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# *DUODENAL ULCER,*

# *HELICOBACTER PYLORI*

# *&*

# *GASTRIC SECRETION*

# DUODENAL ULCER, *HELICOBACTER PYLORI* and GASTRIC SECRETION.

THESIS SUBMITTED TO THE

UNIVERSITY OF LONDON

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

by

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**ABSTRACT**

This study investigated the possibility that *Helicobacter pylori* is an aetiological factor in the pathogenesis of duodenal ulcer. The aim was to establish whether subjects with duodenal ulcer with *Helicobacter pylori* had a maximal gastric secretion that was measurably different from that of subjects with duodenal ulcer without *Helicobacter pylori*. Because *Helicobacter pylori* is a common infection of individuals without duodenal ulcer, it was felt important to control the observations in the duodenal ulcer group with similar observations in subjects without duodenal ulcer.

In 62 subjects with dyspepsia attending for upper gastro-intestinal endoscopy, maximal gastric secretion was measured in the 11 non-duodenal ulcer subjects without *Helicobacter pylori*, 20 non-duodenal ulcer subjects with *Helicobacter pylori*, 21 duodenal ulcer subjects with *Helicobacter pylori* and 10 duodenal ulcer subjects without *Helicobacter pylori*. Thus the incidence of duodenal ulcer was about 50% whether or not *Helicobacter pylori* was present. Several tests were used for identification of *Helicobacter pylori*.

In both groups, duodenal ulcer and non-duodenal ulcer, the presence of *Helicobacter pylori* was associated with a smaller gastric secretion than that in the absence of *Helicobacter pylori*. In subjects with duodenal ulcer the reduction in secretion was 15% and in non-duodenal ulcer it was 18%.

Regression analysis indicated that in the absence of *Helicobacter pylori*, there was a strong positive correlation between dose of chronic smoking and maximal gastric secretion in both duodenal ulcer and non-ulcer subjects. However, in the non-duodenal ulcer and duodenal ulcer subjects in whom *Helicobacter pylori* present, there was no correlation. The enhancing effect of tobacco was apparently nullified by infection with *Helicobacter pylori*. This finding made it unlikely that the association between duodenal ulcer, *Helicobacter pylori* and reduced gastric secretion was due to a cumulation of aetiological effect between acid and organism.

These results lend support to the hypothesis that *Helicobacter pylori* is not a significant factor in the aetiology of duodenal ulcer. The subjects were followed up and the various details of the follow up studies were consistent with this view.

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**DEDICATED**

*To the loving memory of my dearest daughter Sinthuja, who passed away on 16th of May 1990 at the age of six weeks.*

**ACKNOWLEDGEMENTS**

I wish to express my gratitude, appreciation and indebtedness to Professor Michael Hobsley, Professor of Surgery, University College and Middlesex School of Medicine, for his guidance and supervision not only in the preparation of this thesis but throughout the entire course of my postgraduate study. I am also grateful to him who provided me with all the facilities necessary to carry out the work in the Department of Surgical Studies, The Middlesex Hospital. I thank him for the invaluable time, especially after work, he spent with me to put my thoughts in a sequential order and to express them in simple English, and for teaching me statistics from scratch.

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Finally, I wish to thank my wife for tolerating my many hours spent with the computer and for looking after our two kids - Keerthana & Maiuran - alone during this period.

**DECLARATION**

The suggestion for this work came from a discussion with Professor Michael Hobsley on the remarkable lack of evidence for the link between *Helicobacter pylori* and duodenal ulceration, though many investigators have put forward hypotheses and suggestions for the possible mechanism of such a link.

The design and running of the study was the sole work of the author. One hundred and fifty of the oesophagogastro- duodenoscopy procedures and all gastric secretion studies were performed by the author. Thirty of the 62 gastric juice analyses were done by the author. Microbiology and histopathology studies were not carried out by the author, but by the respective senior staff members of those Departments.

It is believed that this thesis makes the following original contributions, i). that chronic *Helicobacter pylori* infection is associated with reduction of gastric secretion, ii). that the positive correlation between chronic smoking and gastric secretion exists only in *Helicobacter pylori* negative subjects and iii) possibly that there is no association between duodenal ulcer and *Helicobacter pylori*, and certainly not through gastric secretion.

'.....the secretion became greatly vitiated, greatly diminished or entirely suppressed.....'

**William Beaumont, 1883**

(commented when he observed the reduction in the gastric secretion of his gastric fistula patient Alexis St. Martin during a febrile illness).

Experiments and Observations on the Gastric Juice and the Physiology of Digestion. Harvard University Press, Boston. p.107.

*CHAPTERS*

# *CHAPTER 1*

# *INTRODUCTION*

# 1. INTRODUCTION

## 1.1 DUODENAL ULCER

Duodenal ulcer is a discontinuity in the mucosa of the duodenum through the muscularis mucosae with an ulcer crater surrounded by acute and chronic inflammatory cells. Typically found within the first 3 cm of the duodenum, the ulceration is chronic and recurrent in nature.

Duodenal ulcer was first described in 1817 (Bouchier 1981) and since then its prevalence has increased steadily until about 1960 (Langman 1979). Over the last 20 years, however the indirect indicators such as hospitalisation, operation and death suggest a marked decrease in duodenal ulcer incidence in England, Europe and the United States (Brown, Langman and Lambert 1976, Langman 1979, Coggon, Lambert and Langman 1981). Duodenal ulcer remains a common disease and an important scurce of morbidity and mortality, especially in the elderly. Roughly 10% of the population can expect to develop this disease during their lifetime and death from ulcer disease is about 8 per 1000 of all deaths (Langman, 1983). This leads to an enormous economic loss in terms of money spent on health care (direct cost) and indirect cost due to loss of productivity and wages due to absenteeism.

There has grown up over the years a general acceptance that duodenal ulcers are in some way related to the secretion by

the stomach of acid and pepsin. However, over the last two decades two interesting ideas have emerged. Firstly it has been agreed that duodenal ulcer belongs to a heterogeneous group of disorders (Lam and Sicrus 1975, Rotter and Rimoin 1977) with multiple aetiological factors. The second view was the suggestion that the gastric microorganism known as *Helicobacter pylori* is a causative factor (Marshall 1983, Warren 1983, Marshall, McGechie, Rogers et al 1985).

Although much is known about the pathophysiology of peptic ulceration, the exact cause of this disease remains obscure. Attempts to elucidate the pathogenesis of duodenal ulcer are hampered by the difficulty of getting a close insight into the morphological, functional and biochemical changes occurring during the development of the ulcer crater.

### 1.1.1 EPIDEMIOLOGY

Epidemiological studies of duodenal ulcer are difficult because estimates of the incidence and prevalence of the disease depend upon the mode of diagnosis such as symptoms, radiology, endoscopy or autopsy. The study is also complicated by the facts that duodenal ulcer is a recurrent disease and that some individuals who are asymptomatic may have ulcers.

There has been a decline in the number of duodenal ulcers diagnosed over the last two decades in the western countries (Langman 1979, Sonnenberg 1987), and starting even before the introduction of H-2 receptor antagonists; but there is a contrasting increased incidence in the Far East (Koo, Ngan and

Lam 1983). In Japan the prevalence rate is 9-11%, in England about 5.2% while in U S A it is approximately 2.5%. However, the survey methods differed in these countries and the results are not directly comparable.

There is a marked variation in the frequency of ulcer disease throughout the world. In the Faroe Islands the annual incidence of first time diagnosed peptic ulcers is as high as 33 out of 1000 inhabitants. There are also significant differences in incidence and prevalence in different part of the same country. The prevalence of duodenal ulcer and the incidence of perforation are higher in Scotland and to a lesser extent in the north of England than in south of England (Langman 1983). Duodenal ulcer is found to be commoner in the southern part compared to the northern part of Scandinavia (Bonnevie 1985). In Australia, the prevalence is much greater in New South Wales than in Victoria, Queensland or Western Australia (Hugh, Coleman, McMamara et al 1984).

Epidemiological studies show that the association of other factors such as smoking (Friedman, Siegelaub, Robbs et al 1974) coffee (Paffenbarger, Wing and Hyde 1974), diet (Malhotra 1978, Tovey 1979, Ryding and Berstad 1986), sex (Bonnevie 1975, Grossman 1980) and racial (Kurata and Haile 1984) differences may play a part in the aetiology of peptic ulcer disease.

Although some foods may cause dyspepsia, there is no conclusive evidence to show that diet is incriminated as a cause of ulcers. But the high unrefined wheat content in the diet has been found to influence the low incidence of ulcers in North India compared with the South (Tovey 1979, Rydning and Berstad 1986). It has been noted as well that the recurrence rate on a wheat diet was 14% over a 5 year period while it was

81% on a rice diet (Malhotra 1978). Similar results were noted in a Norwegian study with a follow-up of 6 months (Rydning, Berstad, Aadland et al 1982).

The distribution of duodenal ulcer has been found to differ significantly among different races in the same country. The incidence of ulcer in full-blood Aboriginal patients is nil (Bateson 1976). The Chinese show a higher incidence of duodenal ulcer than the non-Chinese (Indian and Malayan) in Singapore (Kang, Guan, La Broody et al, 1983). One study carried out in Australia showed that duodenal ulcer is six times more common in patients born in UK and 17 times more common in patients born in other European countries than in patients born in Australia (Bateson 1980).

Coffee is said to be a strong stimulant of acid secretion and causes dyspepsia in some individuals: however, even though the consumption of coffee in the younger age groups is associated with a slightly increased risk of duodenal ulcer in later years, consumption at the time of presentation is not a risk factor (Friedman, Siegelaub, Seltzer 1974).

Drugs, such as aspirin and non-steroidal anti-inflammatory drugs (NSAID) cause acute injury to the duodenal mucosal lining and may exacerbate ulcers, bleeding and perforation but it used to be thought that there was little or no causative effect for chronic duodenal ulcer (Levy 1974). However, more recent work indicates that there is a strong association between NSAID and duodenal ulceration (Freston and Freston 1990, Wilson 1990).

It is estimated that duodenal ulcer is 1.5 to 3 times more common in males than females (Bonnevie 1975, Pulvertaft 1959) and in India the ratio has been reported as 18 : 1 (Jayaraj,

Tovey and Clark 1980) . But the ratio has been decreasing over the last 15 years in the Western countries, even to the extent that the ratio in the U S A is 1.4 : 1 (Elashoff and Grossman 1980); this phenomenon is probably due to the change in smoking habits (Kurata, Haile and Elashoff 1985, Kurata, Elshoff, Negawa 1986).

Cigarette smokers are at increased risk of developing ulcers and cigarette smoking also impairs healing (Friedman, Siegelaub and Seltzer 1974, Korman, Hansky, Eaves 1983, McCarthy1984). Chronic smoking may also increase parietal and chief cells, resulting in a higher gastric and pepsin secretory capacity (Parente, Lazzaroni, Sangaletti et al 1985, Whitfield and Hobsley 1985 and 1987). The increased secretion is not a universal finding in smokers (Walker and Taylor 1979, Whitfield and Hobsley 1987). It is noted in male but not in female smokers with duodenal ulcers (Whitfield and Hobsley 1985). After controlling for variables such as age and height the effect of smoking on acid secretion is no greater in subjects with duodenal ulcers than in normal subjects (Whitfield and Hobsley 1987). Smoking also reduces prostaglandin synthesis in gastric mucosa but not in duodenal mucosa (Quimby 1986)

Psychodynamic factors may be important, but probably only in a very small group of duodenal ulcer subjects. The validity of this concept is difficult to prove but deserves further effort (Fordtran 1979).

Recently, the concept of genetic heterogeneity was proposed to explain the high incidence among siblings. Twenty to 50% of duodenal ulcer subjects have a positive family history. The concordance of peptic ulcer is greater in monozygotic twins than dizygotic twins, a phenomenon that indicates the existence of a genetic factor (McConnell 1980 and Rotter 1983).

Individuals with blood group O have a 1.3 times greater incidence of duodenal ulcer than individuals of other blood groups (Aird, Bentall, Menhign et al 1954) and in non-secretors of group O the incidence increases by 1.5 times compared with secretors (Clarke, Edwards, Haddock et al 1956). The HLA antigens (HLA-B5 and HLA-B12) have also been examined and duodenal ulcer has been found to be associated with only selected sub-groups (Ellis and Woodrow 1979). Serum pepsinogen I is elevated in 30% of DU subjects (Samloff, Liebam and Panitch 1975) and it is believed that the increase in serum pepsinogen I is a highly significant risk factor for duodenal ulcer.

### 1.1.2 PATHOPHYSIOLOGY

Peptic ulcers always tend to occur near, but not in, the acid secreting mucosa. Ninety percent of duodenal ulcers are situated in the first part of the duodenum and most commonly in the anterior wall of the duodenal bulb. The ulcer is circular in shape with a vertical edge and invades the muscular layers, which it tends to penetrate. The base of the ulcer is lined by granulation tissue and the surrounding arteries show evidence of endarteritis obliterans. The ulcer heals spontaneously or with appropriate treatment and it may recur later. The main complications of duodenal ulcer are haemorrhage, perforation or gastric outlet obstruction (pyloric stenosis)

The cause of the duodenal ulcer diathesis has continued to elude investigators in spite of several recent developments and remains controversial. However, the general picture seems to be a genetic predisposition complicated by environmental and

local factors. The integrity of the gastroduodenal mucosa depends on the balance of protective and aggressive factors. The environmental ulcerogens are probably chemical and infective. Duodenal ulcers are strongly linked to the level of gastric acid and Schwarz's dictum - 'no acid, no ulcer', has very few exceptions (Isenberg, Spector, Hootkin 1971).

DU is rare in people who secrete less than 15 mmol of acid per hour (Baron 1972) and healing is enhanced by agents that inhibit acid secretion. However, subjects with DU have on average only 35% greater secretion than controls and only 12 to 30% are hypersecretors (Wormsley and Grossman 1965, Baron 1972, Whitfield 1984). This increase in acid could theoretically be due to an increase in parietal cell mass or an increased response to secretagogues due to enhanced sensitivity. However, recent work by Roxburgh (1990) has clarified this point; there seems no doubt that the enlarged parietal cell mass is a true phenomenon and that there is no alteration in the sensitivity.

Several mechanisms are involved in the maintenance of the integrity of the duodenal mucosa in a deleterious acid environment. The large amount of the acid is neutralised in the duodenum by pancreatic and biliary bicarbonate secretion. Although duodenal mucosa secretes $HCO_3^-$ it is a relatively insignificant contribution, but its site of production may increase its importance (Feldman 1987).

Prostaglandin production by the duodenal mucosa may exert a negative feed back control of acid secretion. But there is no evidence to show that when there is an ulcer, the duodenal mucosa is deficient in prostaglandin production. It is interesting to note that although smoking decreases endogenous prostaglandin synthesis in gastric mucosa, prostaglandin

production by duodenal mucosa is unaffected. However, it has been reported that during treatment with prostaglandin E (1&2) derivatives such as misoprostol and enprostol, the healing curves of smokers and non-smokers are identical (Lam, Lau, Choi et al 1986) and are unaffected by smoking which hampers healing when treating with anti- acid secretory agents.

Both autoimmune and infective mechanisms have been suggested for the pathogenesis of duodenal ulcer. The autoimmune theory suggests that immunological stimulation or destruction of the G -cell population leads to parietal cell hyperplasia with distal extension, predisposing to duodenal ulcer (Kirk 1986).

Numbers of investigators have suggested that ulcers could be caused by infection as far back as before the turn of the century. Since 1983, there has been mounting evidence that mucosal defence is impaired by the newly described microorganism called *Helicobacter pylori* and that it plays a significant role in the pathogenesis of ulcer disease. This organism has been isolated from the gastric antrum and, to a lesser extent and only if there is gastric metaplasia, from the edge of the duodenal ulcer.

The indirect evidence comes from the studies where the investigators have shown that treatment which suppresses or eradicates *Helicobacter pylori* leads to longer remission and that the ulcer rarely relapses before recolonisation occurs (Coghlan, Gilligan, Humphries et al 1987, Marshall, Goodwin, Warren et al 1988, Rauws and Tytgat 1990).

One suggested view of the pathogenesis of DU is that high gastric acidity and / or rapid gastric emptying, by creating a low duodenal pH, results in gastric metaplasia in the duodenum.

This allows *Helicobacter pylori* from an area of antral active chronic gastritis to colonise the duodenum leading to duodenitis and DU, probably in association with other aggressive factors (Wyatt, Rathbone, Dixon et al 1987, Wyatt, Rathbone, Dixon et al 1988, Goodwin 1988, Wyatt 1989, Goodwin, Gordon and Burke 1990). However, against this hypothesis is the fact that Inatsuchi, Tanaka, Treasaki et al (1990) could not recognise any case of duodenitis which progressed to duodenal ulcer.

The other hypothesis is that urease produced by *Helicobacter pylori* splits urea in the gastric juice into ammonia which neutralises the acid in contact with the mucosa overlying the G-cells and parietal cells. This impairs the normal inhibition of gastrin release by intraluminal acid leading to an inappropriate secretion of gastrin in response to food and this increases acid output and the raised acid level which leads to the pathogenesis of the ulcer (Levi, Beardshall, Haddad et al 1989, Levi, Beardshall, Swift et al 1989).

If *Helicobacter pylori* is eradicated by a suitable medication, the postprandial gastrin response return to normal, however the peak acid output remains high. McColl, Fullarton, Chittajalu et al (1991) studied 10 subjects with *Helicobacter pylori* - related antral gastritis and a history of duodenal ulcer and concluded that the marked fall in serum gastrin concentration following eradication of *Helicobacter pylori* was not accompanied by any reduction of gastric acidity whether early or even as as late as 7 months after the eradication. They felt that this is due to an increase in parietal cell mass caused by the elevated gastrin and therefore it takes a longer period for the number of parietal cell to fall to normal; or else to the fact that these subjects naturally had a large parietal cell mass even before they got infected by *Helicobacter pylori.*

### 1.1.3 TREATMENT

The treatment of duodenal ulcer depends on understanding the pathogenesis and recent advances in pharmacology help to improve the treatment of duodenal ulcer.

The natural history of DU varies from spontaneous healing in weeks with many months of remission to a refractory state that persists even after months of treatment. Many measures have been proposed to enhance the healing.

Abercrombie in 1820 recommended a diet consisting of milk and in 1831 Johnson advocated antacids such as sodium bicarbonate. These treatments were aimed at the aggressive factor of acid and this practice was continued until the 1960s. Then the antacids changed with the introduction of aluminium and magnesium antacids. The turning point in the medical treatment of duodenal ulcer came with the emergence of the histamine receptor antagonists in the 1970s.

Duodenal ulcer in the elderly heals less well than in younger subjects (Permutt and Cello 1982)and in females the ulcers heal faster than in males (Sonnenberg, Muller-Lissner, Vogel et al 1981). Smoking impairs healing and it increases the risk of complication and the recurrence rate. Aspirin and NSAIDs weaken the healing capacity and prostaglandin analogues enhance the healing. Alcohol abuse interferes with healing but it has been suggested that in moderate amount the consumption of alcohol may promote healing (Sonnenberg, Muller-Lissner, Vogel et al 1981).

The choice of drug depends on its effectiveness on the healing rate, on the relapse rate and on the side effects. It

should be convenient for the subject to take so as to promote compliance.

The drugs commonly used broadly fall into the following categories:

a) Antacids
b) Mucoprotective agents
c) Anti-secretory agents
    Anticholinergic
    Histamine -2 receptor antagonists
    Gastric proton-pump inhibitor
d) Antibiotics

a) Antacids:

The efficiency depends on the neutralising capacity. The frequently used antacid is Aluminium - Magnesium hydroxide. The healing and relapse rate are not acceptable compared with other drugs but the side effects are minimal.

b) Mucoprotective agents:

Colloidal bismuth subcitrate (CBS) and sucralfate fall into this category. In addition to the mucoprotective action both are said to increase prostaglandin secretion. CBS also increases the $HCO_3^-$ secretion and enhances epithelial regeneration. But the most important action of CBS, recently noted, is its bactericidal effect against *Helicobacter pylori* Marshall, Armstrong, Francis et al 1987). The latter action is proposed by some investigators as an explanation of the fact that duodenal ulcer heals better and faster when treated with CBS.

and that the relapse rate is lower compared with treatment with $H_2$ receptor antagonists (Lee, Samloff and Hardman 1985, Smith, Price, Borriello et al 1988). The disadvantages of CBS are that it lasts longer in the body and it can cause nephrotoxicity and encephalopathy (Martindale 1989).

c) i. Anticholinergic agents:

Pirenzepine, which specifically inhibits gastric acid and pepsin secretion and is not as effective as other anti-secretory drugs (Stockbrugger, Eugenides, Hobsley et al 1982). The side effects such as dry mouth and blurring of vision are severe and rarely it affects the bone marrow (agranulocytosis).

c) ii. Histamine-2 receptor blocking agents:

Cimetidine, ranitidine, nizatidine and famotidine are the freely available drugs. The healing and relapse rates among these drugs varies between studies. In general they are well tolerated with few unwanted effects but drug interaction and metabolic inhibition are the most frequent problems. Ranitidine is popularly used by many physicians since it has fewest side effects.

c) iii. Gastric proton-pump inhibitor:

This is the newest drug in the treatment of peptic ulceration, especially useful in the Zollinger-Ellison syndrome. Omeprazole is transformed by an acid activated mechanism to a derivate which is the active inhibitor of $H^+$, $K^+$ - ATPase, thus

reducing gastric acid secretion (Wallmark, Bradstrom and Larsson 1984). This drug is used to treat patients with ulcer unresponsive to an adequate dose and duration of other drugs.

d) Antibiotics:

The use of antibiotics is based on the supposition that duodenal ulcer could be infective in origin. The first detailed report describing the benefit of metronidazole (vs cimetidine) in the treatment of gastroduodenal ulcers was published in 1986 (Quintero and Sooto). The results in healing DU achieved by metronidazole are similar to those of cimetidine.

Since then, numerous trials and studies have been carried out with other antibiotics. Nitrofurantoin and furazolidone, amoxycillin, tetracycline are the other promising antibiotics used in the treatment of duodenal ulcer. A combination of CBS with one or 2 of these antibiotics ('triple therapy') gives a better clearance of *Helicobacter pylori* and hence faster healing of duodenal ulcer (Borody, Cole, Noonan et al 1988, Borsch, Mai and Muller 1988).

Many antibiotics fail to eliminate *Helicobacter pylori* despite good in-vitro activity. This may be due to the fact that some antibiotics lose activity at low pH or are not secreted in adequate concentration, or else to the emergence of resistant strains of *Helicobacter pylori*

Surgical procedures:

The history of surgery for peptic ulcer over the past century has been characterised by the development of surgical

skill and dexterity, aided by ever safer anaesthesia and postoperative care.

In 1814, the vagus nerves constituted the eighth pair of 10 cranial nerves and it was accepted that division of this nerve inhibited the stimulation of gastric juice by arsenic. This was revealed by the experiments of Benjamin Brodie. Unfortunately, he did bilateral cervical vagotomy and it disturbed the respiration and was followed by death. Later, he repeated his experiments but with the difference: 'by dividing the nerve, from within the abdomen, above the cardiac orifice of the stomach', thus the birth of the 'subdiaphragmatic vagotomy'.

Duodenal ulcers were diagnosed far less frequently than gastric ulcers; thus in the beginning of $19^{th}$ century most of the operations were performed for complications of the gastric ulcers. The surgical treatment of obstructing DU was introduced by Loreta, which was to make a an incision in the antrum and then dilate the pylorus with fingers. Wolfler (1891) introduced gastroenterostomy and this was first performed for duodenal obstruction by Rydygier. Then came pyloroplasty, particularly the one that bears the name of Heineke and Mikulicz (1896). The first successful closure of a perforated DU was done by Dean in1894, 10 years after Mikulicz-Radecki first attempted to close a perforated gastric ulcer and 2 years after the first successful closure of a perforated gastric ulcer. In 1901, Moynihan was able to find 51 patients who had been operated on for perforated DU; only 9 recovered.

In 1852, Guenzburg put forth the idea that the cause of DU was in some way associated with acid gastric juice, but the view was not accepted universally at that time. The surgical treatment for uncomplicated DU by gastroenterostomy was

performed by Codivilla in 1893. Later he modified it to gastroenterostomy-Y, now known as Roux-Y gastroenterostomy and so named by him. The other operations initially used for treatment of DU were pyloroplasty, ulcer excision, pylorectomy and antrectomy.

Theodor Billroth peformed the first successful gastrectomy on a Viennese housewife, Therese Heller in 1881 (Hugh 1985). This procedure, known as Billroth I, consisted of gastric resection followed by gastroduodenal anastomosis. He was not the first to undertake gastric resection; he was preceded by Jules E Pean of Paris (1879) and Ludwik Rydygier of Culm, Poland (1880). Rydygier (1882) performed the Billroth I resection for a benign pyloric ulcer in 1881. In 1885, the Billroth II operation was introduced as a two-stage procedure, more by accident than design. It is nowadays performed as a one stage procedure with more than 40 modifications but the basic principle is partial gastrectomy and gastrojejunostomy. Until 1918, it was not realised that it was essential to excise two thirds of the stomach to effect permanent reduction of acid in order to reduce the risk of gastrojejunal ulcers in subjects who had undergone gastroenterostomy for DU. Finsterer, first recommended the resection of part of the stomach for DU, but did not receive overwhelming support. Only in 1940 did subtotal gastrectomy gain a firm hold in the profession.

Nearly a century after Brodie recognised the link between the vagus and gastric secretion, Rokitansky revived the nuerogenic origin but this was discredited by Virchow (1853). However, Rokitansky's opinion gradually gained acceptance over the years. Later, Andre Latarjet of Lyons accurately described the vagus nerve supply of the stomach (1922). Despite the

generally poor experimental and clinical results associated with vagotomy, the modern era of vagotomy began in 1943 when Dragstedt and Owens performed transthoracic vagotomy without a drainage operation (1943). This was quickly followed by subdiaphragmatic truncal vagotomy and gastroenterostomy and later pyloroplasty to provide adequate gastric emptying. Independently, Jackson in Michigan (1947) and Franksson of Sweden (1948) suggested that a selective vagotomy would provide gastric denervation without the unnecssary vagal denervation of the remaining organs. But it was not recognised by many surgical 'giants'.

The newest operation for DU and one that permits preservation of the pylorus is parietal cell or proximal gastric vagotomy without drainage was proposed by Griffith and Harkins in 1957. The report on their study in dogs was rejected by every major surgical journal, probably because it was surgical heresy to retain vagal innervation of the antrum. The clinical application of the operation in human subjects was independently introduced in (1970) by Amdrup and Jensen in Sweden and Johnston and Wilkinson in England. This procedure was known as proximal gastric vagotomy (PGV), parietal cell vagotomy (PCV) or highly selective vagotomy (HSV). It has many attractive features, has been in clinical use for 20 years and has been critically studied. The associated mortality is less than 0.5%, and it is accompanied by virtually none of the disabling side effects that occur with other gastric operations for DU. The recurrent ulcer rate has varied from 2 to 30%. The main factor that influenced the recurrence rate was the skill and experience of the surgeon. Then came the simpler, easier and quicker procedure known as anterior lesser curve seromyotomy and (right) posterior truncal vagotomy which gives results similar to those of PGV. Now, this procedure can be performed without laparotomy via laparoscopy (videocoelioscopy) (Katkhouda and Mouiel 1991).

## 1.2 GASTRIC SECRETION

One function of gastric acid is to facilitate peptic digestion of dietary protein. There is a close relationship between acid and pepsinogen secretion. In general, a stimulant or inhibitor of acid secretion will have the same effect on pepsinogen secretion. However, there is no evidence that acid secretion regulates pepsinogen secretion or vice versa (Hersey, Miller, May et al 1983). In a normal person, under basal conditions the stomach secretes an average of 7-10% of maximum. A physiological event, a pharmacological or a pathological condition increases the gastric secretion above the basal level by neurohumoral mechanisms.

Gastric secretion of acid is controlled by excitatory and inhibitory stimuli that are neuro-hormonal in nature. There are three phases, namely cephalic, gastric and intestinal and these phases overlap and integrate in a complex manner.

During the cephalic phase secretion may result from the thought, sight or smell of food as well as from the presence of food in the mouth, as in sham-feeding. The cephalic phase is triggered by vagal stimulation and vagotomy eliminates this phase.

The gastric phase is activated either by distension of stomach or by chemicals in the food. Distension of stomach by food stimulates stretch receptors and elicits a local and vagovagal reflexes. Peptides and aminoacids in the food stimulate chemoreceptors in the pyloric area. The later two events cause G-cells of the gastric antrum to release gastrin which is carried by the blood stream to the parietal cells and stimulates them to secrete acid. When the antrum is in contact

with acid, further release of gastrin is inhibited by a negative feed back mechanism. Vagotomy reduces maximal secretion by 50-70% (Payne and Kay 1962, Baron and Spencer 1976) and vagotomy and antrectomy reduces the maximal secretion by 85% (Leth, Elander, Fellenius et al 1984).

The intestinal phase is initiated by distension of the duodenal wall or by chemicals intraluminally in the duodenum (peptides, amino acids). Neuroreceptors are stimulated and intestinal gastrins and other hormones secreted. This phase is complicated by the presence of inhibitory mechanisms.

At least 4 endogenous substances have identified as secretagogues; histamine, gastrin, acetylcholine and calcium. Apart from calcium, the other substances have receptors on the outer membrane of the parietal cells and on interaction they initiate the intracellular biochemical reaction which ultimately activates the proton pump.

## 1.3 *HELICOBACTER PYLORI*

The presence of spiral organisms in the human stomach has been reported at intervals throughout this century, but Marshall and Warren first demonstrated the relationship between this bacteria and gastritis and peptic ulceration. The current world wide interest in *H pylori* is explosive. There is almost universal acceptance that *H pylori* is a pathogen and a dominant cause of non-autoimmune type B active-chronic gastritis (ACG). Its role in the pathogenesis of duodenal ulcer needs further investigation.

*Helicobacter pylori* (*H pylori*) is an S-shaped

Gram-negative micro-organism and measures approximately 3μm by 0.5μm. The organism is found within and below mucus overlying gastric antral type mucosa. The bacteria are rapidly killed by acid pH in vitro, yet most subjects with *H pylori* are said to have normal (Brady, Hadfield, Hyatt et al 1988, Kang and Wee 1991) or higher gastric acidity (Levi, Beardshall, Haddad et al 1989, McColl, Fullarton, Nujumi et al 1989). This organism is found only in gastric type epithelium no matter whether it is found in the oesophagus (Paull and Yardley 1988, Walker, Birch, Stewert 1990), duodenum (Wyatt and Rathbone 1989), or Meckel's diverticulum (Morris, Nicholson, Zwi et al 1989, Fich, Talley, Shorter et al 1990). The natural history of *H pylori* infection is still a puzzle. Approximately 20% of normal asymptomatic volunteers have been found to have *H pylori* gastritis and the incidence rises with age (Barthel, Westblom, Harvey et al 1988, Greenberg and Bank 1990). The organism is rarely found on normal mucosa. It is not clear how infection is usually acquired, though it has been noted that infection has been transmitted by inadequately sterilized endoscopes. *H pylori* organisms produce a large amount of urease and the activity of this enzyme is utilised in the detection of this bacterium. A wide range of antibiotics kill the organism in vitro, but they are generally less effective in vivo, probably because they do not reach the site of infection in a concentration high enough to kill the *H pylori*.

# *CHAPTER 2*

# *HISTORICAL REVIEW*

# 2. HISTORICAL REVIEW

## 2.1 GASTRIC SECRETION

The gastric mucosal epithelium includes cells that line the surface, cells that line the gastric pits and cells composing the gastric glands beneath the pits. Cells that line the surface and the pits are the same throughout the stomach; they are columnar in type and secrete mucus and bicarbonate. The surface cells also maintain a lumen-negative potential difference (McGreevy 1984) and dispose of hydrogen ions (Olender, Fromm, Furukawa et al 1984).

In contrast to surface cells and cells that line the gastric pits, cells composing gastric glands differ from one region of the stomach to another. The glands of the body and fundus have different secretory functions from those of the antrum. The glandular cells of the gastric antrum synthesize and release alimentary polypeptide hormones such as gastrin.

The gastric glands of fundus and body are composed of mucous cells, parietal (oxyntic) cells and chief (peptic) cells. Some of the glands also contain enterochromaffin cells, which contain serotonin, and several types of endocrine cell. The parietal cells secrete acid and intrinsic factor (the latter is needed for the absorption of dietary vitamin $B_{12}$) and the chief cells secrete pepsinogens (I and II) which are converted by acid to pepsins.

## 2.1.1 GASTRIC JUICE

Gastric juice is conveniently regarded as a mixture of an aqueous solution of hydrochloric acid and other electrolytes, and enzymes - a parietal secretion; and a mucous or non-parietal or alkaline secretion. The acid contributes to the greatest part and the hydrochloric acid is secreted from the parietal cells together with other electrolytes such as sodium and potassium. In practice, pure gastric juice is unobtainable because of contamination with the other gastric contents. However, under maximal stimulation in man, parietal secretion appears to contain 170 mmols per litre of chloride and 145 mmols per litre of hydrogen ion in addition to small amounts of potassium (17 mmols $l^{-1}$) and sodium (7 mmols $l^{-1}$) making it approximately isotonic with plasma (Hobsley and Whitfield 1977).

The enzyme secretion of the stomach consists mainly of inactive pepsinogen precursors such as (i) pepsinogen I which is secreted from the mucus neck and chief cells and (ii) pepsinogen II secreted from the cells in the pyloric antrum and Brunner's glands. These precursors are converted by the action of acid into active pepsins which break down the polypeptide links adjacent to the aromatic aminoacids of ingested proteins.

The gastric mucus is secreted by the surface epithelial cells in response to mechanical and chemical irritation of the stomach as well as following sympathetic and parasympathetic stimulation. The mucus has a protective role against acid and peptic digestion of the gastric mucosa.

## 2.1.2 NATURE OF THE ACID

The first attempt to determine the nature of the acid was made by MacQuart in 1786 and he thought the gastric juice contained various salts of acids such as acetic, lactic and phosphoric acid. In 1803 Young also attempted to identify the acid and until 1824 it was thought that the acid in the gastric juice was phosphoric acid. William Prout in 1824 proved that free hydrochloric acid was the only acid present in the gastric juice. It took nearly 30 years to settle the debate in favour of hydrochloric acid and this was concluded by Bidder and Schmidt in 1852, two years after Prout's death.

Pavlov (1902) was the first person to be interested in the acid concentration of gastric juice. His findings were later quantified by Hollander (1932) in the 'Two Component Hypothesis'. The hypothesis suggested that gastric juice is a mixture of pure acid component, secreted by parietal cells and an alkaline component of either surface epithelial cells or mucous neck cells (Makhlouf, McManus and Card 1966). This has been validated and confirmed by Hobsley and Silen (1970). In contrast to this it has been claimed that a primary acid secretion is altered by back diffusion of hydrogen ions in exchange of sodium ions (Teorell 1947) to account for the presence of sodium in the gastric juice. His findings suggested that $H^+$ was lost from the lumen twice as fast as $Na^+$ was gained from the gastric mucosa. Davenport, Warner and Code (1964) suggested a ratio of 0.85:1 for Na/H exchange. But in 1970 it was shown that variations of electrolyte concentrations in gastric aspirate from subjects with pernicious anaemia are probably due to the dilution of the gastric juice by saliva; and the purest specimen of alkaline gastric juice closely resembled the alkaline

component predicted by the 'two-component' hypothesis (Gardham and Hobsley, 1970). In 1974, this hypothesis was modified by Hobsley who showed that the pure gastric juice is also altered by an alkaline contribution from refluxed duodenal contents.

With regard to the acid component of gastric juice, the purest gastric juice has a composition of $[H^+]$ 145, $[Cl^-]$ 170, $[Na^+]$ 7 and $[K^+]$ 17 (Hobsley and Whitfield 1977). In 1978 Hobsley and Whitfield produced evidence that cast doubt on the necessity for the back diffusion hypothesis as an explanation for the apparent alkaline component.

### 2.1.3 ACID SECRETION & DUODENAL ULCER

The part played by acid in the development of duodenal ulcer has been recognised for many years and Schwarz's dictum of 1910, "No acid no ulcer" has held true. Baron (1972) stated that duodenal ulcers are very unlikely to develop with maximal acid secretion of less than 15 mmol per hour and also indicated that excessive acid secretion above the normal range is only found in half of the subjects with duodenal ulcer. Later Hobsley, Whitfield, Faber et al (1975) correcting for pyloric losses found that only a quarter to third were hypersecretors.

In 1926, Galambos demonstrated a greater mean basal secretion in duodenal ulcer subjects than in controls. Polland and Bloomfield (1931) showed that normal subjects produced a continuous though small basal secretion and they confirmed the hypersecretion of subjects with duodenal ulcer. Several workers

reported high acid basal output in subjects with duodenal ulcers. However, basal gastric secretion was regarded as an unreliable measure. In 1943, based on Dragstedt's theory that basal hypersecretion of acid gastric juice in duodenal ulcer subjects was primarily due to vagal drive, vagotomy was advocated in the surgical management of duodenal ulcers (Dragstedt and Owens 1943). Faber and Hobsley in 1977 showed that vagal drive could only be an aetiological factor in a small proportion of subjects and Roxburgh (1989) has completely disproved this hypothesis of increased vagal drive. Hypersecretion has been thought to reflect the increased parietal cell mass and this has been confirmed by Roxburgh (1989). This hypersecretion seems to be an acquired phenomenon. Maximal acid secretion in duodenal ulcer increases with the duration of the disease (Hobsley, Whitfield, Faber et al 1975), although Sircus (1977) did not confirm this observation. In any case, it now seems clear that the hypersecretion is related to cigarette smoking; as time passes and the length of the history increases, so does the total number of cigarettes smoked and so does the maximal secretion increase (Roxburgh 1989). Apart from an increased secretory capacity and the now disproved increased drive on the parietal cells, there has been a suggestion that there is increased sensitivity of the parietal cells to various stimuli in duodenal ulcer subjects. Thus the dose of pentagastrin required to provide half-maximal stimulation in DU subjects was only one-third of that required in normal subjects (Isenberg, Grossman and Maxwell 1975). However, this phenomemon is not observed in all subjects with duodenal ulcers and has been since disproved (Roxburgh 1990, personal communication).

Popielski, in 1920, showed that histamine increased the gastric secretion of acid in dogs and Carnot, Koskowski and Libert (1922) showed the same effect in man using a dose equivalent to 0.03 μmol per kg body weight. In 1953, Kay

introduced a more precise test of the gastric acid secretory function using a large dose of histamine with an anti-histamine. His results confirmed the relationship of gastric secretion of duodenal ulcer subjects and of controls previously carried out by many research workers. Kay found that a subcutaneous bolus dose of 0.13 μmol per kg body weight of histamine acid phosphate gave a maximal secretion of gastric juice in the period 15-45 minutes after injection. Similar results were reproduced by other workers with larger doses of histamine (Songster, Grossman and Ivy 1946, Halpern 1947).

In order to produce a plateau of maximal response, Lawrie, Smith and Forrest in 1964 used a continuous intravenous infusion of histamine and produced a maximal response yielding a mean acid secretion of 24.0 mmol per hour in 26 male controls and 42.3 mmol per hour in male DU subjects. Hassan and Hobsley (1971) using the same technique found a mean of 33.9 mmol per hour in 20 male controls and a mean of 41.17 mmol per hour in 20 DU subjects. Similar results were reproduced by many workers, also using a maximal bolus dose of pentagastrin (Johnston and Jepson in 1967).

The weighted means of all these studies were 29.36 mmols per hour for the control and 43.17 mmol per hour for the DU subjects (Whitfield 1984), that means an increase of 47% above the control mean; thus, the findings were considered to be of great pathophysiological importance for the development of ulcer in the duodenum.

Maximal gastric secretion shows a great variation from individual to individual and from group to group. One reason for these observed group differences may be a relationship between body size and gastric secretion as suggested by Marks in 1961 in his summary of the augmented histamine test. The difference in

maximal acid output before and after gastrectomy is related to the calculated parietal cell mass of the resected portion (Card and Marks 1960, Cheng, Lam and Ong 1977). Maximal acid secretion measurements in children have shown that secretion increased as the age and weight of the children increased. Baron in 1964 found no correlation between body weight and acid secretion in adult male controls or adult male DU. However, the same author did find a correlation between maximal acid output and body weight in female controls but not in female DU. Hassan and Hobsley in 1971 found a significant correlation between acid secretion, and separately, weight, lean body mass, surface area and height in both controls and DU subjects, though the weight was a poor correlate.

Baron in 1969 found a statistically significant effect of sex on maximal gastric secretion over the age of 30. Hassan and Hobsley (1971) using pyloric loss corrected values found significant differences in the maximal gastric secretion between male and female controls and between male and female duodenal ulcer subjects but these differences become non-significant after correction for lean body mass. Thus the differences in secretion appear to be due to the differences in stature and these findings were also confirmed by Anderson, Dotevall, Lingaas et al in 1970.

Males generally have a greater maximal gastric secretion rate than females and the most obvious epidemiological aetiological factor in duodenal ulcers is that the majority of sufferers are male (Brown, Langman and Lambert 1976). These findings of greater secretion could be simply due to the greater height and weight of males. However, the male / female ratio has been decreasing over the last 2 decades and it has been postulated that this change is due to the fact that more females have taken up smoking, and tend to start at an especially young age (Kurata 1985).

## 2.1.4 METHODS USED TO MEASURE GASTRIC SECRETION

Measurement of gastric secretion requires a safe means of obtaining the juice as well as an accurate method of analysis. A major problem of gastric secretion tests is the positioning of the tip of the nasogastric tube in the stomach to ensure the best recovery of the secretion. The problem of positioning of the tube was overcome by the method pioneered by Hassan and Hobsley (1970), called the water-recovery test. The assessment of the completeness of gastric aspiration was measured by the dye dilution technique.

## 2.1.5 NASOGASTRIC TUBE

The development of the nasogastric tube is a matter of considerable interest. The first stomach tube was made by Van Helmont in 1648, from leather soaked in resin. But it was Boerhaave (1744) who first described the use of a stomach tube to give antidote to children who had swallowed hemlock, and were unable to swallow because of the convulsions. In 1793 John Hunter used a stomach tube made from eel skin for intragastric instillation in a subject with dysphagia. Physick in 1812 attached a pump to the end of the stomach tube to aspirate laudanum from twins who had accidently been overdosed. In 1909 Einhorn devised a smaller tube for duodenal intubation and later others ( Rehfuss 1914, Ryle 1920, Levin 1921) made further modifications. Leube (1871) seems to have been first to use the stomach tube to explore the gastric response to a test meal in order to distinguish between varieties of dyspepsia.

The commonly used nasogastric tubes are slight modifications of those described by Ryle (1920). Hobsley and Silen (1969) added a second lumen to allow the infusion of marker dye which was needed to assess accurately the volume of gastric secretion.

### 2.1.6 POSITIONING OF THE NASOGASTRIC TUBE & SUBJECT

The stomach, an irregularly shaped muscular organ with an open distal end, presents special difficulties of complete aspiration. Hence, much attention has been given to the positioning of the nasogastric tube and subjects during the gastric secretion studies in order to obtain an adequate recovery of gastric juice.

(a) Nasogastric Tube

Any positioning of the tube was initially more luck than anything else. James and Pickering (1949) showed that the acidity in a sample of gastric juice might differ according to the position of the tip of the tube within the stomach and therefore used fluoroscopy to check its position. Since then many workers (Kirsner, Bock, Palmer et al 1956, Johnston and McGraw 1958, Royle and Catchpole 1967) have stressed the necessity to use fluoroscopy to obtain a reliable results.

But this procedure is time consuming and involves radiation. Hassan and Hobsley (1970) devised a simpler method

to ensure the correct position of the tube, known as the water recovery test. Findlay, Prescott and Sircus in 1972 compared the results of secretion studies performed on successive days on the same group of subjects using fluoroscopy or the water recovery test to position the nasogastric tube. For every subject each positioning procedure was carried out on one of two consecutive days. No significant difference was found between the secretory results irrespective of the method used for positioning of the nasogastric tube, and this study validated the water recovery test as a method for satisfactory tube placement for gastric secretion studies.

(b) Subject

There has been less controversy about positioning the subject for the secretion study. Kay (1953) stressed that the left lateral position was the optimal position for gastric secretion test. Hector in 1968 stated that there was an increased recovery of gastric juice when subjects were in the left lateral position with the foot end of the bed raised compared with a control group whose stomachs were aspirated while seated. These results cannot be accepted because he did not state whether the two groups were matched for age, sex, stature and clinical state; all of which could have influenced the results. In 1970, Hassan and Hobsley found no difference in the recovery of gastric juice, as determined by phenol red measurements, whether the subject lay in the semi-recumbent position or in the left lateral position.

## 2.1.7 ASPIRATION OF GASTRIC JUICE SAMPLES

The sampling of the gastric juice became easy and practical only after the advent of the flexible gastric tube. Early workers (Rehfuss 1914, and Ryle 1926) used intermittent hand suction for sampling the gastric juice. Lim, Matheson and Schlapp in 1923 recommended the use of continuous mechanical suction which they found more efficient than hand aspiration. This rapidly gained popularity and Johnston and McGraw again compared both methods of aspiration in 1958 and concluded that continuous mechanical suction is better and more efficient than intermittent hand suction.

Though the accurate positioning of the tube and continuous mechanical aspiration increased the recovery of the gastric juice, it was still not complete. This error was eliminated by a very simple method, using the dye dilution technique. A known concentration of a marker in a known volume is instilled into the stomach, allowed to mix well and aspirated after a known time. The concentration of the marker in the aspirated sample is found and hence the actual volume of liquid that has been added to the stomach since the last aspiration can be calculated.

Mathieu (1896) was the first to investigate the factor of dilution in gastric analysis and Gorham in 1923 used phenolsulphonphthalein (phenol red, PSP) as a dilution indicator. When gastric motility is inhibited the recovery of phenol red is 100% (Penner, Post and Hollander 1940). Bloom, Jacobsen and Grossman (1967) found that there were losses of up to 17% after initial instillation but subsequent instillation showed a recovery close to 100%. This initial apparent loss is

due to the sequestration of the PSP in the folds of gastric mucosa. Hobsley and Silen (1969) modified the technique with the use of a continuous infusion of PSP through a double lumen nasogastric tube and overcame the problem of sequestration. The volume (as a plateau average) by which aspiration is incomplete once a steady state has been reached, can be equated to pyloric loss.

### 2.1.8 MEASUREMENT OF THE GASTRIC JUICE

Jaworski and Gluzinski (1886) titrated the gastric juice with N/10 sodium hydroxide using litmus paper as an indicator, and expressed acid concentration as cc N/10 NaOH per 100 cc of gastric juice. The introduction of the pH scale for hydrogen ion concentration by Sorensen (1909) made it possible to titrate to a specific end point. Christiansen (1912) determined the pH ranges of various indicators and recommended that gastric juice should be titrated using Topfer's reagent or Congo red as indicator to determine free acid and then with litmus paper to determine the total acid. In 1931 Hollander recommended a pH of 7 as the end point. The development of electrometric titration methods made the measurement easy and various forms are in use nowadays.

### 2.1.9 CORRECTION FOR PYLORIC LOSS

The pylorus, which has thick and prominent muscle layers marks the gastroduodenal junction. Electrical and

radiographic studies show the pylorus to be a high pressure zone (Fisher and Cohen 1973). The antrum, pylorus and proximal duodenum can function as a single unit in expelling the gastric contents into the duodenum (Heading 1984). When the flow is in the opposite direction, the result will be a gain of duodenal contents within the stomach.

The dye dilution method is used to correct the loss. Recovery of various markers, such as polyethylene glycol, radio-iodinated serum albumin and phenol red (Johnston and McGraw 1958, Bendett, Fritz and Donaldson 1963, Lari, Lister and Duthie 1968) were used. PSP was the most commonly used indicator because it is inert, cheap, easy to measure and stable in the pH range of gastric juice. Panda, Van Peborgh, Elder et al (1974) doubted the inertness of PSP in contact with stomach mucosa, but their experimental model was canine and is not necessarily applicable to human studies.

Some workers have not used dilution indicator markers, presumably because they were not certain that quantifying pyloric losses would improve the accuracy of their tests (Levin, Kirshner and Palmer 1948). However, Hobsley and Silen (1969) have shown that during histamine-stimulated submaximal gastric secretion using PSP as a marker, the coefficient of variation of the samples on a plateau was significantly less if the volume was corrected for pyloric losses than if no correction was made. Hassan, Gardham and Hobsley (1969) found that as much as 20% of the acid output might be lost through the pylorus. Both Hobsley and Silen (1969) and Venables (1972) stated that pyloric loss is probably constant rather than a fixed proportion of secretion, so that the proportional effect of loss is lower at the high rate of gastric secretion associated with duodenal ulcer. Wieman, Whifield and Hobsley (1988) have shown that the PSP estimated pyloric loss

is physiological in the sense that it gives an index of the intrinsic emptying ability of the stomach. Recent work had shown that in any one subject pyloric loss is related to the maximal secretory capacity of that person and has a constant value (Roxburgh 1989).

### 2.1.10 CORRECTION FOR DUODENOGASTRIC REFLUX

The first person to document duodenogastric reflux was Beaumont (1833) and since then many workers (Ewald 1892, Roholm 1930, Faber, Russell, Royston et al 1974) recognised and appreciated the reflux of duodenal contents into the stomach, especially during the insulin test after a vagotomy and drainage procedure.

Duodenogastric reflux is often seen during gastroscopy and the assessment of reflux does not correlate well with the measured bile acid concentration (Domellof, Reddy and Weisberger1980). The method used by Capper, Arith and Kilby (1966) has marked drawbacks and the results cannot be validated. Non-absorbable markers such as polyethylene glycol and PSP were used to quantify the reflux but the technique required duodenal intubation.

Based on the two-component hypothesis (Hollander 1932) Hobsley, Gardham and Hassan (1968) proposed to use sodium as a marker for the measurement of duodenogastric reflux. The two-component hypothesis postulates that the acid component is of constant concentration but of variable volume and the alkaline component is of constant volume and with a composition approximately that of intestitial fluid. Makhlouf,

McManus and Card (1966) found that hydrogen ion concentration was low at low secretion rate but rose in a hyperbolic fashion as secretion was stimulated. Reciprocally, at low secretion rate there was a high concentration of sodium which rapidly decreased as secretion increased.

The theoretical basis of the two component hypothesis was explored and it was shown that the variations in gastric acidity and tonicity were better explained by duodenogastric reflux (Hobsley 1974). Fiddian-Green, Parkin, Faber et al in 1979 further confirmed this by measuring bile labelled with indocyanine green, which was given intravenously, and sodium concentration (Hobsley 1974) in the duodenogastric reflux induced by secretin at the maximal plateau of gastric secretion. Gastric emptying and duodenogastric reflux in healthy volunteers (Muller-Lissner, Fimnel, Sonnenberg et al 1982) and in subjects with peptic ulcer are unaffected by gastric intubation (Wolverson, Sorgi, Mossiman et al 1984).

$Tc^{99m}$-labelled iminodiacetic acid was used to radio-label the bile and the duodenogastric reflux was measured by external scintillation scan. Quantification was possible over regions of interest (stomach, duodenum, liver etc) but an accurate measurement of low volume intermittent reflux was not possible (Thomas 1984). However, sodium as a natural marker is a better label than any artificially labelled substance to study the duodenogastric reflux.

## 2.2 *HELICOBACTER PYLORI*

The spiral organism, initially named as *Campylobacter pyloridis*, then changed to *Campylobacter pylori*, and now known as *Helicobacter pylori* (*H pylori*), is a newly recognised bacterium. This bacterium is an important cause for gastritis and *H pylori* infection is associated with duodenal ulcer, gastric ulcer, gastric cancer and non-ulcer dyspepsia. These associations have prompted an unprecedented amount of active investigation over the last 8 years. *H pylori* infection may be diagnosed either invasively or non-invasively. Invasive methods include obtaining gastric biopsy by endoscopy and performing biochemical, microbiological and histological studies, while non-invasive methods include the urea breath test and the serological technique. With improving methods, the sensitivity and specificity of each techniques are now rated 95%-100%.

### 2.2.1 THE DISCOVERY

This bacterium was first cultured in 1982 by Marshall and Warren in Perth, Australia. However, it is probable that this organism was seen in the stomach by many histopathologists long ago.

In 1874, Bottcher identified bacteria-like organisms in the stomachs of cadavers and thought they might be due to

autolysis (Barber and Franklin 1946). Thereafter Bizzozero in 1893 described spiral organisms in mammalian stomach and this was later confirmed by Salomon (1896). Krienitz from Germany was first to describe spiral organisms in the human stomach, in 1906. Doenges in 1938 examined 242 human stomachs from postmortem specimens and found that 43% contained spirochaetes. Two years later, Freedberg and Baron in Boston found these spiral bacteria in 13 of 35 gastrectomy specimens. Until 1975, it was concluded that the bacteria were natural inhabitants in the stomach, became more prominent when pathology was present and were not associated with the pathogenesis of peptic ulceration.

In 1975, Steer and Colin-Jones from Southampton described the bacilli in human stomachs, closely related to the gastric mucosa in association with gastritis. Warren, in 1979, noted the appearance of spiral bacteria overlying the gastric mucosa and associated with the inflamed gastric tissue (Warren and Marshall 1983). Later, in 1984, Steer showed the spiral bacteria in large numbers on the surface of gastric epithelium and areas of gastric metaplasia in the duodenal bulb but not associated with intestinal type epithelium. Also, these bacteria were found in 73% of subjects with duodenal ulcer.

Since 1980 curved *Campylobacter*-like organisms were observed by Warren in gastric antral biopsies from subjects with gastritis and peptic ulceration. This bacterium stained poorly with haematoxylin and eosin but well with the Warthin-Starry silver method (1922). A prospective study was carried out to culture the bacteria using *Campylobacter* media and non-selective media. The first 34 antral mucosal biopsy specimens cultured for 48 hours failed to grow any colonies. The 35th specimen was incubated during the extended Easter holiday and thus examined only after 5 days. A heavy growth of *Campylobacter*-like

organisms was found on the non-selective media (Marshall 1983, Marshall and Warren 1984). This fortuitous error of delay is reminiscent of Fleming and penicillin. The provisional name of *Campylobacter*-like organisms (CLO's) was proposed because of their morphological similarity to other members of this genus.

Because of the frequency with which CLOs were found in the gastric antrum it was named as *C pyloridis*. Later the name was modified to *C pylori* was given and subsequently in 1989 the name *Helicobacter pylori* was proposed and accepted (see subsection 2.2.3). It is universally accepted as a pathogen and there are sufficient indications of an aetiological role in the causation of non-autoimmune (B-type) gastritis (Marshall 1983, Goodwin, Armstrong and Marshall 1986, Rauws, Langenberg, Houthoff et al 1988). There are hints of a facilitatory role in at least some subjects with duodenal ulcer (Marshall, McGechie, Rogers et al 1985, Coghlan, Gilligam, Humphries et al 1987, Levi, Beardshall, Haddad et al 1989) and this role needs clarification.

### 2.2.2 CHARACTERISTICS OF THE ORGANISM

*H pylori* is detected in the gastric type of epithelium of humans and primates. It is a spiral shaped gram negative motile bacillus and measures approximately 0.8mm in with and has an average length of 3mm. It lives beneath the mucosa of the gastric antrum well away from the acid-secreting cells. *H pylori* is sensitive to pH<5 and is able to hydrolyse urea due to a powerful urease. The latter property has been utilised in diagnosis.

### 2.2.3 MORPHOLOGY OF HELICOBACTER PYLORI

The first successful culture of this bacterium (April 15th 1982) was reported in The Lancet in 1983. The light microscopic features and guanine and cytosine content of the DNA of this organism was found to be similar to the genus *Campylobacter.* However, doubt has existed about the correct classification and it remained controversial. The ultrastructure features, fatty acid composition and amino acids sequence of rRNA of the new bacterium were found to be different from those of the genus *Campylobacter* and the amino acid sequence of rRNA closely resembled *Wolinella succinogenes.* However, the detailed ultrastructure analysis revealed pronounced differences between *C pylori* and *W succinogenes* and in early October 1989 the name *Helicobacter pylori* was proposed and accepted (Editorial: Lancet 1989)

### 2.2.4 MICROSCOPIC FEATURES AND ULTRASTRUCTURE

*H pylori* exists in two forms: curved or spiral bacilli and coccoidal forms. The spiral one is the young and vegetative form. Its size is 3-5μm in length and 0.5-1μm in width. The organism has a smooth coat and 2-6 flagellae are found at one end. In some cells, especially the dividing forms, flagellae may be seen at both ends. The flagella are sheathed and have bulbous ends and an internal filament. Each flagellum is 30-35nm in diameter; the terminal bulb is 100nm in diameter (Geis, Leying, Suerbaum 1989). The flagellum has a cell-anchored basal plate.

There are 2 types of coccoid forms. The larger one has electron-loosened cytoplasma with enlarged periplasmic space and it is the degenerative form commonly found in old cultures. The smaller type has electron-dense cytoplasm and intact cytoplasmic membranes. This type is stable against chemical and physical injuries for about 4 weeks and regrows to a spiral vegetative form in 4-6 weeks in favourable conditions. This form possibly reflects a transformation in response to unfavourable conditions or nutrition and such forms occur in faeces and may be responsible for an oro-faecal mode of transmission between human hosts, but they are difficult to culture.

*H pylori* is often visible on routine haematoxylin and eosin stained biopsies. The Warthin-Starry silver stain (1922) shows *H pylori* clearly (Warren 1983, Marshall 1983) but the disadvantages are (a) that it is technically demanding and (b) may give false positives if the stain is not taken up uniformly. Other stains such as Giemsa (Gray, Wyatt and Rathbone 1986), cresyl violet (Burnett, Brown and Findlay 1987) and acredine orange (Walters, Budin and Paull 1986) are all suitable, quick and simple and correlate well with Warthin-Starry technique.

These stains are not specific for *H pylori*. Immunostains using anti-*H pylori* antibodies will allow positive identification of H pylori, but they are not yet available commercially.

### 2.2.5 GROWTH REQUIREMENT

The organism is specially adapted for life in the environment overlying gastric epithelial cells and it may be located throughout the fundus, body and antrum lying underneath

the mucus layer in close apposition to the surface mucous cells. The bacterium is never present in regions of intestinal metaplasia of the stomach. When found in other organs such as oesophagus, duodenum and Meckel's diverticulum and it occurs only in areas of gastric metaplasia.

Most bacteria are killed in an acid environment of pH less than 4.0 and it grows over a wide range of pH from 5.5 to 8.5 with good growth between pH of 7 and 8. Addition of 5mmol $l^{-1}$ of urea will protect the bacteria even at pH of as low as 1.5 (Marshall, Barrett, Prakesh et al 1987). It is suggested that the production, by the urease activity of *H pylori*, of a local 'cloud' of ammonia reduces the hydrogen ion concentration in the immediate microenvironment and thus enables the bacteria to survive.

The presence of more than 1.5% bile inhibits the growth of the organism (Tompkins and West 1987). This may be the reason *H pylori* is seen less often in subjects with reflux gastritis and following gastric surgery resulting in bile reflux, than in other subjects with chronic gastritis. However, only 25% of strains are killed when *H pylori* is exposed to 5% bile in liquid medium for 30 minutes (unpublished data - personal communication from C S Goodwin & J A Armstrong, Perth).

*H pylori* grows best in a microaerobic environment and a suitable atmosphere is achieved, in an Erlenmeyer flask, by a gas mixture of $O_2$:$CO_2$:$N_2$ at a ratio of 5:10:85 (Morgan, Freedman, Depew et al 1987). A moist atmosphere, approximately 98% humidity, is required for a good growth (Goodwin, Blincow, Warren et al 1985). The organism can survive between 30-40$^{o}$C and maximum growth is observed at 37$^{o}$C (Goodwin, Collins and Blincow 1986, Itoh, Yanagawa, Shingaki et al 1987). *H pylori* in

a conventional medium, such as Trypton soy broth containing 15% glycerol can be stored at -70$^{o}$C and remain viable for more than a year (Ribeiro and Gray 1987).

A wide variety of media have been used for culture and sensitivity testing. These usually incorporate blood or serum, and may be either solid or liquid media. The commonly used solid media are blood agar (Jones, Lessells and Eldridge 1984) and brain heart infusion agar (Goodwin, Blincow, Marshall et al 1985). When incubated at 37$^{o}$C, small (1mm diameter) shiny and convex colonies are seen after 3 days with weakly haemolysed areas surrounding them. The optimum selective media is brain heart infusion agar with 7% horse blood and 1% Isovitalex with antibiotics (vancomycin 6mg/l, nalidixic acid 20mg/l and amphotericin 2mg/l), which prevent the growth of contaminant bacteria. Liquid media that have been described include brain heart infusion broth with 10% horse serum and 0.25% yeast extract (Goodwin, Blake and Blincow 1986), brucella broth with 1-10% fetalcalf serum (Morgan, Freedman, Depew et al 19887) and nutrient broth with 0.25% yeast extract and 5% horse serum (Goodwin, Armstrong and Marshall 1986).

Specimens taken for isolation of *H pylori* should be transported as quickly as possible in appropriate media to prevent the drying out and exposure to atmospheric oxygen. Physiological saline (Prakash, Marshall, Barrett et al 1987) and 20% glucose (Goodwin, Blincow, Warren et al 1985) are the commonest used transport media. It has been reported that gastric biopsies can be stored in these media for up to 5 hours at 4$^{o}$C without any adverse effect on the recovery of *H pylori* (Goodwin, Blincow, Warren et al 1985). Other transport media have been suggested including Brucella broth (Taylor, Hargreaves, Ng et al 1987) with 25% glycerol. The latter maintains the organisms viable for at least six months if kept at -70$^{o}$C.

## 2.2.6 BIOCHEMICAL ACTIVITY

Bacterial enzymes have been studied for the purpose of taxonomy, identification and to determine pathogenetic mechanisms.

*H pylori* exhibits catalase and cytochrome oxidase activity and produces haemolysin, but the production of urease is the key factor and exceeds the highest activity of other bacteria (Mobley, Cortesia, Rosenthal et al 1988). The average rate of hydrolysis of urea by cell lysates is 36 +/- 26 mmol of ammonia/min/mg of protein, which is more than twice that of *Proteus mirabilis* . The large amount of urease produced has been used for rapid detection in the endoscopy room and for indirect non-invasive detection by breath test. The urease is antigenic and it has been proposed as an antigen in the enzyme-linked immunosorbent assay test (Dent, McNulty, Uff et al 1988).

The urease has been considered as a pathogenic factor of *H pylori*. The ammonia, released from urea by urease activity, creates a buffered, favourable microenvironment in the midst of the highly acidic surrounding thus overcoming the first barrier against microrganisms in the stomach (Marshall, Barrett, Prakesh et al 1988). It is also claimed to be toxic to cells and may initiate damage to the gastric mucosa (Barer, Elliot, Berkeley et al 1988). The presence of strong catalase may prevent ingestion of *H pylori* by polymorphs (Lior and Johnston 1985).

## 2.2.7 IMMUNOLOGY

The protein profile of *H pylori* differs considerably from those of other microganisms. Colonization induces both systemic and local immune responses. The serological responses correlate well with the histological detection and culture of *H pylori.*

The systemic response is accompanied by an increase in IgG and IgA levels with little or no effect on IgM, which is not surprising in view of the chronicity of the infection (Rathbone, Wyatt, Worsley et al 1986). Agglutination, complement fixation and ELISA test have all been used to identify the bacteria. The ELISA method is the most sensitive and convenient test. The level of IgG does not correlate with the severity of mucosal inflammation. Serology can be used as a tool for epidemiological study and for monitoring the response to treatment.

Local humoral response leads to an increase of plasma cells and secretion of IgA at the site of *H pylori* infection. Raised specific IgA levels have been detected in the gastric juice of 30% of subjects with *H pylori*-associated gastritis. The functional significance of this response is not known.

## 2.2.8 PATHOLOGY

Many bacteria possess, in addition to specialised adaptive mechanisms, factors which, when produced under appropriate circumstances in a susceptible host, result in disease.

Although adherence factors are not considered pathogenic, they are important in the initial colonisation of the host mucosa which is essential for subsequent pathogenesis. *H pylori* are found in close association with gastric mucosal cells and at intracellular junctions (Hazel, Lee, Brady et al 1986), and thickening of the gastric epithelial cell at the point of attachment has been observed (Graham 1989b, Bode, Malfertheiner and Ditschuneit 1988), suggestive of specific binding of gastric epithelial cell surface with *H pylori* adherence factors. Thomsen, Gavin and Tasman-Jones (1990) showed fine filamentous strands extended between organisms and nearby epithelial cells, with few organisms in membrane-to-membrane contact. *H pylori* were not observed between, beneath or within cells of gastric mucosa.

*H pylori* produces a cytotoxin which is heat-labile with a molecular weight of $>10^5$. This cytotoxin causes vacuolization and cytopathic effects on mammalian cell lines (Leunk, Johnson, David et al 1988). It is interesting to note that there is a statistically significant difference in cytotoxin production by *H pylori* isolates from subjects with peptic ulcers and subjects with active chronic gastritis (Figura, Guglielmetti, Rossolini et al 1989).

The gastric mucin protease produced by *H pylori* degrades gastric mucin (Sarosiek, Slomiany and Slomiany 1988) which is liquefied and loses its structure, thus no longer serving as an effective barrier. The optimum activity of the enzyme is at pH 7.0 and at $37^oC$.

As mentioned above, the potent urease of *H pylori* has been proposed as a pathogenic factor (Hazell, Lee, Brady et al 1986) with the ammonia that accumulates in the protective gastric mucin layer causing ionic disintegration (Thomsen, Tasman-Jones, Morris et al 1989). However, some investigators have contended that ammonia is not toxic to the stomach lining (Graham 1989).

Cave and Vargas (1989) demonstrated that whole or sonicated *H pylori* inhibit stimulated acid secretion from rabbit parietal cells, using the uptake of $^{14}C$- aminopyrine as an indirect assay and found that the non-dialysable protein inhibitor was as effective as $10^{-4}$ mol/l cimetidine. This may be responsible for the hypochlorhydric state seen during acute *H pylori* infection. Defize, Goldie and Hunt (1988) showed a similar effect on isolated guinea-pig parietal cells and the effect is only partially reversible.

### 2.2.9 HISTOLOGICAL EFFECT ON THE CELLULAR ENVIRONMENT

There is a strong link between colonization and gastritis. The presence of *H pylori* is associated with an active chronic gastritis (ACG) which is characterised by the presence of intraepithelial and intestitial neutrophil polymorphs in addition to lymphocytes and plasma cells. The number of *H pylori* declines steadily from surface to foveolar neck; paradoxically, infiltration with polymorphs is greatest in the pit-isthmus region. When the exact topographical relations are considered there are obvious discrepancies between the density of *H pylori* and polymorph infiltration. It may be that the immature foveolar epithelium of the isthmus proliferative zone is more permeable to leucocytic factors (such as complement products) activated by *H pylori* higher up the gastric pits. The tall mucin-filled cells are replaced in patchy fashion by cuboidal cells, and the consistency of the mucus alters. A further aspect of note is the frequent finding of reactive lymphoid follicles.

## 2.2.10 IDENTIFICATION & DIAGNOSIS OF H PYLORI

Approaches to the diagnosis of *H pylori* infection fall into two groups. 1). Invasive, where a biopsy specimen is required, and is obtained by endoscopy. The biopsy material is analysed by a variety of methods, such as an urease test, histology and microbiology. 2) Less invasive or non-invasive methods: these methods are useful in epidemiological studies; they are based upon the high endogenous urease activity of the organism and upon serological methods that identify the presence of antibodies against *H pylori* antigens.

Biopsy of the gastric antrum is the standard procedure for detecting *H pylori*, defining the disease and identifying the organism. However, the distribution of *H pylori* is focal and patchy in the gastric mucosa, hence, mutiple biopsies are necessary for the detection. Disinfectants and chemical agents used during endoscopy can be bactericidal to *H pylori*. Benzocaine and simethicone are inhibitory to *H pylori* but lidocaine is not. Gluteraldehyde, contaminated with biopsy forceps, is also bactericidal to *H pylori*.

The urease activity of *H pylori* was first described by Langenberg, Tytgat, Schnipper et al (1984) and is about 20 to 70 times as great as that of proteus (Ferrero, Hazell and Lee 1988). This property has been utilised in the simple and rapid urease test which is now a well recognised endoscopy room test. The test detects the presence of urease produced by *H pylori* in the biopsy specimen. The urease hydrolyses urea, producing ammonium ions; this raises the pH, which can be detected by a pH indicator. The indicator most commonly used is phenol red, which changes colour from yellow at pH of 6.8 to pink at pH of 8.4.

Two percent Christensen's urea broth was the first broth used for this test (McNaulty and Wise 1985). Since then, to increase the predictive value and speed of the biopsy urease test, many centres have modified the broth by excluding the nutrients or the buffer, adding bacteriostatic agents or increasing the concentration of urea etc (Hazel, Borody, Gal et al 1987, Marshall, Warren, Francis et al 1987, Vaira, Holton, Cairns et al 1988a & 1988b and Arvind, Cook, Tabaqchali et al 1988).

The sensitivity of different urease tests varies from 58% to 98% in the hands of different investigators (Morris, McIntyre, Rose et al 1986, Vaira, Holton, Cairns et al 1988b). The minimum concentration of organisms required is $10^4$ colony forming unit (CFU)/ml (Vaira, Holton, Cairns et al 1988a). Thus the main limitation of the urease test lies in the number of organisms present in the biopsy specimen. Less than $10^4$ CFU/ml may give a positive culture result but not a positive urease test (Glupcznski, Thibaumont, Labbe et al 1988). False positive results are a potential problem, but the investigators claim that if the tests are performed according to the instructions the specificity is near 100%.

Histological staining followed by microscopic study is the standard method for detection of *H pylori* in a biopsy specimen (Warren 1983). There are many techniques proposed. The first and most frequently reported is the Warthin - Starry silver stain, but this method is time consuming and difficult to perform. Some other workers (Jones, Lesselle and Eldridge et al 1984, Taylor, Hargreaves, Ng et al 1987, Madan, Kemp, Westblom et al 1988) found standard haematoxylin and eosin stain to be as good as the Warthin - Starry stain. However, these two techniques are less effective than Giemsa (Gray, Wyatt and Rathbone 1986) or acridine orange (Walters, Budin, Paull 1986) staining. The acridine orange fluorescent method is rapid and easy to perform,

and Giemsa staining offers the advantage of preservation of the morphology. There are other non-specific methods for the detection of *H pylori* which include staining with carbol-fuchsin (Rocha, Queiroz, Mendes et al 1989), modified Gram technique(Trowell, Yoong, Saul et al 1987), or Giemenza method (McMullen, Walker, Bais et al 1987) and the use of phase-contrast microscopy (Pinkard, Harrison, Capstick et al 1986).

The above methods are not specific to *H pylori* though all enhance the visualization of this bacterium. Specific immunofluorescent methods, such as polyclonal antisera (Steer and Newell 1985) and monoclonal antibodies (Engstrand, Pahlson, Schwan et al 1986 and 1988) are now available.

Brush cytology with rapid staining by Giemsa stain has been reported to have a good sensitivity and has an 86% success rate (Debongine, Legros, Wautelet et al 1987) in identification of *H pylori*; however other workers found that the sensitivity of this test was only 27% (Vaira, Holton, Falzon et al 1988a). Thus the test is probably valuable only in the hands of a well trained pathologist.

The histological detection of *H pylori* depends on the distribution of the organism in the section examined, and also on the expertise of the pathologist. It has been noted that more than 3% of *H pylori* positive specimens are found not to have antral gastritis. Two antral biopsies are sufficient to avoid sampling error and establish *H pylori* status.

Culturing may also be a less sensitive method because of the many difficulties encountered in the process. Potential sampling error, prior use of antibiotics or bismuth compound by the subjects, use of antiseptic and / or antibacterial agents during

the endoscopy, and inappropriate handling, transport and laboratory processing of the sample determine the positive culture rate. Despite these problems, recovery rates of more than 90% have been reported by Jones, Lesselle and Eldridge et al (1984), Goodwin, Blincow, Warren et al (1985) and Schnell and Schubert (1989).

*H pylori* was first isolated from gastric antral mucosa using a non-selective culture medium consisting of brain-heart infusion chocolate agar with 7% horse blood (Marshall, Royce, Annear et al 1984). Since then, blood-based media (either selective or non-selective chocolate media prepared with fresh blood) have remained the choice of most investigators. Selective blood agar media inhibit the growth of the contaminating flora from the mouth and respiratory tract. Skirrow's medium (Skirrow, 1977) which is supplemented with vancomycin, trimethoprim and polymyxin B is a very suitable medium for isolation of *H pylori* strains (Krajden, Bohnen, Anerson et al 1987). Other selective media contain vancomycin 6mg/l, nalidixic acid 20mg/l, and amphotericin 2mg/l. The culture gives the best result in a microaerobic environment achieved by a gas mixture of $O_2$:$CO_2$:$N_2$ at a ratio of 5:10:85 in an Erlenmeyer flask. It is important to maintain a moist atmosphere of approximately 98% humidity and an optimal temperature of 37$^{o}$C. Incubation of primary cultures requires between 3 and 5 days.

The accuracy of the urease test, microbiology and culture depends on *H pylori* density. For histological and microbiological studies a highly motivated and experienced pathologist is required. The combined results of urease test and histology are similar to results obtained by culture. Only culture provides a reliable definite diagnosis of *H pylori* infection, when

the biopsy specimen is rapidly and correctly transported and processed in an appropriate medium, and culture therefore remains the gold standard in the identification of *H pylori.*

Detection of gastric infection in the cat by the urea breath test was first reported by Korenberg, Davis and Wood (1954). They showed that the urease was bacterial in origin; and that this hydrolysed the administered $^{14}C$-labelled urea, and released $^{14}CO_2$ which was detectable in the expired air. Currently there are two methods, named according to the labelled carbon incorporated in the urea. Graham, Klein, Evans et al (1987) devised the test using $^{13}C$-urea while Marshall and Surveyor (1988) independently developed the test using $^{14}C$-urea.

$^{13}C$-urea is a stable and naturally occurring non-radioactive substance, but a mass spectrometer is required to measure $^{13}C$ in the expired air, and $^{13}CO_2$ also fluctuates in response to dietary consumption of $^{13}C$-enriched food, such as corn or cane sugar. $^{14}C$ is a radioactive substance, and the required amount for the test is 0.4Mbq (10mCi) (Bell, Weil, Harrison et al 1987, Marshall and Surveyor 1988); this is one-tenth of the radiation dose to a subject undergoing a single plain abdominal X-ray. However, satisfactory results can be obtained with 0.11MBq (3mCi) of $^{14}C$. The advantage of using $^{14}C$ is that it only requires a simple scintillation beta counter which costs less than one-tenth of the cost of a mass spectrometer. Both tests are reasonably sensitive and reproducible.

A known amount of carbon-labelled urea is given orally, after an overnight fast, and breath samples are collected every 10 minutes for at least 2 hours. If *H pylori* is present in the stomach, it splits the urea and the labelled carbon will appear as carbon dioxide in the expired air.

In addition to being non-invasive, the urea breath test has other potential advantages when compared with endoscopic examination and biopsy. *H pylori* infection is patchy and its successful detection requires multiple biopsies, which must also be transported rapidly in a proper medium. A dedicated and experienced pathologist is necessary for identification of the organism in histology specimens. The urea breath test effectively integrates the response of the entire gastric mucosa, and hence even a low level of infection can be detected.

By 1984, association between gastritis and the presence of serum *H pylori* antibodies had been established (Eldridge, Lessells and Jones 1984). In 1985, Kaldor, Tee, McCarthy et al showed a significant association between the presence of *H pylori* antibodies and peptic ulceration. Whole bacteria or partially purified antigen frequently gave false-positive and false-negative results (Newell, Johnston, Ali et al 1988; Gnarpe 1988). This is because the preparations contained antigens that cross-reacted with antibodies directed against other bacteria (*C jejuni, C fetus, E coli*). Good results have recently been reported for an enzyme-linked immunosorbent based on the urease produced by *H pylori* and it was correlated closely with the presence of active chronic gastritis (Dent, McNulty, Uff et al 1988). However, at present no single antigen has been detected which consistently reacts with all positive sera investigated. This supports the contention that the variation of antibody specificity is host-mediated rather than a reflection of antigenic differences between the infective agents. The most commonly used antigen is an acid-extractable material eluted from the surface of whole organisms in 0.2 M glycine buffer (pH 2.2).

Serum anti-*H pylori* antibody can be detected by a complement fixation test, haemagglutination, bacterial agglutination, immunofluorescence and enzyme linked

immunosorbent assay (ELISA) (Jones, Lessells and Eldridge 1984; Marshall, McGechie, Francis et al 1984; Kaldor, Tee, McCarthy et al 1985; Newell and Rathbone 1989). ELISA is currently the technique of choice in most laboratories, because of its speed, low cost, simplicity and reproducibility.

The majority of people with *H pylori* colonisation exhibit a specific antibody response. The IgG response remain constant throughout the course of the infection (Langenberg, Rauws, Houthoff, et al 1988, Perez-Perez, Dworkin, 1988) and is present even in the absence of raised IgA. The IgG level declines after eradication of *H pylori*, but the fall is usually slow and it may take upto 6 months to attain the normal level (Von Wulffen and Grote 1988, Evans Jr, Graham, Lew 1991). Re-infection is accompanied by a rapid rise in titre (Langenberg, Rauws, Houthoff et al 1988).

Serodiagnosis can be used effectively for epidemiological study and to assess the effectiveness of the treatment and the possibility of re-infection.

Many techniques have been developed to identify *H pylori* infection; however none shows 100% specificity and sensitivity. Unfortunately, the specificity and sensitivity of each test varies between investigators: thus it is essential to perform two or more tests to confirm the diagnosis of *H pylori* infection.

### 2.2.11 TRANSMISSION

No natural animal host has yet been found for *H pylori*. Laboratory experiments have shown that this organism will

colonise only in the stomach of the germ-free piglet. Thus, one would postulate person-to-person spread as the most likely means of transmission of *H pylori*. The voluteer studies (Marshall, Armstrong, McGechie et al 1985, Morris and Nicholson 1987) and the reported epidemic outbreak of hypochlorhydria (Ramsay, Carey, Peterson et al 1979, Gledhill. Leicester, Addis et al 1984) show that infection can be transmitted from person-to-person.

Infectious diseases which are spread from person to person by close contact are commonly found to have a higher incidence in institutions for the mentally handicapped. Berkowicz and Lee (1987) studied the sera of subjects in an Australian institution by the ELISA method and the results showed that there was a greater prevalence of *H pylori* among these subjects compared with blood donors. Family studies have shown a high incidence of active *H pylori* infection in consanguineous contacts of children infected with *H pylori* (Mitchell, Bohane, Berkovicz et al 1987). The prevalence of *H pylori* in low socio-economic countries is found to be very high and subjects in those countries become infected with *H pylori* earlier in life than those living in the developed countries. While faecal-to-oral spread has been suggested, it is unlikely as *H pylori* has never been isolated from the stools of subjects with gastritis. Rawles, Harris, Paul et al (1987) found a higher frequency of antibody to *H pylori* in endoscopy staff when compared with blood donors and Mitchell, Lee and Carrick (1989) found that endoscopists have a significantly higher incidence of *H pylori* infection compared to general practitioners and blood donors. This suggests the most likely route is via stomach secretion, possibly by regurgitation and then with sputum. These results are consistent with the hypothesis of person-to-person spread of *H pylori*.

### 2.2.12 CLINICAL and EPIDEMIOLOGY EVIDENCE

The epidemiological studies show that the incidence of *H pylori* infection is greater in the developing countries, that prevalence increase with age and that there is no gender predilection. There is no significant association between *H pylori* and ABO blood group and secretor status. The prevalence studies are mostly concentrated on subjects with dyspepsia. Morris, Maher, Thomsen et al (1988) studied the prevalence of *H pylori* in the gastric material obtained from post-mortem and they found that the infection was present in 60% of Polynesians and 20% of Caucasians. A prevalence study using the isotopically labelled urea breath test showed that the Mexican nationals have a similar rate of infection to US nationals (33%), while Indian nationals showed a higher rate (46%) (Graham, Klien, Opekun et al 1988). Serology has proved a valuable tool for measuring the prevalence rate. More often than not the surveys have been done on blood donors. These studies showed that there was a gradient in seroprevalence which was age-related. Dwyer, Sun, Kaldor et al (1988) found that *H pylori* in Aborigines from northern Australia is rare (<1%), but the rate was 50% among the Aborigines from southern Australia. It is also noted that that there was a distinct differences in antibody rates between people of different races or cultures living in the same area (Nafeeza, Shahim, Kudva et al 1989).

The human ingestion, treatment, and epidemiological studies are suggestive of a very strong association between *H pylori*, gastritis, peptic ulceration, and even gastric cancer. However, *H pylori* was isolated from 10-20% of the asymptomatic subjects in whom no endoscopic abnormalities

were noted and no histopathological abnormalities noted in the antral biopsies (Buck, Gourley, Lee et al 1986; Jiang, Zhang, Shi et al 1987, Raskov, Lanng, Gaarslev et al 1987).

The frequency of *H pylori* in chronic type B gastritis is approximately 70% and it is as high as 100% in active chronic gastritis (Goodwin, Armstrong and Marshall 1986; Andersen, Holck, Povlsen et al 1987). Results from different centres are not strictly comparable because the methods of detection of *H pylori* differ and the criteria for histological diagnosis also vary. However, the overall prevalence data point to a very close link between *H pylori* and the predominantly antral form of active chronic gastritis.

Many subjects with duodenal ulcer have active chronic gastritis which involves the antral mucosa and the gastritis is closely associated with *H pylori* infection of the antrum. Many studies show a strong (70% - 100%) association between duodenal ulcer and *H pylori* -associated gastritis (Marshall, McGechie, Rogers et al 1985; Booth, Holdstock, MacBride et al 1986. Jiang, Liu, Zhang et al 1987. Raskov, Lanng, Gaarslve et al 1987, Rauws, Langenberg, Houthoff et al 1988 and Goodwin, Marshall, Blincow et al 1988). Arborigines in Northern Australia do not develop DU and the incidence of *H pylori* was lowest when compared to any groups studied so far. A Few studies have shown that *H pylori* is found in the duodenum of subjects with duodenal ulcer and duodenitis (Steer 1984, Wyatt, Rathbone, Dixon et al 1987 and Caselli, Bovolenta, Aleotti et al 1988). The reported frequency of the finding is between 29% and 100%.

However, there is no clear-cut direct causal relationship between *H pylori* and duodenal ulcer. Only a minor fraction of people harbouring *H pylori* will apparently develop DU and DU is not invariably associated with *H pylori* colonisation. Approximately 10% to 30% of DU are not associated with *H pylori*.

The presence of gastric epithelium in the duodenum is essential for *H pylori* to colonise in the duodenum. This can present in 2 forms; (a) gastric heterotopia, characterised by fully developed fundic mucosa, is rare and (b) gastric metaplasia which is more common and extensive in DU subjects. The presence of gastric metaplasia is seen only in subjects with low pH and correlates well with maximal acid output. Thus, the hypothesis is that an increased acid load into the duodenum leads to gastric metaplasia (James 1964) which in turn is colonised by *H pylori*; this produces duodenitis and ultimately DU (Wyatt, Rathbone, Dixon et al 1988; Goodwin 1988 and Wyatt, Rathbone, Dixon et al 1989).

There is another hypothesis put forward by Levi, Beardshall, Haddad et al (1989) to link *H pylori* and DU. It is often known as 'gastrin link' theory. Urease produced by *H pylori* splits the urea in the gastric juice into ammonia which neutralises the acid in contact with the mucosa overlying the G-cells and parietal cells. This impairs the normal inhibition of gastrin release by intraluminal acid leading to an inappropriate secretion of gastrin in response to food; this increases acid output and the raised acid level leads to the pathogenesis of the ulcer.

In Brazil the incident of *H pylori* among the dyspepsia subjects is nearly 100% but the incidence of DU is not very high when compared with Western countries. Indians of Fiji appear to have rates of DU at least twice that seen in ethnic Fijians (Parshu 1975) but Beg, Oldmeadow, Morris et al (1988) found that *H pylori* is present with equal frequency in both races. The prevalence of *H pylori* in Tibetan monks is approximately 52% but the incidence of DU is one of the highest in the world. Moreover, in Malaysia the incidence of *H pylori* is greater (75%) in the Indians compared to (45% in the) Chinese (Nafeeza,

Shahimi, Kudva et al 1989) but DU is common in Chinese and less common in Indians (Ti 1983). These findings cast doubts about the role played by *H pylori* in the pathogenesis of DU.

The strongest evidence that *H pylori* is causally related to DU is mainly derived from therapeutic eradication trials. When *H pylori* infection was eradicated, follow-up for 1-2 years showed no DU relapse, but a high relapse rate was observed when the infection was not eradicated (Coghlan, Gilligan, Humphries et al 1987, Marshall, Goodwin, Warren et al 1988, Rauws, Langernber, Houthoff et al 1988). It is tempting to speculate that because cure of the ulcer is associated with removal of the organism, then the original ulcer may well have been due to the presence of the organism, but proof of this causal role is presently lacking.

There have been some reports of association of *H pylori* with gastric carcinoma ( Marshall, McGechie, Rogers et al 1985). However, *H pylori* does not colonise on cancer cells and *H pylori* are found to colonise in the remaining foveolar epithelium of the stomach. No good evidence to support *H pylori* as a causative factor is yet available.

### 2.2.13 EFFECTS OF DRUGS ON *HELICOBACTER PYLORI*

Many antimicrobial agents are effective *in vitro* but their activity was reduced *in vivo*. This is due to the lack of stability and activity at low pH (McNulty and Dent 1988), decreased tissue penetration (Allen 1981) and the presence or absence of food and / or other drugs.

Bismuth salts are found to be bactericidal to *H pylori* and also some reports indicate that omeprazole eradicates *H pylori* in the gastric antrum (Tessaro, Mario, Rugge et al1990) but other reports contradict this finding (Mainguet, Delmee and Debongnie 1989, Termini, Scialaba, Pisciotta et al 1990). Metronidazole is unaffected by pH and is bactericidal but acquired resistance had been reported. Combination with bismuth salts reduces the development of resistance. Amoxycillin is less ionised at low pH and retains good activity at acidic pH, although the concentration in gastric mucus that can be achieved with this agent is much lower than with many antibiotics,. Tetracycline is another antibiotic which is active *in vitro* as well as *in vivo.*

The aim of the treatment is to achieve eradication, rather than clearance, of the bacteria. Clearance means failure to identify the bacteria at the end of the treatment and eradication means failure to identify the bacteria for at least 4 weeks after the cessation of the treatment.

Bismuth monotherapy achieved a clearance of 45-100% but the long term eradication was only 10-30% (Coghlan, Gilligan, Humphries et al 1987, Lambert 1988, and Borsch, Mai and Muller1988). Monotherapy with amoxycillin gives good clearance and eradication rates (Glupczynski, Burette, Labbe et al 1988), but there is always a possibility of acquired resistance taking place. This is true also with metronidazole (Hirschl, Hentschel, Schutze et al 1988, Glupczynski, Burette, DeKoster et al 1990). Combinations of bismuth salts with various antibiotics have been tried (Marshall, Goodwin, Warren et al 1988, Rauws, Langenber, Houthoff et al 1988) in an attempt to improve the rate of long term eradication. Even triple therapy has been used (Borody, Cole, Noonan et al 1988, Borsch, Mai and Opferkuch 1988 ) for the same purpose. Currently, a highly effective drug

combination is bismuth salt, amoxycillin (or tetracycline) and metronidazole; this gives an eradication rate of 80-95% and was recommended at the 4th World Congresses of Gastroenterology in Sydney, 1990.

## 2.3.1 ENDOSCOPY

The modern endoscopy unit comprises patients and relatives reception and waiting areas; a nursing and consultation office; an instrument cleaning room; a storage room or rooms and an endoscopy procedure room. It is important that the procedure room should have easy access to the instrument storage room. The recovery bay is situated near the nursing station with facilities for a relative to be with the subject.

The endoscopy procedure room usually has a large floor area with smooth and washable flooring. The room is equipped with an alarm call system and resuscitation equipment including ECG monitor, defibrillator, intubation facilities and emergency drugs.

### 2.3.1.1 INTRODUCTION

Endoscopy is derived from Greek words endo = "within" and skopeo = "to view". Several attempts have been made over

the last 200 years to achieve practical endoscopes, but the enabling technology has really only been available in the last 40 years.

A leaflet from KeyMed reproduces a 16th century picture showing the use of a proctoscope and this may have been possibly the first endoscope ever to be used. But it appears that Phillip Bozzini, in 1806, was the first person who attempted to visualise the urinary tract with a tin tube and candle. Adolf Kussmal (1868) used a rigid metal tube to examine the upper gastrointestinal tract, after watching a sword-swallower in action. In Munich, in 1932, Rudolf Schindler used a semi-flexible gastroscope, made for him by George Wolf. Preoccupation with their gastric cancer problem led the Japanese to develop the blind gastric camera in the 1950s.

In 1949, Hopkins proposed and devised an optical unit to convey images along the flexible axis. Baird had proposed much the same idea in 1927, though he failed to devise a proper working system. The essential mechanism is that the unit consists of bundles of fine glass fibres that transmit the image by continuous internal reflexion. The first flexible fibreoptic endoscope was used by Hirschowitz in 1957 in a patient at the University of Michigan Hospital (Salmon 1975).

#### 2.3.1.2 FIBREOPTIC UPPER GASTROINTESTINAL INSTRUMENT

There are three basic types of gastroscopes: rigid, semiflexible and flexible. The rigid gastroscope was a relatively

dangerous instrument and caused a lot of fatal injuries and it is of medical historical interest only. High technology progress has also made the semiflexible upper gastrointestinal endoscope obsolete.

The greatest advance in the field of endoscopy was the ability to direct light around corners and thus to manipulate the instrument under direct vision; the birth of the fibreoptic endoscope. During the past decade considerable advances have been made in the field of gastrointestinal endoscopy. Newer and narrower bodied endoscopes are available for the more comprehensive examination of the upper gastrointestinal tract. Gastrointestinal endoscopy is now considered as a basic diagnostic technique and it has virtually replaced radiology as the primary approach to structural disorders of the gut. Endoscopes themselves have been developed and modified to increase their diagnostic and therapeutic potentials and so have large, sometimes multiple channels and elevating bridges to assist in the placing of biopsy forceps and other tools.

The modern gastrointestinal fibrescope basically consists of a head with eyepiece and controls, and a flexible shaft which has a manoeuvrable tip. The head is connected to a light source via a connecting 'umbilical' cord, through which pass other tubes transmitting air and suction. There is a separate channel for the passage of accessories including flexible biopsy forceps. The modern endoscope is narrow, with a working length of 1,025mm (total length : 1,345mm) and diameter of 9.8mm and with an instrument channel 2.8mm wide. It has versatile flexibility and can be easily manoeuvred.

### 2.3.1.3 BIOPSY FORCEPS

The ability to take a satisfactory specimen is a crucial part of the fibreoptic endoscopy. Wood et al (KeyMed 1983) developed gastric suction biopsy in Australia in 1949 and it revolutionised the idea of gastric mucosal morphology. Benedict built a delicate biopsy forcep to be used under direct vision (KeyMed 1983).

The modern biopsy forceps consist of a pair of sharpened cups, is controlled by a flexible cable and can be easily introduced through the channel in the gastroscope. The biopsy forceps tip is visible as soon as it has passed 3mm beyond the distal end of the gastroscope.

# *CHAPTER 3*

# *METHODS*

# 3 METHODS

## 3.1 ENDOSCOPY

When the clinical indication for endoscopy was established the procedure was fully explained to the subjects. Every detail was explained in layman's terms and a written explanation was also given or sent to the subject.

It is the responsibility of the endoscopist to protect the subjects and the ancillary staff from cross infection with micro-organisms. In the past sufficient attention has not been paid to infection and subjects as well as staff have suffered from infection transmitted by endoscopes and accessories. In the UK these problems are now actively addressed and advice is given by the Endoscopy Committe of the British Society of Gastroenterology (BSG).

The examination was carried out by using an Olympus GIF-XQ 20 fibreoptic gastroduodenoscope. The channels of the instrument, air insufflation, suction and biopsy were checked at the beginning of the examination. The biopsy forceps and the suction pump were also checked for any defect.

### 3.1.1 SUBJECT SELECTION

Patients who were referred to the Gastrointestinal Endoscopy Department at The Middlesex Hospital for upper gastrointestinal endoscopy as part of the investigation for complaint of dyspepsia were approached. Patients who were on steroids, non-steroidal anti-inflammatory drugs, antibiotics or colloidal bismuth subcitrate within the last three months were excluded.

### 3.1.2 PATIENT PREPRATION

Patient education, some sedation and a good technique of passing the instrument are essential to perform a satisfactory endoscopic examination.

Although with the modern flexible fibrescope complications are rare, subjects with any contraindications such as cardiac or respiratory embarrassment were excluded. Any potential hazard to the unit staff and to subsequent subjects from infectious subjects was looked into and each case was individually assessed.

The upper gastrointestinal endoscopy was performed in the morning, as it was more convenient for the subject to fast overnight. The subject was instructed not to eat or drink for at least 6 hours before endoscopy. Patients with suspected oesophageal or gastric outlet obstruction were advised not to eat any solid food for 24 hours and only to take liquids.

The subject was advised to arrange a relative or friend to accompany him or her home after the test. When the subjects arrived at the department, the nursing staff received them and registered them in the entry book. Patients were given a sympathetic approach and the procedure was explained again and a reassuring friendly atmosphere created in order to gain the confidence of the subject. The formal written consent was obtained and countersigned by the endoscopist.

The subject was requested to take off the top half of his or her clothes and put on a gown. Dentures and spectacles were removed and stored safely. The subject was allowed to lie on the trolley and rest in the reception area until his or her turn came.

### 3.1.3 INTRAVENOUS ACCESS

The subject was wheeled into the endoscopy room and a 22G Butterfly needle was inserted into a vein, usually on the dorsum of the hand. This access was used to give the sedation.

### 3.1.4 POSITIONING THE PATIENT

The positioning of the subject varied according to the examiners but most endoscopists had the subject in the left lateral position with the back supported by a pillow against the trolley side. The head lay on a single pillow, which was covered with a disposable towel, and a nurse was standing at the patient's

head to ensure an adequate airway and access for the use of suction when necessary. The nurse also helped to keep the mouth guard in position and to hold or move the endoscope when instructed. The nurse ensured that the subject rested his head on the pillow properly and held his hands to prevent the subject interfering during process of intubation.

### 3.1.5 SEDATION

Opinion and practice concerning sedation and analgesia vary widely. The use of pharyngeal anaesthesia is also controversial.

In this study pethidine 50mg (Roche) was given intravenously and followed by 10mg of emulsion diazepam (Diazemuls - Dumex) via the butterfly needle. Both pethidine and Diazemuls can cause respiratory depression, particularly in the elderly, and every precaution was taken to prevent this potentially fatal event. Sometimes, lignocaine aerosol (Xylocaine - Astra) was sprayed into the throat if the subject had a marked gag reflex.

### 3.1.6 EXAMINATION

The examiner stood on the left side of the subject, facing the subject and holding the head and tip of the endoscope close

to each other. The mouthguard was placed in position and the nurse held the patient's head flexed slightly forward. The tip of the endoscope was passed through the mouthguard and over the back of the tongue into the pharynx. The tip was advanced over the back of the tongue and larynx under direct vision, maintaining a correct central axis. The subject was asked to swallow as the tip was passed through the sphincter. A constant conversation of reassurance and encouragement helped to achieve an easy passage of the tip through the cricopharyngeus sphincter. During this procedure any secretion accumulated in the mouth was sucked out by another nurse.

A systematic routine examination was adopted to survey the entire oesophagus, stomach and proximal duodenum, minimizing the possibility of missing any area. The examination was carried out as the instrument was advanced under direct vision, using air insufflation and suction as required.

The two golden rules of endoscope examinations are 'do not advance without vision' and 'if in doubt, withdraw', and these were observed. The oesophagus was thoroughly examined and then the stomach was examined including the lesser and greater curvatures and the fundus by retroversion or J-manoeuvring the tip by $180^0$ upward angulation. During all these manoeuvres the shaft of the instrument was kept relatively straight from the patient's teeth to the examiner's hand.

Then the pyloric ring was approached for passage into the duodenum. The tip of the instrument was kept in the correct axis and advanced. The bulb was scanned by circumferential manipulation of the tip. Hyoscine butylbromide (Buscopan - Boehringer Ingelheim) 20mg intravenously was given when visualisation was impaired by pyloric or duodenal motility. The endoscope was advanced into the superior angle of the

duodenum and the shaft was rotated by 90$^{0}$ and gently advanced so that a tunnel view of the descending duodenum was seen.

The duodenum, stomach and oesophagus were carefully surveyed and the insufflated air was sucked out during withdrawal. The main and common problem is subject distress and the endoscopy was terminated if reassurance did not calm the subject. In this series I met no case of severe pain during endoscopy.

### 3.1.7 TISSUE BIOPSY

Once a recognised lesion was noted biopsies were taken for appropriate laboratory investigations. The biopsies were taken with the cupped forceps passed through the appopriate channel. The forceps cups were held in closed position by the assistant when the forceps was advanced and withdrawn. When the tip of the forcep was seen at the distal end of the endoscope, it was further pushed forward under direct vision until the lesion was approached. The assistant was asked to open the cup, the endoscopist advanced the forceps preferably 'face-on', so that firm and direct pressure could be applied to it with the cups; the cups were closed and the forcep withdrawn.

At least 4 antral biopsies were taken from all the subjects. One was used for the endoscopy room urease test (CP-urease test), the specimen for histology was immediately fixed in 10% buffered formalin and the others for microbiology were placed in 0.5 ml of 20% sterile glucose and were transported to the laboratory as soon as possible.

The staff of the endoscopic unit were protected from aerosol emission, which may be a mode of transmission of infection, during the insertion and removal of the biopsy forceps thorough the channel.

### 3.1.8 TRANSPORTATION OF TISSUES

The tissues were placed in water-proof bottles, secured and labelled. These specimens together with the duly filled request forms were kept in a polyethylene bag and sent to the respective departments as soon as the endoscopy session was over.

### 3.1.9 PATIENT CARE AFTER GASTROSCOPY

The subject was kept in the recovery area on the trolley (or stretcher) in view of the nurse for about 30-45 minutes and usually a relative was allowed to sit by the side of the subject. When the subject felt comfortable after this period, he or she was asked to sit up and usually given a hot drink. If pharyngeal anaesthesia had been used then the drink was delayed by another 30 minutes.

When the subject was fit to go home, usually 2 hours after the procedure, the findings and treatment were told in great detail and explanations were also given of the nature of a gastric

secretion study and the reasons for wishing to undertake such a study. Those patients who accepted the invitation were given an appointment for the gastric secretion test.

The subjects were advised not to operate machinery and to rest at home. They were warned about the symptoms (sore throat, abdominal discomfort, flatulence or discomfort in the vein where the injection was given) they might experience. This information was also given in writing, together with names and telephone numbers to contact if the subject was worried.

The details of the subject, the endoscopy findings, urease test result and the treatment given to the subject were fed into the computer and stored. A print out of the letter to the family practitioner and a summary of findings were retrieved and given to the subject to hand over to the GP. Also a follow up appointment for endoscopy was given to the subject.

Flexible upper endoscopy is a safe procedure; though there are many potential hazards, in this study none was experienced.

### 3.1.10 DISINFECTION OF THE INSTUMENT

All advice and instructions issued by the British Society of Gastroenterology and the Local Health Authority were strictly observed.

Cleaning and disinfection were undertaken before the endoscopy list and between each examination and at the end of

the list. Protein is a physical and chemical barrier to the disinfectants and renders them less effective against the micro-organisms. Thus assiduous manual cleaning of the instrument and internal channels was done before the use of disinfectants. The decontamination of the endoscope was a specialised procedure and was carried out by a specially trained staff in a specified area.

Disinfection was carried out according to the Bloomsbury Health Authority policy. At the beginning and end of the list the endoscope was exposed to 8% dettol for 20 minutes followed by 70% methylated spirit . Between patients the endoscope was exposed to dettol for a minimum of 2 minutes followed by immersion in methylated spirit for 4 minutes. In the case of suspected TB or HIV positive cases the endoscope was exposed to gluteraldehyde for 1 hour.

As soon as the endoscope was removed from the subject, mucus was wiped off the insertion tube and the latter was placed on a specially reserved working surface. The detergent and water were aspirated through the suction channel and blown through the air channel and the insertion tube was washed with soapy water. The distal tip of the endoscope was cleaned with a brush and the biopsy channel was cleaned with a brush passed through it and more soapy water was aspirated through the suction channel.

Dettol was aspirated through the suction channel and the endoscope was immersed in dettol and left in it for 2 minutes. Then, the channel was filled with methylated spirit and the insertion tube was immersed in methylated spirit for 4 minutes. The biopsy valve was disconnected and disinfected with dettol followed by methylated spirit.

## 3.2 TREATMENT

Subjects with DU were treated with ranitidine or colloidal bismuth subcitrate (CBS). All subjects were advised to start the treatment after the gastric secretion test. Initially, subjects without *H pylori* infection were given 300 mg of ranitidine (Zantac, Glaxo) nocte for a maximum of 8 weeks and those with *H pylori* infection 240 mg of CBS (Gist-Brocades) twice daily for maximum of a 8 weeks. In the ranitidine treatment group, if the ulcer had healed at the end of 8 weeks, they were given a maintenance dose of 150 mg of ranitidine nocte for a further 8 weeks. In the CBS treatment group no further treatment was prescribed. If the DU had not healed at the end of 8 weeks, then the treatment was altered. Those who had had CBS received ranitidine and the others received CBS. This altered treatment was given for 8 weeks. If the change to ranitidine resulted in healing of the ulcer by eight weeks, maintenance therapy with ranitidine was continued as above. In the case of those who were changed to CBS, if the ulcers were healed by 8 weeks treatment was stopped. Subjects whose ulcers were failed to heal after both CBS and ranitidine were continued on therapeutic doses of ranitidine for further 12 weeks and if the ulcers remained unhealed, they were referred for surgery. Proximal gastric vagotomy was performed in subjects whose DU failed to heal.

## 3.3 FOLLOW UP

All subjects were re-endoscoped between 4-6 weeks after the treatment was started and then between 8-10 weeks.

In subjects whose DU had healed, repeat endoscopy was performed at 6 months and 1 year or earlier if dyspepsia recurred. In subjects in whom the DU had not healed, the endoscopy was repeated in 4-6 weeks after the altered treatment and again in 8-10 weeks and then as above. The repeat endoscopy was carried out in the same way as described above and biopsies from the antrum were taken for microbiology and histopathology.

## 3.4 GASTRIC SECRETION STUDY

The technique used has been gradually developed and modified over many years in this Department and the details were described by Whitfield and Hobsley, in 1979. The procedure followed eliminates observer bias in the selection of plateaux, and uses techniques for reducing collection errors by the use of two markers; one artificial (PSP) and other natural (Na). This study was approved by the Clinical Investigation Panel of The Middlesex Hospital and Medical School (now of University College & Middlesex School of Medicine).

### 3.4.1 PATIENT SELECTION

Most of the subjects were recruited from patients undergoing upper gastrointestinal endoscopy at The Middlesex Hospital, although 9 had undergone endoscopy elsewhere. Only

those subjects who had biopsies taken from the gastric antrum for the CP-urease test, histology and microbiology were included. Patients who had been on non-steroidal anti-inflammatory drugs, steroids, antibiotics or colloidal bismuth subcitrate within the last three months and who had had gastric surgery were excluded.

### 3.4.2 EQUIPMENT

All equipment used satisfied the Health and Safety Standards. Since 1970, more than 1500 gastric secretion tests have been carried out in this department, with this standard equipment and without any mishap, as far as histamine infusion tests were concerned.

#### 3.4.2.1 THE NASOGASTRIC TUBE

This was a double lumen nasogastric tube; the narrower lumen was for instillation of phenol red and the larger for the aspiration of gastric contents. The tube was specially made (Portex Ltd). This tube has a smooth contour and its passage is more easily tolerated by the subjects. The tube was weighted and marked at 10cm. intervals from the tip. The aspiration channel has a standard 6mm sleeve connector. A Luer-lock connector was attached to the infusion arm.

### 3.4.2.2 SUCTION PUMP

A technique of continuous mechanical suction with intermittent blowback was used. The pump used was designed specifically for gastric secretion tests (Sycopel Scientific Ltd) and generated suction pressures of up to 100 mm of Hg. It has a safety valve that automatically cuts off the pump when the pressure rises above this level. The pump is pre-set so that it clears the tube of any blockage by blowing back at regular intervals (3 min) for a fixed period of time (30 sec); this blowback pressure was adjusted not to exceed 160 mm of Hg. The nasogastric tube was connected to another tube that emptied into the collecting flask. The inlet was at the neck of the flask, the outlet was at the base on the opposite side. A side port was connected by fine tubing to the mercury manometers, allowing continual pressure readings. The outlet tubing was connected to a soft plastic tubing which ran through the roller pump mechanism and so to the final collecting flask.

### 3.4.2.3 INFUSION PUMPS

These were used for the infusion of phenol red, histamine phosphate and promethazine (Phenergan - May & Baker Ltd). Three pumps of the exactly the same design (Model 352, Sage Instruments) were used throughout the test; they could be set for both varying syringe size and varying infusion rate. A standard 60cc syringe (BD Plastipak) with an infusion rate of 10.2ml $h^{-1}$ was used in all the syringe pumps. The calibration of the pumps was checked at regular intervals.

### 3.4.2.4 TIMER

The gastric juice was collected in 10 minute aliquots. To ensure that the investigator changed over between samples at the same time and received advanced warning that a collection period was ending, a specially constructed timer was used. This was an electrical device connected to a pair of coloured lamps and a buzzer, which were activated in a standard sequence.

### 3.4.2.5 PHENOL RED (Phenolsulphonphthalein, PSP)

The phenol red was made up as a stock solution in the following manner. Six grammes was dissolved in a litre of distilled water and allowed to settle for one month. This allowed the optical properties of the phenol red to stabilise (Hobsley and Silen 1969). At the start of each test 60 ml was drawn up into the syringe through a CVP manometer line (Portex) secured to the syringe by a Luer-lock. The whole assembly was freed of air bubbles and fitted into the syringe pump. The pump was run at a maximum speed until the syringe driver was flush against the piston of the syringe. Then the pump was switched down to its normal rate (10.2 ml $h^{-1}$) and allowed to run for several minutes until PSP appeared at the other end of the manometer tube. A 10 minute aliquot was collected into a standard 10 ml collecting flask. The infusion pump was stopped and was now ready for the start of the test. The flask was then capped and labelled as the pre-test standard and with the name of the subject and date of the test. Another 10 minute aliquot was collected at the end of the test and labelled as the post-test standard.

### 3.4.3 SUBJECT PREPRATION

The subject was advised to have nothing by mouth for at least eight hours, and smoking and alcohol were proscribed for at least 12 hours. For more than 72 hours the subjects were not allowed to take $H_2$ antagonists and / or any other medication that alters the gastric motility or secretion. The subject was asked to come to one of the wards (Lord Athlone or Bland Sutton) in The Middlesex Hospital, at 0800 hrs and also advised to arrange for someone to come and collect him or her from the ward after 1700 hrs. The subject was brought to the gastric secretion laboratory at 0830 hrs, and weight and height were measured. A full gastric history was taken and all information including age and smoking habits was entered onto a standard form. The subject was again given the details of the test. Only one gastric secretion test was performed on a single day.

#### 3.4.3.1 PASSAGE OF THE NASOGASTRIC TUBE

The subject was encouraged to relax as far as possible and then asked to sit in an upright position, local anaesthetic gel (lignocaine 1%) was squeezed into the nostril; the subject sniffed the gel up into the nose. Five minutes was usually sufficient for this agent to take effect. The subject was allowed to swallow a small quantity of water to moisten the dry throat and help swallowing when the tube was passed. The approximate length of the nasogastric tube required to pass was initially assessed by external measurement. The nasogastric tube passed through the nostril to the back of the throat and a small mouthful

of water was taken: too much water could lead to choking when the tube was passed. The tube was passed further in, the subject swallowed and the tube passed into the upper oesophagus. Coughing suggested laryngeal irritation: tipping the head forwards overcame this problem by directing the tube into the pharynx. Once the tube was in the oesophagus the subject swallowed a few mouthfuls of water to ease the passage of the tube into the stomach. After insertion of a previously measured length aspiration was attempted. Adjustments were made to the tube until aspiration was achieved. Aspiration of gastric content confirmed that the tube was in the stomach. The tube was passed in up to its furthest mark: if resistance was met this implied that the tube had been passed in too far and was coiling up upon itself. Once the tube was so positioned the stomach was emptied of the overnight secretions and the swallowed water.

#### 3.4.3.2 THE WATER RECOVERY TEST

When the initial aspiration was complete and the stomach assumed to be empty, the subject swallowed 20 ml of water and aspiration was immediately attempted. If between 16 and 20 ml was recovered then the tube was said to have passed the water recovery test (Hassan and Hobsley 1970). The tube was then withdrawn 2.5 cm at a time and the test was repeated until the aspiration was unsuccessful. Then the tube was advanced 2.5 cm and secured by micropore to the nose of the subject.

### 3.4.3.3 POSITION OF THE SUBJECT

Once the tube was secured the subject lay in the semi recumbent position, with the legs flat and the hips flexed to $45^{o}$. There is no difference in recoveries between this position and the left- lateral (Hassan and Hobsley 1970); and subjects were allowed to adopt this position for short periods of time during the test for reasons of comfort alone.

### 3.4.3.4 INTRAVENOUS ACCESS

A large bore cannula was used; the large bore was not necessary for the histamine/promethazine hydrochloride infusions, but for venous access in case of emergency. A 16G cannula (Wallace) was inserted under local anaesthesia (lignocaine 1% - plain) into a large forearm or antecubital vein. This was connected by a three way tap to a slow running infusion of 0.9% saline. The cannula was secured with Micropore in the standard manner.

### 3.4.3.5 PREPRATION OF THE INTRAVENOUS INFUSION

Maximal stimulation was achieved with histamine acid

phosphate infused intravenously at the rate of 0.13 mmol $kg^{-1}$ $h^{-1}$ (0.04 mg $kg^{-1}$ $h^{-1}$ ). This dose used was based on earlier work (Lawrie et al 1964) on the infusion test. The period of maximal stimulation was for two hours and a dose sufficient for two and a half hours of infusion was prepared. The dose of histamine was calculated (0.04mg x 2.5 x wt in kg) and drawn up into an insulin syringe (U100). This was transferred to a 60ml syringe and made upto 25.5 ml with 0.9% saline. The dose of anti-histamine ($H_1$ receptor) used was 25 mg of promethazine hydrochloride (Phenergan) and was made up to 25.5 ml, the same volume as the histamine, with 9% saline. A separate CVP manometer line was attached to each syringe and a 25G needle fixed to the other end. Syringes were labelled accordingly and placed in the double syringe pump and secured in position. The pump was run at a maximum speed until the syringe driver was flush against the piston of the syringe. Then the pump was switched down to its normal rate (10.2 ml $h^{-1}$) and allowed to run for several minutes until the fluid appeared at the other end of the manometer tubes. The pump was turned off and the free ends of the manometer tube were connected to the three way tap, which was connected to the venous access.

### 3.4.4 GASTRIC SECRETION TEST

The gastric secretion test included a basal period for one hour and a two-hour period of maximal stimulation. After the pre-test standard of PSP was collected the free end of the tube was connected to the narrower lumen, which is meant for the instillation of PSP, of the nasogastric tube and the infusion pump was started. The suction pump was switched on. The gastric

juice collected at the first 20 minutes time was discarded to overcome the possible error of loss through adsorption and to allow for thorough mixing of PSP with gastric juice.

### 3.4.4.1 COLLECTION OF SAMPLES

The samples were collected in consecutive 10-minute intervals as signalled by the timer. The volume of secretion was measured; if the volume was less than 10 ml it was returned to the collecting flask and pooled together with the subsequent 10 minutes collection(s) until the collected volume exceeded 10 ml. This pooling procedure was permitted wholly only within each period; i.e either basal or maximal. The volume of the sample was recorded in the data sheet and the sample was filtered (Whatman's No 1 Paper) and at least 8ml of filtered specimen was saved in an air tight bottle. Each bottle was labelled carefully (name of the subject, date and specimen number). The pump bearing the histamine and that for the anti-histamine were switched on a minute before the end of the collection of last basal period sample. At the end of the test all the bottles were sealed and stored at $4^{o}C$ to await analysis. The PSP instillation tube was disconnected from the nasogastric tube and the post-test standard PSP was collected over next 10 minutes.

### 3.4.4.2 CARE OF PATIENT AFTER TEST

The subject was returned to the ward, a small meal was given and the subject advised to rest for four hours or longer until

most of the effect of the Phenergan worn off. The nursing staff of the ward was told about the test and requested to observe the subject for any unexpected reaction. All subjects were visited by me after 4 hours and checked for the general condition and any after effect. They were advised not to drive a car or operate any machinery for 24 hours, allowing time for the anti-histamine to fully wear off.

### 3.4.5 ANALYSIS OF THE GASTRIC SECRETION SAMPLES

Usually all samples were analysed within a week. The samples were removed from the refrigerator and allowed to warm up to the room temperature. From the sample, chloride, potassium, hydrogen and sodium in ionic forms and PSP were analysed. The hydrogen ion was measured in order to calculate acid output, sodium was measured for the calculation of duodenogastric reflux and the other two ions in order to check that the sums of cations and anions balanced. If the anion/ cation difference was more than 4 mmol $l^{-1}$ then a repeat analysis of the sample was performed.

#### 3.5.5.1 PSP CONCENTRATION

The concentration of PSP was measured by spectrophotometer (Corning Spectrophotometer Model 256).

The filtered aspirate was taken up by a 1 in 200 dilutor, and passed to a flow cell in the spectrophotometer. The diluent contained ammonium hydroxide, in order to make the sample alkaline and so develop the colour. The pre- and post-test standards were carefully and completely washed out of their bottles into separate 10 ml flasks, and measured as aspirate samples. The means of each pair of 558nm and each pair of 410nm readings were used in the calculations.

### 3.5.5.2 CHLORIDE IONS

This was measured electrochemically using a silver electrode chloride meter (EE1 Chloride Meter Model 96, Radiometer Copenhagen). 0.2 ml of the aspirate was pipetted into a beaker of buffer, and titrated to electrical neutrality and a direct reading obtained in mmol $l^{-1}$.

### 3.4.5.3 HYDROGEN IONS

Titratable hydrogen ion was measured by pH meter and automatic burette (pH meter, Autoburette ABU 12 Radiometer Copenhagen), using 1ml of sample titrated against 0.1N NaOH to pH 7.0. The result in ml of 0.1 N NaOH was converted to mmol $l^{-1}$ of hydrogen ion by multiplication by 100.

### 3.4.5.4 SODIUM and POTASSIUM IONS

These were measured by flame photometry (FLM3 Radiometer Copenhagen), using a 1 in 200 dilution of the aspirate, the results being read directly in mmol $l^{-1}$. Since the same diluent (ammonium hydroxide) was used for PSP readings it was possible to semi-automate the readings of phenol red, sodium ions and potassium ions.

## 3.4.6 COMPUTER ANALYSIS

The patient's data and measured readings were fed into a BBC computer and sent to a main frame computer at Imperial College. The data were analysed using a specially written Fortran programme run on the main frame. The results were stored in the master file at Imperial College and a printout was received at laboratory terminal.

### 3.4.6.1 CALCULATION FOR PYLORIC LOSS

The probable contaminants were blood and bile and they have small absorbance at 558 nm and peak at 410 nm. A

correction for this absorbance has been calculated, though the correction was never more than 7% of the initial readings (Crawford and Hobsley 1968). It was found to have a linear regression.

$$PSP_{corr} = PSP_{558} - 0.135 \times PSP_{410} + 0.004$$

Using this value the aspirated volumes were corrected for pyloric loss. The PSP was adjusted to allow for the 1 in 200 dilution that the aspirated sample underwent (PSPadj). The total volume (Vtot) of gastric content was calculated as follows:

$$Vtot = (PSPadj / PSPasp) \times Vasp$$

The volume of infused PSP per minute (1.7 ml) was deducted from Vtot to give the corrected volume (Vcor), this being the volume of gastric juice corrected for incomplete aspiration and pyloric loss. The volume of aspirated juice was divided by Vtot to give the fractional recovery of gastric juice and hence of PSP. This same fraction of 1.7 ml was deducted from the aspirated volume to give the observed volume (Vobs) and the difference between Vcor and Vobs was the pyloric loss.

### 3.4.6.2 CORRECTION FOR ELECTROLYTE CONCENTRATION

Electrolyte concentration had been reduced by the diluting effect of the infused PSP. Correction for this effect was made by multiplying the observed concentration by the fraction Vtot/Vobs, which at low volumes (< 20 ml $min^{-1}$) of secretion can be appreciable.

### 3.4.6.3 CALCULATION FOR ACID OUTPUT

Hydrogen ion output was calculated from the product of the corrected hydrogen ion concentration and Vobs multiplied by 0.006 to give the output in ml $h^{-1}$.

### 3.4.6.4 CALCULATION FOR DUODENOGASTRIC REFLUX

No correction is yet possible for swallowed saliva and in the calculation of duodenogastric reflux it was assumed that the swallowed saliva was negligible. The concentration of sodium was assumed to be constant 143 mmol $l^{-1}$. The electrolyte composition, all expressed in mmol $l^{-1}$, (Hobsley and Whitfield 1977) of 'pure gastric juice' is as follows:

$[Cl^-] = 170$, $[H^+] = 145$, $[Na^+] = 7$ and $[K^+] = 17$

The volume of duodenogastric reflux (Vr) can be calculated by the following formula (see Appendix for derivation):

$$Vr = \{Vcor \times ([Na^+] - 7)\} / 143$$

The Vg, which was the volume of pure gastric secretion corrected for pyloric loss and duodenogastric reflux is calculated as:

$$Vg = Vcor - Vr$$

### 3.4.6.5 SELECTION OF PLATEAU

This was done by the computer programme (Whitfield and Hobsley 1979), selecting a plateau of maximal length using the latest possible samples in the maximal secretion period. The PSP recovery of each sample had to be within 15% of the mean of the proposed plateau. No plateau could start from the first period nor could it last for less than 20 minutes.

### 3.4.6.6 STATISTICAL ANALYSIS

Data were expressed in means and standard deviation. An unpaired Student's *t* test was used to compare the means. A non-parametric test, the Wilcoxon signed-rank test, was also used to compare the data, because gastric secretion was not normally distributed in the population. Contingency-table analysis with chi-squared ($\chi^2$) with Yates's correction or Fisher's exact test was used depending on the expected numbers within the smallest cells. Kendall's t was used to assess the non-parametric correlation between smoking and gastric secretion. $P < 0.05$ was considered to be statistically significant.

# *CHAPTER 4*

# *SUBJECTS & RESULTS*

# 4. SUBJECTS AND STRUCTURE OF INVESTIGATION & RESULTS

## 4.1 Subjects and Diagnosis

Two hundred and fifty two patients underwent endoscopy (Table1 & Fig. 1). Oesophagitis was diagnosed in 30 (12%); 23 (9%) patients had miscellaneous findings, including 5 with gastric ulcer. These 53 patients were not approached for gastric secretion studies. A duodenal ulcer (DU) was diagnosed in 76 (30%) and no abnormality, that is no ulcer disease (NUD) was found in 123 (49%).

The CP-urease test was positive in 145 (57%) and negative in 107 (43%). The results of the CP- urease tests by diagnostic category are given in Table 2.

When the culture and histology results for *H pylori* were obtained, none of the CP-urease positive patients was classified as *H pylori* negative but 14 (13%) of the 107 CP-urease negative patients were positive for *H pylori* by the more searching tests. Subdividing the patient groups according to the new decision on whether or not *H pylori* was present gave the results also shown in Table 5& 8 and Fig. 2 . It was clear that the percentage prevalence of *H pylori* was similar in all diagnostic categories except that it was smaller in reflux oesophagitis patients than in the others (for DU vs NUD $\chi^2 = 0.099$, $p > 0.7$ n. s; for miscellaneous findings vs DU & NUD $\chi^2 = 1.604$, $p > 0.30$ n.s and for oesophagitis vs DU & NUD $\chi^2 = 16.51$, $p < 0.0005$, s).

Soon after the endoscopy, when the results of the CP-urease test but not the other tests for *H pylori* were known, 46

(60%) of 76 DU and 93 (76%) of 123 NUD patients were approached for gastric secretion. The remainder were excluded because of the criteria previously described. Thirty-six (78%) of the 46 DU patients and 58 (62%) of the 93 NUD patients agreed to undergo the test. Secretion data became available in 62 (60%) of the 94 subjects who agreed to the secretion studies; the remainder failed to attend. Secretion was measured in 31 (86%) of the 36 DU, 31 (53%) of the 58 NUD; that is in 37 (64%) of the CP-urease test positive and 25 (64%) of the 39 CP-urease test negative subjects; in 41 (65%) of the 63 positive by the more searching tests for *H pylori*, and in 21 (68%) of the 31 negative by those tests. The flow chart in Figure 3 demonstrates the structure of the investigation.

Active chronic antral gastritis was present in sections of mucosa in all subjects finally assigned to the *H pylori* positive division, but was absent in all *H pylori* negative patients. The proportion of patients infected with *H pylori* increased with age (Table11 & Fig. 4&5).

## 4.2 Sex

Oesophagogastroduodenoscopy was done on 100 male and 152 female subjects (Tables 12 & 13). NUD was diagnosed in 37 male and 75 female subjects; 39 male and 48 female subjects had an active DU; reflux oesophagitis was noted in 13 male and 17 female subjects and 10 male and 13 female subjects had miscellaneous findings. Sixty (60%) of the male subjects and 99 (65%) of the female subjects were *H pylori* positive.

A total of 39 males and 23 females underwent a gastric

secretion test. There were 19 males and 12 females in the NUD group and 20 males and 11 females in the DU group. *H pylori* was present in 25 males and 16 females. Twelve of the males and 8 of the females in the NUD group and 13 of males and 8 of the females in the DU group were positive for *H pylori* (Tables 14 - 16)

## 4.3 Age

The mean age for NUD was 52 years (14 sd), for DU was 46 years (13 sd), 56 years (8 sd) for oesophagitis and 54 years (11 sd) for those subjects who had miscellaneous findings. In general the proportion of subjects infected with *H pylori* increased with age (Table 11 & Fig. 4 & 5).

The mean age for the 61 subjects attended the gastric secretion test was 54 years and out of the 61 subjects 23 (74%) of the 31 NUD were either 54 years or older than 54 years and only 11 (36%) of the 31 DU were either 54 or older than 54 years (Table 19).

The mean age for subjects with *H pylori* was 54 (14 sd) and for subjects negative for *H pylori* was 40 (13 sd). Twenty five (81%) of the 31 subjects with *H pylori* were either 54 years or older than 54 years and only 16 (51%) of the 31subjects with no *H pylori* were either 54 years or older than 54 years (Tables 21).

## 4.4 Height

The mean height of subjects in the NUD group was 168 cm (8 sd) and in the DU group was 169 cm (10 sd) (Table 22).

## 4.5 Smoking Habits

Thirty three of the 61 subjects, who underwent a gastric secretion test were smokers; 17 were male and 16 female subjects. There were 11 smokers in the NUD group of which 7 were females; there were 22 smokers in the DU group of which 9 were females. When classified according to the *H pylori* status it was noted that there were 22 smokers among the subjects with *H pylori* and 11 smokers among the subjects without *H pylori* (Tables 23 - 25 & Fig. 8 & 9)

## 4.6 Gastric secretion study

Maximal gastric secretion was higher in patients with DU than in those without DU no matter how secretion was expressed (Table 32 & Fig. 14 & 15)), and within each of these major groups was 15 -18% lower in those with *H pylori* compared to those without (Table 33 & Fig. 16 & 17). This second statement was correct when secretion was expressed as height-standardised Vg, but the effect of using the less accurate indices (Vg unstandardised, acid output unstandardised and standardised) is shown in Tables 32 - 36. These tables also show that the Wilcoxon rank sum test can be more sensitive than the t-test.

In smokers, positive correlations between smoking factor (SMF), that is the square root of the product of the number of cigarettes smoked per day and the number of years smoked, and maximal gastric secretion were demonstrated in *H pylori* negative (both ulcer and non-ulcer) groups, but once again only if the secretion was expressed as Vg (Table 37). However in patients who were *H pylori* positive, there was no correlation between gastric secretion and smoking, no matter how the secretion rate was expressed or whether the patients had a DU.

## 4.7 Treatment & Follow-up

All 20 NUD subjects with *H pylori* were treated with colloidal bismuth subcitrate (CBS). In 15 (75%) subjects *H pylori* was cleared and the ACG was improved after 6-8 weeks of treatment. In 5 subjects CBS failed to clear *H pylori* and these 5 subjects were later treated with antibiotics (ampicillin and metronidazole) and *H pylori* was cleared in 3 subjects with improvement of ACG. *H pylori* was still present in 2 subjects and on follow up though ACG was present no DU was diagnosed in these two patients. *H pylori* was isolated in 5 of the 18 subjects between 9 and 12 months.

All 21 DU subjects with *H pylori* were treated with CBS and all 10 DU subjects without *H pylori* were treated with ranitidine. After 4-6 weeks of treatment the ulcer did not heal in 6 subjects who were taking CBS and in 6 who were taking ranitidine. The ulcer had healed in 3 subjects in the CBS

treatment group in spite of the presence of *H pylori* and failed to heal in 4 subjects even though *H pylori* was cleared with CBS (Fig. 24& 25).

At the end of eight weeks of treatment in the CBS treatment group, the ulcer had not healed in the 6 patients but in the ranitidine treatment group the DU had healed in 2 more subjects. CBS was given to those whose ulcer had failed to heal with ranitidine and ranitidine was given to those whose ulcer had failed to heal with CBS. Four weeks after this revised treatment it was found that 4 of the ulcers which failed to heal with CBS healed with ranitidine but 2 failed to heal and 3 of the ulcers which failed to heal with ranitidine healed with CBS but 1 failed to heal.

The 3 subjects whose ulcer failed to heal were continued with the same treatment for another 4 weeks but remained unhealed and they required proximal gastric vagotomy. The repeat oesophagogastroduodenoscopy performed on these 3 subjects after 6 weeks showed the ulcer had healed and 2 of these 3 subjects were found to have *H pylori* in the antrum.

Twenty six (84%) of the 31 NUD and 28 (90%) of the 31 DU subjects completed the 12 month follow-up. Two (10%) of the 20 NUD with *H pylori*, 2 (10%) of the 21 DU with *H pylori*; 3 (27%) of the 11 NUD with no *H pylori* and 1 (10%) of the 10 DU with no *H pylori* failed to complete the 12 month follow-up.

Nineteen (90%) of the 21 DU subjects who originally had *H pylori* and 9 (90%) of the 10 DU subjects who did not have *H pylori* completed the 12 month follow up. At the end of the followed up period, DU had recurred in 4 of the 9 subjects who never had *H pylori* and in 6 of the 15 subjects whose

*H pylori* infection was cleared. In the latter group of 6, *H pylori* was positive in 2 subjects and negative in 4. Two of the 5 subjects whose DU healed in spite of the presence of *H pylori* had recurrence of DU.

DU did not recur in 5 (1 had PGV) of the 9 subjects who never had *H pylori*, 10 of the 15 subjects whose *H pylori* infection was cleared and in 2 (both had PGV) of the 4 subjects whose *H pylori* infection was not being cleared.

Two subjects, 1 of the 10 DU subjects who never had *H pylori* and 1 of the 16 subjects on whom *H pylori* was cleared, failed to attend the 12 month follow up but were seen 6 months after the ulcer was healed and no recurrence was found. One of the 16 patients with DU, in whom *H pylori* had been cleared failed to attend the 12 month follow up clinic but was seen 15 months after the ulcer was healed, found to have a DU and *H pylori* was isolated.

Twenty six (84%) of the 31 NUD and 28 (90%) of the 31 DU subjects completed the 12 month follow-up. Two (10%) of the 20 NUD with *H pylori*, 2 (10%) of the 21 DU with *H pylori*; 3 (27%) of the 11 NUD with no *H pylori* and 1 (10%) of the 10 DU with no *H pylori* failed to complete the 12 month follow-up (Table 38).

***TABLES & FIGURES***

Table 1.

MACROSCOPIC DIAGNOSIS OF PATIENTS UNDERGOING ENDOSCOPY

| | | |
|---|---|---|
| **Total Number** | **252** | |
| Non-Ulcer Dyspepsia (NUD) | 123 | (49%) |
| Duodenal Ulcer (DU) | 76 | (30%) |
| Oesophagitis | 30 | (12%) |
| Miscellaneous | 23 | (9%) |

Table 2.

CP-UREASE TEST RESULTS

| Group | CP-Urease test positive | CP-Urease test negative | Total |
|---|---|---|---|
| **NUD** | 75 (61%) | 48 (39%) | 123 |
| **DU** | 49 (64%) | 27 (36%) | 76 |
| **Oesophagitis** | 8 (28%) | 22 (74%) | 30 |
| **Miscellaneous** | 13 (57%) | 10 (43%) | 23 |
| **TOTAL** | **145 (58%)** | **107 (42%)** | **252** |

Table 3.

*HELICOBACTER PYLORI* IN GASTRIC BIOPSIES
IDENTIFIED WITH GIEMSA STAINING

| Group | Giemsa stain positive for *H pylori* | Giemsa stain negative for *H pylori* | Total |
|---|---|---|---|
| **NUD** | 80 (65%) | 43 (35%) | 123 |
| **DU** | 51 (67%) | 25 (33%) | 76 |
| **Oesophagitis** | 9 (30%) | 21 (70%) | 30 |
| **Miscellaneous** | 13 (57%) | 10 (43%) | 23 |
| **TOTAL** | **153 (61%)** | **99 (39%)** | **252** |

Table 4.

*HELICOBACTER PYLORI* IDENTIFIED WITH CULTURE TECHNIQUE IN THE FOUR GROUPS

| Group | Culture positive for *H pylori* | Culture negative for *H pylori* | Total |
|---|---|---|---|
| **NUD** | 81 (66%) | 42 (34%) | 123 |
| **DU** | 52 (68%) | 24 (32%) | 76 |
| **Oesophagitis** | 7 (23%) | 23 (77%) | 30 |
| **Miscellaneous** | 12 (52%) | 11 (48%) | 23 |
| **TOTAL** | **154 (61%)** | **98 (39%)** | **252** |

Table 5.

DISTRIBUTION OF
*HELICOBACTER PYLORI* IN THE FOUR GROUPS

| **Group** | ***H pylori* positive** | | ***H pylori* negative** | | **Total** |
|---|---|---|---|---|---|
| **NUD** | 83 | (67%) | 40 | (33%) | 123 |
| **DU** | 53 | (70%) | 23 | (30%) | 76 |
| **Oesophagitis** | 9 | (30%) | 21 | (70%) | 30 |
| **Miscellaneous** | 14 | (61%) | 9 | (39%) | 23 |
| **TOTAL** | **159** | **(63%)** | **93** | **(37%)** | **252** |

Figure 1.

MACROSCOPIC DIAGNOSIS OF PATIENTS UNDERGOING ENDOSCOPY

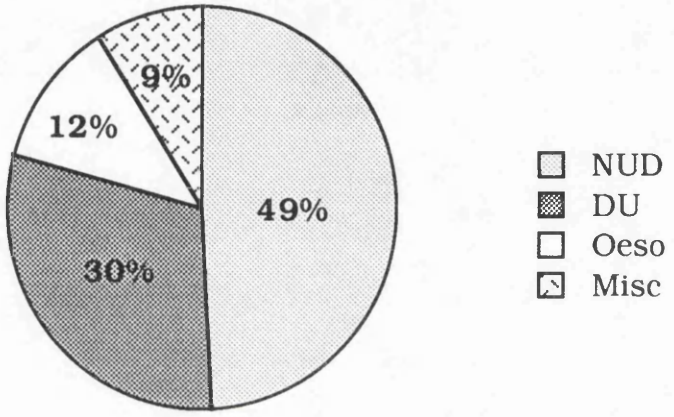



Figure 2.

FREQUENCY OF HELICOBACTER PYLORI IN THE FOUR GROUPS

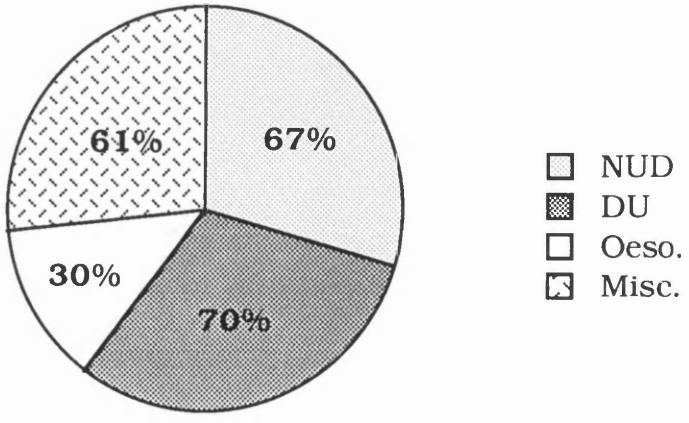
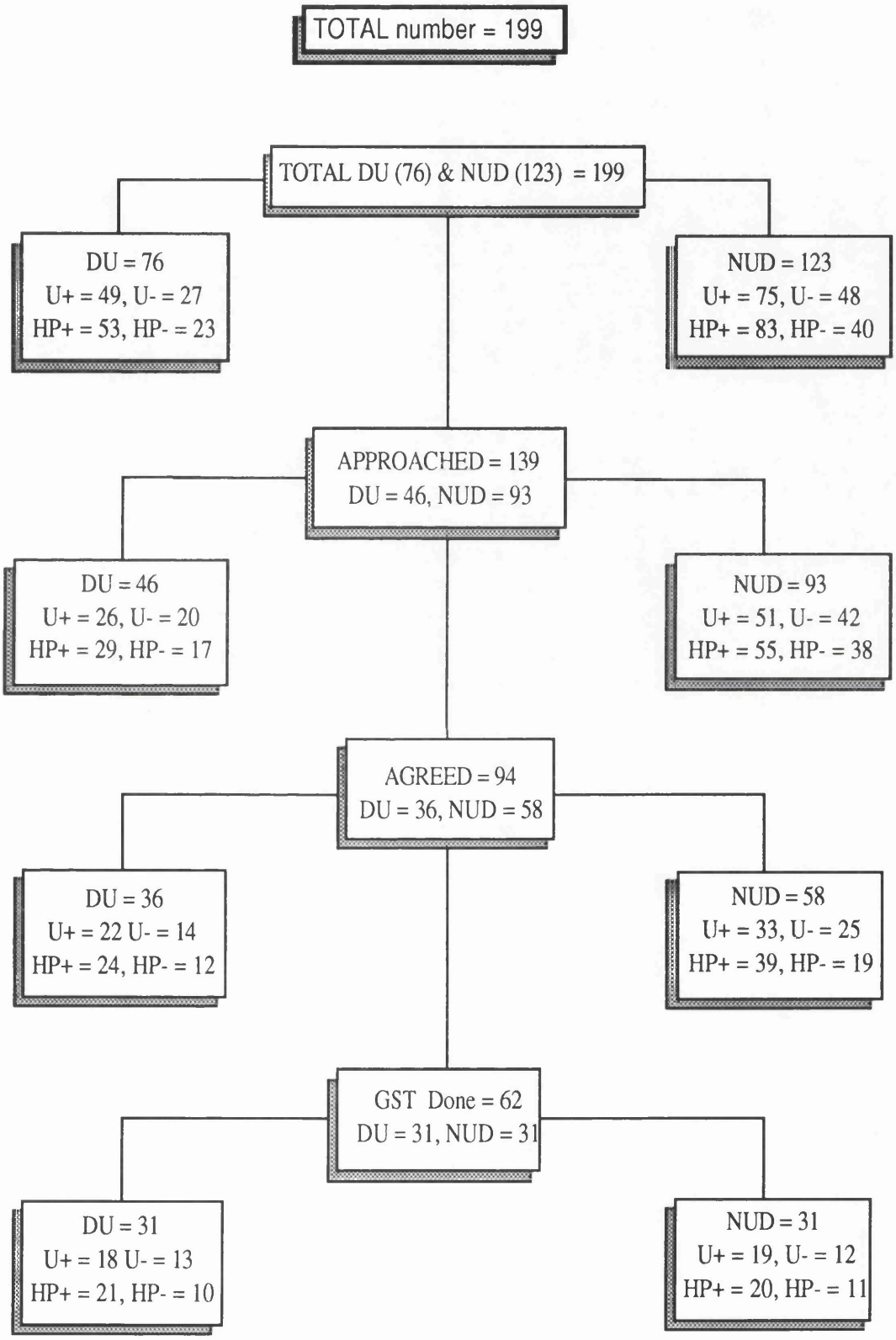


Figure 3. Chart shows the patients who were invited and those who attended for the Gastric Secretion Test.




*(* DU = duodenal ulcer, GST = gastric secretion test, HP = Helicobacter pylori, NUD = non-ulcer dyspepsia, U = urease test, + / - = positive / negative)*

Table 6.

COMPARISON OF THE THREE TESTS USED FOR THE IDENTIFICATION OF *HELICOBACTER PYLORI*

| **Test** | ***H pylori* positive** | | ***H pylori* negative** | | **Total** |
|---|---|---|---|---|---|
| **CP-urease test** | 145 | (0 false pos.) | 107 | (14 false neg.) | 252 |
| **Giemsa stain** | 153 | (2 false pos.) | 99 | (8 false neg.) | 252 |
| **Culture** | 154 | (1 false pos.) | 98 | (6 false neg.) | 252 |
| **True *H pylori* positivity** | **159** | | **93** | | **252** |

Table 7.

COMPARISON OF THE RELIABLITY OF THE THREE TESTS USED FOR THE IDENTIFICATION OF *HELICOBACTER PYLORI*

| **INDEX** | **CP-Urease test** | **Gram stain** | **Culture** |
|---|---|---|---|
| **Sensitivity** | 91.2% | 94.9% | 96.2% |
| **Specificity** | 100% | 97.8% | 98.9% |
| **Accuracy** | 94.4% | 96.0% | 97.2% |
| **Positive predictive value** | 100% | 98.7% | 99.4% |
| **Negative predictive value** | 86.9% | 91.9% | 93.9% |

Table 8.

COMPARISON OF

CP-UREASE TEST POSITIVITY & *HELICOBACTER PYLORI*

IN THE FOUR GROUPS

| **Group** | **CP-urease test positive** | | ***H pylori* positive** | |
|---|---|---|---|---|
| **NUD** | 75 | (61%) | 83 | (67%) |
| **DU** | 49 | (64%) | 53 | (70%) |
| **Oesophagitis** | 8 | (28%) | 9 | (30%) |
| **Miscellaneous** | 13 | (57%) | 14 | (61%) |
| **TOTAL** | **145** | **(58%)** | **159** | **(63%)** |

Table 9.

THE ASSOCIATION OF ACTIVE CHRONIC GASTRITIS IN THE DIFFERENT GROUPS

| Group | Active Chronic (antral) Gastritis present | | Active Chronic (antral) Gastritis absent | | Total |
|---|---|---|---|---|---|
| **NUD** | 83 | (67%) | 40 | (33%) | 123 |
| **DU** | 53 | (70%) | 23 | (30%) | 76 |
| **Oesophagitis** | 9 | (30%) | 21 | (70%) | 30 |
| **Miscellaneous** | 14 | (61%) | 9 | (39%) | 23 |
| **TOTAL** | **159** | **(63%)** | **93** | **(37%)** | **252** |

Table 10.

FREQUENCY OF *HELICOBACTER PYLORI*
IN DU & NUD SUBJECTS
(who underwent Gastric Secretion Test)

| | ***H pylori* present** | ***H pylori* absent** |
|---|---|---|
| **Duodenal Ulcer subjects** | 21 | 10 |
| **Non-Ulcer Dyspepsia subjects** | 20 | 11 |
| **TOTAL** | 41 | 21 |

The difference in distribution of H pylori in the duodenal ulcer group and non-duodenal ulcer group is non significant ($\chi^2 = 0.720$, $p > 0.40$).

Table 11.

PREVALENCE OF *HELICOBACTER PYLORI* STRATIFIED ACCORDING TO AGE

| Age | NUD HP positive | | DU HP positive | | Oeso. HP positive | | Misc. HP positive | | Total HP positive | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) | n | (%) | n | (%) |
| 16 -25 | 2 | (40) | 2 | (50) | - | - | - | - | 4 | (44) |
| 26-35 | 6 | (55) | 7 | (53) | - | - | - | - | 13 | (52) |
| 36-45 | 14 | (78) | 13 | (72) | 2 | (25) | 2 | (66) | 31 | (66) |
| 46-55 | 28 | (72) | 17 | (68) | 3 | (23) | 4 | (57) | 52 | (62) |
| 56-65 | 21 | (68) | 11 | (91) | 2 | (50) | 7 | (64) | 41 | (68) |
| 66-75 | 10 | (67) | 3 | (75) | 1 | (33) | - | - | 14 | (64) |
| 76-85 | 2 | (50) | - | - | 1 | (100) | 1 | (100) | 4 | (57) |

Figure 4.

THE PREVALENCE OF *HELICOBACTER PYLORI* vs AGE

(with simple curve fit)

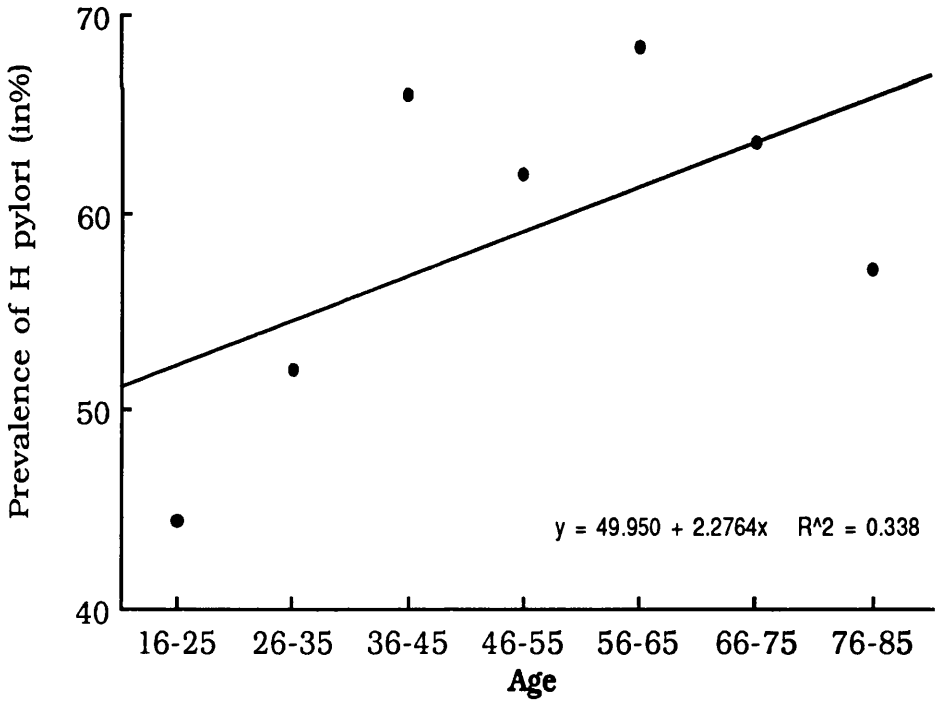


Figure 5.

THE PREVALENCE OF *HELICOBACTER PYLORI* vs AGE

(with binomial curve fit)

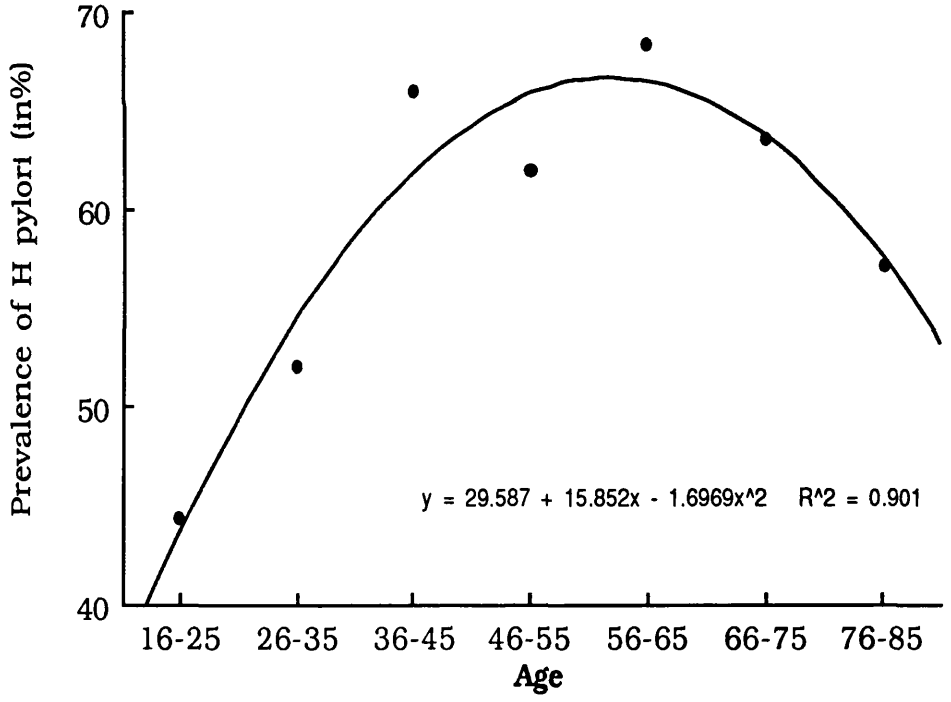


Table 12.

THE ENDOSCOPIC DIAGNOSIS &
DISTRIBUTION OF THE SEXES

| | MALE | FEMALE | TOTAL |
|---|---|---|---|
| **Non-Ulcer Dyspeptic subjects** | 41 | 82 | 123 |
| **Duodenal Ulcer subjects** | 36 | 40 | 76 |
| **Oesophagitis subjects** | 13 | 17 | 30 |
| **Subjects with Misc. findings** | 10 | 13 | 23 |
| **TOTAL** | **100** | **152** | **252** |

Table 13.

COMPARISON OF SEXES &
FREQUENCY OF *HELICOBACTER PYLORI*

| | **MALE** | **FEMALE** | **TOTAL** |
|---|---|---|---|
| ***H pylori* present** | 60 | 99 | 159 |
| ***H pylori* absent** | 40 | 53 | 93 |
| **TOTAL** | **100** | **152** | **252** |

Table 14.

COMPARISON OF SEXES IN THE DU & NUD SUBJECTS

(who underwent Gastric Secretion Test)

| | **MALE** | **FEMALE** | **TOTAL** |
|---|---|---|---|
| **Duodenal Ulcer subjects** | 20 | 11 | 31 |
| **Non-Ulcer Dyspepitc patients** | 19 | 12 | 20 |
| **TOTAL** | 39 | 23 | 62 |

The difference in distribution of DU & NUD between male and female subjects is not significant ($\chi^2 = 0.069$, $p > 0.7$).

Table 15.

COMPARISON OF SEXES &

FREQUENCY OF*HELICOBACTER PYLORI*

(in subjects who underwent Gastric Secretion Test)

| | **MALE** | **FEMALE** | **TOTAL** |
|---|---|---|---|
| ***Helicobacter pylori* present** | 25 | 16 | 41 |
| ***Helicobacter pylori* absent** | 14 | 7 | 21 |
| **TOTAL** | 39 | 23 | 62 |

The difference in distribution of *H pylori* positive and negative subjects is non significant between the 2 sexes ($\chi^2 = 0.196$, $p > 0.06$).

TABLE 16.

COMPARISON OF SEXES, ENDOSCOPIC DIAGNOSIS &
*HELICOBACTER PYLORI* IN SUBJECTS
(who underwent Gastric Secretion Test)

| | | MALE | FEMALE | TOTAL |
|---|---|---|---|---|
| **Duodenal Ulcer subjects** | *H pylori* present | 13 | 8 | 21 |
| | *H pylori* absent | 7 | 3 | 10 |
| **Non-Ulcer Dyspepsia subjects** | *H pylori* present | 12 | 8 | 20 |
| | *H pylori* absent | 7 | 4 | 11 |
| **TOTAL** | | 39 | 23 | 62 |

Table 17.

THE MEAN AGE FOR THE FOUR GROUPS
DIAGNOSED ENDOSCOPICALLY

| | AGE | RANGE | SD |
|---|---|---|---|
| **Non-Ulcer Dyspeptic Subjects** | 52 | 24-79 | 14 |
| **Duodenal Ulcer Subjects** | 46 | 16-70 | 13 |
| **Oesophagitis Subjects** | 56 | 32-80 | 8 |
| **Subjects with Misc. findings** | 54 | 37-76 | 11 |

Table 18.

DISTRIBUTION OF FOUR GROUPS
(ENDOSCOPICALLY DIAGNOSED)
BELOW 53 YEARS AND 54 YEARS & MORE

| | **< 53 YRS** | **54 YRS & more** | **TOTAL** |
|---|---|---|---|
| **Non-Duodenal Ulcer subjects** | 53 | 70 | 123 |
| **Duodenal Ulcer subjects** | 47 | 29 | 76 |
| **Oesophagitis subjects** | 14 | 16 | 30 |
| **Subjects with Misc. findings** | 7 | 16 | 23 |
| **TOTAL** | **127** | **125** | **252** |

Table 19.

DISTRIBUTION OF DU & NUD SUBJECTS
BELOW 53 YEARS AND 54 YEARS & MORE
(who underwent Gastric Secretion Test)

| | **< 53 YRS** | **54 YRS & more** | **TOTAL** |
|---|---|---|---|
| **Duodenal Ulcer Subjects** | 20 | 11 | 31 |
| **Non-Ulcer Dyspeptic Subjects** | 8 | 23 | 31 |
| **TOTAL** | **28** | **34** | **62** |

There is a significant difference in the distribution of duodenal ulcer subjects between the two age groups.
($\chi^2 = 9.378$, $p < 0.0050$).

Figure 6.

DISTRIBUTION OF DU & NUD SUBJECTS
BELOW 53 YEARS AND 54 YEARS & MORE

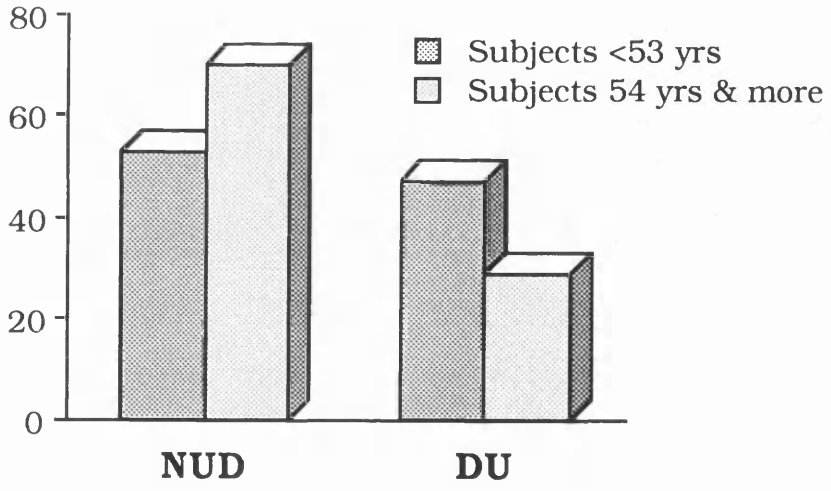


Table 20.

DISTRIBUTION OF *HELICOBACTER PYLORI*
SUBJECTS BELOW 53 YEARS AND 54 YEARS & MORE

| **GROUP** | **< 53 YRS** | **54 YRS & more** | **TOTAL** |
|---|---|---|---|
| ***H pylori* present** | 72 | 87 | 159 |
| ***H pylori* absent** | 55 | 38 | 93 |
| **TOTAL** | **127** | **125** | **252** |

The differences in the distridution of *H pylori* positive and negative subjects between the two age groups are significant ($\chi^2 = 4.362$, $p < 0.050$).

Figure 7.

DISTRIBUTION OF *HELICOBACTER PYLORI*
SUBJECTS BELOW 53 YEARS AND 54 YEARS & MORE

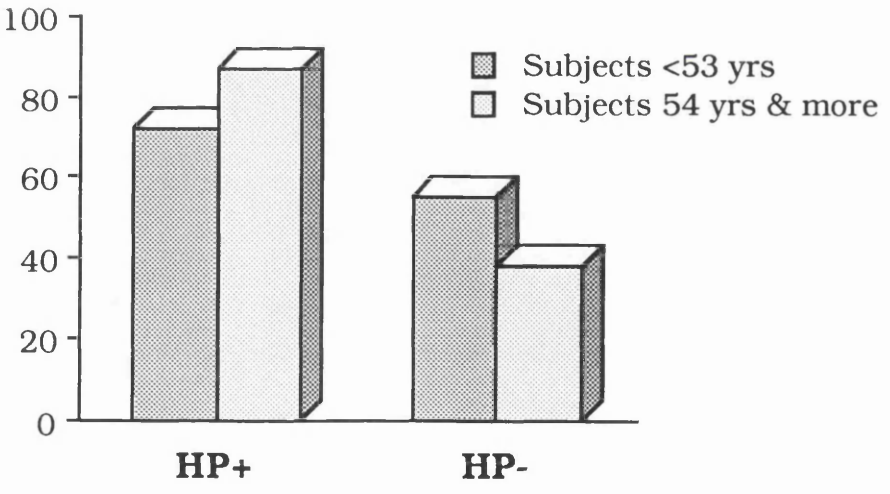


Table 21.

DISTRIBUTION OF *HELICOBACTER PYLORI*
SUBJECTS BELOW 53 YEARS AND 54 YEARS & MORE
(who underwent Gastric Secretion Test)

| | **< 53 YRS** | **54 YRS & more** | **TOTAL** |
|---|---|---|---|
| ***H pylori* present** | 16 | 25 | 41 |
| ***H pylori* absent** | 12 | 9 | 21 |
| **TOTAL** | **28** | **34** | **62** |

The differences in distribution of *H pylori* positive and negative between the two age groups are significant. ($\chi^2 = 5.272$, $p < 0.0250$).

Table 22.

MEAN HEIGHT OF SUBJECTS

(who underwent Gastric Secretion Test)

| | | HEIGHT | RANGE | SD |
|---|---|---|---|---|
| **Duodenal Ulcer subjects** | *H pylori* present | 169 | 158-183 | 9 |
| | *H pylori* absent | 170 | 152-184 | 11 |
| **Non-Ulcer Dyspeptic subjects** | *H pylor* present | 168 | 152-183 | 6 |
| | *H pylori* absent | 168 | 152-182 | 10 |

Table 23.

FREQUENCY OF SMOKERS & NON-SMOKERS IN BOTH SEXES
(who underwent Gastric Secretion Test)

| | SMOKERS | NON-SMOKERS | TOTAL |
|---|---|---|---|
| **Male subjects** | 18 | 21 | 39 |
| **Female subjects** | 15 | 8 | 23 |
| **TOTAL** | **33** | **29** | **62** |

There is no significant difference in the distribution of smokers and non-smokers between the 2 sexes ($\chi^2 = 2.115$, $p > 0.20$).

Table 24.

DISTRIBUTION OF SMOKERS & NON-SMOKERS
DU & NUD SUBJECTS
(who underwent Gastric Secretion Test)

| | **SMOKERS** | **NON-SMOKERS** | **TOTAL** |
|---|---|---|---|
| **Duodenal Ulcer subjects** | 22 | 9 | 31 |
| **Non-Ulcer Dyspeptic subjects** | 11 | 20 | 31 |
| **TOTAL** | **33** | **29** | **62** |

There is a significant difference in the distribution of duodenal ulcer in the smoking subjects when compared with non-smokers ($\chi^2 = 7.839$, $p < 0.010$).

Figure 8.

DISTRIBUTION OF SMOKERS & NON-SMOKERS
DU & NUD SUBJECTS
(who underwent Gastric Secretion Test)

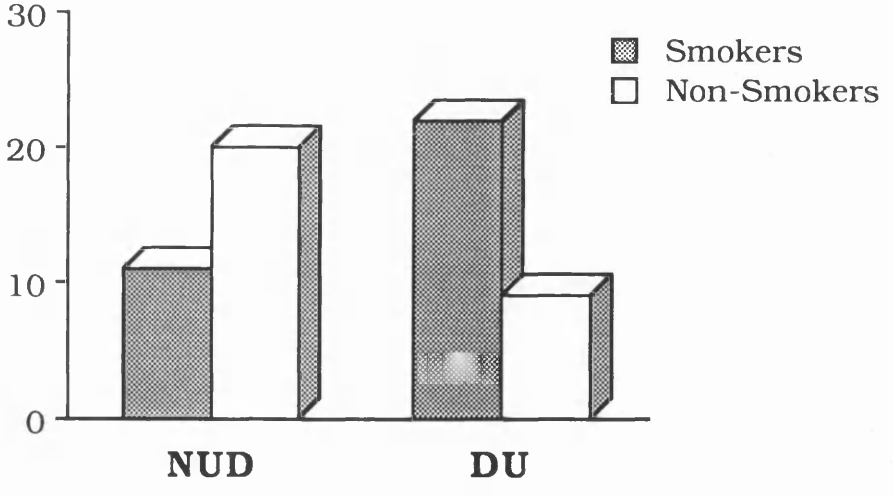


Table 25.

FREQUENCY OF *HELICOBACTER PYLORI*
AMONG THE SMOKERS & NON-SMOKERS SUBJECT
(who underwent Gastric Secretion Test)

| | **SMOKERS** | **NON-SMOKERS** | **TOTAL** |
|---|---|---|---|
| ***H pylori* present** | 22 | 19 | 41 |
| ***H pylori* absent** | 11 | 10 | 21 |
| **TOTAL** | **33** | **29** | **62** |

There is no significant differences in the distribution of *H pylori* in the 2 groups ($\chi^2 = 0.0780$, $p > 0.40$).

Figure 9.

FREQUENCY OF *HELICOBACTER PYLORI*
AMONG THE SMOKERS & NON-SMOKERS SUBIECT
(who underwent Gastric Secretion Test)

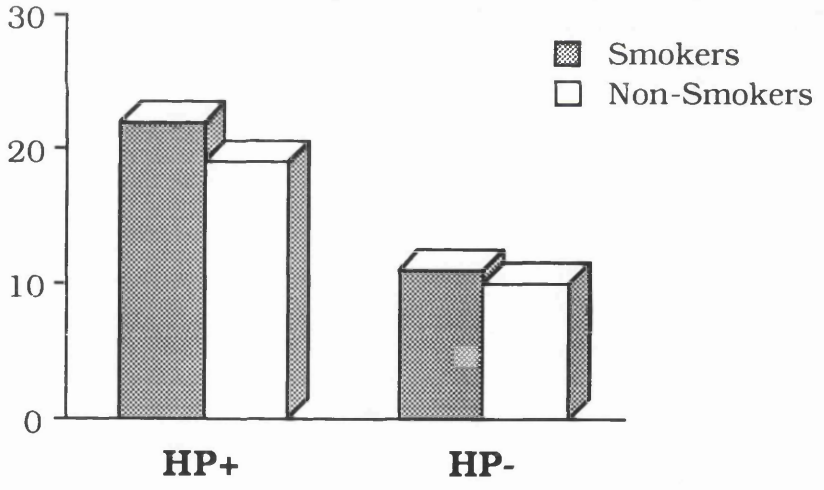


Table 26.

GASTRIC SECRETION STUDY

BASAL ACID OUTPUT ($BAO_{pl}$) &
BASAL VOLUME OF ACID SECRETED
($Vg_{baspl}$, corrected for pyloric loss & duodenogastric reflux)
by DU & NUD SUBJECTS

| | **$Vg_{baspl}$** ml $h^{-1}$ mean (sd) | **$BAO_{pl}$** mmol $h^{-1}$ mean (sd) |
|---|---|---|
| **Duodenal Ulcer Subjects n=31** | 122 (42) | 7.33 (5.13) |
| **Non-Ulcer Dyspeptic Subjects n=31** | 100 (52) | 2.8 (3.67) |

Table 27.

GASTRIC SECRETION STUDY

MAXIMAL ACID OUTPUT ($MAO_{pl}$) AND
MAXIMAL VOLUME OF ACID SECRETED
($Vg_{maxpl}$, corrected for pyloric loss & duodenogastric reflux)
by DU & NUD SUBJECTS

| | **$Vg_{maxpl}$** ml $h^{-1}$ mean (sd) | **$MAO_{pl}$** mmol $h^{-1}$ mean (sd) |
|---|---|---|
| **Duodenal Ulcer subjects n=31** | 383 (77) | 47.90 (14.96) |
| **Non-Ulcer Dyspeptic subjects n=31** | 188 (72) | 25.75 (12.14) |

Table 28.

GASTRIC SECRETION STUDY

HEIGHT-STANDARDISED
MAXIMAL ACID OUTPUT ($MAO_{pl.s}$) AND
MAXIMAL VOLUME OF ACID SECRETED
($Vg_{maxpl.s}$, corrected for pyloric loss & duodenogastric reflux)
by DU & NUD SUBJECTS

| | **Vgmaxpl.s** ml $h^{-1}$ mean (sd) | **MAOpl.s** mmol $h^{-1}$ mean (sd) |
|---|---|---|
| **Duodenal Ulcer Subjects n=31** | 395 (87) | 49.60 (14.35) |
| **Non-Duodenal Ulcer Subjects n=32** | 203 (68) | 27.31 (11.51) |

Figure 10.

ACID OUTPUT (mmol $l^{-1}$)
(BASAL, MAXIMAL & HEIGHT-STANDARDISED)
OF DU AND NUD SUBJECTS.

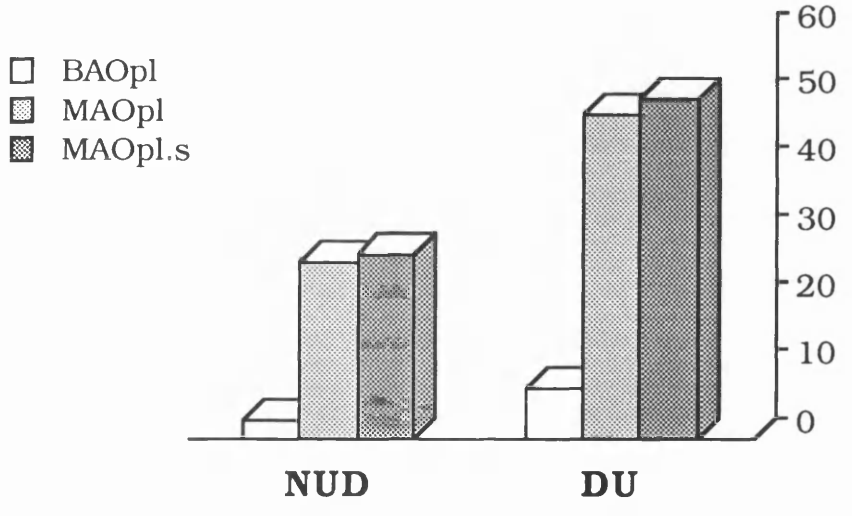


Figure 11.

VOLUME OF ACID SECRETED (ml $h^{-1}$)
(Vg, corrected for pyloric loss & duodenogastric reflux)
(BASAL, MAXIMAL & HEIGHT-STANDARDISED)
by DU AND NUD SUBJECTS.

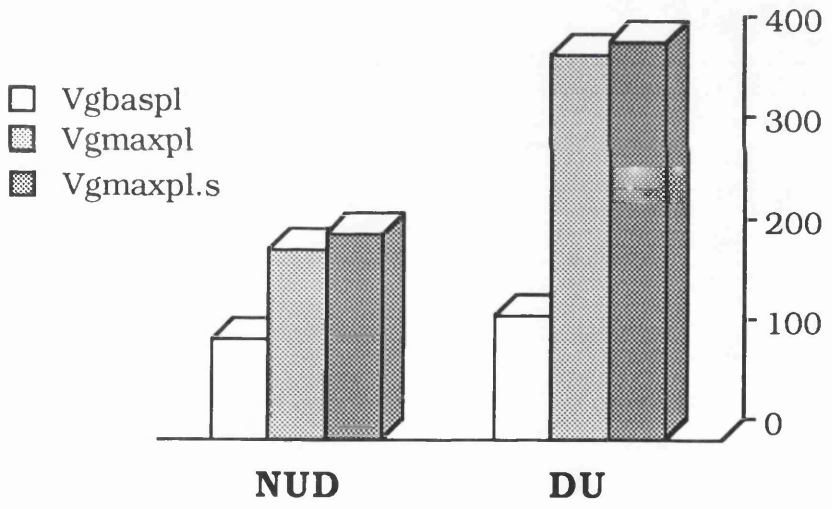


Table 29.

GASTRIC SECRETION STUDY

BASAL ACID OUTPUT ($BAO_{pl}$) AND
BASAL VOLUME OF ACID SECRETED
($Vg_{baspl}$, corrected for pyloric loss & duodenogastric reflux)
by *H PYLORI* POSITIVE & NEGATIVE SUBJECTS

| | **$Vg_{baspl}$** ml $h^{-1}$ mean (sd) | **$BAO_{pl}$** mmol $h^{-1}$ mean (sd) |
|---|---|---|
| ***Helicobacter pylori*** **positive Subjects** **n=31** | 111 (51) | 5.14 (5.56) |
| ***Helicobacter pylori*** **negative Subjects** **n=31** | 110 (41) | 4.97 (3.68) |

Table 30.

GASTRIC SECRETION STUDY

MAXIMAL ACID OUTPUT ($MAO_{pl}$) AND
MAXIMAL VOLUME OF ACID SECRETED
($Vg_{maxpl}$, corrected for pyloric loss & duodenogastric reflux)
by*H PYLORI* POSITIVE & NEGATIVE SUBJECTS

| | **$Vg_{maxpl}$** ml $h^{-1}$ mean (sd) | **$MAO_{pl}$** mmol $h^{-1}$ mean (sd) |
|---|---|---|
| ***Helicobacter pylori* positive subjects n=31** | 267 (120) | 34.34 (17.33) |
| ***Helicobacter pylori* negative subjects n=31** | 323 (122) | 41.68 (17.29) |

Table 31.

GASTRIC SECRETION STUDY

HEIGHT-STANDARDISED
MAXIMAL ACID OUTPUT ($MAO_{pl.s}$) AND
MAXIMAL VOLUME OF ACID SECRETED
($Vg_{maxpl.s}$, corrected for pyloric loss & duodenogastric reflux)
by *H PYLORI* POSITIVE & NEGATIVE SUBJECTS

| | **$Vg_{maxpl.s}$** ml $h^{-1}$ mean (sd) | **$MAO_{pl.s}$** mmol $h^{-1}$ mean (sd) |
|---|---|---|
| ***Helicobacter pylori* positive subjects n=31** | 284 (119) | 36.58 (17.32) |
| ***Helicobacter pylori* negative subjects n=31** | 330 (122) | 42.13 (16.49) |

Figure 12.

ACID OUTPUT (mmol $l^{-1}$)
(BASAL, MAXIMAL & HEIGHT-STANDARDISED)
OF *H PYLORI* POSITIVE & NEGATIVE SUBJECTS.

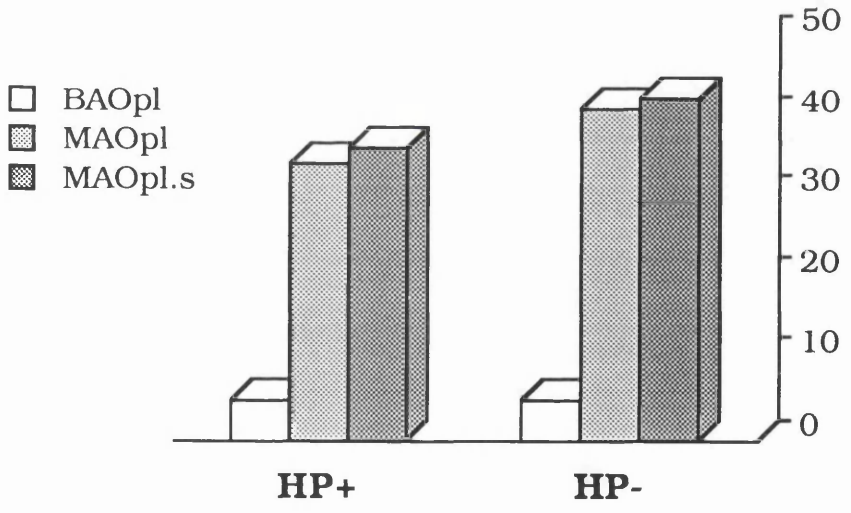


Figure 13.

VOLUME OF ACID SECRETED (ml $h^{-1}$)
(Vg, corrected for pyloric loss & duodenogastric reflux)
(BASAL, MAXIMAL & HEIGHT-STANDARDISED)
BY *H PYLORI* POSITIVE & NEGATIVE SUBJECTS.

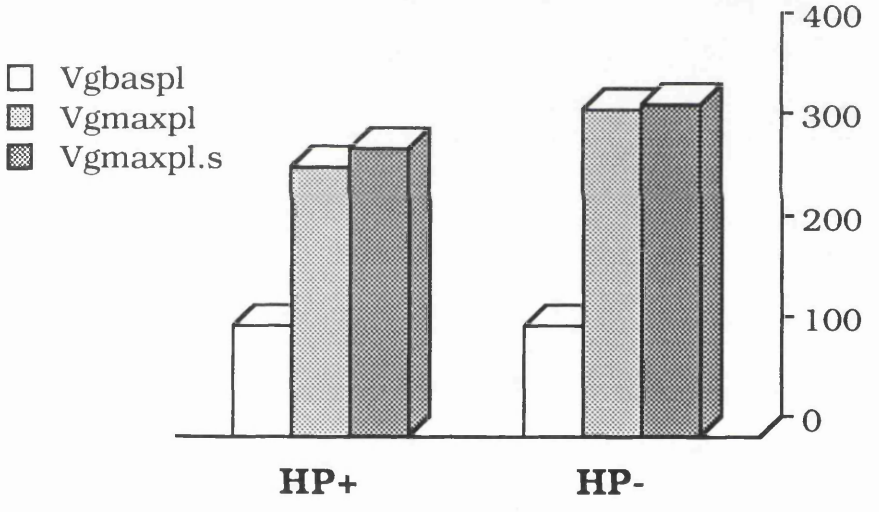


Table 32.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR DU & NDU SUBJECTS

| Group | MAOpl mmol $h^{-1}$ | MAOpl.s mmol $h^{-1}$ | Vgmaxpl ml $h^{-1}$ | Vgmaxpl.s ml $h^{-1}$ |
|---|---|---|---|---|
| **D U** (n=31) | 47.90 | 49.60 | 383 | 395 |
| **Non-D U** (n=31) | 25.75 | 27.31 | 188 | 203 |
| % difference | 46.24 | 44.93 | 50.9 | 48.6 |
| t-value | 6.399 S | 6.746 S | 10.187 S | 8.883 S |

Figure 14.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR DU & NDU SUBJECTS

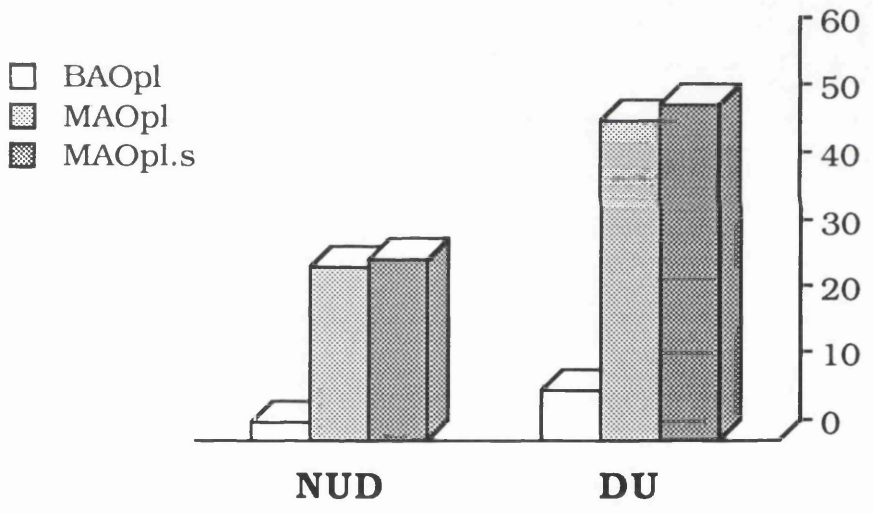


Figure 15.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR DU & NDU SUBJECTS

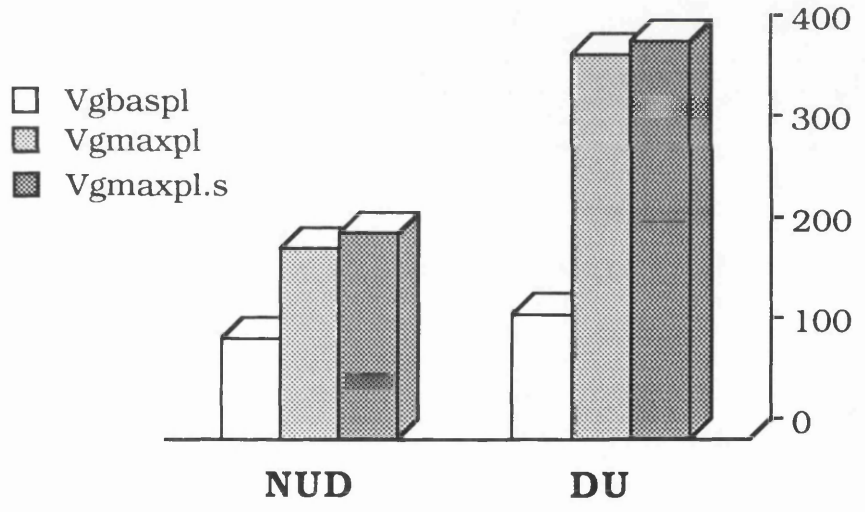


Table 33.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE SUBJECTS

| **Group** | **MAOpl** $mmol\ h^{-1}$ | **MAOpl.s** $mmol\ h^{-1}$ | **Vgmaxpl** $ml\ h^{-1}$ | **Vgmaxpl.s** $ml\ h^{-1}$ |
|---|---|---|---|---|
| **HP neg.** (n=21) | 39.68 | 42.13 | 323 | 339 |
| **HP pos.** (n=21) | 34.34 | 36.58 | 267 | 274 |
| % difference | 13.45 | 13.17 | 17.3 | 19.1 |
| t-value | 1.578 NS | 1.213 NS | 1.654 NS | 1.7 NS |
| Wilcoxon rank sum | 738 NS | 743 NS | 751 NS | 774 S |

Figure 16.

THE RELATIONSHIP BETWEEN MAXIMAL ACID OUTPUT, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE SUBJECTS

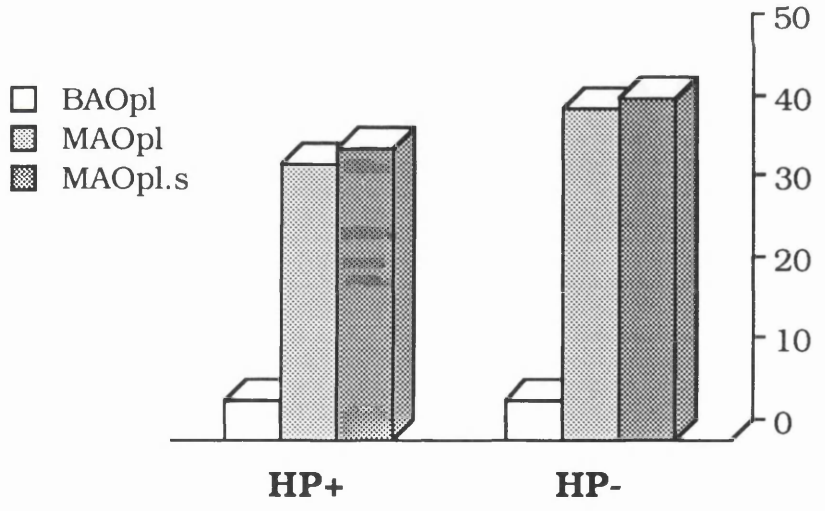


Figure 17.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE SUBJECTS

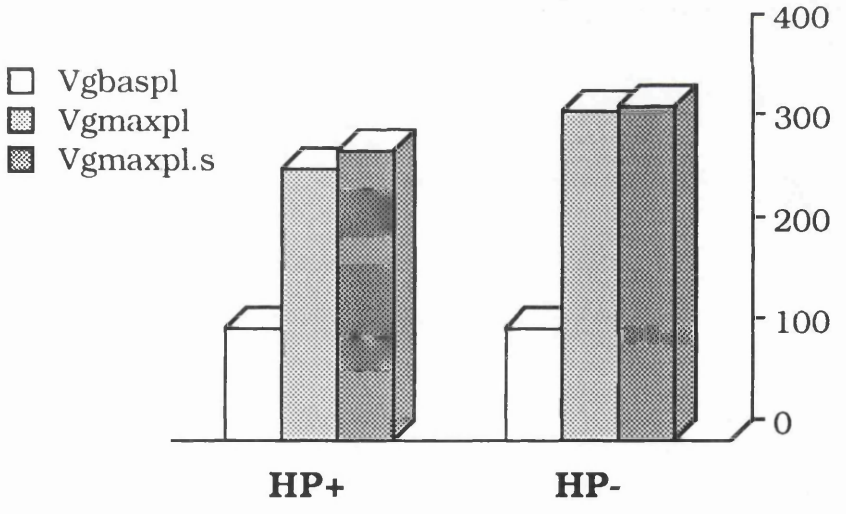


Table 35.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE DU SUBJECTS

| | **$MAO_{pl}$** mmol $h^{-1}$ mean (sd) | **$MAO_{pl.s}$** mmol $h^{-1}$ mean (sd) | **$Vg_{max}$** ml $h^{-1}$ mean (sd) | **$Vg_{max.s}$** ml $h^{-1}$ mean (sd) |
|---|---|---|---|---|
| **HP(+)** n=21 | 45.28 (15.12) | 47.82 (14.08) | 361 (68) | 374 (73) |
| **HP(-)** n=10 | 53.41 (13.75) | 53.35 (14.94) | 429 (79) | 439 (100) |
| % Reduction | 15.0 | 10.3 | 15.8 | 14.8 |
| t-value | 1.439 NS | 1.003 NS | 2.463 S | 2.340 S |
| Wilcoxon rank sum | 182 NS | 194 NS | 202 S | 206 S |

Figure 18.

THE RELATIONSHIP BETWEEN MAXIMAL ACID OUTPUT,
EXPRESSED IN FOUR DIFFERENT WAYS
FOR *H PYLORI* POSITIVE & NEGATIVE DU SUBJECTS

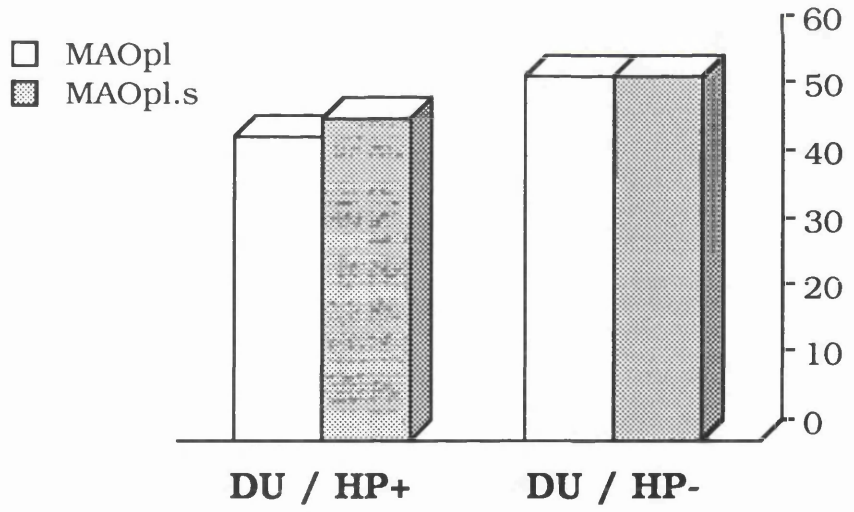


Figure 19.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE DU SUBJECTS

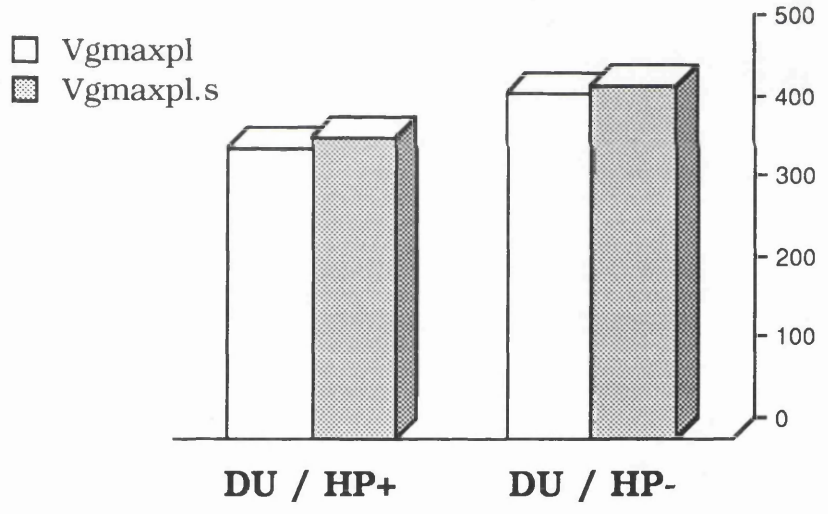


Table 36.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE NUD SUBJECTS

| | **$MAO_{pl}$** mmol $h^{-1}$ mean (sd) | **$MAO_{pl.s}$** mmol $h^{-1}$ mean (sd) | **$Vg_{maxpl}$** ml $h^{-1}$ mean (sd) | **$Vg_{maxpl.s}$** ml $h^{-}$ mean (sd) |
|---|---|---|---|---|
| **HP(+)** n=20 | 22.86 (10.90) | 24.77 (11.67) | 168 (75) | 188 (74) |
| **HP(-)** n=11 | 31.01 (12.86) | 31.92 (10.11) | 225 (51) | 230 (46) |
| % Reduction | 26.2 | 22.3 | 25.3 | 18.2 |
| t-value | 1.861 NS | 1.706 NS | 2.231 S | 2.401 S |
| Wilcoxon rank sum | 229 S | 221 S | 219 S | 266 S |

Figure 20.

THE RELATIONSHIP BETWEEN MAXIMAL ACID OUTPUT, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE NUD SUBJECTS

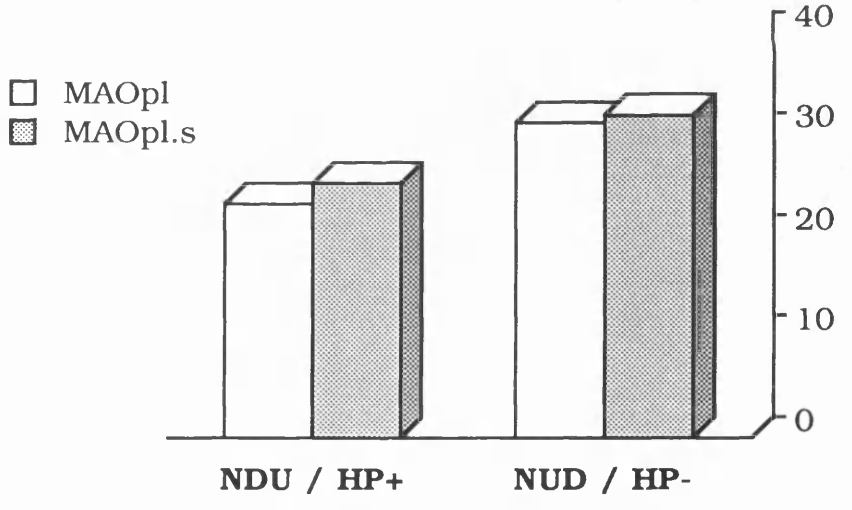


Figure 21.

THE RELATIONSHIP BETWEEN MAXIMAL GASTRIC SECRETION, EXPRESSED IN FOUR DIFFERENT WAYS FOR *H PYLORI* POSITIVE & NEGATIVE NUD SUBJECTS

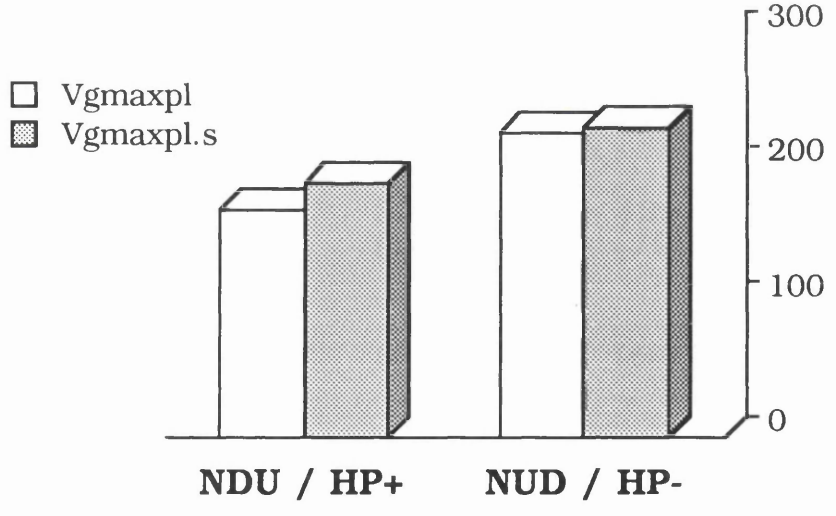


Table 37.

Helicobacter pylori Negative Patients

Kendall's rank correlation ($\tau$)

(with Smoking Factor)

| | $MAO_{pl}$ (sd) | $MAO_{pl.s}$ (sd) | $Vg_{maxpl}$ (sd) | $Vg_{maxpl.s}$ (sd) |
|---|---|---|---|---|
| **DU** n=10 | 53.41 (13.75) | 53.35 (14.94) | 429 (79) | 439 (100) |
| | ($\tau$=0.198,ns) | ($\tau$=0.188,ns) | ($\tau$=0.288,s) | ($\tau$=0.301,s) |
| **non-DU** n=11 | 31.01 (12.86) | 31.92 (10.11) | 225 (51) | 230 (46) |
| | ($\tau$=0.303,ns) | ($\tau$=0.361,ns) | ($\tau$=0.410,s) | ($\tau$=0.441,s) |

Table 38.

FOLLOW-UP OF SUBJECTS
(who underwent Gastric Secretion Test)

| | **CONTROL no ulcer or ACG** (*H pylori* negative) | **ACG only** (*H pylori* positive) | **ACG with DU** (*H pylori* positive) | **DU no ACG** (*H pylori* negative) |
|---|---|---|---|---|
| **GSS Done** | 11 | 20 | 21 | 10 |
| **FU attended** | | | | |
| **4-6 Weeks** | * | 20 | 20 | * |
| **8-12 Weeks** | * | 17 | 21 | 9 |
| **24-26 Weeks** | 10 | 19 | 19 | 10 |
| **52-60 Weeks** | 8 | 18 | 19 | 9 |

* subjects were not invited for endoscopy for that period.

Figure 22.

THE RISK CURVE OF DUODENAL ULCER
IN RELATION TO MAXIMAL GASTRIC SECRETION
& THE DISTRIBUTION OF *H PYLORI* POSITIVE &
NEGATIVE DU SUBJECTS)

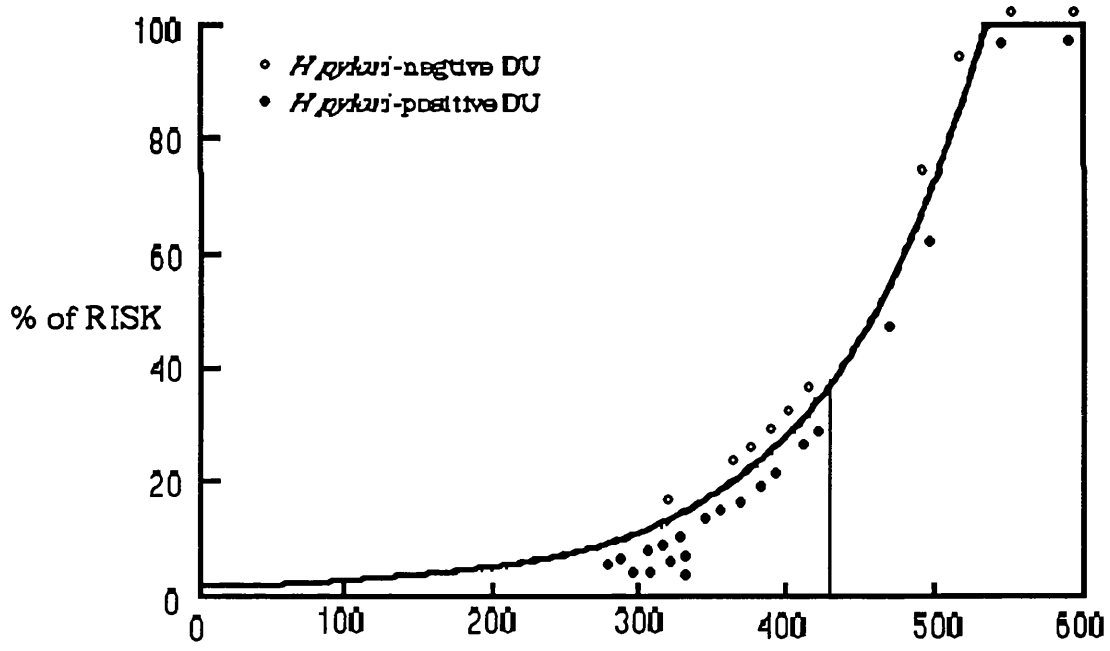


Figure 23.

THE RISK CURVE OF DUODENAL ULCER
IN RELATION TO MAXIMAL GASTRIC SECRETION
& THE DISTRIBUTION OF *H PYLORI* POSITIVE &
NEGATIVE DU SUBJECTS)
(after the Vgmaxpl.s of *H pylori* positive DU subjects was raised by 17%)

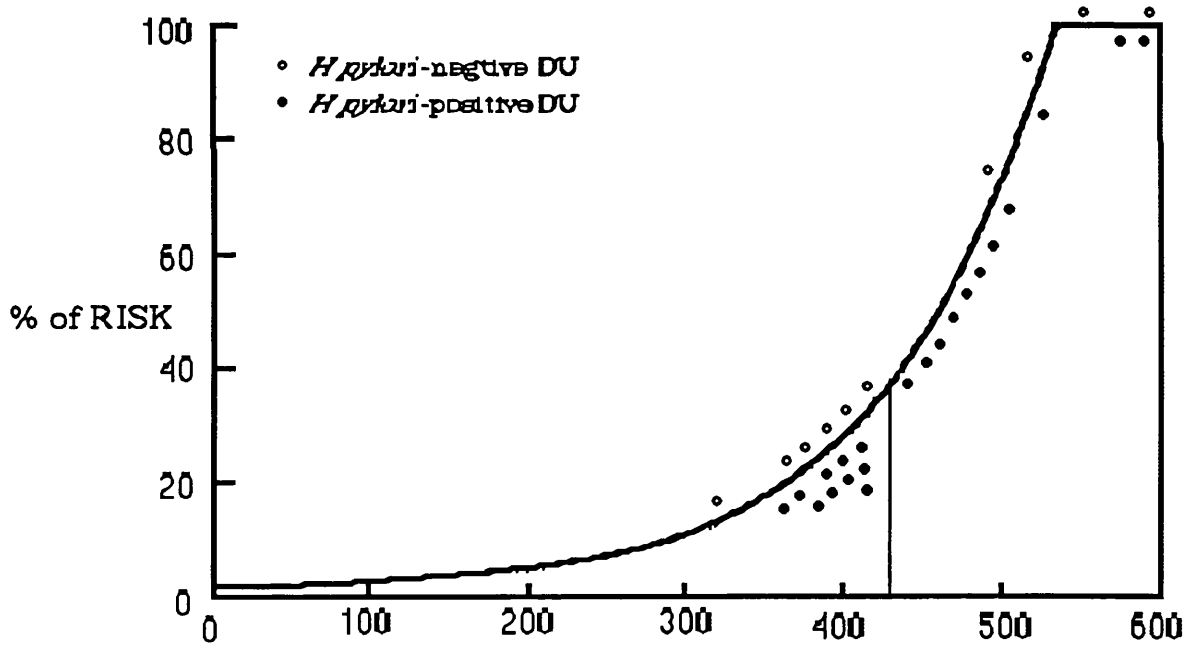


Figure 24.

THE CHART SHOWS THE TREATMENT RESPONSE OF *HELICOBACTER PYLORI NEGATIVE* - DUODENAL ULCER SUBJECTS.

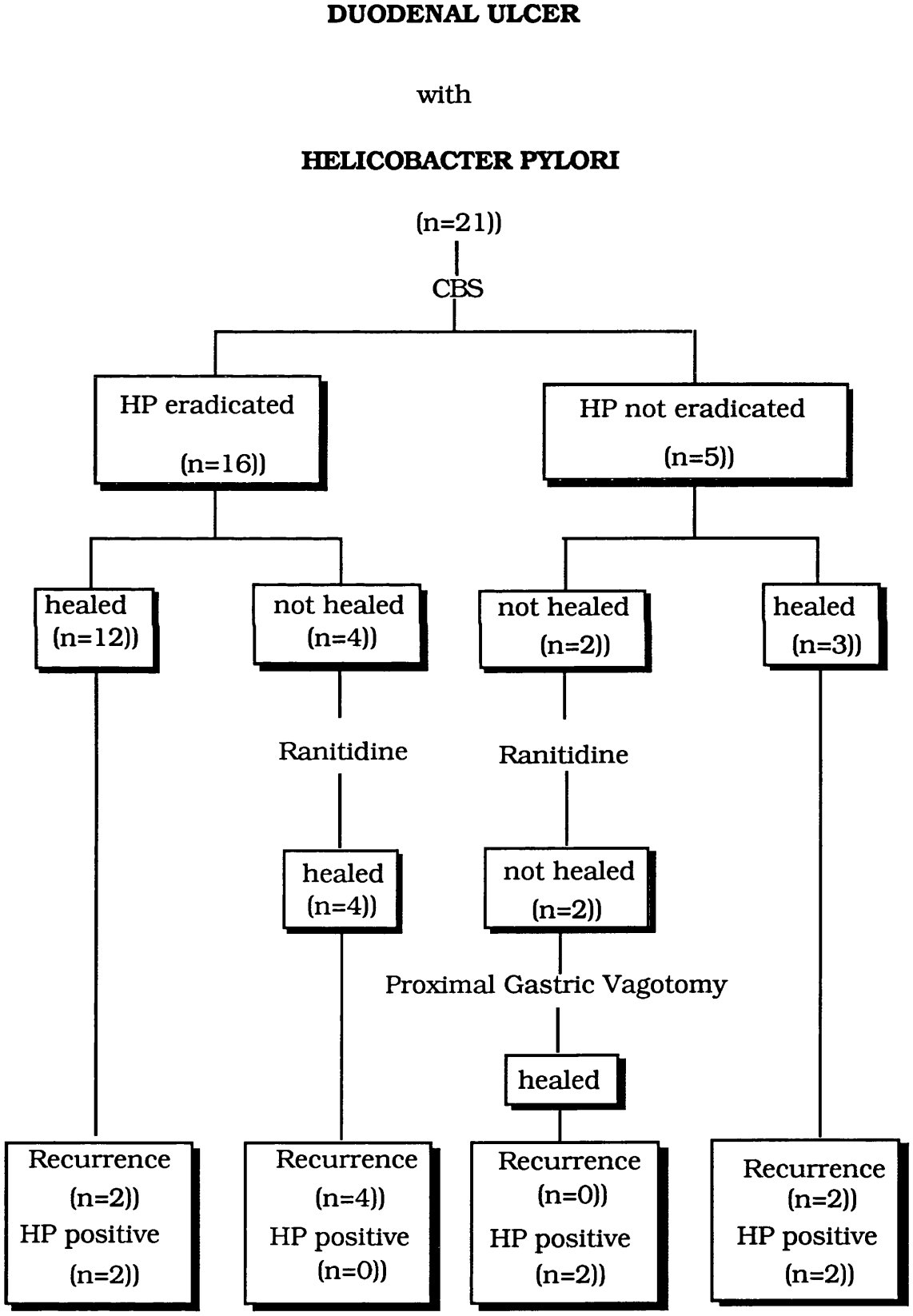
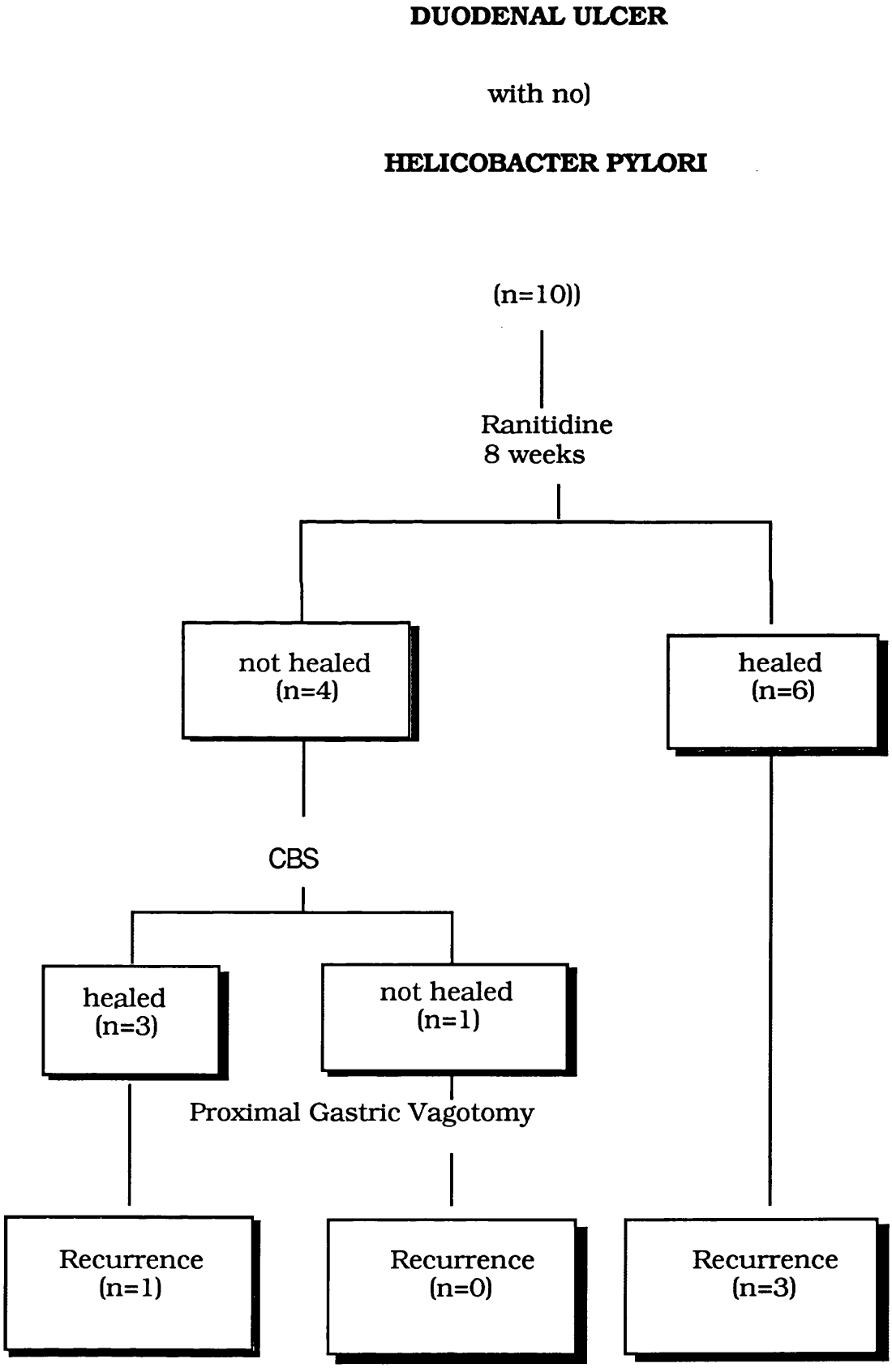


Figure 25.

THE CHART SHOWS THE TREATMENT RESPONSE OF

*HELICOBACTER PYLORI NEGATIVE* - DUODENAL ULCER SUBJECTS.



# *CHAPTER 5*

# *DISCUSSION*

# 5. DISCUSSION

The discussion of this thesis will be presented under the headings: introduction, subjects, methods and results.

## 5.1 Introduction

Duodenal ulcers are strongly linked to the level of gastric acid output, but the recent isolation of the spiral gastric bacterium *Helicobacter pylori* (Warren and Marshall 1983) has led to an explosion of worldwide research and the current data have been interpreted as strongly suggesting that *H pylori* is the causative agent for DU (Marshall, McGehie, Rogers et al 1985, Rauws, Langenberg, Houthoff et al, 1988 and Goodwin, Marshall, Blincow et al 1988)). However, the available results are inconclusive and sometimes conflicting. Those who accept the evidence for a causal relationship have spent considerable effort on exploring the mechanism by which the organism might produce the ulcer. At present there are 2 popular hypotheses: (i) patients with hypersecretion of acid and / or rapid gatstric emptying with an increased acid load in the duodenum get gastric metaplasia which encourages colonisation of the duodenum with *H pylori* and produces duodenitis of the duodenum and finally an ulcer (Wyatt, Rathbone, Dixon et al 1987 and Goodwin 1988), and (ii) patients with antral infection with the organism have an antral milieu which is less acid than usual, because the urea-splitting activity of the organism results in production ofammonia. The relative antral hypoacidity

stimulates the secretion of gastrin which results in an increased output of acid (Levi, Haddad, Beardshall et al 1989) in what would otherwise be submaximal secretory circumstances, and in the long term an increase in parietal cell mass.

If both hypotheses are put together, it appears that *H pylori* infection causes an inappropriate secretion of gastrin which in turn leads to hypersecretion of gastric acid; this creates a low duodenal pH, results in gastric metaplasia in the duodenum and permits the *H pylori to* colonise this area of gastric metaplasia leading to duodenitis and then to DU. It must be stressed that both these hypotheses require increased acid delivery to the duodenum, either due to an increased parietal cell mass or to increased gastric emptying. However, the evidence about the relationship between the presence of the organism and either acid secretion or gastric emptying is conflicting and will be discussed later.

The aim of the study was to investigate whether there was any link between *H pylori* infection of the antrum and the capacity of the stomach to secrete acid when maximally stimulated. At the same time it was felt important to explore the incidence of the organism in DU patients and in a contrasting group. The closest control appeared to be dyspeptic patients without a DU and indeed without any endoscopic evidence of disease, that is non - ulcer dyspepsia or NUD. During the period of study many other patients underwent endoscopy and were found to have lesions other than DU; the opportunity was therefore taken to define the incidence of *H pylori* in such patients. For reasons which became evident during the study, this group of patients has been subdivided into reflux oesophagitis and other miscellaneous condition.

## 5.2 Subjects:

The subjects in this study consisted of patients with dyspepsia, who were referred to the Department of Gastroenterology for oesophagogastro- duodenoscopy. The type of patients with dyspepsia referred to gastrointestinal departments has changed. In the past, especially with the wide-spread use of $H_2$-receptor antagonists, patients were usually referred only if the treatment had failed or relapse had occurred. In other words, they were a selected group with severe symptoms and not necessarily representative of the majority of patients with dyspepsia in the community. However, during the period of this study, and presumably because of the greatly increased availability of endoscopic services, many of the referred patients had relatively mild symptoms. Thus the subjects studied have probably represented a good sample of the patients with dyspepsia in the population.

This investigation started with the hypothesis that *H pylori* is a causative agent for DU. It was therefore necessary to determine the incidence of the organism in the DU group and in a contrast or control group. The closest control was the group of patients with dyspepsia who had no organic lesion, that is NUD. Other organic lesions might themselves have been associated with *H pylori* and it was necessary to exclude them. At first I therefore had 3 groups: DU, NUD and miscellaneous findings. However, during the analysis of the results it became clear that patients with oesophagitis should be segregated as a separate group and so the results have been presented in terms of 4 groups.

In this study dyspepsia was defined as chronic or recurrent upper abdominal pain of at least 6 months' duration, with or without nausea and not necessarily related to meals. Duodenal ulcer subjects were those with endoscopic evidence in the duodenum of a chronic ulcer, including the scar of a healed ulcer. Oesophagitis subjects were those with macroscopic inflammatory changes in the lower end of oesophagus and in whom biopsy and microscopy had failed to show any evidence of malignancy. Subjects with miscellaneous findings included patients with malignant lesions in the oesophagus and stomach, oesophageal varices and gastric ulcer, erosion, polyp or diverticulum. Non Ulcer Dyspepsia subjects were those with no (macroscopic) organic lesions noted endoscopically.

## 5.3 Methods

### 5.3.1 Endoscopy:

The 252 endoscopy examinations were carried out with the help of an experienced endoscopist. The modern narrow and flexible GIF-XQ 20 gastroscope was easy to use and my findings of macroscopic lesions (51%) in consecutive endoscopy examinations were no different from other major centres (Jiang, Liu, Zhang et al 1987). This eliminated any bias arising from inexperience and the failure to identify macroscopic lesions. The endoscope was disinfected according to the Health Authority policy and there was no evidence that infection was ever passed on to the subsequent subjects.

Duodenal ulcer was noted in 76 (30% of total 252) subjects, oesophagitis was diagnosed in 30 (12%) subjects, there were miscellaneous findings in 23 (9%) and in 123 (49%) no macroscopic lesions were identified (Table 1 & Fig. 1).

5.3.2 Microbiology:

It was accepted that the distribution of *H pylori* was patchy in the gastric antrum and hence 3 specimens (biopsies) were taken randomly from the antrum for 3 separate tests to identify the *H pylori*.

The CP-urease test used in this study was invented by the Department of Microbiology of The Middlesex Hospital Medical School (Vaira, Holton, Cairns et al 1988). This test has been compared with other urease tests and found to yield results consistent with those tests. Moreover, it has been shown that its sensitivity in comparison with more powerful tests like culture and histology is equally as good as other urease tests (Vaira, Holton, Falzon et al 1988). In this study the CP-urease test had a sensitivity of 91.2%, specificity of 100%, and accuracy of 94.4% with a positive predictive value of 100% and a negative predictive value of 86.9% (Tables 2 & 7). These results were also similar to other studies (Vaira, Holton, Falzon et al 1988, Arvind, Cook, Tabaqchali et al 1988).

The microbiological culture study was performed not by me but by an experienced senior staff member of the Dept. of Microbiology. The blood agar medium with 6mg/ml of amphotericin was used for culture and the results had a sensitivity of 96.2%, specificity of 98.9%, and accuracy of 97.2% with a positive predictive value of 99.4% and a negative

predictive value of 93.9% (Tables 3 & 7). These results were comparable to the results other investigators ( Tytgat, Rauws, Langenberg 1986).

The histopathology studies were also not performed by me but by another senior member of the Dept. of Histopathology. Multiple cuts of the specimen were used and Giemsa staining performed to identify *H pylori*. Sections were stained with haemotoxylin and eosin to detect the ACG. The identification of *H pylori* results had a sensitivity of 94.9%, specificity of 97.8%, and accuracy of 96.0% with a positive predictive value of 98.7% and a negative predictive value of 91.9% (Tables 4 & 7). These results were similar to other studies (Montgomery, Martin and Peura 1988).

It was noted that ACG was present in all specimens where *H pylori* was identified (Tables 5 & 9). Though many factors influence (as described in Chapter 2) the isolation of *H pylori*, in this study it was noted that *H pylori* was identified by at least 2 tests and always associated with ACG. Subjects who were CP-urease positive were positive by microbiology and / or culture.

The percentage incidences of a positive CP-urease test in DU, oesophagitis, subjects with miscellaneous findings and NUD were 64, 28, 57 and 61 respectively and the incidences of *H pylori* after consideration of all methods were 70, 30, 61 and 67 respectively (Table 8). The latter four results were similar to those found in other clinics. The ratios of CP-urease positive and *H pylori* positive subjects were similar in all four groups and similar to those found by others investigators. The sum of all this evidence supports the accuracy of the microbiology tests.

### 5.3.3 Selection of subjects for Gastric Secretion Test.

The patients I studied by gastric secretion tests were less than half of the total number undergoing endoscopy. It was important therefore to consider the possibility that the selection for secretion studies was in some way biased.

At the time of inviting the subjects for gastric secretion tests, only the results of the CP-urease test were available. However, the results of the other tests for *H pylori* became available later and usually after the gastric secretion test had been performed.

Sixty (30%) of the 199 DU and NUD subjects were excluded because of the criteria (Chapter 4) previously described. The remaining 139 (70%) subjects (46 of DU and 93 of NUD) were approached for gastric secretion tests.

The ratios of CP-urease positive and negative subjects were similar in the 76 DU (49/27 or 64% - 36%) and in the123 NUD (75/48 or 60% - 40%); in those who were approached for a gastric secretion test (DU: 26/20 or 56% - 44% and NUD: 51/42 or 55% - 45%), and in those who attended for the gastric secretion test (DU: 18/13 or 58% - 42% and NUD: 19/12 or 61% - 39%). It was interesting to note how similar the proportion of *H pylori* infected subjects remained in the 2 groups(DU: 21/10 or 68% - 32% and NUD: 20/11 or 65% - 35%) at all the stages of the selection process (Table 8 & Fig. 3). Thus there was no evidence of bias, either in the selection of patients for the gastric secretion test or in those who attended for the gastric secretion test. However, it was also interesting that DU subjects were more likely to attend the gastric secretion test than NUD

subjects. Though 93 NUD patients, nearly twice the DU (46), were approached, only equal numbers of patients (31) from each group attended the gastric secretion test (Fig. 3). This difference in the ratio attending was possibly due to the fact that the DU patients had the knowledge that a serious disease had been diagnosed and this made them more likely to volunteer for further investigation than the NUD patients who had been reassured that no serious organic disease had been found.

### 5.3.4 Gastric Secretion Test.

Only 62 subjects attended for the gastric secretion test and a careful detailed recording of each patient's age, smoking history, height and weight was kept. For smoking history, the maximum period of smoking and stated average rate of smoking per day was used. All non-smokers in this study confirmed that they had been life-long non-smokers. Subjects may understate their cigarette consumption or deny being smokers for fear of disapproval; thus in the main it had to be taken on trust that the subjects had told the truth.

The method of stimulating maximal gastric secretion was at variance with common practice, the most popular stimulatory method being that of a single bolus of pentagastrin. The method used in this study was that of Lawrie, Smith and Forrest (1964) and modified by Whitfield and Hobsley (1979): a continuous intravenous infusion of histamine, with the use of an anti-histamine to reduce the side effects. The infusion was continued long enough to produce a relatively stable period to select a plateau. The plateau was preferable to a peak response

for this reason of greater stability, and also because it facilitated the detection of, and correction for, sequestration and poor aspiration. Histamine was used since it does not have a 'fade' effect as had been found with pentagastrin (Aubrey 1970 and Emas and Svensson, 1972).

As expected, exogenous histamine increased acid output in all subjects. The maximal gastric secretion was expressed in 4 different ways, namely maximal acid output (mmol $h^{-1}$, $MAO_{pl}$), maximal acid output standardised for a height of 170 cm ($MAOs_{pl}$), volume corrected for pyloric loss and duodenogastric reflux (ml $h^{-1}$, $Vg_{max}$) and volume corrected for pyloric loss and duodenogastric reflux and standardised for height of 170 cm ($Vgs_{max}$). The $Vgs_{max}$. is theoretically the most accurate index and evidence to emphasise this point will be shown later.

In this study the mean secretion of gastric acid in DU subjects was greater than in non-DU subjects, and consistent with other published reports. However, it is important to emphasise that the gastric secretion increased as the index of smoking (SMF) increased (Table 38). This positive correlation was found only in the *H pylori* negative subjects and it was clearly present despite the small number of individuals in this *H pylori* negative group. This finding attested to the sensitivity of the gastric secretion studies. Similar findings in other studies have required much larger groups (Whitfield and Hobsley 1985 & 1987).

## 5.4 Results

### 5.4.1 Diagnosis

Oesophagogastroduodenoscopy was done on 100 male and 152 female subjects. Thirtymine male and 48 female subjects had a DU; reflux oesophagitis was noted in 13 male and 17 female subjects and 10 male and 13 female subjects had miscellaneous findings; NUD was diagnosed in 37 male and 75 female subjects. There was no difference between the distribution of sexes in the four groups (Table13).

Sixty (60%) of the male subjects and 99 (65%) of the female subjects were *H pylori* positive and there was no significant difference in the distribution of the organisms between the sexes (Table14, $\chi^2 = 0.196$, $p > 0.06$). These data suggest that there is no gender predilection and the published data of others confirm this finding.

The mean age for NUD was 52 years, for DU was 46 years, 56 years for oesophagitis and 54 years for those subjects who had miscellaneous findings (Table 17).

The incidence of *H pylori* in this study increased with age. The mean age for subjects with *H pylori* was 54 and for subjects negative for *H pylori* was 40. Eighty-seven (55%) of the 159 *H pylori* positive subjects and 38 (41%) of the *H pylori* negative subjects were either 54 years or older. But 72 (45%) of the 159 *H pylori* positive subjects and 55 (59%) of the *H pylori*

negative subjects were younger than 54 years. The differences in frequency of *H pylori* positive and negative between these 2 age groups were significant (Table19, $\chi^2 = 9.378$, $p < 0.0050$).

However, it is interesting that the peak incidence of *H pylori* was in the sixth decade and there was a decline after that. This was suggested by eye when a polynomial rather than a straight fit was used. A similar phenomenon can be seen in the tables of other workers (Barthel, Westblom, Havey et al 1988, Graham, Klien, Opkun et al 1988, Al-Moagel, Evans, Abdulghani et al 190) and in a report for an asymptomatic control population (Cheng, Bermanski, Silversmith et al 1989). However, those authors, with exception of Pretolani, Bonvicini, Brocchi et al (1989), did not mention the decline in incidence. In my study, with the small number of subjects it was not possible to show any statistical significance in the decline; but combining my results with those of other workers it may be reasonable to accept that there is one.

The smoking history was available only for those 62 subjects who underwent a gastric secretion test. Thirty three (53%) of the subjects were smokers; 18 were male and 15 were female subjects. There were 11 (35%) smokers in the NUD group and 22 (71%) smokers in the DU group. Among the 41 *H pylori* positive subjects 22 (54%) were smokers and 11 (52%) of the 21 *H pylori* negative subjects were smokers. There was no significant difference in the distribution of smokers either between the two sexes or between *H pylori* positive or negative subjects (Table 25, $\chi 2 = 0.0780$, $p > 0.4$). But significantly more of the subjects in the DU group were smokers when compared with NUD subjects (Table 24, $\chi 2 = 7.839$, $p < 0.010$). Thus my results confirm that smoking is a risk factor for DU; it is

interesting that the distribution of DU between the two sexes in the Western population nowadays is nearly the same, perhaps because the number of smokers among men is declining while among women it has been increasing over the last decade (Kurata, Haile and Elashoff 1985).

### 5.4.2 Acid output.

#### 5.4.2.1 Effect of *H pylori* on Gastric Secretion.

The maximal gastric secretion was higher in subjects with DU than in NUD subjects, no matter how secretion was expressed (Table 33). This fact was obvious even without any correction for pyloric loss and duodenogastric reflux or standardisation for height. This is because the difference was so great. There was also a difference in maximal gastric secretion between the *H pylori* positive and negative subjects, the former secreting the larger volume. It was interesting that this difference became apparent only when the secretion was expressed as height-standardised Vg and the statistical test used was the Wilcoxon rank sum test (Tables 32-36). If correction and standardisation had not been made and an appropriate statistical test had not been used, this valuable information would have been lost. The reduction of gastric secretion associated with *H pylori* suggests that infection with *H pylori* may be the cause for the reduction of gastric secretion noted in elderly subjects (Baron 1969, Hassan and Hobsley 1971).

In the NUD subjects the gastric secretion was 18-25% lower in those with *H pylori* compared to those without, but

again this difference was significant only when expressed in Vg or height-standardised Vg. Among the DU subjects the reduction was about 15% and here too, the significance became manifest only when secretion was expressed as Vg or height-standardised Vg. This point again illustrates the fact that correction had to be done to avoid any misleading conclusions.

In smokers, a positive correlation between smoking factor (SMF), that is the square root of the product of cigarettes smoked per day and number of years smoked, and maximal gastric secretion was demonstrated in the *H pylori* negative patients (both DU and NUD groups) (Table 37) but there was no correlation between gastric secretion and smoking in the *H pylori* positive subjects. Again, the correction was not statistically significant in the *H pylori* negative group unless the corrections for collection errors were made. The question arises, why was there no correlation between dose of smoking and maximal secretion in *H pylori* positive subjects? Smoking increases parietal cell mass and so the maximal gastric secretion but *H pylori* decreases the secretion. These effects are identical in DU and NUD subjects. It is known that there is a positive correlation between SMF and maximal gastric secretion in all DU and control subjects (Whitfield and Hobsley 1987). It is also known from this present study that *H pylori* infection was associated with reduced gastric secretion. Thus, both DU and NUD subjects who smoke and have *H pylori* infection are subject to 2 forces, the one increasing and the other reducing the maximal secretion. This counteraction would seem to explain why the correlation is clear-cut in the *H pylori* negative group but not in the *H pylori* positive subjects. It seems likely that these two factors (smoking and *H pylori*) cancel each other out, and the link with smoking is lost in the *H pylori* positive group.

The results obtained in my study show that the DU subjects secrete more acid when maximally secreted. This is in concordance with other studies which also show that maximal (and meal stimulated) acid secretion rates were higher in subjects with DU than in normal subjects (Wormsley and Grossman 1965, Hassan and Hobsley 1971). The most important finding in my study was that the *H pylori* is associated with reduced gastric secretion whether the subjects had DU or not.

A review of the literature about the effect of *H pylori* on gastric secretion must start with the acute infection with the organism. It is an established fact that the acute infection of the stomach with *H pylori* leads to hypochlorhydria. There were two instances of epidemic hypochlorhydria (Ramsey, Carey, Peterson et al 1979, Gledhill, Leicester, Addis et al 1985) in subjects participating in the gastric secretion studies. In both instances the healthy volunteers became rapidly and profoundly hypochlorhydric after a transient illness with abdominal pain and nausea. It was thought that an unidentified pathogen had been transmitted by the contaminated pH electrode. These studies were carried out before *H pylori* was identified by Warren and Marshall in 1983. (Gledhill, Leicester, Addis et al studied the gastric secretion in volunteers in 1981/82). Later Peterson et al (1987) tested the serum samples taken during and after the illness in subjects reported by Ramsey (1979) and found the antibody titres for *H pylori* raised, suggesting that the organism played a role in the epidemic hypochlorhydria due to gastritis. In the case of Gledhill's study the gastric biopsies of the volunteers subsequently proved to contain *H pylori*. The gastric secretion returned to near normal level when the infection was cleared. There was another case documented (Graham, Alpert, Smith et al 1988) that iatrogenic *H pylori* infection was the

cause for hypochlorhydria in a volunteer who underwent repeated gastric analyses in the study of gastric adaptation to aspirin. Further evidence was provided by a volunteer who ingested *H pylori* (Morris and Nicholson 1987); the pH rose to 7.6 on the 8$^{th}$ day of ingestion but it had returned to 1.6 by the 29$^{th}$ day. The infection persisted in a chronic form for more than 3 years and it would have been interesting to know whether the chronic state produced its own tendency to hypoacidity, but sadly the gastric secretion studies were not carried out in the chronic stage (Morris, Ali, Nicholson et al 1991).

So much for gastric secretion results for <u>acute</u> infection with *H pylori*. It is important to consider now the relationship between <u>chronic</u> infection with the organism and gastric secretion in subjects with DU and NUD. Barthel, Westblom, Havey et al (1988) studied the hydrogen ion concentration of the fasting gastric secretion in the subjects with and without *H pylori* infection and found that volunteers without *H pylori* infection secreted significantly greater amounts of hydrogen ion than those with *H pylori* infection. Maximally stimulated acid secretion in response to pentagastrin or histamine was not undertaken in these studies. In Brazil, the incidence of *H pylori* is very high and among the poor Brazilians it is nearly 100%: a study shows that asymptomatic Brazilians with *H pylori* were hypochlorhydric and 20% of them were histamine-fast achlorhydric (Braga, Marshall, Moreno et al 1990). Furthermore, Peterson, Barnett, Evans et al (1991) showed that normal volunteers with *H pylori* infection had significantly lower basal acid outputs than voluteers without *H pylori* infection. In all these studies the volume of gastric secretion was either not measured or not published; however, they confirm my finding that NUD subjects with *H pylori* infection secrete a lesser amount of gastric juice than NUD subjects without *H pylori* infection.

With regard to gastric secretion in DU subjects, a number of studies are available. Steer (1984) found that there was a significant negative correlation between the total number of bacteria (Campylobacter - like organisms) and either the basal acid output or the maximal acid output after pentagastrin in DU patients. One study (Saeed, Evans Jr, Evans et al 1991) showed that in Zollinger-Ellison syndrome the *H pylori* positive subjects secreted less acid than the negative subjects; and it was also reported in another case that the DU in a Z-E syndrome had healed following an intercurrent illness without any alteration of the gastrin level (Wiersingh and Tytgat 1977). No antibodies against parietal cells could be detected nor infarction of the gastrinoma documented. Again, in these studies the gastric secretion was measured in terms of acid output rather than in corrected volume; however these studies are in concordance with my finding that DU subjects with *H pylori* secrete less acid.

It must be pointed out that some studies of infection with *H pylori* and gastric secretion have shown no clear-cut relationship between the two. Wagner, Gebel, Freise et al (1990) found DU subjects irrespective of whether they were *H pylori* positive or negative had lower pH than patients with gastritis or normal healthy subjects; and did not find any difference between *H pylori* positive and negative DU subjects. Brady, Hadfield, Hyatt et al (1988) found no consistent correlation between gastric secretion and *H pylori* in DU subjects. Again, in both these studies the acid output was measured without correction for collection error or standardisation for stature; moreover, in Brady's study the subjects were not stringently advised to stop $H_2$ blockers for at least 72 hours prior to the gastric secretion test. I am, therefore, not altogether surprised that these studies showed no consistent findings.

5.4.2.2 *H pylori* and gastrin

Finally, there is one study which claims that DU subjects with *H pylori* secrete more acid than those free of infection (Levi, Beardshall, Haddad et al 1989). It is particular important to note that their method of measuring gastric secretion is similar to mine and yet the results were contrasting. The reason for this I cannot explain. However, in my study the gastric secretion was reduced in the presence of *H pylori* infection, not only in the DU subjects but also in the NUD subjects. This finding suggests that the phenomenon of reduction of gastric secretion is of wider significance than that of a link with the aetiology of DU.

Levi, Beardshall, Haddad et al (1989) suggested the 'gastrin link' theory, that is that the presence of *H pylori* induces an increased basal gastrin level and an increased response to food, thereby producing increased gastric secretion and ultimately increased parietal cell mass. A few studies have reproduced similar results (Graham, Opekun, Lew et al 1989 & 1990, McColl, Fullarton, Nujumi et al 1989). Though eradication of *H pylori* led to the fall of the gastrin level to normal, the acid secretion remained unchanged even as late as 7 months after eradication (McColl, Fullarton, Chittajalu et al 1991). They presumed that this reflected the need for a longer period of time to elapse before a significant reduction of parietal cell numbers occurs. Peters, Feldman, Walsh et al (1983) had shown that there would be an increase of serum gastrin in healthy and DU subjects when the antral pH was maintained between 6.0 and 7.0. Also it is now known that prolonged alkalinisation of the antrum produces an exaggerated gastrin release as seen in pernicious anaemia. McGuin and Trudeau (1973) recorded that the mean fasting serum gastrin concentration for DU subjects did not differ from that in control

subjects but the post-prandial levels were significantly greater than those of the control subjects. A study comprising 58 patients with chronic DU and 91 normal subjects showed that the basal gastrin, basal acid output and maximal acid output were higher in DU patients compared to normal subjects. But the post-prandial increases of gastrin and gastric secretion were similar in both groups (Blair, Feldman, Barnett et al 1987). Montbriand, Appleman, Cotner et al (1989) studied 12 NUD subjects with *H pylori* infection and also found no difference in gastric acid secretion before and after the clearance of *H pylori*. However, in none of these studies was acid secretion measured with all the corrections described in this Thesis.

Chittajallu, Neithercut, MacDonald et al (1991) studied the effect on plasma gastrin of increasing ammonia production within the stomach by infusing urea in 8 *H pylori* positive DU subjects. They found that augmenting the *H pylori* ammonia production did not cause any early change in plasma gastrin. Walsh,Richardson and Fordtran (1975) described that in DU subjects there was a defective inhibition of gastrin release by a low intragastric pH. Wyatt, Rathbone, Green et al (1989) suggested the hypergastrinaemia induced by *H pylori* was a result of inflammatory response stimulated by the organisms in the antral mucosa. Later, they showed that serum gastrin was higher in gastritis patients with or without *H pylori* than in subjects with normal mucosa and was similar in subjects with *H pylori* whether or not they had DU. Asymptomatic subjects infected with *H pylori* (diagnosed by ELISA test) were found to have higher integrated 24 hour plasma gastrin than those without *H pylori* infection. But there was no significance difference in the median integrated 24 hour intragastric acidity (Smith, Pounder, Nwokolo et al 1990). Similar results were reproduced by Goldschmiedt, Karnes and Feldman(1989) and Peterson, Barnett, Evans Jr et al (1991). In the the last three studies

*H pylori* status was assessed by serology and one should be cautious when accepting this finding because ELISA test will not detect the infection until the immune reaction has taken place and the titre remains high for a period even after the eradication of the *H pylori* . Furthermore, it is a well known fact that serum gastrin concentrations are elevated in patients who have low acid levels due either to pernicious anaemia or to vagotomy. Levi, Beardshall, Hddad et al (1989) suggested that the raised gastrin concentration was a primary phenomenon due to *H pylori* but I believe it is a secondary to the damage to the acid-secreting cells by *H pylori.*

The basic principle of the 'gastrin link' theory was that the urease of *H pylori* splits the urea to produce ammonia and this would produce a microenvironment of elevated pH within the mucus layer. In my study, apart from measuring hydrogen, sodium and chloride I also measured the potassium ion concentration and found that was no significant difference between the chloride ions and the sum of cations in the *H pylori* positive and negative groups. One would have expected to find a positive cation difference in subjects with *H pylori* infection, but I did not find such a difference. The available data about the intragastric ammonia levels with and without *H pylori* are conflicting. Andreica, Dumitrascu, Suciu et al (1990) and Imota, Shida, Yoshida et al (1990) showed that the intragastric ammonia decreased significantly after the eradication of *H pylori* but Kato, Kano, Sato et al (1990) studied the ammonia levels in *H pylori* positive and negative patients and found that the presence of *H pylori* did not affect the ammonia level in gastric juice. Lawson, Taylor, Dresner et al (1990) found that there were no significant differences in the intragastric ammonia levels of *H pylori* infected subjects with different variety of gastroduodenal diseases.

The relative antral hypoacidity produced by *H pylori* could fit the observed tendency to hypergastrinaemia, and in this respect, it is instructive to compare the effect of *H pylori* with that of acute cigarette smoking, which similarly produces hypergastrinaemia. However, chronic cigarette smoking gives rise to an increased parietal cell mass and hyperacidity. This does not occur with *H pylori*. The reason may be that *H pylori* produces its hypoacidity not only by pharmacological (Cave and Vargas) but also by pathological effects (Wiersinga and Tytgat 1977 and Defize, Goldie and Hunt 1988). This suggestion is supported by my observation that the increase in maximal gastric secretion with overall dose of cigarettes (SMF) was demonstrable in *H pylori* negative subjects, but not in *H pylori* positive subjects (Table 37).

5.4.2.3 *H pylori* in the Duodenum

If *H pylori* is the final and immediate cause of DU it must be found in the duodenum. *H pylori* is only able to colonise on the gastric type of epithelium.

The hypothesis proposed by Wyatt et al (1989) was based on the fact that *H pylori* can colonise the gastric type of epithelium in the duodenum, and they suggested that the presence of the organism then led to duodenitis and ultimately to DU. Johnson, Reed and Ali et al (1986) and Wyatt, Rathbone, Dixon et al (1987) observed *H pylori* in the duodenal biopsies of 92% and 55% of patients with duodenitis. Gastric epithelium is present in the duodenum either over areas of heterotopic gastric body type mucosa or as a metaplastic change in the duodenal epithelium.

However, this hypothesis is unlikely to be true in this very simple form because there is a considerable body of evidence against it. Firstly, gastric metaplasia is commoner than heterotopia and is present in up to 64% of normal individuals (Kreuning, Bosman, Kuiper et al 1978) and has been correlated with maximal acid output (Wyatt, Rathbone, Dixon et al 1987). The occurrence of gastric metaplasia in the duodenum in subjects with duodenal ulcer ranges in the literature from 41-90% (Fitzgibbons, Dooley, Cihen et al 1988) but it is not uncommon in normal subjects in whom the prevalence ranges from 22 -64%. These high prevalence rates may be overestimates resulting from inclusion of biopsies taken from the pyloroduodenal transition zone (Whitehead 1979). Secondly, a detailed study of 32 patients by James (1964) concluded that 'gastric' epithelium appears when the duodenal acidity is high and it confers resistance to ulceration rather than predisposing towards it. Thirdly, in some studies it has been noted that the incidence of gastric metaplasia in the duodenum of DU subjects was less than 8% (Gad 1989). Fourthly, there have been a few reports which show that in Meckel's diverticulum the heterotopic gastric epithelium can become inflamed and sometimes progresses to ulcer in the absence of *H pylori* . Finally, "duodenitis" remains a controversial entity. The definition of duodenitis has proved to be extremly difficult and a satisfactory definition is impossible. Inatsuchi et al studied 484 patients and found the frequency was 7.2% and they could not recognise any cases of duodenitis which advanced to DU. It is estimated that duodenitis is 3-4 times common in males than females but the duodenal ulcer is nearly of 1:1 ratio (Johnsen, Bernersen, Straume et al 1991). The incidence of *H pylori* in duodenitis (0-33%) is comparatively lower than in DU and DU healed without any improvement of the duodenitis. The presence or absence of duodenitis has no effect on subsequent effect on relapse (Pettengell, Spitael and Simjee 1985). The

basal acid output of subjects with DU was significantly higher than subjects with duodenitis (Collen and Loebenberg 1989). Thus it is unlikely that duodenitis progresses to DU and it does not appear that duodenitis and DU are two different parts of the same spectrum.

#### 5.4.2.4 *H pylori* and gastric emptying

Wyatt et al believed that development of gastric metaplasia in the duodenum requires an increased load of acid delivered to the duodenum, either by rapid emptying or by naturally increased gastric secretion; the present study found no evidence for increased acid output, but has not addressed the subject of gastric emptying.

One study (Masci, Tosi, Colombo et al 1990) had shown that there was no alteration in the gastric emptying in *H pylori* positive subjects compared with *H pylori* negative subjects. But in DU there is an increased load of acid to duodenum (Roxburgh 1990); he showed that pyloric loss is proportional to the parietal cell mass but demonstrated that it is not related to the presence of DU as such but only to increased gastric secretory capacity.

#### 5.4.2.5 Risk curve and *Helicobacter pylori*

I believe that DU belongs to a heterogeneous group of disorders with multiple aetiological factors. Before the recent

interest in *H pylori*, the straight evidence for an aetiological factor was that for gastric hyperacidity. My study demonstrates that *H pylori* is associated not with an increase of maximal gastric secretion but with a decrease. Under these circumstances, it is impossible to postulate an aetiological link between *H pylori* and DU, that acts in a way that involves hypersecretion of acid.

There is, however, one way that the hyposecretion in association with *H pylori* might be linked with duodenal ulcer. Hobsley and Whitfield (1987) used the properties of Gaussian distribution curves for the control and DU population, to deduce a risk curve which showed that at low levels of secretion, factors other than hyperacidity must play a role in the aetiology of DU. If *H pylori* is a factor in the pathogenesis of DU, one would expect to find that DU subjects with lower rather than higher secretion rates would have a greater rather than a lower incidence of *H pylori* positivity.

At first the results obtained suggested that this hypothesis might be correct: it was indeed the case that *H pylori* positive DU negative patients had a significantly lower gastric secretion than *H pylori* negative patients. If one accepts that *H pylori* is summating with maximal gastric secretion to produce duodenal ulcer, then my finding of low gastric secretion in *H pylori* positive DU could fit very well with this hypothesis. However, there is no reason why *H pylori* is also associated with low gastric secretion in NUD subjects. In other words, it seems likely that there is a true association between *H pylori* and reduced maximal gastric secretion, manifest not only in the DU but also in the NUD subjects. Thus the summation hypothesis cannot be true.

In the range of standardised Vg of 270-321ml there was an overlap between the DU and NUD groups consisting of 6 DU

and 6 NUD subjects. All 6 of the DU, but also 4 of the NUD, were *H pylori* positive . Subdividing the DU group into those lying within the normal range previously reported from this department, that is below 425ml, and those lying above this range and therefore statistically hypersecretors, there were 23 in the normal range and 8 hypersecretors. The *H pylori* positive subjects were distributed as 17 out of the 23 in the normal range (74%) and 4 out of 8 in the hypersecretors (50%) (Fig. 22). This difference is not significant. If one accepts that *H pylori* reduces gastric secretion by 17% and increases the secretion values of the *H pylori* positive DU patients approximately by 17%, they now split as 11 hypersecretors and 10 hyposecretors (Fig. 23). This emphasise the fact that *H pylori* is not apparently summating with low gastric secretion to produce a DU. The fact that NUD subjects with *H pylori* do not have ulcers also lends support to the above view that it is not a risk factor for the aetiology of DU.

The patients in the DU and NUD groups who had overlapping gastric secretion showed no evidence of any predilection of the organism for the DU group. Again, this argues against an aetiological role for *H pylori* but I accept that the numbers were small.

5.4.2.6 Is *H pylori* associated with DU?

In my study I found that there was no significant difference in the distribution of *H pylori* among the dyspeptic patients, except those with oesophagitis. Many investigators noted similar incidences, but many others found that the incidence of *H pylori* was more than 90% (even up to 100%) in DU.

The incidence of DU in the Aborigines of North Autralia is virtually zero and they have a very low incidence of *H pylori* (Dwyer, Sun, Kaldor et al 1988). This finding is consistent with the hypothesis that *H pylori* is an important factor in the pathogenesis of DU. However the incidence of *H pylori* in South-West Indians in USA is approximately, 58% but the incidence of DU is very low and not one subject had a DU in the 53 cases studied (Gogel, Crook, Merlin et al et al 1989). Also it is noted that in the closed community of monks in Tibet the DU incidence is very high (more than 3 times that of developed countries) but that of *H pylori* is similar to that in the developed countries (Katelaris, Tippet, Brennan et al et al 1991). Moreover, in South America and in developing countries, especially among people of low socio-economic status and institutionalised subjects, the incidence of *H pylori* is very high, nearly 90%, but the incidence of DU is no higher than reported in the Western countries. In Singapore / Malaysia the prevalence of DU in Chinese is greater than the Indians (Ti 1983) but the prevalence of *H pylori* infection is higher in Indians than Chinese. Also, Parshu (1975) noted that Indians of Fiji appeared to have a rate of DU at least twice that in ethnic Fijians; but Beg and co-workers (1988) have shown *H pylori* is present with equal frequency in both Fijian and Indian patients with DU. These findings are in sharp contrast to Dwyer's finding.

Only a small subset of individuals with *H pylori* gastritis develop DU. Some epidemiological studies showed that different ethnic groups living in the same area have different rates of prevalence of DU, even though the prevalence of *H pylori* is the same. Other studies showed that the prevalence of DU is higher in some races in whom the *H pylori* infection rate is lesser than another ethnic group in whom the prevalence rate of DU is less, in spite of the fact they live in same area.

The prevalence of *H pylori* increases with age but DU is not predominantly a disease of the elderly. DU is more common among the smokers while the prevalence rate of *H pylori* is similar between smokers and non smokers. *H pylori* has no gender predilection, and at first sight this fact fits neatly with the present situation for DU which in Western countries show little predilection for gender. However, it should be recalled that DU used to show a marked predilection for males and the recent lowering of the imbalance may well be associated with the change in smoking habits - females now smoke almost as commonly as males. Indeed, in countries where females do not smoke the prevalence of DU among males is very high even though the prevalence of *H pylori* is the same in both sexes. Again, there is a strong association between non-secretor blood group O and DU but *H pylori* was found equally commonly in secretors and in non-secretors as well as in each of the blood groups (Mohiuddin, Rhodes and Rhodes (1989).

One study which followed up, for 12 to 28 months, patients with untreated ACG due to *H pylori* found no spontaneous disappearance of *H pylori* nor anyone who developed DU.

Thus, the ability to dissociate between the presence of *H pylori* and the presence of DU suggests that *H pylori* is neither a necessary nor a sufficient factor in the pathogenisis of DU.

5.4.2.7 *H pylori* and Treatment, Eradication & Relapse

Many studies have shown both that ulcers have healed even when the *H pylori* had not been cleared and that ulcers had not healed when the *H pylori* had been eradicated (Chandra-Mam

1990, Jia, Hu and He 1990, Wang, Chen, Jan et al 1990). Thus healing of DU is not necessarily influenced by the presence of *H pylori*. My data are consistent with these findings. There is usually a significant reduction in *H pylori* colonisation after surgical treatment and ulcer healing (Steer 1988). At the same time Petkaneshov, Mitova, Michova et al (1990) showed that proximal gastric vagotomy as well as truncal vagotomy with pyloroplasty do not influence the incidence rate of *H pylori* gastritis.

Over the last 7 years several studies have shown that the relapse rate of DU is lower in those initially treated with colloidal bismuth subcitrate (CBS) than those healed with H2 antagonists. This finding was attributed to the antibacterial effect of CBS, nevertheless one should bear in mind that CBS has many ulcer healing properties which may be the reason that patients treated with CBS have less recurrence of DU. Lately, when the antibiotics which are bactericidal (metronidazole, tetracycline, amoxycillin) to *H pylori* were used to treat DU, many investigators found that the recurrence rate was low if the *H pylori* was eradicated.

It is difficult to be certain how these findings should be interpreted. If on further assessment of this zone of conflicting evidence, it was to be established that the recurrence rate of ulcers healed with antibiotics is lower than that of ulcers healed with, say, $H_2$- antagonists, the conclusion will probably have to be that the phenomenon is due not to the effect of the antibiotics on *H pylori* but to some effect that promotes the healing of the ulcers.

# *CHAPTER 6*

# *CONCLUSIONS*

# 6 CONCLUSIONS:

(a) *H pylori* was found in the antrum of dyspeptic subjects with the same frequency whether the subjects had a duodenal ulcer (DU) or no endoscopically diagnosable lesion (NUD) or any other diagnosable lesions except reflux oesophagitis. It was much less common in subjects with reflux oesophagitis.

(b) Smoking was commoner in DU than in NUD.

(c) The average age of DU in this study was less than the average age of subjects with *H pylori* infection.

(d) The DU subjects secreted more acid than NUD subjects.

(e) The presence of *H pylori* was associated with a smaller maximal gastric secretion in both DU and NUD groups.

(f) Gastric secretion studies in which corrections had been made for pyloric loss and duodenogastric reflux, and values had been standardised for height were less likely to miss valuable information.

(g) There was a positive correlation between smokers and gastric gastric secretion in *H pylori*-negative subjects, but not in *H pylori*-positve subjects.

(h) I have failed to find any link between *H pylori* and DU.

I accept that my study pertains only to the duodenal ulcer group in contrast with the group in whom no disease could be diagnosed from endoscopic appearance. However, it is a point to note that only 10% of the population has an absence of dyspepsia, normal endoscopic findings, and no microscopic abnormalities (Johnsen et al 1991). This makes it difficult to get adequate numbers of control subjects to perform a study similar to mine; until such time the fascinating saga of *Helicobacter pylori* will continue.

# *REFERENCES*

**REFERENCES:**


Amdrup-E and Jensen-HE 1970
Selective vagotomy of the parietal cell mass preserving innervation of the undrained antrum: a preliminary report of results in patients with duodenal ulcer.
Gastroenterology. 59: 522-527.

Aird-I, Bentall-HH, Mehigan-JA, and Roberts-JAF. 1954.
The blood groups in relation to peptic ulceration and carcinoma of colon, rectum, heart and bronchus.
Br Med J 2 : 315-321.

Allen-A. 1981.
Structure and function of gastrointestinal mucus.
In Jhonson (ed.) Physiology of the gastrointestinal Tract. Raven Press, NewYork. (21) : p.617-639.

Andreica-V, Dummitrascu-D, Suciu-A, Veresezen-A, Draghici-A, Pascu-O and Suciu-M. 1990
Biochemicalchanges in the gastric juice of patients with *Campylobacter pylori* infection treated with metronidazole.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PD 127.

Andersen-LP, Holck-S, Povlsen-CO, Elsborg-L, and Justesen-T. 1987.
*Campylobacter pyloridis* in peptic ulcer disease. I. Gastric and duodenal infection caused by C. pyloridis: histopathologic and microbiologic findings.
Scand J Gastroenterol. 2 22(2): 219-224

Anderson-H, Dotevall-G, Lingaas-H, and Walan-A. 1970.
Correlation between radiological findings in the stomach and duodenum and gastric secretion in patients with peptic ulcer disease.
Scand J Gastroenterol. 5 : 123-125.

Andrews-T, Borody-T, Brandy-J, Lenne-J and Devine-M. 1991.
Comparison of gastric ammonia / pH levels: DU vs NUD.
Gastroenterology 1991; (5 Pt2): A25.

Arvind-AS, Cook-RS, Tabaqchali-S, and Farthing-MJ. 1988.
One-minute endoscopy room test for *Campylobacter pylori* [letter]. Lancet. 1 (8587) : 704.

Aubery-DA 1970
The effects on human gastric secretion of prolonged continuous intravenous infusions of maximal and supramaximal doses of histamine phosphate and pentagastrin.
Gut 11: 392-394.

Barber-M, and Franklin-RH. 1946.
Bacteriology of stomach and duodenum in cases of peptic ulcer and gastric carcinoma.
Br Med J 1 : 951-953.

Barer-MR, Elliott-TS, Berkeley-D, Thomas-JE, and Eastham-EJ. 1988. Cytopathic effects of *Campylobacter pylori urease* [letter].
J Clin Pathol. 41 (5) : 597.

Baron-JH. 1969.
Lean body mass, gastric acid and peptic ulcer.
Gut 10 : 637-642.

Baron-JH. 1972.
Aetiology.
In Westell-C (ed). Chronic duodenal ulcer. London: Butterworth. pp. 19-52.

Baron-JH, and Spencer-J. 1976.
Facts and heresises about vagotomy.
Surg Clin N Am 56 : 1297-1312.

Barthel-JS, Westblom-TU, Havey-AD; Gonzalez-F, and Everett-ED. 1988.
Gastritis and *Campylobacter pylori* in healthy, asymptomatic volunteers.
Arch Intern Med. 148 (5) : 1149-51.

Bateson-EM. 1976.
Duodenal ulcer - does it exist in Australian Aborigines?
Aust N Z J Med 6 : 545.

Bateson-EM. 1980.
Duodenal ulcer and migrants.
Aust Radiol 24 : 131-135.

Beaumont-W. 1883.
Experiments and observations on the gastric juice and the physiology of digestion.
Harvard University Press, Boston.

Beg-F, Oldmeadow-M, Morris-A, Miller-M, and Nicholson-G. 1988.
*Campylobacter pylori* infection in patients undergoing endoscopy in Fiji.
N Z Med J. 101 (842) : 140-1.

Bell-GD, Weil-J, Harrison-G, Morden-A, Jones-PH, Gant-PW, Trowell-JE, Yoong-AK, Daneshmend-TK, and Logan-RF. 1987.
14C-urea breath analysis, a non-invasive test for *Campylobacter pylori* in the stomach [letter] .
Lancet.1987 Jun 13; i (8546): 1367-81.

Bendett-RJ, Fritz-HL, and Donaldson-RM. 1963.
Gastric acid secretion after parenterally and intragstrically adminstered histamine in patients with portacaval shunt.
N Eng J Med 268 : 511-516.

Berkowicz-J, and Lee-A. 1987.
Person-to-person transmission of *Campylobacter pylori* [letter].
Lancet. 2 (8560) : 680-1.

Biasco-G, Miglioli-M, Barbara-L, CorinaldesiR, and Di Febo-G. 1989. Omeprazole, *Helicobacter pylori*, gastritis and duodenal ulcer. Lancet ii : 1403.

Bidder-FW, and Schmidt-K. 1852.
Die Verdauungssaefte und der Stoffwechsel eine physiologisch-chemische Untersuchung. Mittau and Leipzing : 44.

Bizzozero-G. 1893.
Uber die schlauchformigen Drusen des Magendarmkanals und die Bezienhungren ihres Epithels zu dem Oberflachenepithel der Schleimhaut. Arch F Mikr Anat 42 : 82.

Blair-III-JA, Feldman-M, Barnett-C, Walsh-JH, and Richardson-CT. 1987. Detailed comparison of basal and food stimulated gastric acid secretion rates and serum gastrin concentrations in duodenal ulcer patients and normal subjects.
J Clin Invest 79 : 582-586.

Blomberg-B, Jarnerot-G, Kjellander-J, Danielsson-D, and Kraaz-W. 1988. Prevalence of *Campylobacter pylori* in an unselected Swedish population of patients referred for gastroscopy. Scand-J-Gastroenterol. 23 (3) : 358-62.

Bloom-DS, Jacobsen-ED, and Grossman-MI. 1967.
Validation of dilution indicators in the stomach.
Gastroenterology 52 : 205-210.

Bode-G, Malfertheiner-P, and Ditschuneit-H. 1988.
Pathogenetic implications of ultrastructural findings in *Campylobacter pylori* related gastroduodenal disease.
Scand J Gastroenterol. 142 (23) : 25-39.

Boerhaave-H. 1744.
Praelectiones Academiae in Proprias Institiones rei Medcae. 6 : 388 para 1138 Amstelaedami.

Bonnevie-O. 1975
The incidence of duodenal ulcer in Copenhagen County.
Scan J Gastroenterol

Bonnevie-O. 1985. Changing demographics of peptic ulcer disease.
Dig Dis Sci. 30 (11 uppl) : 8S-14S.

Booth-L, Holdstock-G, MacBride-H, Hawtin-P, Gibson-JR, Ireland-A, Bamforth-J, DuBoulay-CE, Lloyd-RS, and Pearson-AD. 1986.
Clinical importance of *Campylobacter pyloridis* and associated serum IgG and IgA antibody responses in patients undergoing upper gastrointestinal endoscopy.
J Clin Pathol. 39 (2) : 215-9.

Borody-TJ, Cole-P, Noonan-S, Morgan-A, Lenne-J, Hyland-L, Brandl-S, Borody-EG, and George-LL. 1989.
Recurrence of duodenal ulcer and *Campylobacter pylori* infection after eradication.
Med J Aust. 151 (8) : 431-5.

Borsch-G, Mai-U, and Muller-KM. 1988.
Monotherapy or polychemotherapy in the treatment of *Campylobacter pylori*-related gastroduodenal disease.
Scand J Gastroenterol. 142 (23) : 101-6.

Bouchier-IAD. 1981.
Peptic ulcer: The changing scene.
Scott Med J 26 : 297-302.

Brady-III-CE, Hadfield-TL, Hyatt-JR, and Utts-SJ. 1988.
Acid secretion and serum gastrin levels in individuals with *Campylobacter pylori.*
Gastroenterology. 94 (4) : 923-7.

Braga-LL, Marshall-BJ, Moreno-H, Menzes-D, McCallum-RW, and Guerrant-Rl. 1989.
*Helicobacter pylori* and hypochlohydria in asymtomatic Brazillian men.
Gastroenterol 93 (5) : A274.

Britt-DP, Barakat-MH, Tungekar-MF, Painchaud-SM, Adlouni-M, Kern-K, and Malhas-L. 199.
*Helicobacter pylori* in dyspeptic patients in Kuwait. : 987-991.

Brodie-BC. 1814.
Experiments and observations of the influences of the nerves of the eighth pair on the secretions of the stomach.
Phil Trans R Soc London. 104: 102-106

Brown-RC, Langman-MJS, and Lambert-PM. 1976.
Hospital admissions for peptic ulcer during 1958-1972.
Br Med J 1 : 35.

Buck-GE, Gourley-WK, Lee-WK, Subramanyam-K, Latimer-JM, and DiNuzzo-AR. 1986.
Relation of *Campylobacter pyloridis* to gastritis and peptic ulcer.
J Infect Dis. 153 (4) : 664-9.

Burnett-RA, Brown-RA and Findlay-J. 1987.
Cresyl fast violet staining method for *Campylobacter*- like organisms.
J Clin Pathol. 40: 353.

Capper-WM, Airth-GR, and Kilby-JO. 1966.
A test for pyloric regurgitation.
Lancet ii : 621-623.

Card-WI, and Marks-IN. 1960.
The relationship between the acid output of the stomach following "maximal" histamine stimulation and the parietal cell mass.
Clin Sci 19 : 147-163.

Carnot-P, Koskowski-W, and Libert-E. 1922.
Action de l'histamine sur les sucs digestives chez l'home.
Competes rendus hebdomadaires seances et de la societe de biologie et des filiales 86 : 670-673.

Caselli-M, Trevisani-L, Aleotti-A, Bovolenta-MR, and Stabellini-G. 1989.
Gastric metaplasia in duodenal bulb and *Campylobacter*-like organisms in development of duodenal ulcer.
Dig Dis Sci. 34 (9) : 1374-8.

Cave-DR, and Vargas-M. 1989.
Effect of *Campylobacter pylori* protein on acid secretion by parietal cells.
Lancet : 187-189.

Chandra-Mam. 1990.
*Campylobacter pylori* in gastroduodenal diseases
Clinical aspect and effect of treatment.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract I) : PD111.

Cheng-EH, Bermanski-P, Silversmith-M, Valenstein-P, and Kawanishi-H. 1989.
Prevalence of *Campylobacter pylori* in Esophagitis, gastritis and duodenal disease.
Arch Inten Med 149 : 1373-1375.

Cheng-FY, Lam-SK, and Ong-GB. 1977.
Maximum acid output to graded doses of pentagastrin and its relation to parietal cell mass in Chinese pateients with duodenal ulcer.
Gut 18 : 827-832.

Chittajallu-RS, Neithercut-WD, MacDonald-AMI, and McColl-KEL. 1991.
Effect of increasing *Helicobacter pylori* ammonia production by urea infusion on plasma gastrin concentrations.
Gut 32 : 21-24.

Chodos-JE, Dworkin-BM, Smith-F, Van-Horn-K, Weiss-L, and Rosenthal-WS. 1988.
*Campylobacter pylori* and gastroduodenal disease: a prospective endoscopic study and comparison of diagnostic tests.
Am-J-Gastroenterol. 83 (11) : 1226-30.

Christiansen-J. 1912.
Untersuchungen uber freie und gebundenen
Salzaure im Magneinhalt. I, Bestimmung freir Salzaure im
Mageninhalt
Biochem Z 46: 24-49.

Clark-CA, Edwards-JW, Haddock-DRW, Howel-Edvans-AW,
McConnell-RB, and Sheppard-PM. 1956.
ABO blood groups and secretor charecter in duodenal ulcer.
Br M J 2 : 725-731.

Clausen-MR, Franzman-MB, Holst-C, Sorensen-TIA,
Christoffersen-P, Matzen-P, and Krag-E. 1990.
Longitudinal study of the influence of *Campylobacter pylori* on
current risk of duodenal ulcer (Abstract).
The World Congresses of Gastroenterology, Sydney, Australia
(26-31 August) (Abstract I) : PP934.

Codivilla-A. 1889.
Considerations on 40 cases of gastric surgery.
Bull Med Sci 9: 651-717.

Coggon-D, Lambert-P, and Langman-MJS. 1981.
Twenty years of hospital admissions for peptic ulcer in England
and Wales. Lancet 1 : 1302.

Coghlan-JG, Gilligan-D, Humphries-H, McKenna-D, Dooley-C,
Sweeney-E, Keane-C, and O'Morain-C. 1987.
*Campylobacter pylori* and recurrence of duodenal ulcers--a
12-month follow-up study.
Lancet. 2 (8568) : 1109-11.

Collen-MJ, and Loebenberg-MJ. 1989.
Basal gastric acid secretion in nonulcer dyspepsia with or
without duodenitis.
Dig Dis Sci. 32 (2) : 246-50.

Correa-M, Fox-J, Fontham-E, Ruiz-B, Lin-Y, Taylor-N,
MacKinley-D, de Lima-E, Portilla_h and Zarama-G. 1990.
*Helicobacter pylori* and gastric carcinoma.
Cancer 66: 2569-2574.

Crawford-G, and Hobsley-M. 1968.
Spectrometric estimation in gastric juice in the presence of
blood.
Biochme J 107 : 25A.

Davenport-WH, Warner-HA, and Code-CF. 1964.
Functional significance of gastric mucosal barrier to sodium.
Gastroenterology 47 : 142-152.

Dean-HP. 1894.
A case of perforation of a chronic ulcer of the duodenum
successfully treated by excision.
Br Med J 1: 1014-1015

Defize-J, Goldie-J, and Hunt-RH. 1988.
Effect of *Campylobacter pylori* on acid production by isolated guinea pig parietal cells.
Gut 29 : A1435.

Dent-JC, McNulty-CA, Uff-JS, Gear-MW, and Wilkinson-SP. 1988. *Campylobacter pylori* urease: a new serological test [letter].
Lancet. 1 (8592) : 1002

Doenges-JL. 1939.
Spirochetes in the glands of *Macacus* rhesus and of man without related disease. Arch Pathol. 27 : 469-77.

Domellof-L, Reddy-BS, and Weisberger-JH. 1980.
Microflora and deconjugation of bile acids in alkaline reflux after partial reflux. Am J Surg 140 (2) : 291-295.

Dooley-CP, Cohen-H, Fitzgibbons-PL, Bauer-M; Appleman-MD, Perez-Perez-GI, and Blaser-MJ. 1989.
Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons.
N Engl J Med. 321 (23) : 1562-6.

Dragstedt-LR, and Owens-FM Jr. 1943.
Supra-diaphragmatic section of the vagus nerve in the treatment of duodenal ulcer.
Pro Soc Exp Biol Med (N Y) 53 : 152-154.

Dwyer-B, Sun-NX, Kaldor-J, Tee-W, Lambert-J, Luppino-M, and Flannery-G. 1988.
Antibody response to *Campylobacter pylori* in an ethnic group lacking peptic ulceration. Scand J Infect Dis. 20 (1) : 63-8.

Editorial. 1989.
*Campylobacter pylori* becomes Helicobacter pylori.
Lancet ii (8670) : 1019-1020.

Einhorn-M. 1909. A new method for catheterising the pylorus and duodenum.
Med Rec 76 : 595-599.

Elashoff-JD and Grossman-MT. 1980.
Trends in hospital admissions and death rates for peptic ulcer in the United States from 1970 to 1978.
Gastroenterology. 78: 280-285

Eldridge-J, Lessells-AM and Jones-DM. 1984
Antibody to spiral organisms on gastric mucosa.
Lancet. i: 1237.

Ellis-A, and Woodrow-JC. 1979.
HLA and duodenal ulcer.
Gut 20 : 760.

Engstrand-L, Pahlson-C, Gustavsson-S, and Schwan-A. 1986.
Monoclonal antibodies for rapid identification of *Campylobacter pyloridis* [letter].
Lancet i (8520) : 1402-3.

Engstrand-L, Pahlson-C, Schwan-A, and Gustavsson-S. 1988.
Monoclonal antibodies for detection of *Campylobacter pylori* in biopsy smears and frozen sections.
Scand J Gastroenterol Suppl. 142 (23): 50-52

Evans Jr-DJ, Graham-DY, Lew-GM, Evans-DG and Malaty-HM. 1991.
Can one use the results of serology testing to monitor the results of therapy of *Helicobacter pylori*?
Gastroenterology 100 (5 Pt 2) A62.

Ewald-CA. 1892.
Lectures on Diseases of the Digestive System ii, (Trans by R Saunby).
New Sydenham society, London.

Faber-RG, Russell-RCG, Royston-CMS, Whitfield-P, and Hobsley-M. 1974.
Duodenal reflux during insulin-stimulated secretion.
Gut 15 : 880-884.

Faber-RG, and Hobsley-M. 1977.
Basal gastric secretion: reproducibility and relationship with duodenal ulcers.
Gut 18 : 57-63.

Feldman-M. 1987
Bicarbonate, acid and duodenal ulcer.
N Eng J Med. 316 (7): 408.

Ferrero-RL, Hazell-SL, and Lee-A. 1988.
The urease enzymes of *Campylobacter pylori* and a related bacterium.
J Med Microbiol. 27 (1) : 33-40.

Fich-A, Talley-NJ et-al. 1990.
Does *Helicobacter pylori* colonise gastric mucosa of Mickel's diverticulum.
Mayo Clin Proc 65 : 187-191.

Fich-A, Talley-NJ, Shorter-RG, and Phillips-SF. 1991.
Zollinger-Ellison Syndrome: Relation to *Helicobacter pylori* - associated chronic gastritis and gastric acid secretion.
Dig Dis Sci 36 (1) : 10-14.

Fiddian-Green-RG, Parkin-JV, Faber-RG, Russell-RCG, Whitfield-PF and Hobsley-M. 1979.
The quantification in human gastric juice of duodenogastric reflux by sodium output and bile lablling using idocyanine green.
Klin Wochenschr 57: 815-824.

Figura-RL, Guglielmetti-P, Rossolini-A, Barberi-A, and Cusi-G. 1989.
Cytotoxin production by *Campylobacter pylori* strains isolated from patients with peptic ulcers and patients with chronic gastritis only.
J Clin Microbiol 27 (1) : 225-226.

Findlay-J, Prescott-T, and Sircus-W. 1972.
A comparative evaluation of the water recovery test and fluoroscopic screening in positioning a nasogastric tube during gastric secretory studies.
Br med J 4 : 458-461.

Fisher-R, and Cohen-S. 1973.
Physiological characteristics of the human pyloric sphincter.
Gastroenterology 64 : 67-75.

Fitzgibbons-PL, Dooley-CP, Cohen-H, and Appleman-MD. 1988.
Prevalence of gastric metaplasia, inflammation, and *Campylobacter pylori* in the duodenum of members of a normal population.
Am J Clin Pathol. 90 (6) : 711-4.

Fordtran-JS. 1979.
Psycological factors in duodenal ulcer.
Pract Gastroenterol 3 : 24.

Forman-D, Sitas-F, Newell-Dg, Stacey-AR, Boreham-J, Peto-R, Campbell-TC, Li-J, and Cen-J. 1990.
Geographic association of *Helicobacter pylori* antibody prevalence and gastric cancer mortality in rural China.
Int J Cancer 608-611

Fox-JG, Correa-P, Taylor-NS, Zavala-D, Fontham-E, Janney-F, Rodriguez-E, Hunter-F, and Diavolitsis-S. 1989.
*Campylobacter pylori* - associated gastritis and immune response in a population at increased risk of gastric carcinaoma.
Am J Gastroenterol 84 (7) : 775-781.

Frankson-C. 1948.
Selective abdominal vagotomy.
Acta Chir Scand 96: 409-412.

Freedberg-AS, and Barron-LE. 1940.
The presence of spirochetes in human gastric mucosa.
Am J Digest Dis 7 : 443-5.

Freston-MS and Freston-JW. 1990.
Peptic ulcers in the elderly: unique features and management.
Geriatrics. 45 (1): 39-42, 45.

Friedman-GD, Siegelaub-AB, and Seltzer-CC. 1974. Cigarettes, alcohol, coffee and peptic ulcer.
N Eng J Med 290 : 469.

Frommer-DJ, Carrick-J, Lee-A, and Hazell-SL. 1988. Acute presentation of *Campylobacter pylori* gastritis.
Am J Gastroenterol. 83 (10) : 1168-71.

Fronmuller-F. 1886.
Operation der pylorusstenose Erlangung der Doktorwurde der median Fakultat der Universitat Erlangen.

Gad-A, Hradsky-M, Furugard-K, and Malmodin-B. 1989.
*Campylobacter pylori* and gastroduodenal ulcer disease. A prospective study in a Swedish population.
Scand J Gastroenterol 167 (Suppl) : 81-5.

Gardham-JRC, Hassan-MA, and Hobsley-M. 1968.
The recognition and measurement of pyloric loss in aspirated gastric juice.
Br J Surg 55 (abstract) SRS : 865 (37).

Gardham-JRC, and Hobsley-m. 1970.
The electrolytes of alkaline human gastric juice.
Clinical Science 39 : 77-87.

Geis-G, Leying-H, Suerbaum-S, Mai-U, and Opferkuch-W. 1989.
Ultrastructure and chemical analysis of of *Campylobacter pylori* flagella.
J Clin Microbiol 27 (3) : 436-441.

Gledhill-T, Leicester-RJ, Addis-B, Lightfoot-N, Barnard-J, Viney-N, Darkin-D, and Hunt-RH. 1985.
Epidemic hypochlohydria.
Br Med J 290 : 1383-1386.

Glupczynski-Y, Thibaumont-F, and Labbe-M. 1988.
The effect of delayed processing of gastric specimens on the validity of *Campylobacter pylori* and lack of relation between bacterial colony count and rapid urease test.
Proc Brussels. 132.

Glupczynski-Y, Burette-A, DeKoster-E et al. 1990
Metronidazole resistance *Helicobacter pylori.*
Lancet. 335; i; 976.

Glupczynski-Y, Burette-A, Nyst-JF, De-Prez-C, De-Koster-E, and Deltenre-M. 1989.
*Campylobacter pylori*-associated gastritis: attempts to eradicate the bacteria by various antibiotics and anti-ulcer regimens.
Acta Gastroenterol Belg. 51 (4-5) : 329-37.

Gnarpe-H, Unge-P, Blomqvist-C, and Makitalo-S. 1988.
*Campylobacter pylori* in Swedish patients referred for gastroscopy.
APMIS. 96 (2) : 128-32.

Goldschmiendt-M, Karnes-WE, and Feldman-M.
Relationship between *Helicobacter pylori* (HP) and secretion / serum gastrin concentration in healthy humans.
Gastroenterol 98 (5) : A50.

Gogel-HK, Crook-L, Merlin-TL, Black-W, Brown-K, Herman-D, Ming-R and Strickland-RG. 1989.
*Campylobacter pylori* in Southwest Indians.
Gastroenterology 98; (5 Pt 2): A173.

Goodwin-CS, Blincow-ED, Warren-JR, Waters-TE, Sanderson-CR, and Easton-L. 1985.
Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa.
J-Clin-Pathol. 38 (10) : 1127-31.

Goodwin-CS, Blake-P, and Blincow-E. 1986.
The minimum inhibitory and bactericidal concentrations of antibiotics and anti-ulcer agents against *Campylobacter pyloridis*.
J-Antimicrob-Chemother. 17 (3) : 309-14.

Goodwin-CS, Armstrong-JA, and Marshall-BJ. 1986 .
Campylobacter pyloridis, gastritis, and peptic ulceration.
J-Clin-Pathol. 39 (4) : 353-65.

Goodwin-CS, Collins-MD and Blincow-E. 1986.
The absence of thermoplasmaquinones in *Campylobacter pyloridis*, and its temperature and pH growth range,
Microbios Lett 32: 137-140.

Goodwin-CS. 1988.
Duodenal ulcer, *Campylobacter pylori*, and the "leaking roof" concept.
Lancet. 2 (8626-8627) : 1467-9.

Goodwin-CS, Gordon-A and Burke-V. 1990.
*Helicobacter pylori (Campylobacter pylori)* and duodenal ulcer [Editorial].
Med J Aust 153: 66-67.

Graham-DY, Smith-JL, Alpert-LC, and Yoshimura-HH. 1983.
Epidemic achlohydria is not viral but is caused by *Campylobacter pyloridis*.
Gastroenterology. 92 (5 Pt 2) : 1412.

Graham-DY, Klein-PD, Evans-DJ Jr, Evans-DG, Alpert-LC, Opekun-AR, and Boutton-TW. 1987.
*Campylobacter pylori* detected noninvasively by the 13C-urea breath test.
Lancet. 1 (8543) : 1174-7.

Graham-DY, Klein-PD, Opekun-AR, Boutton-TW, Evans-DJ Jr, Evans-DG, Alpert-LC, Michaletz-PA, Yoshimura-HH, and Adam-E. 1988.
Epidemiology of *Campylobacter pylori* infection: ethnic considerations.
Scand J Gastroenterol (Suppl.) 142 : 9-13.

Graham-DY, Alpert-LC, Smith-JL, and Yoshimura-HH. 1988.
Iatrogenic *Campylobacter pylori* infection is a cause of epidemic achlorhydria.
Am J Gastroenterol. 83 (9) : 974-80.

Graham-DY, Klein-PD, Opekun-AR and Boutton-TW. 1988
Effect of age on the frequency of active *Campylobacter pylori* infection diagnosed by the [13C] urea breath test in normal subjects and patients with peptic ulcer.
J Infect Dis 157: 777-789.

Graham-DY, Klein-PD, Opekun-AR, Smith-KE, Polasani-RR, Evans-DJ Jr, Evans-DG, Alpert-LC, Michaletz-PA, Yoshimura-HH, et-al. 1989.
In vivo susceptibility of *Campylobacter pylori*.
Am J Gastroenterol. 84 (3) : 233-8.

Graham-DY. 1989a.
*Campylobacter pylori* as a pathogenetic factor in duodenal ulcer: the case for.
Scand J Gastroenterol-Suppl. 160 : 46-52.

Graham-DY. 1989b.
*Campylobacter pylori* and peptic ulcer disease.
Gastroenterology. 96 (2 Pt 2 Suppl) : 615-25.

Graham-DY, Opekun-AR, Lew-GM, Klien-PD, and Walsh-JH. 1989.
*Helicobacter pylori* infection and gastrin release in duodenal ulcer.
Gatroenterology. 98 (5 Pt 2) : A52.

Graham-DY, Opekun-A, Lew-GM, Evans-DJ Jr, Klein-PD, and Evans-DG. 1990.
Ablation of exaggerated meal-stimulated gastrin release in duodenal ulcer patients after clearance of *Helicobacter* (*Campylobacter*) pylori infection.
Am J Gastroenterol. 85 (4) : 394-8.

Graham-DY, Opekun-A, Lew-GM, Klein-PD, and Walsh-JH. 1991.
*Helicobacter pylori*-associated exaggerated gastrin release in duodenal ulcer patients.
Gastroenterology. 100 : 1571-1575.

Gray-SF, Wyatt-JI, and Rathbone-BJ. 1986.
Simplified techniques for identifying *Campylobacter pyloridis* [letter] .
J Clin Pathol. 39 (11) : 1279.

Greenberg-RE, and Bank-S. 1990.
The prevalence of *Helicobacter pylori* in non-ulcer dyspepsia.
Arch Intern Med 150 : 2053-2055.

Greig-MA, Neithercut-WD, Hossack-M, and McColl-KEL. 1991.
Harnessing of urease activity of *Helicobacter pylori* to induce self-destruction of the bacterium. J Clin Pathol 44 : 157-159.

Griffith-CA and Harkins-HN. 1957.
Partial gastric vagotomy: an experimental study.
Gastroenterology. 32: 96-102.

Grossman-MI. 1980.
Peptic ulcer.
In Epidemiology of Chronic Digestive Disease.
Year Book Medical Publishers, Inc. p 9.

Hacki-WH, Staub-P, Jost-R, Ch. Eberle, Stamm-B, and Wust-J. 1988.
*Campylobacter pylori* infection of the antrum does not affect fasting plasma levels of gastrin, somatostatin, pancreatic polypeptide and neurotensin in man.
First Meeting of the European *Campylobacter* Study Group.
Workshop Gastroduodenal Pathology & *Campylobacter pylori.*
Bordeaux, France. (October 7-8) : P165.

Halpern-BN. 1947.
Reserches sur un nouvelle serie chimique de corps doues de proprietes antihistaminiques et anti anaphytactiques: les derives de la thiodiphenylalanine.
Arch Int Pharmacodyn Ther 74 : 314-333.

Hassan-MA, Gardham JRC, and Hobsley-M. 1969.
Evaluation of errors in gastric secretion studies.
Brit J Surg 56 (5) : 384 (20).

Hassan-MA, and Hobsley-M. 1970.
Positioning of subjects and of nasogastric tube during a gastric secretion test.
Br Med J 1 : 458-460.

Hassan-MA, and Hobsley-H. 1971.
The accurate assessment of maximal secretion in control subjects and patients with duodenal ulcer.
Brit J Surg 58 (3) : 171-179.

Hazell-SL, Borody-TJ, Gal-A and Lee-A. 1987.
*Campylobacter pyloridis*-gastritis I: Detection of urease as a marker of bacterial colonozation and gastritis.
Am J Gastroenterol. 82 (4): 292-296.

Hazell-SL, Lee-A, Brady-L, and Hennessy-W. 1986.
*Campylobacter pyloridis* and gastritis: association with intercellular spaces and adaptation to an environment of mucus as import ant factors in colonization of the gastric epithelium. .
J-Infect-Dis. 153 (4) : 658-63.

Heading-RC. 1984. Role of motility in the upper digestive tract.
Scand J Gastroenterol 19 (96) : 39-44.

Hersey-SJ, Miller-M, May-D, and Norris-SH. 1983.
Lack of interaction between acid and pepsinogen secretion in isolated gastric glands.
Am J Physiol. 245 : G775.

Hirschl-AM, Hentschel-E, Schutze-K, Nemec-H, Potzi-R, Gangl-A, Weiss-W, Pletschette-M, Stanek-G, and Rotter-ML. 1988.
The efficacy of antimicrobial treatment in *Campylobacter pylori*-associated gastritis and duodenal ulcer.
Scand J Gastroenterol (Suppl.) 76-81

Ho-J, Hui-MW, Ng-I, and Lam-SK. 1989.
Natural history of *Campylobacter pylori* in duodenal ulceration treated with an H2- antagonist.
Aliment Pharmacol Therap 3 : 315-320.

Hobsley-M, and Silen-W. 1969.
Use of an inert marker (phenol red) to improve accuracy in gastric secretion studies.
Gut 10 : 787-795.

Hobsley-M, Gardham-JRC, and Hassan-MA. 1969.
The charcteristics of human alkaline gastric secretion (A study of 30 patients with pepticulceration).
In: Grgegor-O and Riedl-O (Eds.); Modern Gastroenterology. Proceedings. VIIIth International Congress of Gastroenterology.
New York : 151-152.

Hobsley-M, and Silen-W. 1970.
The relation between the rate of production of gastric juice and its electrolyte concentrations.
Clinical Science 39 : 61-75.

Hobsley-M. 1974.
Pyloric reflux: A modification of the two - component hypothesis of gastric secretion.
Clinical Science and Molecular Medicine 47 : 131-141.

Hobsley-M, Whitfield-Pf, Faber-RG, and Parkin-JV. 1975.
Hypersecretion and length of history of duodenal ulceration.
Lancet : 101-110.

Hobsley-M. and Whitfield-PF. 1977.
The electrolyte composition of pure gastric juice.
J Physio 271 : 57-58P.

Hobsley-M, and Whitfield-PF. 1978.
Electrolyte changes in human gastric juice during steady-state secretion.
Journal of Physiology 281 : 39P.

Hobsley-M, and Whitfield-PF. 1987.
The likelihood of a disease in relation to the magnitude of a risk factor: the example of duodenal ulcer.
Theor Surg 2 : 106-109.

Hollander-F. 1931.
Studies in gastric secretion II. A comparison of criteria of acidity used in this investigation.
J Biol Chem 91 : 481-492.

Hollander-F. 1932.
Studies in gastric secretion IV. Variations in chlorine concentration of gastric juice and their significance.
J Biol Chem 97 : 585-604.

Holscher-AH, Bollschweiler-E, Bumm-R and Becker-K. 1990.
Effect of proximal gastric vagotomy (PGV) on gastriv colonisation of *Campylobacter pylori.*
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PD 927..

Hook-Nikanne-J, Sistonen-P, and Kosunen-TU. 1990.
Effect of ABO blood group and secretor status on the frequency of *Helicobacter pylori antibodies.*
Scand J Gastroenterol 25: 815-818

Hopkins-HH and Kapany-NS. 1954.
A flexible fibrescope, using static scanning.
Nature 173: 39-40.

Hugh-TB, Coleman-MJ, McNamara-ME, Norman-JR, and Howell-C. 1984.
Epidemiology of peptic ulcer in Australia. A study based on government statistics in four states.
Med J Aust. 141 (2) : 81-5.

Hugh-TB. 1985.
Peptic ulcer surgery.
Med J Aust. 143 (1) : 37-9.

Hui-WM, Lam-SH, Chau-PY, Ho-J, Lui-I, Lai-CL, Lok-ASF, and NG-MMT. 1987.
Persistance of *Campylobacter pyloridis* despite healing of duodenal ulcer and improvement of accompanying duodenitis and gastritis.
Dig Dis Sci 32 (11) : 1255-1260.

Hui-WM, and Lam-SK. 1989.
Etiology and management of chronic gastritis.
Dig Dis. Sci 7 (1) : 51-60.

Humphreys-H, Bourke-S, Dooley-C, McKenna-D, Power-B, Keane-CT, Sweeney-EC, and O'Morain-C. 1988.
Effect of treatment on *Campylobacter pylori* in peptic disease: a randomised prospective trial.
Gut. 29 (3) : 279-83.

Hunter-J. 1793.
A case of paralysis of the muscles of deglutination cured by artificial mode of conveying food and medicine into the stomach.
Transactions of a Society for the Improvement of Medical and Chirurgical Knowledge. London : p.182.

Imato-I, Shida-Y, Yoshida-M, Fukukita-S, Taguchi-Y ans Suzuki-S. 1990
Gastric juice ammonia and mucin production of the *Helicobacter pylori* infected gastric mucosa.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PP 951.

Inatsuchi-S, Tanaka-M, Treasaki-T, Wakabayasi-H, Kubota-Y, and Watanabe-A. 1990.
Clinical analysis of duodenitis (Abstract).
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract II) : P649.

Itoh-T, Yanagawa-Y, Shingaki-M, Takahashi-M, Kai-A, Ohashi-M and Hamana-G. 1987.
Isolation of *Campylobacter pyloridis* from human gastric mucosa and characterization of the isolates,
Microbiol Immunol 31: 603-614.

Isenberg-JI, Spector-HJ, and Hootkin -LA. 1971.
An apparent exception to Schwarz's dictum 'No acid - No ulcer'.
N Eng J Med 285 : 620.

Isenberg-JI, Grossman-MI, and Maxwell-V. 1975.
Increased sensitivity to stimulation of acid secretion by pentagastrin in duodenal ulcer.
J Clin Invest 55 : 330-337.

Jackson-RG 1948.
Anatomic study of the vagus nerve: with a technique of transabdominal selective gastric vagus resection.
Arch Surg 57: 333-352.

James-AH, and Pickering-GW. 1949.
Role of gastric acidity in pathogenesis of peptic ulcer.
Clin Sci 8 : 181-210.

James-AH. 1964.
Gastric epithelium in the duodenum.
Gut 5 : 285-294.

James-AH and Pickering-GW. 1949
Role of gastric acidity in pathogenesis of peptic ulcer.
Clin Sci 8: 181-121.

Jaworski-W, and Gluzinski-A. 1886.
Experimentall-klinische Untersuchungen uber der Chemissus und Mechanismus der Verdauungsfunction des menschilichen Magens im physiologischen und pathologischen Zustande, nesbst einer methde zur klinischen Prufung der Magenfunction fur diagnostiche und therepeutische Zwecke.
Z Klin Med 11 : 50-98 & 270-293.

Jeyaraj-AP, Tovey-FI and Clark-CG. 1980.
Possible dietary factors in relation to the distribution of duodenal ulcer in India and Bangaladesh.
Gut. 21: 1067-1076.

Jeyaraj-AP, Tovey-FI, Clark-CG, Rees-KR, White-JS, and Lewin-MR. 1987.
The ulcerogenic and protective action of rice and rice fractions in experimental peptic ulceration.
Clin Sci. 72 (4) : 463-6.

Jia-BQ, Hu-FL, and He-CH. 1990.
Ranitidine and antibacterial drugs in the treatment of *Campylobacter pylori* associated duodenal ulcer disease (Abstract).
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract II)

Jiang-SJ, Liu-WZ, Zhang-DZ, Shi-Y, Xiao-SD, Zhang-ZH, and Lu-DY. 1987.
*Campylobacter*-like organisms in chronic gastritis, peptic ulcer, and gastric carcinoma.
Scand J Gastroenterol. 22 (5) : 553-8.

Johnsen-R, Bernersen-B, Straume-B, Forde-OH, Bostad-L, and Burhol-PG. 1991.
Prevalence of endoscopic and histological findings in subjects with and without dyspepsia.
Br Med J 302 : 749-752.

Johnson-BJ, Reed-PI, and Ali-MH. 1986.
*Campylobacter*-like organisms in duodenal and antral endoscopic biopsies: relationship to inflamation.
Gut 27 : 1132-1137.

Johnston-D and Wilkinson-AR 1970.
Highly selective vagotomy without a drainage procedure in the treatment of duodenal ulcer.
Br J Surg 57: 289-296.

Johnston-BJ, Reed-PI, and Ali-MH. 1988.
Prevalence of *Campylobacter pylori* in duodenal and gastric mucosa--relationship to inflammation.
Scand J Gastroenterol Suppl. 142 : 69-75.

Johnston-D, and Jepson-K. 1967.
Use of pentagastrin in a test of gastric acid secretion.
Lancet ii : 585-588.

Johnston-DH, and McGraw-BH. 1958.
Gastric analysis: Evaluation of collection technique.
Gastroenterology. 35 : 512-516.

Jones-DM, Lessells-AM, and Eldrige-J. 1984.
*Campylobacter* like organisms on the gastric mucosa; culture, histological and serological studies.
J Clin Pathol 37 : 1002-6.

Kaldor-J, Tee-W, McCarthy-P, Watson-J, and Dwyer-B. 1985.
Immune response to *Campylobacter pyloridis* in patients with peptic ulceration [letter].
Lancet. 1 (8434) : 921.

Kaldor-J, Tee-W, Nicolacopolous-C, Demirtzoglou-K, Noonan-D, and Dwyer-B. 1986.
Immunoblot confirmation of immune response to *Campylobacter pyloridis* in patients with duodenal ulcers.
Med J Aust. 145 (3-4) : 133-5.

Kang-JY, Guan-R, LaBrooy-SJ, Lim-KP, and Yap-I. 1983.
The influence of sex, race and dialect on peptic ulcer and non-ulcer dyspepsia in Singapore.
Ann Acad Med Singapore. 12 (4) : 527-31.

Kang-JY, and Wee-A. 1991.
*Helicobacter pylori* and gastric acid output in peptic ulcer disease.
Dig Dis Sci 36 (1) : 5-9.

Kasper-G, and Dickgiesser-N. 1984.
Antibiotic sensitivity of *Campylobacter pyloridis*.
Eur J Clin Microbiol 5 : 444.

Katelaris-PH, Tippet-G, Brennan-R, Norbu-P and Farthing-MJG. 1991.
Lack of correlation of dyspepsia with *Helicobacter pylori* infection in a population with high ulcer prevalence.
Gastroenterology 100 (5 - Pt2): 94.

Kathouda-N, and Mouiel-J. 1991.
A new technique of surgical treatment of chronic duodenal ulcer without laparotomy by videocoelioscopy.
Am J Surgery 161 (3) : 361-365.

Kato-C, Kano-A, Sato-K, Sugawara-M, Nakano-O, Sekino-W, Nakamura-Y and Sato-S. 1990.
Indirect markers for *Helicobacter pylori* infection.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PP930.

Katschinski-BD, Logan-RFA, Edmond-M, and Langman-MSJ. 1990.
Duodenal ulcer and refined carbohydrate intake: a case control study assessing dietary fibre and refined sugar intake.
Gut 31 : 993-996.

Kay-AW. 1953.
Effects of large doses of histamine on gastric secretion of HCl.
Br Med J 2 : 77-80.

KeyMed. 1983
History of flexible endoscopy.
Facsmile edition. KeyMed.
Contributors: Salmon-PR, Sivak-MV and Ravenscroft-MM & Swan-CHS.

Kirk-RM. 1986.
Could chronic peptic ulcers be localised areas of acid susceptibility generated by autoimmunity?
Lancet. 1 (8484) : 772-5.

Kirsner-JB, Bock-D, Palmer-WL, Levin-E, and Ford-H. 1956.
Variations in basal gastric secretion in man and the evaluation of gastric secretory stimulants.
Gastroenterology 30 : 779-789.

Koo-J, Ngan-YK and Lam-SK. 1983.
Trends in hospital admission, perforation and motility of peptic ulcer in Hong Kong frm 1970-1980.
Gastroenterology, 84: 1558-1562

Korman-MG, Hansky-J, EavesER and Schmidt-Gt. 1983.
Influence of cigarette smoking on healing and relapse in duodenal ulcer disease.
Gastroenterology. 85: 871-874.

Kornberg-HL and Davies-RE. 1955.
The activity and function of gastric urease in cat.
Biochem J 54: 363-372

Kosunen-TU, Hook-J, Rautelin-HI, and Myllyla-G. 1989.
Age - dependent increase of *Campylobacter pylori* antibodies in blood donors.
Scand J Gastroenterol 24 : 110-114.

Krajden-S, Bohnen-J, Anderson-J, Kempston-J, Fuksa-M, Matlow-A, Marcon-N, Haber-G, Kortan-P, Karmali-M, Corey-P, Petrea-C, Babida-C, and Hayman-S. 1987.
Comparison of selective and nonselective media for recovery of *Campylobacter pylori* from antral biopsies.
J Clin Microbiol. 25 (6) : 1117-8.

Kreuning-J, Bosman-FT, Kuiper-G, Wal-AM v d, and Lideman. 1978.
Gastric and duodenal mucosa in 'healthy' individuals.
J Clin Pathol 31 : 69-77.

Krienitz-W. 1906.
Uber das Aufreten von Mageninhalt bei carcinoma Ventriculi.
Dtsch med Wochenschr 22 : 872.

Kurata-JH, and Haile-BM. 1984.
Epidemiology of peptic ulcer disease.
Clin Gastroenterol. 13 (2) : 289-307.

Kurata-JH, Haile-BM, and Elashoff-JD. 1985.
Sex differences in peptic ulcer disease.
Gastroenterology. 88 (1 Pt 1) : 96-100.

Lam-SK, and Sircus-W. 1975. Studies on duodenal ulcers I. The clinical evidence for the existence of two populations. Q J Med 44 : 369-387.

Lam-SK, Lau-WY, Choi-TK, Lai-CL, Lok-AS, Hui-WM and Choi-SK. 1986
Prostaglandin E1 (misoprostol) overcame the adverse effect of chronic cigarette smokingg on duodenal ulcer healing.
Dig Dis Sci. 31 (2 Suppl.): 68S-74S.

Lambert-T, Megraud-F, Gerbaud-G, and Courvalin-P. 1986.
Susceptibility of *Campylobacter pyloridis* to 20 antimicrobial agents.
Antimicrob-Agents-Chemother. 30 (3) : 510-1.

Langdale-Brown-B, and Haqqanu-MT. 1990.
Acridine orange fluorescence, *Campylobacter pylori*, and chronic gastritis.
Scand J Gasroenterol 25 : 127-133.

Langenberg-ML, Tytgat-GNJ, Schipper-MEI et al. 1984.
*Campylobacter*-like organisms in the stomach of patients and healthy individuals. Lancet i : 1348.

Langenberg-W, Rauws-EA, Houthoff-HJ, Oudbier-JH, van-Bohemen-CG, Tytgat-GN, and Rietra-PJ. 1988.
Follow-up study of individuals with untreated *Campylobacter pylori*-associated gastritis and of noninfected persons with non-ulcer dyspepsia.
J Infect Dis. 157 (6) : 1245-9.

Langman-MJS. 1979.
Peptic ulcer.
In The Epidemiology of Chronic Digestive Disease. Chicago. Year Book Publishers. Inc. : p9.

Langman-MJS. 1983.
Epidemiology. Clinical Surgery International
Volume 7.
Peptic Ulcer Ed. Carter-DC. Churchill Livingstone : 1-13.

Lari-J, Lister-J, and Duthie-HL. 1968.
Response to gastrin pentapeptide in children.
J Paediat Surg 3 : 682-690.

Lawrie-JH, Smith-GMR, and Forrest-AMP. 1964.
The histamine infusion test.
Lancet : 270-273.

Lawson-JM, Taylor-WZ, Dresner-DM and Jones-DM. 1990.
Role of amnmonia in the pathogenesis of *Helicobacter pylori* associated gastrointestinal injury.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract I) : PD925.

Lee-FI, Samloff-IM, and Hardman. 1985.
Comparison of tri-potassium dicitrato bismuthate tablets with ranitidine in healing and relapse of duodenal ulcers.
Lancet 290 : 1299-1302.

Lessells-AM, and Martin-DF. 1982.
Heterotopic gastric mucosa in the duodenum.
J Clin Pathol 35 : 591-595.

Leth-R, Elander-B, Fellenius-E, Olbe-L and Haglund-U. 1984.
Effects of proximal gastric vagotomy and andrectomy on parietal cell function in human.
Gastroenterology 87 (6): 1277-82.

Leube-WO. 1871.
Sitz Phys Med Soc. Erlangen. 3: 106-108.

Leunk-RD, Johnson-PT, David-BC, Kraft-WG, and Morgan-DR. 1988.
Cytotoxic activity in broth culture filtrates of Campylobacter pylori.
J Med Microbiol 26 : 414-417.

Levi-S, Dollery-CT, Bloom-SR, Calam-J; Cox-TM, Hodgson-HJ, Losowsky-MS, Pepys-MB, Wright-NA, and Wrong-OM. 1989.
Campylobacter pylori, duodenal ulcer disease, and gastrin [clinical conference].
Br Med J 299 (6707) : 1093-4.

Levi-S, Beardshall-K, Haddad-G, Playford-R, Gosh-P, and Calam-J. 1989. Campylobacter pylori and duodenal ulcer gastrin link. Lancet : 1167-1168.

Levi-S, Beardshall-K, Swift-I, Foulkes-W, Playford-R, Gosh-P, and Calam-J. 1989.
Antral Helicobacter pylori, hypergastrinaemia, and gastric ulcer: effect of eradicating the organism.
Br Med J 299 : 1504-1505.

Levin-AL. 1921.
A new gastroduodenal catheter.
JAMA 76 : 1007.

Levin-E, Kirsner-JB, and Palmer-WL. 1948.
Twelve hour nocturnal gastric secretion in uncomplicated duodenal ulcer patients before and after surgery.
Pro Soc Exp Biol Med` 69 : 153-156.

Levy-M. 1974. Aspirin use in patients with major upper gastrointestinal bleeding and peptic-ulcer disease. N Eng J Med 290 (21) : 1158.

Lim RKS, Matheson-ARand Schlapp-W 1923
An improved method for investigating the secretory function of the stomach and duodenum in human subjects.
Q J Exp Physiol 13: 361-391.

Lior-H, and Johnson-WM. 1985.
Catalase, peroxidase and superoxide dismutase activities in *Campylobacter* spp.
In Pearson AD, Skirrow MB, Lior H and Rowe E (eds.) - *Campylobacter* III. Health Laboratory Service, London. : pp.226-227.

Luck-JM, and Seth-TN. 1924.
Gastric urease.
J Biol Chem 18 : 1227-31.

MacQuart-LCH. 1786.
Sur le suc gastrique des animaux.
Historie et memoirs de la Societe Royal. Paris : p. 355.

Madan-E, Kemp-J, and Westblom-U. 1988.
Evaluation of staining methods for identifying *Campylobacter pylori.*
Am J Clin Pathol 90 : 450-453.

Mainguet-P, Delmee-M, and Debongnie-J-E. 1989.
Omeprazole, *Campylobacter pylori* and duodenal ulcer (letter).
Lancet : 389-390.

Makhlouf-GM, McManus-JPA, and Card-WI. 1966.
A quantitative statement of the two component hypothesis of gastric secretion.
Gastroenterology 51 : 149-171.

Malhotra. 1978.
A comaprison of unrefined wheat and rice diets in the management of duodenal ulcer.
Postgrad Med J 54 : 6-9.

Marks-IN. 1961. The augmented histamine test.
Gastroenterology 41 : 599-603.

Marshall-BJ. 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis (letter).
Lancet i : 1273-1275.

Marshall-BJ, and Warren-JR. 1984.
Unidentified curved bacilli inthe stomach of patients with gastritis and peptic ulceration.
Lancet i : 1311-15.

Marshall-BL, Royce-H, Annear-DI et al. 1984.
Original isolation of *Campylobacter pyloridis* from human gastric mucosa.
Microbios Letters 25: 83-88.

Marshall-BJ, McGechie-DB, Francis-GJ, and Utley-PJ. 1984.
Pyloric *Campylobacter* serology.
Lancet ii : 281.

Marshall-BJ, McGechie-DB, Rogers-PA, and Glancy-RJ. 1985.
Pyloric *Campylobacter* infection and gastroduodenal disease.
Med J Aust 142 (439-444)

Marshall-BL, Armstrong-JA, Francis-GJ, Nokes-NT and Wee-SH 1987
Antibacterial action of bismuth in relation to *Campylobacter pyloridis* colonisation and gastritis.
Digestion 37 (2): 16-30.

Marshall-BJ, Armstrong-JA, McGechie-DB, and Glancy-RJ. 1985.
Attempt to fulfil Koch's postulates for pyloric *Campylobacter.*
Med J Aust. 142 (8) : 436-9.

Marshall-BJ, Warren-JR, Francis-GJ, Langton-SR, Goodwin-CS and Blincow-ED. 1987.
Rapid urease test in the management of *Campylobacter pyridis* - associated gastritis. 1987.
Am J Gastroenterol. 82 (3): 200-210.

Marshall-BJ, Goodwin-CS, Warren-JR, Murray-R, Blincow-ED, Blackbourn-SJ, Phillips-M, Waters-TE, and Sanderson-CR. 1988.
Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori.*
Lancet. 1988 2 (8626-8627) : 1439-42.

Marshall-BL and Surveyor-I. 1988.
Carbon-14 urea breath test for diagnosis of *Campylobacter pylori* associated gastritis,
Clin Sci 29: 11-26.

Martindale- 1989.
The Extra Pharmacopia. 29th Edition. Renolds-JEF (ed.).
The Pharmaceutical Press, London p. 111-112 (3778-t).

Mathieu-A. 1896.
Note sur une methode permettant de mesurer la motricite de l'estomac et le transit des liquides dans son cavite.
C R Soc Biol 3 : 74-76.

Masci-E, Tosi-T, Colombo-E, Bagnolo, Testoni-PA, Fanti-L and Tittobello-A. 1990.
*Helicobacter pylori* and dyspepsia: role of motility disorders.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PP 914.

McCarthy-DM. 1984.
Smoking and ulcers--time to quit [editorial].
N Engl J Med. 311 (11) : 726-8.

McColl-KEL, Fullarton-GM, El Nujumi-AM, Brown-IL, and Hilditch-TE. 1989.
Lowered gastrin and gastric acidity after eradication of *Campylobacter pylori* in duodenal ulcer (letter).
Lancet : 499-500.

McColl-KEL, Fullarton-GM, Chittajalu-R, El Nujumi-AM, MacDonald-AMI, Dahill-SW, and Hilditch-TE. 1991.
Plasma gastrin, daytime intragastric pH, and nocturnal acid output before and at 1 and 7 months after eradication of *Helicobacter pylori* in duodenal ulcer subjects.
Scand J Gastroenterol 26 : 339-346.

McConnell-RB. 1980.
Peptic ulcer: Early genetic evidence - families, twins and markers.
In Rotter-JI, Samloff-IM and Rimoin-DL (eds.): The Genetics and Heterogeneity of Common Gastrointestinal Disorders.
New York. Academic Press : 31.

McGreevy-JM. 1984.
Gastric surface cell function: potential difference and mucosal barrier.
Am J Physiol 247 : G79.

McGuigan-JE, and Trudeau-WL. 1973.
Differences in rates of gastrin release in normal persons and patients with duodenal ulcer disease.
N Eng J Medicine 288 (2) : 64-66.

McMullen-L, Walker-MM, Bain-LA, Karim-QN and Baron-JH. 1987.
Histological identification of *Campylobacter* using Gimenez technique in gastritic antral mucosa.
J Clin Pathol. 40: 464-465.

McNulty-CAM, and Dent-JC. 1988.
Susceptiblity of *Campylobacter pylori* to twenty-one antimicrobial agents.
Eur J clin Microbiol Infect Dis 7 : 566-569.

Mitchell-HM, Bohane-TD, Berkowicz-J, Hazell-SL, and Lee-A. 1987.
Antibody to *Campylobacter pylori* in families of index children with gastrointestinal illness due to *C pylori* [letter].
Lancet. 2 (8560) : 681-2.

Mitchell-HM, Lee-A, Berkowicz-J, and Borody-T. 1988.
The use of serology to diagnose active *Campylobacter pylori* infection.
Med J Aust. 149 (11-12) : 604-9.

Mitchell-HM, Lee-A, and Carrick-J. 1989.
Increased incidence of *Campylobacter pylori* infection in gastroenterologists: further evidence to support person-to-person transmission of C. pylori.
Scand J Gastroenterol 24 (4) : 396-400.

Mobley-HL, Cortesia-MJ, Rosenthal-LE, and Jones-BD. 1988.
Characterization of urease from *Campylobacter pylori.*
J Clin Microbiol. 26 (5) : 831-6.

Mohiuddin-J, Rhodes-EG, and Rhodes-JM. 1989.
Nonsecretors and peptic ulceration. No link with *Campylobacter pylori* [letter].
Dig Dis Sci. 34 (5) : 804.

Monis-A, and Mabrouk-S. 1990.
Role of *Campylobacter pylori* in pathogenesis of gastritis, duodenitis, non-ulcer dyspepsis and recurrent duodenal ulcer (Abstract).
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract I) : PD117.

Montbriand-JR, Appleman-HD, Cotner-EK, Nostrant-TT, and Elta-GH. 1989. Treatment of *Campylobacter pylori* does not alter gastric acid secretion. Am J Gastroenterol.

Montgomery-EA, Martin-DF, and Peura-DA. 1988. Rapid diagnosis of *Campylobacter pylori* by Gram's stain.
Am-J-Clin-Pathol. 90 (5) : 606-9.

Moore-JG, and Halberg-F. 1986. Circadian rhythm of gastric acid secretion in men with active duodenal ulcer. Dig Dis Sci 31 (11) : 1185-1191.

Morgan-DR, Freedman-R, Depew-CE, and Kraft-WG. 1987.
Growth of *Campylobacter pylori* in liquid media. .
J-Clin-Microbiol. 25 (11) : 2123-5.

Morris-A, Lloyd-G, and Nicholson-G. 1986.
*Campylobacter pyloridis* serology among gastroendoscopy clinic staff [letter].
N Z Med J. 99 (812) : 819-20.

Morris-A, Arthur-J, and Nicholson-G. 1986.
*Campylobacter pyloridis* infection in Auckland patients with gastritis.
N Z Med J. 99 (802) : 353-5.

Morris-A, Nicholson-G, Lloyd-G, Haines-D, Rogers-A, and Taylor-D. 1986.
Seroepidemiology of *Campylobacter pyloridis*.
N Z Med J. 99 (809) : 657-9.

Morris-A, Gillies-M, and Patton-K. 1986.
Detection of *Campylobacter pyloridis* infection [letter] .
N Z Med J. 99 (801) : 336.

Morris-A, McIntyre-D, Rose-T, and Nicholson-G. 1986.
Rapid diagnosis of *Campylobacter pyloridis* infection [letter].
Lancet. 1 (8473) : 149.

Morris-A, and Nicholson-G. 1987.
Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH.
Am J Gastroenterol 82 (3) : 192-199.

Morris-A, Maher-K, Thomsen-L, Miller-M, Nicholson-G, and Tasman-Jones-C. 1988.
Distribution of *Campylobacter pylori* in the human stomach obtained at postmortem.
Scand J Gastroenterol. 23 (3) : 257-64.

Morris-AJ, Ali-MR, Nicholson-GI, Perez-Perez-GI, and Blaser-MJ. 1991.
Long term follow-up of voluntary ingestion of *Helicobacter pylori*.
Ann Int Medicine 114 (8) : 662-663.

Moshal-MG, Gregory-MA, Pillay-C, et al. 1979.
Does the duodenal cell ever return to normal? A comparison between treatment with cimetidine and DeNol.
Scand J Gastroenterol 14 (Suppl 54) : 48-51.

Moynihan-BGA 1901.
On duodenal ulcer and its surgical treatment.
Lancet ii: 1656-1663.

Muller-Lisser-SA, Funnell-CJ, Sonnenberg-A, Will-N, Muller-Duysing-W, Heinzel-F, and Blum-AL. 1983.
Novel approach to quantify duodenogastric reflux in healthy volunteers and in patients with type 1 gastric ulcer.
Gut 24 : 510-518.

Munshi-S, Khuroo-MS; Mahajan-R; Zargar-SA; Javid-G;. 1989.
Prevalence of peptic ulcer in India: an endoscopic and epidemiological study in urban Kashmir.
Gut. 30 (7) : 930-4.

Nafeeza-MI, Shahimi-MM, Kudva-MV, Mazlam-MZ, and Isa-MR. 1989.
*Campylobacter pylori*, non-ulcer dyspepsia, and race in Malaysia.
Lancet : 1018.

Nedenskov-Sorensen-P, Bjorneklett-A, Fausa-O, Bukholm-G, Aase-S, and Jantzen-E. 1988.
*Campylobacter pylori* infection and its relation to chronic gastritis. An endoscopic, bacteriologic, and histomorphologic study.
Scand J Gastroenterol. 23 (7) : 867-74.

Newell-DG, Johnston-BJ, Ali-MH, and Reed-PI. 1988.
An enzyme-linked immunosorbent assay for the serodiagnosis of *Campylobacter pylori*-associated gastritis.
Scand J Gastroenterol (Suppl.) 142 : 53-7.

Newell-DG, and Rathbone-BJ. 1989.
The serodiagnosis of *Campylobacter pylori* infections.
Serodign Immunother 3 : 1-6.

O'Brien-PA, Cotter-J, and Whelton-MJ. 1987.
Duodenal ulcer incidence.
Lancet (1278-1279)

O'Morain-C, Humphreys-H;. 1988.
Culture of the organisms and histochemical identification.
Scand J Gastroenterol (Suppl.) 142 : 16-20.

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

O'Riordan-T, Leen-E, Keane-C, and O'Morian-C. 1990.
Non ulcer dyspepsia and *Helicobacter pylori* infection - A prospective study (Abstract).
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract II) : PP947.

Olender-EJ, Fromm-D, Furukawa-T, and Kolis-M. 1984.
H+ disposal by rabbit gastric mucosal surface cells.
Gastroenterology 86 : 698.

Otasevic-M, Katic-V, Olujic-M, Milovanovio-J, Otasevic-V, and Nagorni-A. 1990.
*Campylobacter pylori (CP)* in gastric diseases.
Bacteriologic and histopathologic study (Abstract).
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract I) : PD121.

Paffenbarger Jr-RS, Wing-AL and Hyde-RT. 1974
Chronic disease in former college students. XIII. Early precursors of peptic ulcer.
Am J Epidemiol. 100: 307.

Palmer-ED. 1954.
Investigation of the gastric mucosa spirochetes of the human.
Gastroenterology 27 : 218-20.

Panda-AP, Van Peborgh-P, Elder-JB, and Gillespie-IE. 1974.
Comparison of back diffusion of ions and antral and fundic mucosa and effect of phenol red on ion fluxes.
Br J Surg 61 : 912.

Parente-F, Lazzaroni-M, Sangaletti-O, Baroni-S, and Porro-GB. 1985.
Cigarette smoking, gastric acid secretion, and serum pepsinogen I concentrations in duodenal ulcer patients.
Gut 26 : 1327-1332.

Parshu-R. 1975.
Peptic ulcer in Fiji - a retrospective study.
Fiji Med J 3 : 148-153.

Paull-G and Yardley-JH. 1988.
Gastric and esophageal *Campylobacter pylori* in patients with Barett's esophagus.
Gastroenterology. 95: 216-218

Pavlov-IP. 1902.
The work of the digestive glands.
Transl. Thompson-WH . 2nd ed. C G Griffin and Co, London.
p. 12.

Payne-RA, and Kay-AW. 1962.
The effect of vagotomy on the maximal acid secretory response to histamine in man.
Clin Sci 22 : 373.

Penner-A, Post-A, and Hollander-F. 1940.
The use of phenol red as a dilution indicator in gastric analysis.
Am J Dig Dis 7 : 202-205.

Perez-Perez-GI, Dworkin-BM, Chodos-JE, and Blaser-MJ. 1988.
*Campylobacter pylori* antibodies in humans.
Ann Intern Med. 109 (1) : 11-7.

Permutt-RP and Cello-JP. 1982.
Duodenal ulcer disease in the hospitalised elderly patients.
Dig Dis Sci 27 (1): 1.

Peters-MN, Feldman-M, Walsh-JH, and Richardson-CT. 1983.
Effect of gastric alkalinization on serum gastrin concentrations in humans.
Gastroenterology 85 : 35-9.

Peterson-WL, Barnett-C, Evans Jr-DJ, Feldman-M, Richardson-c, Walsh-J, and Graham-D. 1991.
Role of H pylori in gastric acid secretion and serum gastrin concentrations in healthy subjects and patients with duodenal ulcer (Abstract).
Gastroenterology 100 (5) : A140.

Petkaneshov-G, Mitova-R, Michova-A, and Petrov-P. 1990.
Effect of PGV and PGV with pyloroplasty on gastri colonisation with *Campylobacter*-like organisms.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August) (Abstract I) : PD96.

Pettengell-KE, Spitaels-JM and Simjee-AE 1985.
Duodenitis and ulcer healing. (letter)
Lancet ii: 394-395.

Physick-PS. 1812.
Description of an apparatus for removing poison from the stomach.
Eclectic Repertory. (Philadelphia) : p. 3.

Pinkard-KJ, Harrison-B, Capstick-JA, Medley-G, and Lambert-JR. 1986.
Detection of *Campylobacter pyloridis* in gastric mucosa by phase contrast microscopy.
J Clin Pathol. 39 (1) : 112-3.

Polland-WC, and Bloomfield-AL. 1931.
Normal standards of gastric function.
J Clin Invest 9 : 651-658.

Popielski-L. 1920.
Uber die physiologischen und chemischen eigenschaften des peptons wite.
Pflug Arch Ges Physiol 126 : 483.

Prakash-C, Marshall-B, Barrett-L, Guerrant-R, and MacCallum-R. 1987.
Transportation media for gastric biopsies to isolate *Campylobacter pylori.*
Am J Gastroenterol 82 : 936.

Pretolani-S, Bonvicini-F, Brocchi-E, Funaro-S, Careddu-N, Cilla-D, and Gasbarrini-G. 1989.
Campylobacter pylori Gastritis in geriatric patients (Abstract).
Klin Wochenschr 67 (XVIII) : 55.

Prout-W. 1824.
On the nature of the acid and saline matters usually existing in stomach of animals.
Phil Trans R Soc 1 : 45-49.

Pulvertaft-CN. 1959.
Peptic ulcer in town and country.
Br J Prev Soc Med 13: 131.

Queiroz-DM, Barbosa-AJ, Mendes-EN, Rocha-GA, Cisalpino-EO, Lima-GF Jr, and Oliveira-CA. 1988.
Distribution of *Campylobacter pylori* and gastritis in the stomach of patients with and without duodenal ulcer.
Am J Gastroenterol. 83 (12) : 1368-70.

Quintero-M, and Sooto-A. 1986.
Metronidazole versus cimetidine in treatment of gastroduodenal ulcer (letter).
Lancet i (8486) : 907.

Ramsey-EJ, Carey-KV, Peterson-WL, Jackson-JJ, Murphy-FK, Read-NW, Taylor-KB, Trier-JS, and Fordtran-JS. 1979.
Epidemic gastritis with hypochlohydria.
Gastroenterology 76 : 1449-1457.

Raskov-H, Lanng-C, Gaarslev-K, Fischer-Hansen-B, and Hauch-O. 1987.
Screening for *Campylobacter pyloridis* in patients with upper dyspepsia and relation to inflammation of human gastric antrum.
Scand J Gastroenterol 22 : 568-572.

Rathbone-BJ, Wyatt-JI, Worsley-BW, Shires-SE, Trejdosiewicz-LK, Heatley-RV, and Losowsky-MS. 1986.
Systemic and local antibody responses to gastric *Campylobacter pyloridis* in non-ulcer dyspepsia.
Gut. 27 (6) : 642-7.

Rathbone-BJ, Wyatt-JI, and Heatley-RV. 1988.
Possible pathogenetic pathways of *Campylobacter pylori* in gastro-duodenal disease.
Scand J Gastroenterol (Suppl.) 142 (40-3)

Rauws-EAJ, Langenberg-W, Houthoff-HJ, Zanen-HC, and Tytgat-GNJ. 1988.
*Campylobacter pyloridis* - associated chronic ctive antral gastritis: A prospective study of its prevalence and the effects of antibacterial and antiulcer treatment.
Gastroenterology 94 : 33-40.

Rauws-EAJ, Royen-EAV, Langenberg-W, Wonsel-JV, Vrij-AA, and Tytgat-GNJ. 1989.
14C-urea breath test in *C pylori* gastritis.
Gut 30 : 798-803.

Rauws-EAJ, and Tytgat-GNJ. 1990.
Cure of duodenal ulcer associated with eradication of Helicobacter pylori.
Lancet 335 : 1233-1235.

Rauws-EAL, Langenberg-W, Bosma-A, Dankert-J, and Tytgat-GN. 1991.
Lack of eradication of *Helicobacter pylori* after omeprazole (letter). Lancet : 1093.

Rawles-JW, Harris-ML, and Paul-G. 1987. Antibody to *Campylobacter pyloridis* in endoscopy personnel, patients and controls.
Gastroenterology 92 : 1559A.

Recavarren-S, Leon-Barua-R, Cok-j, Rodriguez-C, Berendson-R, Gilman-RM, Ramirez-Ramos-A, and Watanabe-J. 1991.
Low prevalence rate of gastric metaplasia in the duodenal mucosa of dyspeptic patients from a developing country with low prevalence of duodenal ulcer and high prevalence of infection by *H pylori*.
Gastroenterology 100 (5) : A147.

Rehfuss-ME. 1914.
A new method gastric testing with a description of a method for the fractional testing of gastric juice.
Am J Med Sci 147 : 848-855.

Rocha-GA, Queiroz-DM, Mendes-EN, Lage-AP, and Barbosa-AJ. 1989.
Simple carbolfuchsin staining for showing *C pylori* and other spiral bacteria in gastric mucosa [letter].
J Clin Pathol. 42 (9) : 1004-5.

Ribeiro-CD and Gray-SJ. 1987.
Long term freeze storage of *Campylobacter pyloridis*.
J Clin Pathol 40: 1265.

Ross-IN, Nair-S, and Jayakumar-CR. 1985. The incidence of upper gastro-intestinal disease in a Malaysian community.
Singapore-Med-J. 26 (3) : 271-8.

Rotter-JI, and Rimoin-DL. 1977.
Peptic ulcer disease - a heterogeneous group of disorders?
Gastroenterology 73 : 604-607.

Rotter-JI. 1983.
Peptic ulcer. In Emery-AEH and Rimoin-DL (eds.): The Principles and Practice of Medical genetics
New York. Churchill Livingstone : 863-878.

Roxbourgh-JC. 1989.
The acute and chronic effects of cigarette smoking upon variuos aspects of gastric secretion. (M.S. Thesis - University of London)

Royle-JP, and Cathpole-J. 1967. Critical evaluation of the assessment of maximal gastric secretion stimulated by histamine. Br J surg 54 : 56-63.

Rydning-A, and Berstad-A. 1986. Dietary fibre and peptic ulcer. Scand J Gastroenterol. 21: 1.

Ryding-A, Berstad-A, Aadland-E and Odegaard-B. 1982. Prophylactic effect of dietary fibre in duodenal ulcer disease. Lancet. 2: 736.

Rydygier-LV. 1882.
Die erste Mangenusekiton fur Geschwur.
Berl Klin Wochenschr. 19: 32.

Rydyger-LV. 1884.
Der erste Fall von Gastroentestomie bei Stenose des Duodenum in Foige eines Geschwurs (mit Krankenvorstellung)
Zentralbl Chir. 11: 63-5

Saeed-ZA, Evans-Jr-DJ, Evans-DG, Cornelius-MJ, Maton-PN, Jensen-RT, and Graham-DY. 1991.
Helicobacter pylori and Zollinger-Ellison syndrome.
Dig Dis Sci 36 (1) : 15-18.

Salomon-H. 1896.
Uber das spirillum des Saugetiermagens und sein Verhalten zu den Belegzellen.
Centralbl f Bakt 19 : 433-442.

Salmon-PR. 1975.
In Truelove-SC and Trowell-J (eds.)
Topics in Gastroenterology
Blackwell Scientific Publication. London
pp. 2

Samloff-IM, Liebman-WM, and Panitch-NM. 1975. Serum group I pepsinogens by radioimmunoassay in control subjects and subjects with peptic ulcer. Gastroenterology 69 : 83.

Sarosiek-J, Slomiany-A, and Slomiany-BL. 1988.
Evidence for weakening of gastric mucus integrity by *Campylobacter pylori.*
*Scand J Gastroenterol.* 23 (5) : 585-90.

Satti-MB, Twum-Danso-K, Al-Freihi-MH, Ibrahim-EM, Al-Gindan-Y, Al-Quorain-A, Al-Ghassab-G, Al-Hamdan-A, and Al-Idrissi-H. 1990. *Helicobacter pylori* - associated upper gastrointestinal disease in Saudi Arabia: A pathologic evaluation of 98 endoscopic biopsies from 201 consecutive patients. Am J Gastroenterology 85 (5) : 527-534.

Schnell-GA, and Schubert-TT. 1989. Usefulness of culture, histology, and urease testing in the detection of *Campylobacter pylori*. Am-J-Gastroenterol. 84 (2) : 133-7.

Schoetensack-C, Malfertheiner-P, Nilius-M, Baczako-K, and Ditschuneit. 1988.
Influence of *Campylobacter pylori (CP)* positive chronic gastritis on gastrin and pepsinogen I (Abstract).
First Meeting of the European *Campylobacter pylori* Study Group. Workshop Gastroduodenal Pathology and Campylobacter pylori, Bordeaux, France. (7-8 October) : P166.

Schubert-ML. 1990.
Pathogenesis of duodenal ulcer.
Gastroenterology. 98 (5 Pt 1) : 1388-9.

Schubert-TT, and Schnell-GA. 1989.
Prevalence of *Campylobacter pylori* in patients undergoing upper endoscopy.
Am-J-Gastroenterol. 84 (6) : 637-42.

Sethi-P, Banerjee-AK, Jones-DM, Eldridge-J, and Hollanders-D. 1987.
Gastritis and gastric campylobacter-like organisms in patients without peptic ulcer.
Postgrad Med J. 63 (741) : 543-5.

Shirai-T, Harasawa-S, and Miwa-T. 1990.
*Helicobacter pylori* and gastric metaplasi in the duodenum.
The World Congress of Gastroenterology. Sydney, Australia. (26-31 August) (Abstract II) : PP956.

Sipponen-P, Varis-K, Cederberg-A, Salmi-HA, Seppala-K, Ihamaki-T, and Kosunen-TU. 1988.
*Campylobacter pylori* is associated with chronic gastritis but not with active peptic ulcer disease.
APMIS. 96 (1) : 84-8.

Sircus-W. 1977.
Maximal acid output and risk of ulcer.
Lancet i : 594-595.

Sircus-W. 1985.
Duodenitis: A clinical, endoscopic and histopathologic study.
Quart J Medicine 56 (221) : 593-600.

Skirrow-WB. 1977.
*Campylobacter* enteritis: a 'new' disease.
Br Med J 2 : 9-11.

Smith-AC, Price-AB, Borriello-P and Levi-AJ. 1988.
Comparison of ranitidine and tripotassium dicitratobismuth in relapse rate of duodenal ulcer.
Gastroenterology 94; A431.

Smith-JTL, Pounder-RE, Nwokolo-CU, Lanzon-Miller-S, Evans-DG, Graham-DY, and Evans Jr-DJ. 1990.
Inappropriate hypergastrinaemia in asymptomatic healthy subjects infected with *Helicobacter pylori.*
Gut 31 : 522-525.

Smithuis-RHM, and Vos-CG. 1988.
Heterotropic gastric mucosa in the duodenal bulb: relationship to peptic ulcer.
AJR 152 : 59-61.

Songster-W, Grossman-MI, and Ivy-AC. 1946.
The effect of two new histamine antagonists (benadoyl and compound 63).
Gastroenterology 6 : 436-438.

Sonnenberg-A, Muller-Lissner-SA, Vogel-E, Schmid-P, Gonvers-JJ, Peter-P, Strohmeyer-G and Blum-AL. 1987.
Predictors of duodenal ulcer healing and relapse.
Gastroenterology 81: 1061.

Sonnenberg-A. 1987.
Changes in physician visits for gastric and duodenal ulcer in the United States during 1958-1984 as shown by National Disease and Therapeutic Index (NDTI).
Dig Dis Sci 32 (1) : 1.

Sorensen-SPl. 1909.
Engymstudien II. Mitteilung. Uber die Messung und die bedeutung der wasserstoffionen. Konzentration bie enzymatischen prozessen.
Biochem Z 21 : 131-304.

Steer-HW, and Colin-Jones-DG. 1975.
Mucosal changes in gastric ulceration and their response to carbenoxolone.
Gut 16 : 590-7.

Steer-HW. 1984.
Mucosa -related bacteria in the stomach.
Lancet : 528.

Steer-HW, and Newell-DG. 1985.
Immunological identification of *Campylobacter pyloridis* in gastric biopsy tissue [letter].
Lancet. 2 (8445) : 38.

Steer-HW, Hawtin-P, and Newell-DG. 1988.
The effect of surgical treatment of chronic duodenal ulceration on *Campylobacter pylori* colonisation of the stomach (Abstract).
First Meeting of the European Campylobacter pylori Study Group.
Workshop Gastroduodenal Pathology and Campylobacter pylori, Bordeaux, France. (7-8 October) : P153.

Stockbrugger-RW, Eugenides-N, Hobsley-M, and Cotton-PB. 1982.
Pirenzepine inhibits histamine-stimulated gastric secretion.
Scand J Gastroenterol 72 ((Suppl.)) : 43A.

Strickland-RG, Bhathal-PS, Korman-MG, and Hansky-J. 1971.
Serum gastrin and the antral mucosa in atrophic gastritis.
Br Med J 4 : 451-453.

Surveyor-I, Goodwin-CS, Mullan-BP, Geelhoed-E, Warren-JR, Murray-RN, Waters-TE, and Sanderson-CR. 1989.
The 14C-urea breath-test for the detection of gastric *Campylobacter pylori* infection.
Med J Aust. 151 (8) : 435-9.

Szeto-ML, Pounder-RE, Hamilton-Dutoit-SJ, and Dhillon-AP. 1988. Rapid urease test provides specific identification of Campylobacter pylori in antral mucosal biopsies.
Postgrad-Med-J. 64 (758) : 935-6.

Taylor-DE, Hargreaves-JA, Ng-L, Sherbaniuk-RW, and Jewell-LD. 1987.
Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies.
Am J Clin Pathol 87 : 49-54.

Teorell-T. 1947.
Electrolyte diffusion in relation to the acidity regulation of gastric juice.
Gastroenterology 9 : 425-443.

Tessaro-P, Di Mario-F, Rugge-M, Baffa-R, Pasquqletti-P, Vianella-F, Glorioso-S and Naccarato-R. 1990.
Efficacy of omeprazole in eradicating *Helicobacter pylori* from gastric mucosa.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PD 109.

Thomas-WEG. 1984.
The possible role of duodenogastric reflux in the pathogenesis of both gastric and duodenal ulcers.
Scand J Gastroenterol. 19 (Suppl 92) : 151-5.

Thomsen-L, Tasman-Jones-C, Morris-A, Wiggins-P, Lee-S, and Forlong-C. 1989.
Ammonia produced by *Campylobacter pylori* neutralizes H+ moving through gastri mucosa.
Scand J gastroenterol 24 : 761-768.

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

Ti-TK. 1983.
Gastric acid secretion in duodenal ulcer disease in the Malaysia-Singapore region.
Ann Acad Med Singapore. 12 (4) : 507-17.

Tompkins-DS, and West-AP. 1987. *Campylobacter pylori*, acid, and bile [letter] . J Clin Pathol. 40 (11) : 1387.

Tovey-FI 1979.
Peptic ulcer in India and Bangaladesh.
Gut 20-329.

Trowell-JE, Yoon-AKH, Sall-KJ, Gant-PW and Bell-GD. 1987.
Simple half-Gram stain for showing presence of *Campylobacter pyloridis* in sections.
J Clin Pathol 40: 702

Tytgat-GN. 1988.
*Campylobacter pylori.*
Scand J Gastroenterol 155 (Suppl) : 58-66.

Tytgat-GN, Rauws-EA, and De-Koster-E. 1988.
*Campylobacter pylori.*
Scand J Gastroenterol-Suppl. 155 : 68-81.

Tytgat-GN. 1989.
*Campylobacter pylori*: epidemiological considerations.
Scand-J-Gastroenterol 160 (Suppl) : 160: 1-2.

Tytgat-GN, Rauws-EA, and de-Koster-EH. 1989.
*Campylobacter pylori.* Diagnosis and treatment.
J-Clin-Gastroenterol. 11 (Suppl 1) : S49-53.

Tytgat-GNJ, Rauws-EAJ, Langenberg-ML, and Houthoff-HJ. 1986.
*Campylobacter pyloridis*:
The Amstradam Study. Proceedings of the World Congress of Gastroenterology. Sao Paolo. 1986. Dig Dis Sci 31 : 585.

Vaira-D, Holton-J, Cairns-S, Polydorou-A, Falzon-M, Dowsett-J, and Salmon-PR. 1988. Urease tests for *Campylobacter pylori*: care in interpretation [letter]. J Clin Pathol. 41 (7) : 812-3.

Vaira-D, Holton-J, Cairns-SR, Polydorou-A, Falzon-M, Dowsett-JF, and Salmon-PR. 1988.
A novel 10 min urease test for *Campylobacter pylori* (CP).
Proc Sirmione. 21: 136.

Vaira-D, Holton-J, Falzon-M, Cairns-S, Dowsett-J, Polydorou-A, and Salmon-P. 1988.
Investigation of *Campylobacter pylori* associated gstritis by histology, culture, urease test, brushing and antibody levels.
Ital J Gastroenterol 20 : 299-.

Van-Caekenberghe-DL, and Breyssens-J. 1987.
In vitro synergistic activity between bismuth subcitrate and various antimicrobial agents against *Campylobacter pyloridis* (C. pylori).
Antimicrob Agents Chemother. 31 (9) : 1429-30.

Van-Helmont-IB. 1648.
Sextuplex Digestio Alimenti Humani
Ortus Medicinae. Amsterodam : p.208 para 2.

Veldhuyzen-van-Zanten-SJ, Tytgat-KM, Hollingsworth-J, Jalali-S, Rshid-FA, Bowen-BM, Goldie-J, Goodacre-RL, Riddell-RH, and Hunt-RH. 1990.
14C-urea breath test for the detection of *Helicobacter pylori.*
Am J Gastroenterol 85 (4) : 399-403.

Venables-CW. 1972.
An assessment of the value of measuring uncollected gastric secretion during routine secretion studies in man.
Br J Surg 59 : 473-477.

von-Wulffen-H, Heesemann-J, Butzow-GH, Loning-T, and Laufs-R. 1986.
Detection of *Campylobacter pyloridis* in patients with antrum gastritis and peptic ulcers by culture, complement fixation test, and immunoblot.
J Clin Microbiol. 24 (5) : 716-20.

Wagner-S, Freise-J, Beholz-S, and Schmidt-FW. 1988.
Gastric acid secretion and *Campylobacter pylori* [letter].
Gastroenterology. 6 (1695-6)

Wagner-S, Schuler-A, Gebel-M, Freise-J, and Schmidt-FW. 1989.
Campylobacter pylori and acid secretion.
Lancet i: 562.

Wagner-S, Gebel-M, Freise-J, Bar-W, and Schidt-FW. 1990.
Effect of chronic *Helicobacter pylori* infection on gastric acidity with gastritis and duodenal ulcer. (Abstract).
The World Congresses of Gastroenterology. Sydney, Australia. (26-31 August) (Abstract II) : PP943.

Wagner-S, Varrentrapp-M, Haruma-K, Gebel-M, and Schmidt-FW. 1991.
Serum gastrin, pepsinogen I and II in *Helicobacter pylori* positive gastritis and peptic ulcer: Effect of bacterial eradication. (Abstract).
Gastroenterology 100 (5) : A181.

Walker-SJ, Birch-PJ, Stewart-M, Stoddard-CJ, Hart-CA, and Day-DW. 1990. Patterns of colonisation of *Campylobacter pylori* in oesophagus, stomach and duodenum. Gut 30 : 1334-1338.

Walker-V, and Taylor-WH. 1979. Cigarette smoking, chronic peptic ulceration and pepsin I secretion. Gut 20 : 971-976.

Wallmark-B, Bradstrom-A, and Larsson-H. 1984.
Evidence for acid-induced transformation of omeprazole into an active inhibitor of (H+ + K+) - ATPase within the parietal cell.
Biochem Biophys Acta 778 : 549.

Walsh-JH, Richardson-CT and Fordtran-JS. 1975.
pH dependence of acid secretion and gastrin release in normal and ulcer patients.
J Clin Invest 55: 462-469.

Walters-LL, Budin-RE, and Paull-G. 1986.
Acridine-orange to identify *Campylobacter pyloridis* in formalin fixed, paraffin-embedded gastric biopsies [letter].
Lancet. 1 (8471) : 42.

Wang-WM, Chen-CY, Jan-CM, Chen-LT, and Lin-SR. 1990.
The effecacy of different regimens on the treatment of duodenal ulcer associated with Campylobacter pylori infection.
The World Congresses of Gastroenterology. Sydney, Australia. (26-31 August) (Abstract I) : PD106.

Wagner-S, Gebel-M, Freise-J Bar-W and Schmidt-FW. 1990.
Effect of chronic *Helicobacter pylori* infection on gastric acidity in patients with gastritis and duodenal ulcer.
The World Congresses of Gastroenterology, Sydney, Australia (26-31 August): PP943.

Warren-JR, and Marshall-B. 1983.
Unidentified curved bacilli on gastric epithelium in active chronic gastritis.
Lancet i : 1273.

Warren-JR. 1984.
Spiral bacteria of the gastric antrum [letter].
Med J Aust. 141 (7) : 477-8.

Warthin-AS, and Starry-AC. 1922.
The staining of spirochetes in cover glass smears by silver agar method.
J Infectious Dis : 592-600.

Westblom-TU, Madan-E, Kemp-J, and Subik-MA. 1988.
Evaluation of a rapid urease test to detect *Campylobacter pylori* infection.
J Clin Microbiol. 26 (7) : 1393-4.

Whitehead-R, Truelove-SC, and Gear-MWL. 1972.
The histological diagnosis of chronic gastritis in fibreoptic gastroscope biopsy specimens.
J Clin Pathol 25 : 1-11.

Whitfield-PF, and Hobsley-M. 1979.
A standardised technique for the perfomance of accurate gastric secretion studies.
Agents and Action 9/4 : 327-332.

Whitfield-PF. 1984.
The effects of stature, sex, age and smoking on maximal gastric secretion in controls and duodenal ulcer subjects, with the implications for the aetiology and the treatment of duodenal ulcer. (Ph. D. Thesis - University of London)

Whitfield-PF, and Hobsley-M. 1985.
Maximal gastric secretion in smokers and non-smokers with duodenal ulcer.
Br J Surg 72 : 955-957.

Whitfield-PF, and Hobsley-M. 1987.
Comparison of maximal gastric secretion in smokers and non-smokers with and without duodenal ulcer.
Gut 28 (5) : 557-60.

Wieman-TJ, Whitfield-PF, and Hobsley-M. 1988.
The non-random nature of pyloric loss during continuous gastric aspiration.
Surg Res Comm 3 : 27-30.

Wiersingha-WM, and Tytgat-GNJ. 1977.
Clinical recovery owing to target parietal cell failure in a patient with Zollinger-Ellison syndrome.
Gastroenterology 73 (6) : 1413-1417.

Wilson-DE. 1990.
Gastroduodenal ulcers: causes, diagnosis, prevention, and treatment.
Comp Ther 16(5): 43-53.

Wolfler-A. 1881.
Gastro-enterostomie.
Zentralbl Chir 45: 705-708.

Wolverson-RL, Sorgi-M, Mossimann-F, Donovan-IA, and Harding-LK. 1984.
The incidence of duodenogastric reflux in peptic ulcer disease.
Scand J gastroenterol 19 (92) : 149-150.

Wolverson-RL, Sorgi-M, Mossimann-F, Donovan-IA, Harding-LK, and Alexander-Williams-J. 1984. Does entubation cause enterogastric reflux? Scand J gastroenterol 19 (92) : 41-43.

Wormsley-KG and Grossman-MI. 1965.
Masimal Histolog test in control subjects and patients with peptic ulcer.
Gut. 6: 427-435.

Wormsley-KG. 1978. Smoking and duodenal ulcer.
Gastroenterology. 75 (1) : 139-42.
Wyatt-JI, Rathbone-BJ, Dixon-MF and Heatley. 1987.
*Campylobacter pylori* and acid induced gastric metaplasia in the pathogenesis of duodenitis.
J Clin Pathol 40: 840-848.

Wyatt-JI, Rathbone-BJ, Green-DM, and Primrose-J. 1988.
Raised fasting gastrin in chronic gastritis is independent of *Campylobacter pylori* status and duodenal ulceration.
Gut : A1483.

Wyatt-JI, and Rathbone-BJ. 1988. Immune response of the gastric mucosa to *Campylobacter pylori.*
Scand-J-Gastroenterol-Suppl. 142 : 44-9.

Wyatt-JI, Rathbone-BJ, Dixon-MF, Heatley-RV, and Axon-AT. 1988.
*Campylobacter pylori* and development of duodenal ulcer [letter].
Lancet. 1 (8577) : 118-9.

Wyatt-JI. 1988. *Campylobacter pylori* in Meckel's diverticulum.
First Meeting of the European Campylobacter pylori Study Group.
Workshop Gastroduodenal Pathology and Campylobacter pylori, Bordeaux, France. (7-8 October) : P150.

Wyatt-JI, and Rathbone-BJ. 1988. Gastric metaplasia in the duodenum and *C pylori* (Abstract).
First Meeting of the European *Campylobacter pylori* Study Group.
Workshop Gastroduodenal Pathology and Campylobacter pylori, Bordeaux, France. (7-8 October) : L17.

Wyatt-JI, and Rathbone-BJ. 1989.
Gastric metaplasia in the duodenum and *Campylobacter pylori.*
Gastroenterol Clin Biol. 13 (1 Pt1) : 78B-82B.

Wyatt-JI, and Rathbone-BJ. 1989.
The role of serology in the diagnosis of *Campylobacter pylori* infection.
Scand J Gastroenterol 160 (Suppl) : 27-34.

Wyatt-JI. 1989. The role of *Campylobacter pylori* in the pathogenesis of peptic ulcer disease.
Scand J Gastroenterol-Suppl. 157; 7-11 (discussion) : 21-2.

Young-JR. 1803. An experimental inquiry into the principles of nutrition and the digestive process,
(Thesis - University of Pennsylvania), Philadelphia, Eaber and Mecum : 40.