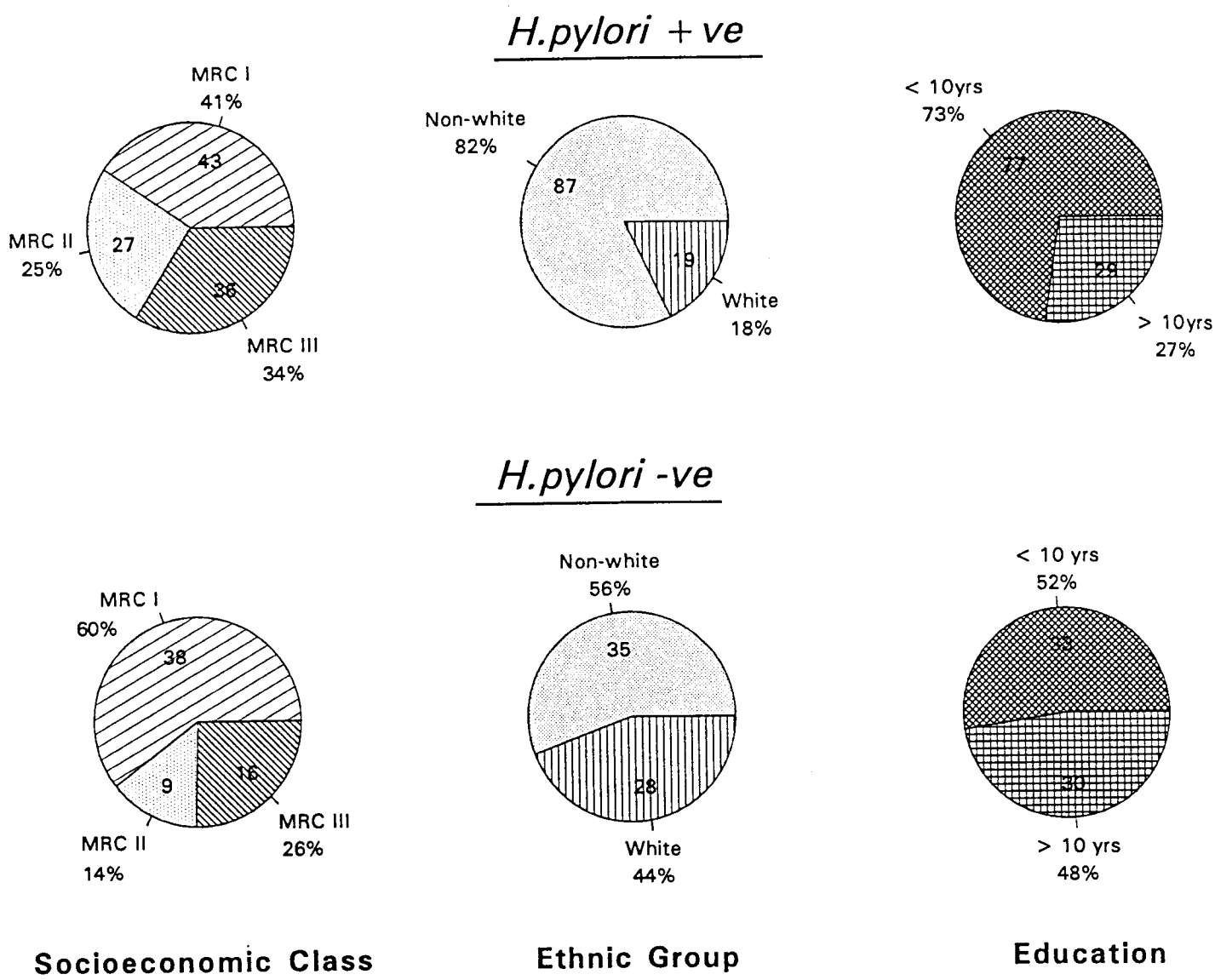


Table 6.2: Summary of the main differences in study characteristics between ethnic groups:

	White n (% of sample)	Non-white n (% of sample)	p
Age: mean ±SD	56 ±16	44 ±14	$<0.001^{\#}$
Education:			
10 years or less:	4 (9)	106 (87)	
>10 years:	43 (91)	16 (13)	$<10^{-6}$*
Persons sharing accommodation:			
3 or less:	42 (89)	42 (34)	
>3:	5 (11)	80 (66)	$<10^{-6}$*
Socioeconomic classification:			
MRC I:	46 (98)	35 (29)	
MRC II:	1 (2)	35 (29)	$<10^{-6}$*
MRC III:	0	52 (42)	

\# Student's t-test
* Chi-square test.

6.5 DISCUSSION:

Our findings, in the first study from Southern Africa to be reported in a population with a single, defined clinical presentation (Louw 1993), confirm that the prevalence of *H. pylori* infection in the socioeconomically disadvantaged communities of our population is high, while the prevalence rate in the socioeconomically advantaged, as assessed by socioeconomic classification and educational level, is significantly lower and comparable to that reported from developed, Western communities.

These findings in dyspeptic subjects have since been confirmed in a volunteer sample of 117 asymptomatic subjects in a study undertaken in our center (van Wyk 1993). The findings in asymptomatic subjects were

essentially the same as those reported in this study on dyspeptic subjects. The ethnic difference was once again marked, 37% of 41 white subjects being *H. pylori* positive, compared to 91% of 76 non-white subjects. The ethnic difference was once again expressed as differences between the three socioconomic levels reported, while a significantly larger number of non-white than white subjects were infected in the younger age group (20-29 years): 31% of 13 white and 93% of 14 non-white subjects being infected.

The finding of a marked ethnic variation in our study population is in keeping with the findings of the Houston (Graham 1991c) and Singapore (Kang 1991) groups. It is unlikely that this is the result of a genetic predisposition to the infection in a particular racial group. The apparent ethnic difference in prevalence of the infection is probably caused by the confounding linkage of poor socioeconomic status and the non-white ethnic group. While this study was unable to separate the influence of race and socioeconomic status, thereby making it impossible to infer a causal relationship, others have had the opportunity to investigate the apparent association between race and *H. pylori* infection. Malaty et al were able to match Blacks, Whites and Hispanics on a 1:1:1 basis for age and socioeconomic status (Malaty 1992). They found that the prevalence of *H. pylori* was almost identical in Hispanics and Blacks, the prevalence in both being significantly higher than in Whites. There was, on the other hand, a clear inverse relationship between educational level (used as an assessment of socioeconomic class) and *H. pylori* infection, which remained after logistic regression analysis for age and gender. Lack of education was also identified, within population groups, as a risk factor for current infection by *H. pylori* by the EUROGAST study group's

prevalence study in over 3000 subjects from 17 defined populations in Europe, North Africa, North America and Japan (Eurogast 1993).

Malaty et al concluded that as Hispanics do not constitute a "race", the increased prevalence in this group, and by inference the Blacks, was unlikely to be caused by differences in the genetic predisposition to infection between racial groups. They argued that the difference in prevalence therefore reflected a "generational cohort phenomenon" related to the generational distance from poor socioeconomic status. It is likely that the findings in our sample reflect the same influence of socioeconomic status on the prevalence of the infection - that is the prevalence in whites is lower than in non-whites because the whites have enjoyed a higher level of socioeconomic development for several generations. This generational cohort theory finds support in the observation that the serologically determined prevalence of the infection appears to have declined in successive generations of epidemiologists (Parsonnet 1992) and Japanese Americans in Hawaii (Nomura 1991).

The significant differences noted between our racial groups with regard to socioeconomic class, schooling and number of persons sharing accomodation also suggest that these factors may in fact be important in determining the prevalence of the infection, possibly by influencing the exposure risk to the organism by their effect on living habits, personal hygiene standards and practices and the availabilty of basic sanitation and hygienic water supplies, factors known to influence the epidemiology of most infectious diseases. Unfortunately it is impossible to identify a particular factor that would favour the transmission of the infection, as the mode of transmission is not known. There is some agreement that

the organism is spread via interpersonal contact, which would make crowded living conditions a potential risk factor.

The evidence for spread of the infection from person-to-person is based on observations made in institutions and in family members of infected subjects. Using sophisticated DNA analysis techniques, Vincent et al have been able to show strain homology in *H. pylori* infected handicapped children in a long-term care facility (Vincent 1991). Hawtin et al, using the same technique, could identify homologous strains in an extended family of 22 persons (Hawtin 1994). Georgopoulos et al showed that *H. pylori* organisms infecting the spouses of duodenal ulcer subjects showed ribotype homology in 44% of spouses, while the risk for spousal infection was apparently associated with a longer duration of cohabitation (Georgopoulos 1994). Nwokolo and Collins also reported that families appeared to share infection with the same strain, Nwokolo being able to identify significant homology in three generations of a duodenal ulcer disease family (Nwokolo 1992, Collins 1992). Although serology is not able to identify strain homology, serological studies have also suggested transmission in family groupings (Collins 1990, Drumm 1990, Oderda 1991, Mitchell 1993), a high prevalence of the infection being found in the families of infected subjects, suggesting intrafamilial clustering and hence person-to-person spread of the infection, or, alternatively, acquisition of the organism from a common environmental source. These findings, of an increased spousal infection rate, have been questioned by the findings of Mendall et al, who found that in 16 of 38 married couples one of the partners was uninfected (Mendall 1993). This finding was duplicated by Alam et al, in a study of 124 couples, using the ^{13}C-urea breath test (Alam 1993). They could not demonstrate a significantly increased risk for developing the infection in the spouses of

infected subjects. However, these observations cannot be interpreted as representing evidence against the theory that the organism is transmitted via (close) interpersonal contact; it may merely indicate that the infection is primarily acquired during childhood.

The finding in this study that the infected group was younger than the non-infected group in this sample, as well as the lack of a clear difference in the number of cohabitants between positive and negative groups, once again reflects the composition of the sample, the predominantly infected non-white subjects being significantly younger than the predominantly uninfected white subjects. The author does not believe that the findings in this study contradict the finding in Western populations that the prevalence increases with age (Sitas 1991, Dooley 1989) and that the risk of infection is increased by overcrowded living conditions. It has become clear that the infection is probably acquired in childhood, so that adult prevalence rates represent a cohort effect and not ongoing acquisition of the infection during life. This finds support in the observation that childhood living conditions are the dominant factors in determining the likelihood of infection in adults (Mendall 1992, Malaty 1994), as well as in the findings in serological studies indicating that acquisition of the infection is uncommon in adults (Cullen 1993, Kuipers 1993, Banatvala 1994). Cullen et al determined that only 7% of 86 subjects seroconverted (from negative to positive) over a 21 year period, while Kuipers identified an annual infection rate of 0.15% in a cohort of 115 individuals and Banatvala et al demonstrated that the majority of 417 Japanese subjects were infected in childhood, seroconversion being rare in adulthood. The apparent contradiction with regard to the younger age of the infected group in the Western Cape is readily explained by the

cohort theory, while, based on available evidence, living conditions in adulthood may not determine the risk of infection.

There is some evidence that *H. pylori* has the potential to spread through interpersonal contact. The organism has been cultured from dental plaque, and demonstrated to be identical to the strain found in the stomach of the same patient (Shames 1989). Culture of the organism from plaque has not been succesful in other studies (Krajden 1989), but this probably reflects the difficulty in culture techniques, as a number of workers have now documented its presence in dental plaque (Banatvala 1993, Takagi 1994). A classic feco-oral mode of transmission has also been suggested and has found some support in the finding of the organism in the faeces of infected subjects by culture (Thomas 1992, Kelly 1993) and PCR-techniques (Saborio 1994).

Some workers have suggested alternate modes of infection, which would implicate poor sanitation/personal hygiene and poor access to high quality drinking water, based on epidemiological data and on the presence of the organism in sewage samples, the latter observation representing a logical extrapolation of the data with regard to the isolation of the organism from stool samples. Epidemiological data from Peru has identified the water source as a possible source of *H. pylori* infection in young Peruvian children (Klein 1991) implicating external water sources (storage drums or communal taps). This theory is given added plausibility by the finding that the organism is capable of surviving in river water (Shahamat 1989) and the detection of the organism by PCR in raw sewage, also from Peru (Westblom 1993). Mitchell et al have, however, provided epidemiological evidence that community-wide (as opposed to close interpersonal) transmission via the fecal-oral route is

unlikely (Mitchell 1994). These workers, using the same sera, compared the prevalence of hepatitis A (known to be transmitted via the fecal-oral route in the community) and *H. pylori* in 1501 subjects from the Guangdong province in China. Their data once again indicated that infection with *H. pylori* is probably acquired at a younger age than hepatitis A, and the differences in age-related peak incidences led these workers to conclude that community-wide fecal-oral spread is probably not important in the case of *H. pylori*.

In conclusion, this study of 169 subjects has identified a high prevalence of *H. pylori* infection in the socioeconomically disadvantaged communities of the Western Cape, served primarily by Groote Schuur Hospital, while the prevalence of the infection in the socioeconomically privileged community appears to be comparable to that found in studies from developed, Western countries. The finding that the infected population was younger than the non-infected group, and that the majority of non-white subjects - 59% - were infected before the age of 45 years is compatible with the observation in epidemiological studies from developed countries that *H. pylori* infection occurs predominantly in childhood, and that acquisition of infection in adulthood is uncommon.

The data from this study, as well as the findings from the Western Cape with regard to asymptomatic subjects, show a high prevalence in the community we serve. The effect of this high prevalence on re-infection and ulcer relapse rates following eradication remains to be elucidated.

CHAPTER 7

DUODENAL ULCER RELAPSE AND *HELICOBACTER PYLORI* RE-INFECTION FOLLOWING ERADICATION OF THE ORGANISM IN THE AFRICAN SETTING

7.1 INTRODUCTION:

The dramatic disease modifying effect of successful eradication of *H. pylori* on the natural history of duodenal ulcer disease has been mentioned (Paragraph 2.5.3.1.5 and Table 2.1). These results, obtained almost exclusively in communities from First World countries with a developed economy, have been influential in establishing *H. pylori* eradication therapy as an important strategy for the long term management of duodenal ulcer disease. Eradication is now considered warranted in all patients in whom medical maintenance or long-term treatment was once recommended. The enthusiasm for *H. pylori* eradication as a management strategy in peptic ulcer disease is so great that the NIH has recently recommended this approach for all patients with peptic ulceration (NIH 1994).

It should be noted, however, that the beneficial effect of eradication has been studied mainly over the relatively short term of 12 months, and principally in Western communities. There is some indication, however, that the beneficial effect of eradication lasts only for as long as the patient remains uninfected. This is supported by the findings published in the very few longer term (more than 12 months) follow up studies available. This support comes mainly from two studies from Australia. The first, by Borody et al, reports on the follow-up of 197 duodenal ulcer patients eradicated of *H. pylori* and followed for a period of one to six

years, with annual, or symptom precipitated, endoscopic control (Borody 1992). Borody's findings were that patients cured of the infection did not suffer a duodenal ulcer relapse, regardless of their smoking habits, a factor thought to be important in contributing to the incidence of duodenal ulcer relapse (Paragraph 2.5.2.2). A second important observation was the total absence of re-infection of subjects successfully treated for the infection. Further support for an eradication strategy comes from Forbes et al (Forbes 1994). These workers had access to the original patients "eradicated" and reported on by Marshall in 1988 (Marshall 1988b), and their paper reports the outcome in 26 "non-eradicated" and 37 "eradicated" subjects followed up seven years after eradication. Although not endoscopically controlled, their findings (summarised in table 2.2) confirm a low incidence of relapse in the eradicated group but, surprisingly, show a relatively low relapse figure in the non-eradicated group as well, although this may be the result of the retrospective, non-controlled nature of the study. They also report that the re-infection rate over the seven year period was only 1.2% per annum with 95% confidence intervals for the re-infection figure of 0 - 4.8%, a range widely accepted in publications from developed countries (Megraud 1993).

We have referred to the current perception regarding *H. pylori* epidemiology in developed countries, where the prevalence of infection is low and, although age-dependent, is thought to reflect acquisition of the organism in early childhood rather than progressive accrual of the infection - the so-called "cohort-effect" (Chapter 6). The latter view may help explain the rarity of *H. pylori* re-infection following successful eradication in developed countries (Borody 1989, Bell 1993). Fibreoptic endoscopy has been incriminated as a potential source of *H. pylori* re-

infection in such patients (Langenberg 1986, Langenberg 1990). Re-infection rates of the order of 20 to 27%, however, have been reported in at least two studies (Coelho 1992, Sabbatini 1993), one of which was carried out in a developing country (Coelho 1992). Re-infection rates of this magnitude would make it difficult to recommend an eradication strategy even for diseases (such as duodenal ulcer disease) in which *H. pylori* eradication has been shown to be of major benefit.

The characteristics of *H. pylori* prevalence in developing countries in general, and Africa in particular, were discussed in Chapter 6. It is generally acknowledged that there is a high prevalence of *H. pylori* infection in Africa (Glupczynski 1991, Holcombe 1992), and the prevalence of *H. pylori* infection is of the order of 60 - 90% in the community we serve in the Western Cape (Louw 1993b, van Wyk 1993). In view of the high prevalence of the infection in the community, it is essential to determine whether eradication of the organism protects subjects against the recurrence of duodenal ulceration, or whether this advantage is lost due to re-infection.

7.2 AIM OF STUDY:

This study, in adult duodenal ulcer patients, was undertaken to determine the effect of *H.pylori* eradication on the natural history of duodenal ulcer disease and to determine the incidence of re-infection following eradication in a community known to have a high prevalence of the infection.

7.3 PATIENTS AND METHODS:

7.3.1 Patients:

Forty eight patients (Male = 38, Female = 10) with "difficult duodenal ulcer disease" were studied; all but two of the patients were from the socioeconomically disadvantaged non-white population. "Difficult duodenal ulcer disease" was defined as the disease in patients in whom the duodenal ulcer had not healed after eight weeks of conventional therapy, or as those who had suffered at least one relapse in the 12 months following healing of the index ulcer. Because of the potential of NSAIDs to cause duodenal ulceration, patients requiring regular NSAID medication were excluded from the study, while the smoking habit of each subject was recorded.

All patients were *H.pylori* positive prior to ulcer healing. Ulcers were healed with omeprazole, 20mg/day, taken for at least four weeks. Patients were required to give informed consent prior to entry into study, and the study protocol was approved by the Ethics and Research Committee of the University of Cape Town.

7.3.2 Endoscopy schedule:

Patients were endoscoped at entry, four weeks after cessation of eradication therapy, and six, 12 and 24 months after entry, or whenever dyspepsia recurred. All endoscopies were performed using standard Olympus fibreoptic gastroduodenoscopes of the X and P series. Xylocaine spray was used for pharyngeal anaesthesia, and all patients received midazolam 2.5mg IV as sedation, unless contraindications to its use existed.

All endoscopies, with the exception of the initial endoscopy, were done by the same endoscopist, who was unaware of the *H. pylori* status of the patients. A second endoscopist, also blinded to the *H. pylori* status of the patient, was required to confirm all ulcer recurrences.

7.3.3 *H. pylori* status:

H.pylori status was determined prior to healing, a minimum of four weeks following cessation of anti-*H. pylori* therapy, and thereafter at six, 12 and 24 months after cessation of therapy, or whenever patients suffered an endoscopically proven duodenal ulcer relapse.

H.pylori status was determined from antral biopsies, taken within 5 cm of the pylorus. Biopsies were examined by rapid urease test (one biopsy) according to the method of Arvind et al (Arvind 1988), histological assessment of biopsies stained by the modified Giemsa method (2 biopsies), and culture (one biopsy). Histological samples were immediately placed in 10% neutralised formalin before further processing, while samples for culture were immediatelly placed and transported in a jar under microaerophilic conditions (Oxoid Gas Generating BR38, Basingstoke, Hampshire, UK). The specimen was cultured on tryptose blood agar (CM 233; Oxoid Ltd) containing lysed horse blood (5% volume/volume) at 37°C under microaerophilic conditions [12% CO_2, 88% air (6% O_2), 95% humidity] for a minimum of 7 days before being considered negative. *H. pylori* was positively identified by colony morphology and urease reaction (Christensen's urea slope).

Patients were considered *H. pylori* positive if the organism was detected with any of these tests.

7.3.4 *H. pylori* eradication therapy:

All patients were treated with a combination therapy regimen, consisting of a mucosal protective agent (colloidal bismuth subcitrate 120mg q.i.d or sucralfate 1g q.i.d.) metronidazole (400mg tds) and a second antibiotic, either tetracycline or amoxicillin. Patients with a body mass of less than 75kg received 250mg and those with a body mass greater than 75kg received 500mg of amoxicillin or tetracycline. In accordance with local treatment policy at the time, 17 patients were treated for one week and 31 received two weeks of treatment.

Compliance was assessed by tablet count at the post-treatment endoscopy visit. For the purposes of this study, the patient's intake of metronidazole was arbitrarily chosen as a marker for overall antibiotic compliance.

7.3.5 Concurrent medication:

No concurrent acid suppressive or other ulcer healing therapy was allowed during the study period.

7.3.6 Statistics:

Categorical data was analysed by means of the Fisher's exact test, while continuous variables were analysed by means of Student's t-test. A p value of <0.05 was considered to be significant.

7.4 RESULTS:

H. pylori infection was eradicated in 27 patients (male = 24, female = 3; age ± SD = 37.1 ± 12.3 years). The infection persisted in the remaining 21 patients (male = 14, female = 7; age ± SD 37.9 ± 10.6 years). Twenty two patients (81%) in the eradicated group and 19 (90%) in the non-eradicated group were cigarette smokers (p = n/s).

7.4.1 Treatment profile:

Six patients in the eradicated group received one week and 21 received two weeks of combination therapy. In the non-eradicated group, 11 patients received one week, and 10 patients two weeks of therapy ($p<0.05$, one versus two weeks). The average antibiotic compliance was 89% in the eradicated group and 86% in the non-eradicated group.

7.4.2 Duration of follow-up:

In the "eradicated" group, 27 patients completed follow-up. In the non-eradicated group, three patients were lost to follow-up before the six month endoscopy, while 18 patients in this group completed their 24 months of follow-up, or were withdrawn from study.

7.4.3 Re-infection by *H.pylori*:

Of the 27 patients successfully eradicated of the infection, re-infection was documented in two patients: one at the six month visit, and one at the 24 month visit.

7.4.4 *H. pylori* status and duodenal ulcer relapse (Table 7.1):

In total, five patients in whom the organism was initially eradicated relapsed in the 24 month follow-up period (5/27 = 18%). Of these five, one was documented as having been re-infected, while one patient, with an asymptomatic relapse at the 12 month endoscopy, has a gastrinoma (confirmed by diagnostic gastrin levels). There were thus only three (11%) *H.pylori* negative patients with idiopathic or unexplained relapses - two presenting at 12 months and one at 19 months. The two *H. pylori* negative patients who relapsed at twelve months were asymptomatic, and none of the three had more than one documented ulcer relapse during the 24 month follow-up period. Although suspected, salicylate use could not be confirmed in two of the three patients with *H. pylori* negative relapses.

TABLE 7.1: *H. pylori* status and duodenal ulcer relapse:

	Relapsed at 12 Months	Relapsed at 24 Months	Total
***H. pylori* positive group:**	14	1	15*
***H. pylori* negative group:**	4#	1	5*

* $p < 0.001$

\# 1 = Reinfected, 1 = Gastrinoma

In the non-eradicated group on the other hand, 15 of the 21 patients showed endoscopic evidence of relapse during the 24 month follow-up period. Fourteen of these relapses occurred during the first year of follow up and one during the second year. This difference in the incidence of relapse between eradicated and non-eradicated patients was highly significant ($p < 0.001$, Fisher's exact). The beneficial effect of

eradication on ulcer relapse is even greater if the three patients lost to follow-up in the non-eradicated group are excluded from analysis and the re-infected patient, as well as the patient with the gastrinoma are removed from the "eradicated" group (15/18 vs 3/25).

7.5 DISCUSSION:

This study confirms the observation of previous workers regarding the effect of *H. pylori* eradication in reducing the liability to duodenal ulcer relapse and shows, in addition, that *H. pylori* re-infection following successful eradication is uncommon, even in our population with high prevalence of the infection. The latter is in keeping with the low incidence of *H. pylori* re-infection in First World countries (Marshall 1988b, Rauws 1990, Labenz 1992, Forbes 1994), but is at variance with the findings of Coelho et al (Coelho 1992) and Sabbatini et al (Sabbatini 1993), who have reported a 20% and 27% re-infection incidence after eradication (maximum follow-up of 18 months). These data should be interpreted with caution, however. The experience with omeprazole has taught us that, if the organism has been suppressed by therapy, *H. pylori* infection may be difficult to detect, by urease reaction, histology and culture (Louw 1992) as well as urea breath test (Weil 1991). It is possible that these apparently high re-infection rates, or at least the early re-infections, may in fact represent the recrudescence of *H. pylori* not eradicated in the first place. Bell et al have suggested that the so-called re-infections seen after therapy probably represent recrudescence of ineffectively treated infections in the majority of cases, especially if these "re-infections" occur early (< 6 months) after eradication (Bell 1993). This, of course, also applies to our patient re-infected at the 6-month follow-up visit.

One would expect that successfully eradicated patients would escape re-infection only if the exposure risk diminished or, if in the case of exposure, the host had developed the capacity to prevent effective, persistent colonization of the gastric mucosa. While the high prevalence of *H. pylori* infection in the younger age categories supports the theory that the infection occurs primarily at a young age in African subjects, (Holcombe 1992b), there is no information to indicate that the exposure risk changes with aging in the Third World. This may be so in the First World, however, where it is thought that the risk of being infected by the organism reflects the poorer socioeconomic conditions in a cohort of subjects, a risk which has been diminished by increasing affluence and hence an improvement in living standards. The organism has been found in dental plaque and the saliva in adult patients (Shames 1989, Banatvala 1993) and there is also some evidence that the organism can be isolated from stool samples (Thomas 1992, Kelly 1993), making oro-oral, feco-oral transmission, or a combination thereof, the likely vehicle for transmission, However, faecal contamination makes environmentally transmitted infection another possibility, poor water sources having been implicated as a source of the infection in Peruvian children (Klein 1991) and the organism being detected in raw sewage, also in Peru (Westblom 1993). While the risk of exposure may not change in the Third World, it is of course possible that the frequency and magnitude of exposure does diminish in adult life. This may be an important factor, as the limited self-ingestion studies in previously uninfected subjects indicate that the infection may establish itself with some difficulty, even in a previously uninfected host with, one assumes, no acquired immunity (Morris 1987, Marshall 1985).

There is indirect evidence that *H. pylori* is acquired at an early age as a result of household contact (Mendall 1992), while adults cohabiting and exposed to daily household contact do not show a similar tendency (Mendall 1993). The limited capacity for primary adult infection, and thus adult re-infection following eradication, may reflect the host's acquired resistance to re-infection by the organism. There is some indication, although limited, that the human host can mount an effective response against infection by *H. pylori*. This is suggested by the findings of Meyer et al who studied 100 healthy volunteers by means of serology and the ^{13}C-urea breath test (Meyer 1991) and found that 49% of subjects were serologically positive for the infection, while only 24% had active infection as assessed by urea breath test. These findings were interpreted as indicating that a considerable number of patients spontaneously eradicated the organism. The whole question of acquired immunity to *H. pylori* is currently the subject of intensive research, however, and the development of effective acquired immunity to the organism has not been established, although animal work in this regard holds some promise (Buck 1994). The latter would have profound implications for the development of active immunization strategies aimed at preventing *H. pylori* associated disease. Resistance to re-infection may, of course, not be the result of acquired immunity in the classical sense. The organism seems to colonize more readily in the presence of suppressed or impaired acid secretion (Marshall 1985, Morris 1987). It is possible that the high colonization found in young African subjects reflects low acid output, perhaps as a result of an immature acid secretory mechanism and/or malnutrition. These factors may not be relevant in the adult population, making re-infection less likely.

In conclusion, the outcome of this study suggests that *H. pylori* eradication will be an effective long-term management strategy for duodenal ulcer disease, even in developing countries, which tend to have a high incidence of *H. pylori* infection. If confirmed, these findings also augur well for intervention strategies aimed at preventing the other complications of *H. pylori* infection, such as gastric carcinoma.

CHAPTER 8

THE EFFECT OF VARIOUS THERAPEUTIC AGENTS, ALONE OR IN COMBINATION, ON *HELICOBACTER PYLORI* STATUS

8.1 ERADICATION STRATEGIES FOR *H. PYLORI* NOT UTILISING ACID SUPPRESSION:

The finding that successful treatment of *H. pylori* dramatically reduces ulcer relapse rates raised the novel possibility that peptic ulcer disease may be cured by medical therapy alone. This has found expression in the recent recommendations of the NIH (NIH 1994) that patients with proven peptic ulcer disease should be given the benefit of eradication therapy. There is, however, no simple, safe and uniformly effective therapy available at present. In contrast to the excellent *in vitro* activity of many antibiotics against the organism (McNulty 1989), *H. pylori* has proven to be extremely difficult to eradicate. There are a number of possible reasons for this lack of *in vivo* efficacy of most single agents.

8.1.2 Factors determining the success of anti-*H. pylori* therapy:

8.1.2.1 Access to organisms:

H. pylori colonizes gastric mucosa below the mucous layer and can be found deep in the gastric pits (see Chapter 2). To be effective, antibiotics have to penetrate the mucous layer, or be secreted by the gastric mucosa after systemic absorption. One or both of these modes of action may pertain.

Although insufficiently studied, there is some indication that the incorporation of a mucolytic agent in an eradication regimen is not

associated with improved efficacy. McNulty reports that Glupczynski et al were unable to show an advantage when the mucolytic agent N-acetyl cysteine was added to antimicrobial therapy (McNulty 1989). On the other hand, there is indirect evidence that intragastric secretion of an antibiotic after intravenous (IV) administration may be important in determining its eradication efficacy. Adamek et al have shown that the organism can be eradicated following IV administration of amoxicillin in conjunction with omeprazole (Adamek 1994), while Idström et al have found high concentrations of ampicillin in the gastric juice after IV administration of the drug, confirming gastric secretion of systemically administered antibiotic (Idström 1994). It is likely that the luminal concentration of secreted amoxicillin will probably be increased by the antisecretory effect of the omeprazole, as appears to be the case for metronidazole (Kapoor 1994).

8.1.2.2 The galenical form of the antibiotic:

The galenical form of an antibiotic has the potential to influence efficacy in that it determines the distribution of the drug in the stomach, as well as the rate at which the drug is cleared from the stomach. Boixeda et al showed eradication of *H. pylori* in six of 15 patients treated with omeprazole and an amoxicillin suspension, whereas no eradication occurred in any of 21 patients treated with an identical dose capsule formulation (Boixeda 1993). However, this preliminary report also identified a potential drawback of a suspension based therapy: two patients in the suspension arm of the study were withdrawn from analysis because of poor compliance (comparative compliance data for the two legs is unfortunately not given, withdrawals merely mentioned).

8.1.2.3 The acidity of the gastric environment:

McNulty et al have studied the effect of changing pH on the efficacy of various antibiotics *in vitro* (Mc Nulty 1988). Their data shows that the anti-*H. pylori* efficacy, as measured by Minimum Inhibitory Concentration (MIC) varies in relation to the pH of the medium in which it has to function. They demonstrated that most of the antibiotics tested, including ampicillin (amoxicillin) were most effective at neutral pH, the activity of ampicillin increasing 10 fold when the pH was increased from 5.5 to 7.5. The activity of metronidazole was unaffected by pH while tetracycline activity was only marginally affected.

While these findings may explain the therapeutic advantage of tetracycline over amoxicillin in conventional "Triple Therapy" (Chiba 1992), the pH dependency of antibiotic efficacy has to be interpreted with care, since the mucosal uptake of amoxicillin appears to be improved in the presence of a low gastric juice pH (Arena 1993) - increased antibacterial efficacy may therefore be offset by a poorer mucosal absorption. Furthermore, the enhanced bactericidal efficacy caused by raising the ambient pH *in vitro* may not be relevant *in vivo*, as the organism resides in the almost neutral pH environment of the mucus-bicarbonate layer of the stomach and duodenum. This confusing set of data is further complicated by the reported clinical efficacy of antibiotic/proton pump inhibitor dual therapies (see paragraph 8.3 of this chapter).

8.1.2.4 Patient factors affecting eradication efficacy:

Cutler et al reported on the patient (and disease) factors affecting the efficacy of "Triple Therapy" (Cutler 1993). They identified an advanced age and a greater amount of baseline chronic inflammation as factors predicting success, while a lack of compliance as well as the presence of gastric ulceration were associated with a poor outcome in their 96 patients treated with "Triple Therapy" for two weeks. A similar trend for poorer eradication in gastric ulcer disease was seen by Graham et al (Graham 1992b), although the dominant "patient factor" affecting outcome in this study was the compliance of the patient. The poorer outcome in gastric ulcer subjects treated with conventional "Triple Therapy" contrasts with the findings in PPI based eradication therapies, discussed later in this chapter.

8.1.2.5 The presence of a "reservoir" for *H. pylori*:

It has been suggested that *H. pylori* may escape the bactericidal effect of antibacterial therapy by colonizing dental plaque (Rauws 1993), where the organism has been found (Shames 1989, Banatvala 1993, Takagi 1994). The effect of antibacterial therapy on the organism residing in dental plaque has not been studied, however, and the common clinical use of metronidazole in treating gingival disease suggests that susceptible organisms here would probably be effectively treated.

8.1.2.6 Microbial resistance:

Bacterial susceptibility to antibacterial agents is traditionally evaluated by determining the MIC of the antibiotic for the organism. We have alluded to the chemical/physical difficulties which the site of infection poses for

antibiotics and which contributes to the huge discrepancy between *in vitro* and *in vivo* efficacy. McKinlay et al have suggested that the Minimum Bactericidal Concentrations (MBC) of antibiotics against *H. pylori* may be a better predictor of *in vivo* success (McKinlay 1992), but this parameter does not appear to be used often in clinical practice.

In addition to the difficulty that traditional microbiological methodology has in predicting *in vivo* success, the organism does in fact have a significant incidence of primary resistance to certain important antibiotics, while secondary resistance commonly develops in patients in whom antibiotic therapy has failed. This problem is seen particularly with the nitroimidazole compounds (tinidazole and metronidazole) and the macrolides. The importance of this phenomenon is further compounded by the finding of cross resistance within an antibiotic class (Mégraud 1994).

Primary resistance patterns have been best studied for the nitroimidazole compounds and it is clear that resistance is fairly common, being as high as 84% in certain populations in Africa (Glupczynski 1990) and occurs in up to 40% of isolates in European countries(Glupczynski 1991b). In addition, secondary resistance develops in a significant proportion of cases where eradication has been unsuccessful with therapies containing nitroimidazoles as the only antimicrobial agent (Goodwin 1988b). A similar trend has also been noted for the macrolides, but at a much lower level (Mégraud 1994), probably reflecting the lower exposure of populations to these drugs.

The primary and acquired (secondary) resistance characteristics of the organism have rendered certain antibiotics essentially useless in anti-*H.*

pylori monotherapy. This is the case for the fluoroquinolones, where resistance developed in all patients treated with the drug (Glupczynski 1987). Bayerdörffer's experience with fluoroquinolone dual therapy was similar (Bayerdörffer 1987). This lack of improvement in clinical efficacy and resistance pattern when combined with a second antimicrobial agent differentiates the fluoroquinolones from the nitroimidazoles, the latter having an improved efficacy with less acquired resistance when combined with a second, active drug.

These host, organism and environmental factors have made drug selection difficult, leading to an abundance of so-called eradication regimens.

8.1.3 Overview of eradication strategies not based on acid suppression:

The lack of a suitable animal in which the particular problems associated with therapy for *H. pylori* can be evaluated has meant that a plethora of data has accumulated in often empiric clinical trials. Historically, three patterns of treatment have developed.

8.1.3.1 Monotherapy:

The first description in Western literature of the use of an antibiotic in the treatment of peptic ulcer disease antedates the rediscovery of *H. pylori* by several years, with Sancho-Milano et al reporting their (Cuban) experience in 1969 (cited by Diaz 1986). This was followed by further publications in the Cuban literature in 1974 and, eventually, after the prominence given to *H. pylori*, a report appeared in The Lancet of 1986 (Diaz 1986).

Since its rediscovery, various agents have been used in an attempt to eradicate the organism. These monotherapeutic strategies have been reviewed by Chiba et al and although their meta-analysis does not allow evaluation of all possible confounding factors, it is clear that monotherapy is not an effective eradication approach. This is, of course, not surprising if the factors referred to in paragraph 8.1.2 are taken into account. Chiba et al found that monotherapy with bismuth gave eradication rates of 19.6% (387 treated, 76 eradicated), which did not differ from monotherapy rates achieved with amoxicillin - 23%, but was clearly superior to other monotherapeutic strategies, including doxycycline, tinidazole and thiamphenicol, which gave a pooled efficacy rate of 8.2% (Chiba 1992).

Subsequently, the new generation macrolides have been used in eradication studies as monotherapy. Data is limited and almost restricted to pilot studies, but the indications are that monotherapy with azythromycin is ineffective (Glupczynski 1990b) while clarithromycin, to achieve a mediocre 50% eradication rate, needs to be given in mega doses, causing taste perversion in the majority of patients (Peterson 1992).

8.1.3.2 Dual Therapy, not incorporating proton pump inhibitors:

Goodwin et al highlighted the problem of acquired resistance to nitroimidazole antibiotics in 1988, finding acquired resistance to tinidazole in 70% of subjects in whom the organism was not eradicated by a combination of cimetidine and tinidazole, this combination "eliminating" *H. pylori* in only one of 29 patients treated (Goodwin 1988b). At the same time, they could show that the combination of

tinidazole with bismuth protected against the development of nitroimidazole resistance. Therefore, while clearly supplying the theoretical basis for the use of combination therapy in *H. pylori* eradication strategies, the efficacy of dual therapy regimens not incorporating a proton pump inhibitor have been disappointing.

In their meta-analysis, Chiba et al have shown that dual therapy achieves an eradication rate of approximately 48% (550 treated, 265 eradicated) (Chiba 1992). Subgroup analysis indicated that one week of dual therapy was less successful and bismuth and metronidazole combinations were more efficacious than bismuth and amoxicillin. Their analysis could not, however, comment on the influence of primary metronidazole resistance.

The eradication rate achieved (approximately 50%) is, however, unacceptably low. While no prospective cost-effective evaluation for eradication versus other forms of medical management of peptic ulcer disease exists, it is obvious that the benefit is dependent on the eradication rate achieved. Once again, no firm guidelines exist, but the current consensus is that the minimum eradication rate should be of the order of 80%.

8.1.3.3 "Triple Therapy":

At the World Congresses of Gastroenterology in 1990, the Working Party recommended the use of "Triple Therapy" for the eradication of *H. pylori*. This regimen consisted of "CBS (colloidal bismuth subcitrate) or BSS (bismuth subsalicylate), one tablet qds, tetracycline hydrochloride 500mg qds and metronidazole 400mg tds given for two weeks". Amoxicillin 500mg qds could replace the tetracycline (Tytgat 1990).

"Triple Therapy" has remained the reference standard for *H. pylori* eradication, success being determined principally by patient compliance (Graham 1992b) and metronidazole sensitivity (Glupczynski 1990c). This approach is, however, not ideal. Side effects are common, reportedly occurring in approximately 30% of patients taking the treatment, which leads to unacceptable compliance in up to 20% of patients in whom therapy is started (Bell 1993b), a particular problem when treating subjects in clinical practice (Wyeth 1993).

The problems experienced with regard to resistance and side-effects have led to experimentation with the composition of "Triple Therapy" regimens (Al-Assi 1994), dosing frequencies (Borody 1994) and duration of therapy (Burette 1992). We have attempted to determine whether the tetracycline dosage of "Triple Therapy" can be reduced.

8.1.4 THE CAPE TOWN EXPERIENCE WITH "TRIPLE THERAPY" IN *H. PYLORI* ERADICATION:

We have, in a preliminary study, reported our experience with "Triple Therapy" containing sucralfate or bismuth as mucosal protective agent (Louw 1992b) (Table 8.1). In that analysis sucralfate was as effective as bismuth in eradicating *H. pylori* when both were used in combination with metronidazole and a second antibiotic. Our interpretation was made difficult by the use of two antibiotic dosages, but the observation with regard to sucralfate efficacy has recently been confirmed by Stupnicki et al (Stupnicki 1994). Our analysis identified certain trends of practical importance in the management of patients with *H. pylori* infection, but due to the limitation of sample size, these did not achieve significance. These trends were: (i) a strong tendency for superior eradication efficacy

in patients treated for two weeks when compared to those treated for one week (71% vs 43%). As this one week eradication efficacy was considered unacceptably low, no further patients were treated for one week only; (ii) a similar strong tendency favouring treatment with a higher dose of tetracycline - 50% overall efficacy with 250mg qid vs 77% overall efficacy in subjects treated with 500mg qid - irrespective of the duration of treatment.

Table 8.1: Summary of the eradication efficacy of "Triple Therapy" containing sucralfate or bismuth:

	Entered	Completed	Eradicated
	(L/H)	(L/H)	(L/H)
Sucralfate:	20 (11/9)	17 (9/8)	10 (3/7)
Bismuth:	20 (13/7)	18 (13/5)	11 (8/3)

(L = Tetracycline 250mg; H = tetracycline 500mg)

As the eradication efficacy of the low dose therapy appeared to be less than optimal, albeit not statistically significantly so, we elected to continue with our antibiotic dosage allocation policy in studies using "Triple Therapy", so as to investigate the trend referred to above. The findings reported below represent the experience in two "Triple Therapy" eradication studies in our centre, using the same source population, investigator and treatment policy with regard to antibiotic dosage.

8.1.4.1 Patients and Methods:

8.1.4.1.1 Patients:

The data from 42 patients was available for analysis. These patients were recruited in two studies for which the entry criteria required the

presence of active duodenal ulceration (5mm or greater) at the time of enrolment as well as the presence of gastric colonization by *H. pylori* as assessed by urease reaction, histological assessment and/or culture of gastric biopsy specimens. Ulcers were healed by conventional methods (proton pump inhibitor therapy, H_2-receptor antagonist or sucralfate) and eradication therapy started as soon as ulcer healing was documented.

All patients gave written, informed consent to participate in the studies, and both studies were approved by the Ethics and Research Committee of the University of Cape Town.

8.1.4.1.2 Therapy:

Ulcer healing was confirmed endoscopically after which the patients received a mucosal protective agent - either a colloidal bismuth preparation, 120mg, or sucralfate, 1g, both four times a day. All patients received metronidazole, 400mg three times daily. In an attempt to reduce the incidence of side-effects of eradication therapy, the recommended tetracycline or amoxicillin dosage of 500mg qid was used only in patients weighing 75kg or more. Those weighing less than 75kg received 250mg qid. Compliance was assessed by tablet count at the post eradication visit (four weeks following cessation of "Triple Therapy").

Patients were encouraged to report all suspected adverse effects immediately. Therapy was discontinued if symptoms were intolerable or led to work loss. Those with minor adverse effects such as nausea and dysgeusia were encouraged to complete therapy.

8.1.4.1.3 Endoscopy and *H. pylori* status:

Patients were endoscoped at entry to the healing phase, after healing (and before commencement of "Triple Therapy") and a minimum of four weeks after completion of "Triple Therapy", when *H. pylori* status was assessed. All endoscopies, with the exception of the entry endoscopy, were performed by the same endoscopist using standard Olympus PQ and XQ series fibrescopes, Xylocaine local anaesthetic spray for pharyngeal anaesthesia and midazolam 2.5mg IV as sedation, unless contra-indications to its use existed.

H. pylori status was determined at all endoscopy sessions by means of four antral biopsies, taken within 5cm of the pylorus, which were assessed for urease status by means of the Rapid Urease Test (Arvind 1988), histological evaluation of multiple sections of Giemsa stained biopsies (two) and by biopsy culture (one). The biopsy taken for culture was transported in a jar under microaerophilic conditions (Anaerocult C, 13682; Merck) and cultured on tryptose blood agar (CM 233; Oxoid Ltd) containing lysed horse blood (5% vol/vol) at 37^{o}C under microaerophilic conditions (12% CO_2, 88% air, 95% humidity) for a minimum of seven days. *H. pylori* was positively identified by colony morphology and urease reaction (Christensen's urea slope). Patients were considered *H. pylori* positive if a positive urease reaction was confirmed by histology and/or culture.

8.1.4.1.4 Statistical analysis:

The categorical data was analysed by means of the Fisher's exact test and a p value of less than 0.05 was taken to indicate significance.

8.1.4.2 Results:

8.1.4.2.1 Medication distribution:

Thirty patients (males =23, females =7) received 250mg of antibiotic qid for the two week treatment period (amoxicillin = 0, tetracycline = 30) and 12 patients (males 10, females 2) received 500mg qid (amoxicillin = 2, tetracycline = 10). The antibiotic compliance rates in the two dosage groups were similar - 93% for both antibiotic and metronidazole in the 250mg treatment group and 90% for the antibiotic and 86% for the metronidazole in the 500mg group.

8.1.4.2.2 Eradication efficacy:

The eradication efficacy was significantly better in the group receiving 500mg of antibiotic qid. The organism was eradicated in 11 (92%) of the 12 patients in this group and in only 15 (50%) of the 30 subjects in the 250mg group - this represented a significant difference ($p=0.01$).

8.1.4.2.3 Side-effects:

The lack of efficacy in the lower dose treatment group was not associated with a significantly superior side-effect profile. While side-effects were usually mild, they were commonly reported. Side-effects were reported by five (42%) of the 12 subjects in the 500mg group (nausea = 2, stool abnormality = 2, dizziness = 1), and eight (27%) of the 30 subjects in the 250mg group (dysgeusia = 1, nausea = 2, vomiting = 1, dizziness = 3, stool abnormality =3, including *C. difficile* positive diarrhoea in one patient). This was not a statistically significant difference in reporting frequency. The potentially most severe side-effect

(*C. difficile* diarrhoea) occurred in the 250mg group and side effects restricted the compliance to < 60% in two of the subjects in this group, while only one subject in the 500mg group used <60% of the antibiotic component of therapy. Side effects were reported with similar frequency in sucralfate and bismuth treated subjects - 6 (25%) of 24 bismuth treated subjects and 7 (38%) of 18 sucralfate treated subjects ($p > 0.5$).

8.1.4.3 Discussion:

Our experience with "Triple Therapy" indicated that conventional "Triple Therapy", i.e with full dose antibiotics, used for two weeks in a compliant, well motivated study population, is probably as effective in our population as it is in populations from developed countries (Chiba 1992). The policy of reducing the tetracycline dosage was, on the other hand, clearly not effective and held no statistically significant advantage with regard to the side-effect profile. Side-effects occurred commonly in both regimens, and may reduce the efficacy of the regimen in less well-motivated and counselled subjects.

The efficacy of conventional "Triple Therapy", in the presence of an extremely favourable compliance, was not surprising. In contrast to the experience elsewhere in Africa, where *H. pylori* is commonly found to be resistant to metronidazole (Glupczynski 1990), metronidazole resistance in South Africa in general and our population in particular, appears to be relatively uncommon. Dawes et al reported a (primary) metronidazole resistance rate of 32.5% in their population from the Gauteng region (Dawes 1991). Unfortunately the number of isolates is not known. Primary metronidazole resistance appears to be even less common in our population. We studied the antibiotic sensitivity profiles in 42 clinical

isolates from our clinic (Greig 1993) and found an overall resistance rate, defined as a minimum inhibitory concentration equal to or more than 16mg/l, of 20%. This rate compares favourably with that calculated by DeCross et al to be compatible with a successful outcome for empiric "Triple Therapy" in the population. These workers suggested that empiric "Triple Therapy" would achieve cure rates in excess of 80% as long as the population prevalence of metronidazole resistant *H. pylori* was below 30% (DeCross 1993).

The poor efficacy of the regimen when the dose of tetracycline was halved contrasts with that reported by other workers. Thijs et al reported a 93% eradication rate in subjects receiving colloidal bismuth subcitrate 120mg, tetracycline 250mg and metronidazole 250mg, all qid (Thijs 1993). Borody et al also found an acceptable eradication rate with 250mg of tetracycline, the common factor in both studies being an increased frequency of administration of metronidazole (Borody 1994). We believe our finding is compatible, however, with the findings made by others (Graham 1992b) with regard to the influence of compliance on the efficacy of full dose "Triple Therapy" which suggest that subjects have to take >60% of a conventional two week course of "Triple Therapy" for it to be successful. It is, however, difficult to reconcile the poor outcome of the low dose therapy with the apparent efficacy of short course (one week) treatments reported from Hong Kong (Hosking 1994). Further study is clearly needed to define the optimal "Triple Therapy" with regard to composition, optimal dosage and dosing frequency, the timing of intake and the duration of therapy. This is important, as it is probable that "Triple Therapy" will remain an important therapeutic approach, especially in poorer (Third World) communities.

In summary, our experience with "Triple Therapy" suggests that therapy with full dose, conventional "Triple Therapy" can be expected to eradicate *H. pylori* effectively, but the tedious regimen coupled with the significant incidence of side-effects limits its usefulness in clinical practice.

8.2 THE EFFECT OF THE PROTON PUMP INHIBITORS ON GASTRIC *HELICOBACTER PYLORI* STATUS:

8.2.1 Introduction:

After the introduction of omeprazole and its acceptance locally as optimal therapy for peptic ulcer disease, we were surprised to find that it was difficult to identify *H. pylori* in the antrum of patients treated with the drug. Against this background, the reports of an omeprazole effect on *H. pylori* which appeared in the late 1980's and early 1990's were of particular interest (Delmee 1989, Vigneri 1990, Biasco 1989, Catalano 1989, Goh 1991, Hui 1991b). These studies, using varying methodology for the detection of *H. pylori* as well as varying omeprazole dosage schedules, showed that *H. pylori* was not detectable in the stomach in 40 - 100% of patients immediately after therapy (Table 8.2).

Table 8.2: Reported effect of omeprazole monotherapy on gastric *H. pylori* status:

						CLEARANCE%			
		Diagnostic method				omeprazole (mg/day)			
Study	**n**	**Urease**	**Histo**	**Cult**	**UBT**	**10**	**20**	**40**	**Erad**
#Delmee 1989:	9	*	*	*	*			78	NA
Biasco 1989:	18		*				56		NA
Catalano 1989:	12	*	*	*			100		NA
#Mainguet 1989:	9			*					
Vigneri 1990:	26	*	*	*			77		NA
Goh 1991:	5	*						100	NA
Hui 1991:	73			*		40			NA
	67			*			64		NA

Histo Histology
Cult Culture
UBT Urea Breath test
Erad Eradication
NA Not assessed
same centre

Relapse rates following ulcer healing with omeprazole do not, however, differ significantly from those following healing with an H_2-receptor antagonist (Maton 1991), the latter having no documented effect on *H. pylori* status (Rauws 1989). We believed it was reasonable to assume that, if the elimination of *H. pylori* was an important factor in preventing duodenal ulcer relapse, the clinical experience with omeprazole made it unlikely that the drug effectively eradicated the organism from the stomach.

8.2.2 Aim of study:

We therefore set out to determine whether gastric *H. pylori* status is influenced by omeprazole therapy and, if so, whether it clears or eradicates the organism from the gastric antrum.

8.2.3 Patients and Methods:

8.2.3.1 Patients:

Thirty-two *H. pylori* positive patients with non-healing duodenal ulcers (i.e. ulcers unhealed following eight weeks of antacid or H_2-receptor antagonist therapy) or recurrent ulceration (second endoscopically documented ulcer within a 12 month period) were entered into the study. Data from 26 could be analysed (two patients defaulted and in four the ulcers were not healed at the end of therapy; three of these continued with a further four weeks of therapy and one withdrew from the study). Written informed consent was obtained from each patient, and the study was approved by the Ethics and Research Committee of the University of Cape Town.

8.2.3.2 Endoscopy:

Endoscopy was performed at entry to the study, after documented healing with omeprazole (clearance assessment), and again after a minimum of 28 days following cessation of omeprazole therapy (eradication assessment) in patients considered *H. pylori* negative on Rapid Urease Test (RUT) and histology at the post treatment endoscopy. An Olympus PQ20 fibreoptic gastroscope was used. Both gastroscope and biopsy forceps were thoroughly cleaned and sterilised in 2%

gluteraldehyde between procedures. Patients received midazolam 2,5 mg IV as sedation unless contraindications to its use existed, and xylocaine local anaesthetic was used as pharyngeal anaesthetic.

8.2.3.3 *H. pylori* status:

H. pylori status was determined by means of antral biopsy samples, taken within 5 cm of the pylorus, as follows:

Urease reaction: Urease status was determined by means of the rapid urease test (RUT) as described by Arvind et al (Arvind 1988).

Histological assessment: Two antral biopsies were evaluated histologically for *H. pylori* status by the same pathologist who was not aware of the therapy received. *H. pylori* presence was determined in sections of Giemsa stained material, and colonization graded from 0 - 3, where: 0 = absence; 1 = mild (sparsely distributed organisms in the superficial part of the mucosa in isolated sections); 2 = moderate (more numerous bacteria on the mucosal surface and a few in crypts); 3 = severe (abundant organisms present on the surface and in the crypts of all mucosal sections).

Culture: One antral biopsy was taken for *H. pylori* culture and was transported directly to the laboratory in a jar under microaerophilic conditions (Anaerocult C, 13682; Merck). The specimen was cultured on tryptose blood agar (CM 233; Oxoid Ltd) containing lysed horse blood (5% vol/vol) at 37°C under microaerophilic conditions [12% CO_2, 88% air (6% O_2), 95% humidity] for a minimum of 7 days. *H. pylori* was positively identified by colony morphology and urease reaction

(Christensen's urea slope). Growth was visually scored, by the same microbiology technologist as 0 (absent) 1 (sparse), 2 (moderate) or 3 (abundant growth).

8.2.3.4 Therapy:

All patients were treated with omeprazole, 20 mg/day, for four weeks.

8.2.3.5 Statistical analysis:

Data was analysed by means of Mc Nemar's exact test.

8.2.4 Results:

At entry, all patients were *H. pylori* positive on RUT and histology, and all but one on culture.

Table 8.3: Summary of histological and culture score frequencies before and immediately after omeprazole therapy:

	Histology		Culture	
Score	Before	After	Before	After
	n	n	n	n
0	0	13	1	6
1	9	10	3	10
2	9	2	7	2
3	8	1	15	8

Immediately after therapy: The data summarised in Table 8.3, shows that omeprazole therapy significantly affected *H. pylori* status. *H. pylori* could not be histologically detected in 13 patients after therapy, whereas all patients were histologically positive at entry. The histological score

improved in 18 patients, remained unchanged in seven and worsened in only one patient after therapy ($p < 0.001$).

The same trend was noted with regard to *H. pylori* cultures (Table 8.3). *H. pylori* could not be cultured in six patients after therapy. The score improved in 13, was unchanged in 10 and worsened in only three patients ($p < 0.05$).

The results of the RUT also changed significantly after therapy, 11 patients testing negative after omeprazole therapy (26/26 positive before therapy, 15 of 26 positive following therapy, $p<0.001$). These included four patients in whom antral histology was considered negative.

H. pylori was still detectable by RUT, histology and culture in the three patients treated with omeprazole for an additional period of four weeks.

In addition to the improvement in the individual parameters shown, six patients were free of *H. pylori* when assessed by urease reaction, histology and culture immediately after cessation of omeprazole therapy. When the results of the urease reaction and histology alone were used, nine patients were assessed as being free of *H. pylori* infection.

One month after completion of therapy: Repeat studies now showed that these nine patients were again clearly positive for *H. pylori* (RUT = 8, histology = 8, culture = 9) ($p < 0.005$, compared to immediately post omeprazole).

8.2.5 Discussion:

This study showed that short-term omeprazole therapy has an undoubted effect on gastric *H. pylori* status, as assessed by antral urease reaction, histology and culture. It should be noted that at the time, assessment of *H. pylori* in the antrum was thought to be sufficient to determine gastric *H. pylori* status (Tytgat 1990). The study indicated, however, that contrary to the belief of some workers, suppression was a transient phenomenon - i.e. omeprazole was able to clear the organism in a significant proportion of subjects immediately following therapy, but eradication did not occur.

Our findings are in keeping with the results of a number of studies published in the 1990's. Weil et al, using the ^{14}C-urea breath test, showed that the organism appeared to be suppressed following omeprazole therapy in nine of 24 patients, while eradication as defined by urea breath test occurred in only one (Weil 1991). Daw et al, using antral biopsies assessed by urease reaction, culture, histopathology and electron microscopy showed a similar finding - therapy with omeprazole clearing but not eradicating the organism in the 21 subjects studied (Daw 1991). Evidence from these studies, as well as our own, indicates that all current diagnostic modalities lack sensitivity in determining *H. pylori* status following therapy aimed at its eradication, a fact ostensibly conceded by the very definition of "eradication", i.e. the failure to demonstrate the organism on antral biopsy samples, by culture and histology, one month after cessation of therapy (Tytgat 1990). Our findings were limited to short term therapy, but have been confirmed by retrospective analysis in longer term follow-up of patients on

"maintenance therapy" in at least two studies in which omeprazole did not appear to affect *H. pylori* status (Rauws 1991, D'Adda 1992).

It is not immediately clear why omeprazole should suppress *H. pylori* infection. It has been suggested that the inability to detect *H. pylori* after omeprazole therapy is caused by the presence of bacterial overgrowth in the achlorhydric stomach (Rauws 1991). Although this is plausible with respect to the culture of this fastidious organism, it does not explain the improvement in histological grading and urease reaction noted in our study. Omeprazole's effect on *H. pylori* status may, of course, reflect the organism's response to a drastically changed gastric milieu. It has been postulated that *H. pylori* survives in acid by generating ammonia and bicarbonate through its urease dependent hydrolysis of urea present in the gastric juice (Goodwin 1986). *In vitro* studies certainly support this hypothesis (Marshall 1990). This protective mechanism, however, may conceivably contribute to the destruction of the organism as neutralisation of the products of urea hydrolysis may not be possible in the hypochlorhydric stomach and, in this setting, accumulation of these products may prove toxic to the organism itself. This theory is supported by the *in vitro* observations of Greig et al, who could demonstrate a pH-dependent "suicidal" destruction of *H. pylori*, mediated by its urease activity (Greig 1991), and by the clinical observation of a lower than expected infection rate in patients with pernicious anaemia (Fong 1991).

The observations of Vigneri et al offered an alternative explanation for the apparent antral clearance seen with omeprazole therapy (Vigneri 1991). They observed, in a sample of 29 patients, that the organism was cleared from the antrum in 23 of 26 subjects, but that it was found in the body of the stomach in approximately 38% of subjects previously

considered not to harbour the organism in the body. This observation has since been confirmed by a retrospective analysis of patients treated with PPI therapy for duodenal ulcer healing. We could show that treatment with the PPIs omeprazole, lansoprazole and pantoprazole led to a change in the gastric *H. pylori* colonization pattern, the antrum tending to be cleared while the body tended to be colonized by the organism during therapy (Louw 1994). This finding was not limited to a specific proton pump inhibitor. The recently published findings of Logan et al lend further support to a "re-distribution" theory with regard to omeprazole (Logan 1995). These workers reported their findings in 29 patients with gastroesophageal reflux or duodenal ulcer disease, and found, as we did, that omeprazole therapy decreased the incidence of antral colonization, but increased the incidence of colonization in the fundus.

The mechanism by which the PPIs cause the gastric redistribution of *H. pylori* is not clear. It seems unlikely that the effect is mediated by the inherent bactericidal effect which these agents have been shown to possess (Mégraud 1991, Suerbaum 1991, Mégraud 1991b), as the organism appears to be redistributed, rather than dead. It is possible that the redistribution represents a migration of the organism to the acid secreting body of the stomach, where, presumably, the pH is more favourable for its survival in the presence of its own urease activity.

Whatever the mechanism mediating this redistribution, the clinical implications are clear. At present, no test can reliably exclude the presence of *H. pylori* in the stomach immediately following cessation of PPI containing therapy. Although Logan et al suggest that the urea breath test is capable of doing this, their own findings and those of Weil et al clearly indicate that false negative urea breath tests do occur (Logan

1995, Weil 1991). If biopsy techniques are to be used in assessing *H. pylori* status following PPI therapy, adequate biopsies from both the antrum and fundus need to be evaluated. It will, furthermore, be important to determine whether the observed effect of the PPIs on gastric *H. pylori* colonization can be exploited in anti-*H. pylori* combination therapy.

8.3 PROTON PUMP INHIBITOR AND ANTIBIOTIC DUAL THERAPY:

8.3.1 Introduction:

We have already reviewed the use of "Triple Therapy" in eradicating *H. pylori* and have indicated that this approach also appears to be effective in our population (Paragraph 8.1). "Triple Therapy" has its shortcomings, however, especially in clinical practice. These are related essentially to the side-effect profile of the combination, which in turn determines patient compliance. A further shortcoming has been the perceived need first to heal ulcers before eradication is attempted, as recommended by the World Congress Working Party in 1990 (Tytgat 1990), although it should be noted that recent publications from Hong Kong question the need for this (Hosking 1994, Sung 1995).

Unge et al were the first to explore the therapeutic potential of a combination of omeprazole and amoxicillin (Unge 1989). They showed that the combination of omeprazole and amoxicillin increased eradication efficacy in subjects when compared to amoxicillin monotherapy as well as to omeprazole monotherapy.

The combination of an effective ulcer healing agent and an antibiotic makes therapeutic sense, if the combination eradicates the organism. Not

only would this combination heal the ulcer, it would also, if effective, eradicate *H. pylori* thereby curing the diathesis. It is also apparent from the discussion in section 8.1.2 of this Chapter that the combination of a potent suppressor of acid secretion and an antibiotic makes sense theoretically, because of the effect of the acid suppressive agent on the acid gastric environment.

Omeprazole/amoxicillin dual therapy has been extensively used (Table 8.3). Initial reports, emanating from Germany, suggested that eradication rates of the order of 80% could be achieved with this combination. It was generally accepted, and widely recommended, that a twice daily dosage of omeprazole, an amoxicillin dose of at least 500mg tds and a two-week duration of therapy would eradicate the organism in almost all cases. It was further believed that pre-treatment with omeprazole would reduce the efficacy of this regimen (Hunt 1993). The latter recommendation was based on the limited clinical findings of Labenz et al (1993b). It was argued that pre-treatment with the PPIs could induce a persistent, coccal form of *H. pylori.* It is worth noting, however, that while the larger, degenerative coccoid form of *H. pylori* has been noted *in vivo*, the existence *in vivo* of the persistent form - a small, electron dense coccoid - is as controversial now as it was in 1989 (Megraud 1989b).

Table 8.4: Efficacy of omeprazole and amoxicillin dual therapies:

Study	n	Omeprazole dose	Amoxicillin dose	Days co Rx	Erad %
Bell 1991:	16	20mg nocte	250mg tds	14	31
Bayerdörffer 1992b:	27	40mg bd	1G bd	10	82
Labenz 1992:	51	40 or 80 mg/day	500mg qid	7	63
Bell 1992:	28	40mg/day	500mg tds	14	50
	25				48
Labenz 1993b:	31	40mg/day	500mg qid	7	61
	47	40mg bd	500mg qid	7	62
	58	20mg bd	500mg qid	14	83
	21	20mg bd	500mg qid	7*	29
Unge 1993:	157	40mg/day	750mg bd	14	54
Bell 1993b:	67	40mg/day	500mg tds	14	49
Bayerdörffer 1993b:	27	40mg bd	1G bd	10	82
Labenz 1993:	19	20mg bd	500mg qid	14	79
Adamek 1994:	12	20mg bd	500mg 6x/d	14	91
Labenz 1994c:	35	20mg bd	1G bd	14	91
	350	40mg bd			85

* Amoxicillin commenced after 7 days of omeprazole monotherapy

It is clear from the data in table 8.4, however, that the studies with omeprazole-based dual therapy are poorly standardised. Duplicate reporting further complicates the interpretation of results, making it extremely difficult to base recommendations on available studies. The data generated from a single centre may be more suitable for this purpose. Labenz et al attempted to identify factors determining the efficacy of omeprazole/amoxicillin dual therapy (Labenz 1994b). They were able to analyse the outcome of 423 treated patients, and identified lack of compliance, short duration of treatment, smoking and omeprazole pre-treatment as factors predicting a poor outcome, while advanced age,

active gastritis and gastric ulcer disease appeared to be associated with an improved efficacy. However, this data was generated in a centre which achieved extremely high eradication rates with dual therapy.

The dual therapy data is also remarkably inconsistent, with efficacy rates varying from < 50% to > 80%. It is obvious that the dual therapy regimens have to be evaluated in the target population before recommendations regarding its efficacy and use can be made.

8.3.2 THE CAPE TOWN EXPERIENCE WITH REGARD TO OMEPRAZOLE AND AMOXICILLIN DUAL THERAPY:

8.3.2.1 Aim of study:

This study was undertaken to determine the eradication efficacy of omeprazole/amoxicillin dual therapy in the population we serve, and to determine whether pre-treatment with omeprazole affects the outcome of therapy.

8.3.2.2 Patients and Methods:

8.3.2.2.1 Patients:

Adult subjects, with endoscopically confirmed, histologically benign gastric ulceration (smallest diameter >5mm) were eligible for study. In addition, *H. pylori* colonization, initially detected by urease reaction of antral and body gastric biopsy, had to be confirmed by histological assessment of two samples each from the same site.

Sample size was calculated on the basis of Labenz's data (Labenz 1993b). The following assumptions and conditions were used:

(i). Eradication efficacy of dual therapy started concurrently = 80%; (ii). Difference in efficacy with omeprazole pre-treatment = 50%; (iii). Sample size to detect 50% difference with 80% certainty and 95% specificity.

On this basis, a sample size of 14 protocol correct subjects was considered adequate to achieve adequate statistical power.

Patients were interviewed before entry and were enrolled only if they did not require regular, prescribed use of NSAIDs or salicylate products in the month before admission. In addition, smoking and alcohol habits were recorded.

All patients gave written, informed consent and the study was approved by the Ethics and Research Committee of the University of Cape Town.

8.3.2.2.2 Endoscopy Schedule:

All patients were endoscoped at entry, after four weeks of therapy (to confirm ulcer healing) and four weeks later (if the ulcer was not healed at the four week endoscopy). Eradication was assessed four weeks after endoscopically documented healing.

Patients were endoscoped by the enrolling endoscopist, using standard fibreoptic endoscopes of the Olympus PQ20 and XQ20 series, with midazolam 2.5mg IV as sedation and Xylocaine local anaesthetic spray for pharyngeal anaesthesia.

At endoscopy, biopsies were taken as follows:

Entry endoscopy: Two biopsies each from the antrum and body for histological assessment of *H. pylori* status; one biopsy each from the body and antrum for RUT; quadrantic biopsies from the ulcer site.

"Eradication" endoscopy: Biopsies for *H. pylori* assessment as above.

Additional biopsies were taken from the ulcer if it had not healed at the time of the "healing" (four week) endoscopy.

8.3.2.2.3 Therapy:

All patients were treated with omeprazole, 20mg bd for a minimum of four weeks. If after one month the ulcer had not healed, treatment was continued with omeprazole 20mg/day for a further four weeks. If ulcers were not healed after a total of eight weeks of therapy, the patient was withdrawn from study. In addition, patients were randomly allocated to one of two antibiotic treatment schedules. Patients either received placebo during the first two weeks of omeprazole therapy, followed by amoxicillin, 1g bd during the second two week treatment period, or, alternatively, received amoxicillin, 1g bd during the first two weeks of therapy, followed by the placebo during the second two week period.

Compliance was assessed by tablet count at the four week endoscopy visit, at which time patients were encouraged to report any adverse events.

8.3.2.2.4 *H. pylori* status:

One biopsy each from both the antrum and body of the stomach was evaluated for the presence of *H. pylori* by means of the RUT (Arvind 1988). Two biopsies from both the antrum and the body of the stomach were placed in 10% neutral formalin before processing for histological assessment of *H. pylori* status. Haematoxylin (H) and eosin (E) staining was standardly used, and if required (no organism detected on H and E), sections were stained by the modified Giemsa method. The pathologist was not informed as to the treatment status of the subjects.

To be considered *H. pylori* positive, a positive RUT had to be confirmed by the detection of *H. pylori* by histology in the antrum and/or body sections.

8.3.2.3 Results:

8.3.2.3.1 "Intention to treat" analysis:

In total, 49 patients were recruited for the study. Of these, seven were considered to have been entered in violation of protocol: In two patients the ulcer was found to be histologically malignant, despite an apparent "benign" endoscopic appearance, one patient was enrolled despite a negative RUT, while the RUT finding was not confirmed by histology in three subjects. In addition, no follow-up data was available in one subject who absconded after the initial endoscopy.

This left 42 patients for evaluation on an "intention to treat" basis. Their findings are summarised in table 8.5.

Table 8.5: Patient characteristics and patient outcome following randomised dual therapy: "Intention to treat"

	Antibiotic during first 14 days	Antibiotic during second 14 days
N:	21	21
Male:Female:	14:7	14:7
Age: Mean(SD) years:	49.7(8.4)	46.8(8.5)
Smokers:	18	20
Alcohol use:	3	10
Healed at four weeks:	18	14
Healed at eight weeks:	2	5
Antibiotic compliance: % (SD)	96%(7.6)	87%(23.7)
Side-effects reported:	1	1
Eradication efficacy: n(%)::	12/21(57)	10/21(48)

The patients were well matched for age, sex and smoking habit, but significantly more patients in the "second two week" treatment group admitted to using alcohol ($p < 0.05$, Fisher's exact test). Compliance, as assessed by tablet count in 17 of the 21 patients in the first two week and 20 of 21 in the second two week group, tended to be superior in the first two week group. The data showed a wide scatter in the second two week group, however, because of a single patient who took no active antibiotic at all. The treatment was extremely well tolerated by these patients, only one patient experiencing a pruritic reaction to the PPI/placebo combination which led to his discontinuation of treatment, while a second reported an episode of "dizzyness" while on the placebo antibiotic leg in the first week treatment group.

An eradication efficacy difference of 9% was noted between the first and second two week treatment groups. This difference does not approach significance ($p = 0.37$, Fisher's exact test).

Although observations with regard to early relapse are probably premature, we noted that two of the 22 (9%) *H. pylori* negative subjects in this study had already developed an ulcer relapse at the "eradication visit" endoscopy. One of these patients admitted to salicylate use in the period preceding relapse.

8.3.2.3.2 "Per protocol" analysis:

Two patients were removed from the "intention to treat" analysis in the first two week treatment group: one patient was not healed following eight weeks of PPI therapy, while a second was healed after eight weeks, but biopsies were not taken for assessment of *H. pylori* status. Three patients were excluded from analysis in the group treated with antibiotics during the second two weeks of omeprazole therapy: two patients had not healed following eight weeks of omeprazole therapy, while one patient had experienced a pruritic reaction to omeprazole and placebo and had taken none of the amoxicillin therapy.

The exclusion of these five patients from the analysis did not affect the outcome significantly. Compliance in the second two week group improved from 87% to 91%, while it remained unchanged in the first two week treatment group - 96%. The eradication efficacy was similar in the two groups - the organism was eradicated in 12 (63%) of 19 patients in the first two week treatment group, and in 10 (56%) of 18 patients in the second two week treatment group - a difference now of only 7%.

8.3.2.4 Discussion:

The findings indicate that, although superbly tolerated by our patients, omeprazole/amoxicillin dual therapy falls short of the ideal projected eradication efficacy of 80%. We have also been unable to confirm the marked, clinically significant, detrimental effect of omeprazole pre-treatment on the outcome of omeprazole dual therapy eradication efficacy, as suggested by Labenz et al (Labenz 1993b).

The poor eradication rate achieved with omeprazole/amoxicillin dual therapy was not surprising. As can be seen from the data in table 8.4, the eradication efficacy was well within the rates described by others. We have also obtained poor eradication efficacy with the use of amoxicillin in combination with either lansoprazole or pantoprazole in duodenal ulcer subjects. Lansoprazole (30mg bd) and amoxycillin (1g bd) in our population achieved an eradication efficacy of approximately 30% (per protocol and intention to treat) (Louw, unpublished data), while a combination of pantoprazole (40mg bd) and amoxicillin (500mg tds, 10 days) gave an eradication efficacy of approximately 20% (Louw 1994b).

The marked regional differences in efficacy reported with omeprazole/amoxicillin dual therapy are not readily explained. If Labenz's analysis of their data is correct, it is difficult to identify factors for "treatment failure" in our gastric ulcer subjects, other than the high incidence of smoking - 90% of the total sample smoked - found locally (Labenz 1994b). Zala et al also identified smoking as a factor affecting the outcome of omeprazole and amoxicillin dual therapy (Zala 1994). It would be difficult to improve on overall compliance, although it may be argued that the timing of antibiotic intake could be rigidly enforced, for

example, in relation to meals, a factor identified by some as influencing the outcome of therapy, pre-prandial intake being considered to be more effective by some workers (Atherton 1994). A more rigid approach, however, is extremely unlikely to be successful in the outpatient setting. The recommendation also has to be viewed with some scepticism at this stage, as the same group reported previously that the timing of amoxicillin intake in relation to meals did not affect eradication efficacy significantly! (Atherton 1993).

The lack of a significant difference between the outcome of patients treated *ab initio* with dual therapy and those receiving omeprazole pre-treatment is not surprising (a sample size in excess of 450 patients/arm would be needed to confirm that the 9% difference in efficacy is "real", with 80% certainty and 95% specificity). The initial observations made by Labenz were, we believe, made in a clearly non-comparative fashion (table 8.4, Labenz 1993b). The data shows that the poor results in the patients treated following ompeprazole "pre-treatment" were found following only seven days of amoxicillin/omeprazole dual therapy. Our findings are, furthermore, similar to those of Zala et al, who could not demonstrate that omeprazole pre-treatment affected treatment outcome in their patients (Zala 1994). These workers treated a total of 61 patients with dual therapy, the amoxicillin being given for 10 days. In 26 of these patients the amoxicillin was given after 20 days of omeprazole (20mg/day) monotherapy. The outcome was essentially the same - the organism was eradicated in 35% of the patients pre-treated and in 31% of those co-treated *ab initio.*

The constant search for improved eradication therapies bears testimony to the generally perceived inadequacy of omeprazole and amoxicillin dual

therapy. Two alternative PPI based strategies have emerged. On the one hand amoxicillin has been replaced by an alternative antibiotic, while on the other the PPI has been combined with at least two antibiotics.

The most successful replacement for amoxicillin appears to be the new generation macrolide, clarithromycin. This drug has been mentioned in paragraphs 8.1.2.6 and 8.1.3.1 of this chapter, and the ineffectiveness as monotherapy as well as the potential to induce secondary resistance discussed. Clarithromycin has one important theoretical advantage - it is markedly concentrated in tissue, and achieves a much higher concentration in the gastric mucosa than amoxicillin (Chang 1993). Clarithromycin is also, unlike the earlier generation macrolides, stable under acidic conditions. Despite initial concern with regard to the possible induction of (or selection for) antibiotic resistance, an increasing number of authors are claiming success with omeprazole and clarithromycin dual therapy. Mendelson et al, in a preliminary communication, were the first to report success in a sample of 25 subjects (Mendelson 1992). These workers reported an eradication rate of 80% following treatment with omeprazole 40mg/day and clarithromycin 500mg tds for 14 days. One patient was not able to complete therapy because of dysgeusia. The same group, but under different principal authorship, has recently published their final results in 73 patients (which presumably includes the 25 patients reported earlier) (Logan 1994). In this larger series they have confirmed the excellent outcome, with an eradication rate of 78%, while Logan has also published eradication rates of 83% in 69 subjects treated with a similar clarithromycin and omeprazole regimen (Logan 1994b).

These promising results with omeprazole and clarithromycin should be interpreted with caution. The experience with omeprazole/amoxicillin dual therapy has shown that promising results in one centre or region cannot necessarily be extrapolated to other centres or populations. This is probably more important in the case of clarithromycin, a drug for which *H. pylori* has been well documented to have the ability to develop resistance, a factor not applicable in the case of amoxicillin. The warning signs for omeprazole and clarithromycin may already be apparent. Deltenre et al, reporting their experience from Belgium, have noted a low eradication rate of only 47% (intention to treat) in 17 patients treated with a regimen similar to the Logan regimen (Deltenre 1994), while Katelaris et al found an eradication rate of 67% in their 21 patients treated with an identical regimen (Katelaris 1994).

More recently the PPIs have been combined with two antibacterial agents in an attempt to ensure a higher and more predictable eradication efficacy (Table 8.6). This approach has had two important effects. Firstly, the overall efficacy of therapy aimed at *H. pylori* appears to have become more predictably in excess of 80%, while shorter term therapy (7-10 days) has become possible. This would increase compliance. Although the majority of the trials using the PPI based triple therapies have been reported only in abstract form, the investigators report excellent tolerance and compliance with the various regimens.

Table 8.6: The characteristics and eradication efficacy of combinations of omeprazole and two antibiotics (Duplicate communications excluded):

Study	n	Days of Co-Rx	Combination (daily intake)	Erad efficacy	Comments
Bazzoli 1993:	36	7	Om 20mg Cla 250mg bd Tin 500mg bd	100%	Compliance >90%; further experience reported 1994
Lamouliatte 1993:	11	10	Om 20mg bd Am 2G Cla 500mg tds	91%	Removes imidazoles from therapy
Vigneri 1993:	45	7	Om 40mg Am 500mg qid Mnz 250 qid	87%	
Lamouliatte 1993b:	8	14	Lz 30mg Am 500mg qid Tin 500mg bd	88%	Intent to treat = 70%
Burette 1993:	20	14	Lz 30mg Rox 300mg Mnz 500mg bd	80%	All failures Mnz resistant following therapy.
Bell 1993b:	127	14	Om 40mg Am 500mg tds Mnz 400mg tds	84%	Erad efficacy in Mnz sensitive strains = 96% of 55
Lamouliatte 1994:	21	10	Om 20mg Am 2G Cl 500mg tds	95%	Comparative study comparing 4 regimens
Labenz 1994d:	7	40	Om 20mg Cla 250mg bd Mnz 400mg bd	95%	Well tolerated
Labenz 1994d:	7	40	Om 20mg Cla 250mg bd Tetra 500mg bd	65%	Withdrawal because of side-effects in 3
Moayyedi 1994:	47	7	Om 20mg bd Cla 250mg Tin 500mg bd	94%	19% side effects, no withdrawals
Cayla 1994:	19	10	Om 20mg Am 2G Tin 500mg bd	68%	
Cayla 1994:	22	10	Om 20mg Am 2G Cla 500mg tds	91%	
Cayla 1994b:	11	14	Lz 30mg bd Am 1G bd Cla 500mg bd	100%	Side-effects noted in 4 subjects, Rx not stopped

Om = omeprazole
Am = amoxicillin
Mnz = metronidazole
Rox = roxythromycin
Erad = eradication
Lz = lansoprazole
Cla = clarithromycin
Tin = tinidazole
Rx = therapy
Tetra = tetracycline

There have also been developments regarding the use of the H_2-receptor antagonists in *H. pylori* eradication therapy. Although these drugs are considered to have no intrinsic anti-*H. pylori* effect and have been noted to be inferior to omeprazole in dual therapy (Al-Assi 1994b), Hentschell et al have reported excellent eradication success when ranitidine was used in combination with amoxicillin (750mg tds) and metronidazole (500mg tds) for 12 days, eradication being found in 46 (88%) of 52 patients treated in this way (Hentschell 1993). In a further attempt to improve the efficacy of the H_2-receptor antagonists, ranitidine has been combined with bismuth subcitrate, to form the "novel" compound GR122311X (ranitidine bismuth citrate). This compound has been shown to be effective in combination with one or two antibiotics, clarithromycin being the most successful single agent (Wyeth 1994). Ranitidine bismuth citrate, which uses the pH modulating effect of the ranitidine and the antibacterial properties of bismuth, is the first compound manufactured to address specifically the problem of *H. pylori* eradication.

Finally, it should be noted that there are those who have advocated the use of "quadruple therapy" for *H. pylori* eradication (Borody 1994b, Hosking 1994, de Boer 1994 and Labenz 1993). The viability of this approach, with eradication rates in excess of 85% following 7 -14 days of therapy, will have to be established against the background of the excellent efficacy achieved with PPI based triple therapies.

CHAPTER 9

CONCLUDING SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

The rediscovery of *H. pylori*, and especially the increasing awareness of its important role in the etiology and management of peptic ulcer disease, have generated tremendous interest. The goals of therapy have, furthermore, advanced from the healing of the ulcer to the cure of the disease. This is due largely to appreciation of the dominant role of *H. pylori* in peptic ulcer disease and, in particular, to the real prospect of ulcer cure following eradication of the organism.

Research has, however, originated chiefly from economically developed First World countries and may therefore not be relevant in socioeconomically underdeveloped settings. This thesis has set out to investigate the role of *H. pylori* in peptic ulcer disease particularly in the socioeconomically disadvantaged communities of the Western Cape.

The thesis provides an overview regarding historical developments in epidemiology, as well as the classic theories regarding peptic ulcer disease etiology and management. It has, furthermore, attempted to place the pioneering observations of Marshall and Warren in historical perspective, and describes the *Helicobacter* genus. The available data with regard to *H. pylori* in the African, and particularly the South African, setting is also summarised.

The organism's gastroduodenal involvement in peptic ulcer disease, as well as the postulated mechanisms by which *H. pylori* can cause peptic ulceration have been investigated. The predominantly antral nature of the

infection in duodenal ulcer disease is confirmed. At the same time it has been demonstrated that colonization of the body of the stomach occurs as commonly in duodenal ulcer disease as it does in gastric ulcer disease. This distribution of the infection may be interpreted as being at variance with earlier data regarding the gastritic pattern in duodenal ulcer disease. The findings, however, indicate that the inflammatory reaction is less marked in the gastric body of duodenal ulcer subjects, and a lower density of the infection has been identified as a possible reason for this.

The duodenal involvement of *H. pylori* in duodenal ulcer disease has also been investigated. The finding that gastric metaplasia commonly occurs in relation to duodenal ulceration and that *H. pylori* can be found in this metaplastic tissue in the majority of duodenal ulcer subjects is compatible with hypotheses regarding the migration of *H. pylori* from the antrum to the duodenal cap. While these data are similar to those reported in First World populations, they are contrary to the published data from India.

The controversial issue of *H. pylori*'s effect on gastric secretion has been investigated by evaluation of basal and pentagastrin stimulated acid and pepsin secretion before therapy, after healing, shortly after eradication, and 12 months later. Secretory studies showed no changes in gastric secretion which could be attributed to eradication, suggesting that changes in parietal cell mass do not occur following eradication. Other workers, using a more "physiological" stimulant (GRP/bombesin), reported changes in acid secretion following eradication of *H. pylori* and speculated that these reflected changes in parietal cell mass. Our data does not support this interpretation, while review of the physiology of acid secretion suggests, rather, that any changes may reflect subtle alterations in the paracrine control of acid secretion.

The work presented in this thesis demonstrates that the prevalence of *H. pylori* is high in the socioeconomically disadvantaged community of the Western Cape. While this has, in the past, been attributed to ethnic differences, the findings in this thesis support the view that the prevalence is determined by socioeconomic factors.

The effect of the eradication of *H. pylori* on the natural history of duodenal ulcer disease in subjects from a socioeconomically disadvantaged background was investigated. The findings indicate that ulcer recurrence is, in fact, dramatically reduced following successful eradication, and that re-infection is rare, even in a community with such a high prevalence. It is thus evident that an eradication strategy would be successful in our population.

Various treatment strategies for *H. pylori* were investigated. Classic "Triple Therapy", with full-dose tetracycline was demonstrated to be an effective therapeutic approach, but is associated with numerous side-effects. The effect of the proton pump inhibitor, omeprazole on gastric *H. pylori* status was shown to be that of temporary suppression only. Finally, the use of omeprazole and amoxicillin dual therapy was investigated. In our population, the efficacy of this combination is less than the widely accepted values reported in the literature. The findings also question the current dogma that pre-treatment with proton pump inhibitors is associated with poor eradication results, as no significant difference could be demonstrated with regard to eradication in pre-treated subjects.

The data presented in this thesis is supportive of a role for *H. pylori* in the pathogenesis and management of peptic ulcer disease, and provides a rationale for treating patients from socioeconomically disadvantaged communities. However, three broad areas of interest need to be addressed in the future.

1. In view of the high prevalence of the infection in the African setting, detailed epidemiological studies are needed to determine the distribution and prevalence of diseases associated with *H. pylori* infection - i.e peptic ulcer disease, gastric carcinoma and gastric lymphoma of the MALT-type. This will also shed light on the conundrum of "The African enigma" of a high prevalence of *H. pylori* infection, apparently not associated with clinical disease.

2. The role of *H. pylori* as an etiologic agent in the pathogenesis of peptic ulcer disease needs to be placed in the proper context. Inherent in this is the need to investigate the mechanism by which the organism causes, or predisposes to, peptic ulceration. Further studies investigating the effect of the organism on gastric secretion are of particular importance, as these may provide the link between classical Schwarzian teaching and *H. pylori*. The more physiological methods of assessing gastric secretory status may be more appropriate than the conventional direct parietal cell stimulatory tests.

3. Current therapeutic strategies for peptic ulcer disease have to be refined. On the basis of the premise that *H. pylori* is indeed the etiologic agent in peptic ulcer disease, therapeutic strategies in

peptic ulceration will have to focus on effective treatment of the organism, and not merely on the palliation of the disease by acid suppression. Present eradication therapy, consisting of dual or triple combinations, although widely prescribed, is not optimal. Simple, effective and affordable anti-*H. pylori* combination therapy or, preferably, a specific antibiotic or vaccination strategy, still has to be developed.

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