STUDIES OF ESTABLISHED FORMS OF THERAPY FOR PEPTIC ULCER ON THE DEVELOPMENT AND GROWTH OF COLORECTAL CANCER

-

THE INFLUENCE OF PREVIOUS PEPTIC ULCER SURGERY AND OMEPRAZOLE

by

James Robert Duncan

M.B., Ch.B ; F.R.C.S.

Thesis submitted for the Degree of Doctor of Medicine

from

The University Department of Surgery

Western Infirmary

Glasgow

August 1998

ProQuest Number: 11007788

ProQuest 11007788

I dedicate the time and effort contained in these pages to my wife, Anne and

to all my family, past, present and future.

Contents

Chapter 2 - Materials and Methods 113

Chapter 3 - Results   136

Chapter 4 - Discussion and Conclusions 166

List of Figures

Chapter 4 - Discussion and Conclusions

List of Tables

List of Appendices

<u>Acknowledgements</u>

I wish to express my gratitude to a number of people, without whose help, this thesis would not have been possible. First and foremost, I would like to thank Professor David George of the University Department of General Surgery, Western Infirmary, Glasgow, for giving me the opportunity to undertake the period of study leading to this thesis. No less important, I would like to express my sincere gratitude to Mr Patrick O'Dwyer (Senior Lecturer) and Mr John McGregor (Lecturer)* of the above department, for their excellent guidance and advice on the experiments and clinical study undertaken for this thesis. I would particularly like to commend John McGregor for his unstinting support and patience throughout the period of study and writing of this thesis, even at times of difficulty and disillusionment.

I am also indebted to Dr. Jane Plumb and Patricia Thomson at the Cancer Research Campaign Beatson Laboratories, Garscube Estate, Glasgow for instruction and guidance with the clonogenic assays. Thanks also to Dr. Karin Øein of the Department of Pathology, Western Infirmary, Glasgow, for her prompt and expert preparation and histological examination of the colonic tumour specimens, and to Professor Joy Ardill of the University Department of Medicine, Queen's University, Belfast, for analysis of the 225 blood samples for plasma gastrin. Thanks also to Mr Hugh Shannon, John Laurie and all animal husbandry staff of the University of Glasgow, for their excellent care of the animals utilised in the in vivo studies, and also for their assistance with the administration of omeprazole and azoxymethane. Thanks also to Elaine Hall and Kay Pollock, Staff Pharmacists at the Western Infirmary, for preparation of omeprazole suspension and drug

---

* now Consultant Surgeon, Crosshouse Hospital, Kilmarnock, KA2 0BE.

Declaration

I declare that the foregoing thesis has been entirely researched and written by me alone.

With the exception of expert technical assistance provided by those persons that I have already duly acknowledged, I declare that I was the major instigator and participant in all aspects of the studies undertaken for this thesis. I also declare that all cited literature references have been personally consulted by me, with the exception of a few mainly historical references that have proved impossible to obtain. Where possible, reliable sources of citation for these references have been given.

The undertaking of all the experimental studies, and the collection of data for the retrospective clinical study, were performed within a two-year period, from August 1993 to August 1995, whilst I was employed as a research fellow in the University Department of Surgery at the Western Infirmary, Glasgow. Most of the computation and statistical analysis on the collected data, particularly that of the clinical study, has been performed since then.

Preface

Colorectal cancer and peptic ulcer are two of the most commonly encountered diseases in present day medical practice. The work which is reported in this thesis links these two very different diseases, by investigation of the effects of various forms of treatment for one, on the the incidence and behaviour of the other, both in a deleterious and potentially beneficial fashion to the patient.

Many reports have suggested that patients who have previously undergone definitive surgery for peptic ulcer are left with the legacy of an increased propensity for developing malignant disease approximately 15 to 20 years later. Gastric cancer has regularly been identified as being one such cancer. To a much lesser extent, colorectal cancer has also been reported as occurring with undue frequency in a number of patient series studied. With few exceptions, the majority of patients studied have previously undergone partial gastric resection as opposed to vagotomy. The latter procedure, although reducing gastric acid output to a similar degree as partial gastrectomy, differs markedly from it by virtue of the chronic elevation of circulating gastrin levels, secreted from the non-resected stomach, which occurs post-operatively. This may be an important factor in the development of colorectal cancer, as a significant body of evidence suggests that gastrin is trophic for a proportion of such neoplasms. There may therefore be implications for the prolonged use of the latest pharmaceutical agents used against acid-peptic disorders, namely the proton-pump inhibitors of which omeprazole is the original. Similar to vagotomy, these drugs induce a chronic elevation of circulating gastrin, secondary to the reduction in acid output. In view of this association, one of the main purposes of the work which follows was to study the cancer incidence, in particular that of colorectal

cancer, in a group of patients who had undergone surgery for peptic ulcer disease, the majority of whom had vagotomy as opposed to antrectomy.

Somewhat conversely, the remainder of the work in this thesis concentrates on investigating a possible inhibitory action of omeprazole on colorectal tumour growth, despite the chronic hypergastrinaemia which it produces. This is in response to two recent reports which suggest that the drug may have such an effect on these tumours, under experimental conditions.

Hence, it seems appropriate to open this account with a brief overview of the diseases under scrutiny; namely colorectal cancer and peptic ulcer; and to continue by explaining the rationale behind both surgical and medical treatment for the latter. This should give the reader a clear understanding of the pathophysiological changes that occur in response to such treatment, and why they may have potentially deleterious effects, or otherwise, elsewhere in the gastrointestinal tract. In the latter part of the introductory chapter, the evidence, both for and against the phenomena under investigation, will be reviewed, and this will illustrate the stimulus to the work carried out for this thesis.

Chapter 1 - Introduction

1. Colorectal Cancer - an overview

Colorectal cancer is the fourth commonest malignancy worldwide with the third highest mortality of all cancers (1). An estimated 468,000 people died from the disease globally in 1993 (1). It is more prevalent in countries of the developed world (United States, Western Europe, Australasia and Scandinavia) (2). In Scotland, in 1993, it was the third most frequently diagnosed cancer in both males and females, accounting for 13.4% of all cancer registrations in their respective sex category (Figure 1) (3). Only lung and prostate cancer in males (25.1% and 13.6% respectively) and breast and lung cancer in females (24.9% and 14.1% respectively) exceeded it in terms of incidence (3). It is therefore appropriate that such common diseases are being targeted by research, health promotion and population screening in an effort to reduce the incidence and improve upon current survival rates. This is indeed the case with colorectal cancer but as yet there has been no significant impact on survival rates.

Colorectal cancer is a multifactorial disease with several genetic and environmental risk factors identified (4). At one end of the spectrum are the inherited forms of the disease, Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC)(5), which account for 1% and 5 to 15% of all colorectal cancers respectively (2). Familial Adenomatous Polyposis is inherited in an autosomal dominant fashion. Affected individuals exhibit a phenotypic marker in the form of hundreds, often thousands, of benign colorectal adenomatous polyps developing in their teens, which invariably go on to develop malignant change in the second or third decades. The mutated gene

Figure 1 - The ten most frequently diagnosed cancers in Scotland in 1993

Males

Lung	25.1%
Prostate	13.6%
Large bowel	13.4%
Bladder	7.7%
Stomach	4.8%
Oesophagus	3.6%
Non-Hodgkin's lymphoma	3.2%
Kidney	2.4%
Pancreas	2.3%
Leukaemia	2.3%

0   500   1 000   1 500   2 000   2 500   3 000   3 500
Number of registrations

Females

Breast	24.9%
Lung	14.1%
Large bowel	13.4%
Ovary	4.2%
Bladder	3.4%
Stomach	3.2%
Non-Hodgkin's lymphoma	3.1%
Cervix	3.1%
Oesophagus	3.0%
Uterus	2.7%

0   500   1 000   1 500   2 000   2 500   3 000   3 500
Number of registrations

Reproduced, with permission, from Scottish Health Statistics 1995. ISD Vol 37 Section 3 - Morbidity - Scottish Cancer Registration Statistics.

responsible, named APC (abbreviation for Adenomatous Polyposis Coli), is a tumour suppressor gene and has been identified on the long arm of chromosome 5 and it is now possible to screen for this genetically. Unlike Familial Adenomatous Polyposis, Hereditary Non-Polyposis Colorectal Cancer does not have any phenotypic marker and diagnosis of this condition is based on family history, for which a list of criteria, known as the Amsterdam criteria, have been laid out (6). These stipulate that at least three members of the family within two generations be affected; that at least two of the affected individuals be first degree relatives of the other; and that at least one of the affected individuals have developed the disease before the age of fifty. Four genes, hMLH 1, hMSH 2, on the short arms of chromosome 2 and 3 respectively and hPMS1 (long arm of chromosome 2) and hPMS2 (short arm of chromosome 7) have subsequently been identified (7,8). These are mismatch repair genes and germline mutations of these have been identified in such families. Both these forms of colorectal cancer exhibit an "all or none" effect with those family members inheriting the mutated genes invariably going on to develop the disease.

"Sporadic" colorectal cancer accounts for the remaining 85% of cases. Even here, genetic predisposition to the disease is thought to play an important role, as a number of mutated genes have been frequently identified in those sporadic tumours studied (9). These include the APC gene responsible for Familial Adenomatous Polyposis as described above, and others such as DCC (abbreviation for Deleted in Colorectal Cancer), K-ras and p53 (9). Furthermore, within this group, familial clustering of the disease is evident, and those with an affected first degree relative run a substantially increasd risk, of the order of two to four-fold, over the general population of developing bowel cancer, suggesting an underlying genetic aetiology (5). Environmental factors undoubtedly also have a role to play. For instance, it has been shown that immigrants from a region with low disease

incidence settling in an area of relatively high incidence, acquire an increased risk for the disease (10,11). Strong associations exist for a number of environmental factors (4). Increased risk is seen in association with obesity, sedentary lifestyle and lack of physical exercise (4) as well as high consumption of animal fat, protein and red meat (12-14). An inverse association exists for increased consumption of dietary fibre, fruit and vegetables (15). A number of chemicals in the diet are also thought to be protective against colorectal neoplasia, among them being calcium (4), folic acid (16) and vitamins A (16), C (4) and E (16). Also, there is evidence to suggest that aspirin and Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) such as piroxicam and sulindac have an anti-neoplastic action on the colorectal mucosa (17-19).

It has long been recognised that a marked survival benefit to the patient accompanies the earlier the stage of disease at presentation, diagnosis and treatment. Indeed, treatment of the earliest stages of colorectal cancer can in some cases be confidently predicted to be curative. The Dukes' classification, first described in 1932 for cancers of the rectum, but now applied to all adenocarcinomas of the large bowel, bears this out (20). Dukes defined three stages of the disease, namely stage A, stage B and stage C. Dukes' A lesions, the earliest stage, are limited to the wall of the bowel. Dukes' B tumours traverse the bowel wall completely, either through to serosa or perirectal tissue, but no regional lymph node metastases are present. Dukes' C disease is that in which there is involvement of the draining mesenteric lymph nodes with metastatic disease. Dukes later sub-divided stage C into C_1 and C_2, the latter describing metastatic nodes at the level of ligature of the main arterial supply to the diseased segment of bowel (21). Quoted five-year survival figures for each of these respective pathological stages of disease after "curative" surgical resection are as follows : 80-100%, 45-78% and 25-60% (2,22-24). Although not one of Dukes' originally described stages, Dukes' D is now often used to describe metastatic

spread to the liver and beyond, which has a dismal prognosis. Modifications to Dukes' original classification, such as those of Astler and Coller (25), Kirklin (26) and Turnbull (27), have not superceded its use, as none have been shown to be any more beneficial in predicting outcome (28,29). As a result of this, and also its simplicity of use, Dukes' staging system remains the gold standard against which all other prognostic classifications should be assessed (30), although a significant number of workers in the field have adopted and now apply the universal Tumour : Node : Metastasis (TNM) classification of cancer to the disease (31).

Surgical resection remains the mainstay of treatment for colorectal cancer. Despite intensive research and an ever increasing understanding of the disease over the years, it is somewhat depressing to realise that the survival figures quoted above have changed little over several previous decades (32). As outlined above, and as with most malignancies, colorectal cancer is a multifactorial disease with multiple genetic and environmental factors cited in its aetiology. As such there exists the potential to modify the disease at several levels, from the intranuclear genetic makeup of an individual to the risk factors to which he or she may be exposed on a daily basis, such as dietary components and drugs.

2. Peptic Ulcer Disease - an overview

Peptic ulcer disease has afflicted mankind for many centuries. For instance, the well preserved corpse of a Chinese man who died in 167 B.C. demonstrated that the cause of death was peritonitis as a result of a perforated pre-pyloric ulcer (33). The first documented reports of gastric and duodenal ulcer in the medical literature were in 1586 (34) and 1688 (35) respectively. Until the beginning of this century, duodenal ulcer was considered to be a relatively rare disease, with gastric ulcer being the commoner of the two, and the latter being more prevalent in females (34). However, in the first half of the twentieth century, the incidence of doudenal ulcer was seen to markedly increase, predominantly among the male population (36). Since 1950 onwards however, a definite decline in the natural incidence of the disease has been evident, which has pre-dated any of the therapeutic advances introduced during this period (37). Analysis of birth cohorts has shown that those born around the turn of the century had the highest risk for the disease and in all subsequent birth cohorts this risk has steadily decreased (38). It has been postulated that the death and senescence of that particular group may be responsible for the now observed decline and that exposure of this cohort of patients to some evironmental risk factor at the time of their birth and early life was responsible for the initial increase in frequency of the disease (37,38). Despite this natural decline, the incidence of complications of peptic ulcer, such as perforation and haemorrhage, and the mortality rates attendant upon them, have remained relatively constant, although the latter now tend to affect more aged patients (39,40). If one also considers that peptic ulcer has an estimated incidence of 2 to 3 per 1000, a one year prevalence of 17 per 1000, and a lifetime prevalence of 5-10% (39), and that present day treatment also confers a considerable financial burden on the economy (for instance, prescriptions for anti-ulcer

drugs cost an estimated two billion dollars per year in the United States) (41), then it can be appreciated that it continues to represent a significant portion of both general practicioner and specialist gastroenterologist physicians and surgeons workloads.

3. Pathophysiology of Peptic Ulcer

In order to understand why treatment for peptic ulcer may adversely affect patients by increasing their propensity to develop colorectal cancer and other malignancies, it is necessary to know the main underlying mechanisms for ulcerogenesis, and the physiological alterations that occur with various forms of treatment.

Ulceration in the stomach or duodenum occurs when the physiological balance between protective mucosal defence mechanisms and the potential for damage by intraluminal acid and pepsin is disrupted (42). Several aetiological factors are implicated.

3.1. Mucosal Protection

Protective mechanisms include the secretion of mucus by the epithelium, which forms an unstirred layer adjacent to it and is resistant to the action of pepsin (42). There is also continuous secretion of bicarbonate which creates a pH gradient across the unstirred mucus layer, so that when luminal pH is as low as 1.5, the fluid bathing the surface of the epithelial cells is pH 6 or greater (43). In addition, tight apical cellular junctions prevent back-diffusion of acid through the gastric mucosa (44). Rapid epithelial turnover and adequate mucosal blood flow are also thought to contribute to the defence of the gastroduodenal mucosa (42). The "aggressors" against these protective features of the gastroduodenal mucosa include the acid and pepsin secreted by the body of the stomach for the purposes of digestion of luminal food contents. It is not necessary for these secretions to be in excess of their "normal" physiological range to lead to mucosal damage and subsequent ulceration (42). Although duodenal ulcer sufferers as a whole are known to have elevated acid secretion relative to the general population, in fact this difference occurs in only one third of such patients, the so called "true hypersecretors" (45). It is

also noteworthy that 10% of Zollinger-Ellison syndrome patients (characterised by significantly excessive acid and pepsin secretion, secondary to a gastrin producing tumour) do not develop the typical ulceration so characteristic of the condition (45). Furthermore, the majority of gastric ulcers, unlike duodenal ulcers, develop in conditions of relative acid lack (hypochlorhydria) (45). However, in stating this, it should be appreciated that acid must be present to some degree for ulceration to occur, as it has been shown that ulceration does not develop in conditions of complete absence of acid (achlorhydria) (46). It follows from this that factors which exert a deleterious action on the mucosal protective mechanisms outlined above, must have a significant bearing on disease development. It is by this mechanism that the two most commonly implicated ulcerogenic agents, the spiral bacterium, Helicobacter pylori, and aspirin and the related Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), are believed to act.

3.2. Helicobacter pylori.

Helicobacter pylori has generated a plethora of research and literature since its description in 1983 by Marshall and Warren in association with histologically confirmed active inflammation of the gastric mucosa (47). However colonisation of the human stomach by such organisms had been reported previously by others, as early as 1896 (48-50). It is now known that Helicobacter is present in virtually all cases of chronic active gastritis, which usually affects the antral mucosa of the stomach, but can occasionally affect the entire stomach (pangastritis) (51). It is not implicated in autoimmune chronic atrophic gastritis, which targets the acid-secreting parietal cells of the body of the stomach, and is characterised by production of auto-antibodies to the parietal cells, leading to their destruction, and resultant achlorhydria (52). It has also been widely shown that the majority of patients, approximately 95%, with duodenal ulcer, harbour the organism, as

do about 70% of those with gastric ulcer (excluding those with NSAID-induced gastric ulcers) (42). The means by which the organism exerts its pathogenic action has not been fully identified, although a number of hypotheses have been proposed. Production of ammonia from urea by the organism's characteristic urease enzyme may interfere with the passage of hydrogen ions from the secretory canaliculi, and ultimately lead to their back diffusion through the gastric mucosa, with resultant mucosal injury (52). Similar injury may also result from damage to the mucus barrier, again leading to its increased permeability to hydrogen ions, and this may be mediated by protease enzymes produced by the bacterium (53). Direct cytotoxic activity of a protein produced by Helicobacter organisms, has been demonstrated in mammalian cells in vitro (54). The gene which encodes for this cytotoxin has been identified and is known as the cag-A gene (cytotoxin associated gene) (55). Infection with the organism leads to chronic elevation of endogenous gastrin levels (56), possibly due to the alkalisation of the mucosal surface by ammonia produced by it's urease enzyme (57), or by interference with local mucosal anti-secretory mechanisms such as somatostatin release (58). This results in significantly increased basal and maximal gastric acid output compared to non-infected persons, a difference which resolves after eradication of the organism (58). Whatever the mechanism of injury, and although the association of the organism to peptic ulceration is very strong, on current evidence, it cannot be conclusively stated that Helicobacter pylori is the sole cause of such ulceration as there are many more asymptomatic and disease free Helicobacter positive individuals who will never develop the disease, than there are peptic ulcer sufferers (52). Furthermore, it cannot be implicated in Helicobacter negative patients with ulceration secondary to Zollinger-Ellison syndrome or aspirin/NSAID use (41). The organism's pivotal role in peptic ulcer disease is inferred from the observation of dramatically reduced recurrent ulceration rates after its eradication by appropriate

antibiotic therapy in conjunction with acid secretory inhibitors, as opposed to those patients treated by acid-suppresing agents alone (52,59).

3.3. Aspirin and Non-Steroidal Anti-inflammatory Drugs (NSAIDs)

The association between the use of aspirin and non-steroidal anti-inflammatory drugs and peptic ulcer, particularly its complications of perforation and haemorrhage, is now widely recognised (60). A positive correlation exists between the use of such agents and the incidence of complicated ulcer disease, and it is perhaps not surprising that the elderly population, who are afflicted by chronic inflammatory disorders such as arthritis and who therefore are prescribed the bulk of such agents, are the group predominantly affected. Treatment is associated with two distinct macroscopic forms of mucosal injury which are the result of direct and systemic effects of these agents respectively (60). Direct injury results in small mucosal haemorrhages and erosions and is due to topical solubility of the drug into the gastric epithelium. More important are the systemic effects of such agents, which lead to the development of ulceration. This occurs as a result of inhibition of mucosal prostaglandin synthesis, which are necessary for maintenance of mucosal protective functions including mucus production, bicarbonate secretion and mucosal blood flow. Indeed, concurrent treatment with the prostaglandin analogue, misoprostil, results in significantly fewer NSAID ulcers (61), and has been commercially available, both alone and in combination with anti-inflammatory agents, for the past few years.

3.4. Zollinger-Ellison Syndrome

Less common than the previous two forms of peptic ulceration are those cases of peptic ulceration occuring in the setting of the Zollinger-Ellison syndrome, briefly referred to earlier. This uncommon disease is characterised by massive hypersecretion of gastric acid

in response to autonomous production of the peptide hormone, gastrin, by a neuro-endocrine cell tumour, most commonly situated in the head of the pancreas. Gastrin, which is the major stimulant to post-prandial gastric acid secretion, will be discussed in more detail later. It is normally secreted from neuro-endocrine G-cells situated in the mucosa of the gastric antrum and proximal duodenum. Its secretion is subject to a negative feedback mechanism, whereby further release is inhibited as intraluminal acid increases and gastric pH falls. Tumour gastrin production is not under such physiologic control and as a result, in response to the chronic circulating hypergastrinaemia so produced, there occurs excessive acid and pepsin secretion. This results in peptic ulceration in the stomach, duodenum and proximal jejunum. Not only is the amount of acid secretion increased by maximal stimulation of existing parietal cells, but the capacity for the stomach to produce more acid is increased due to an expansion in the gastric parietal cell mass (62), secondary to the trophic action of gastrin on the gastro-intestinal tract mucosa. The syndrome takes its name from the clinicians who first described it in two of their patients (63). They correctly postulated that the clinical features were the result of a circulating humoral factor produced from the pancreatic tumour, although it was to take another twelve years before that factor was definitely confirmed as gastrin (64). A complete resumé of the disorder is outwith the scope of this thesis, but a number of salient features are presented to summarise (65-67). It is a relatively rare condition with reported incidence rates of between 0.2 to 0.4 cases per million of the general population per year, and accounts for an estimated 0.1 to 1% of all peptic ulcer patients. Twenty five percent of all cases occur within the spectrum of the hereditary, autosomal dominant, Multiple Endocrine Neoplasia (MEN) Type I syndrome with adenomas elsewhere, most commonly of the parathyroid glands, but also in the pituitary, adrenals, and rarely, thyroid. Gastrinomas in this group are commonly multifocal. The remaining

75% are termed "sporadic" and are usually solitary. They are usually slow growing lesions, but up to 50% of gastrinomas are locally invasive and metastasise. Often such behaviour only becomes apparent retrospectively as it is notoriously difficult to ascertain histologically, benign from malignant lesions. Treatment is aimed at both control of acid hypersecretion and removal of the primary problem itself. Latterly the former was performed by subtotal gastrectomy to excise the parietal cell mass, but with the advent of powerful anti-secretory agents such as omeprazole, this has largely become obsolete. As these tumours can often be only a few millimetres in diameter, attempts are made to accurately localise them pre-operatively and intra-operatively using modalities such as ultrasound, computerised tomography, magnetic resonance imaging, selective mesenteric angiography and selective portal venous tributary blood sampling. These techniques combined with careful and complete inspection and palpation at laparotomy, have varying degrees of specificity, sensitivity and success. Despite this, even in the hands of the most experienced surgeons, up to 50% of laparotomies fail to identify the tumour. Apart from pre-operative tumour localisation, it is also useful to attempt to identify the presence of inoperable metastases as, already mentioned, adequate control of acid secretion can now be obtained with safe and simple medication and furthermore, the patient avoids an unnecessary operation. Where surgery is possible and the tumour is identified, local enucleation of the tumour is the usual method of removal, and more extensive procedures such as excision of the entire pancreatic head by way of a Whipple's procedure are not advocated. Treatment of non-resectable and metastatic gastrinoma by cytotoxic chemotherapy and also the somatostatin analogue, octreotide, have been reported with varying results. At present, control of acid secretion by omeprazole and related compounds remains the therapeutic mainstay. Overall survival figures for Zollinger-Ellison syndrome vary from 62 to 75% at five years and 47 to 53% at ten years.

3.5. Associated factors

Finally, mention must be made of other factors which are associated with peptic ulcer disease. These include familial propensity, possession of blood group O, seasonal and geographic variability, smoking, alcohol, diet, stress and the psychological profile of the individual patient (42,45).

3.6. Summary

In summary, peptic ulcer disease clearly has a multifactorial aetiology, with strong associations to a number of factors. Since the latter part of the nineteenth century until very recently, therapeutic intervention, for the most part, has been aimed at ways of reducing the secretion of acid. However, it should now be clear from the foregoing discussion that the disorder is not solely caused by its excess secretion. A discussion of the various forms of treatment for peptic ulcer now follows, with particular attention to surgical therapy. It is the latter that has been conflictingly reported as rendering its recipients at increased risk for developing malignant disease in the years following surgery. However, in order to appreciate the rationale behind the various surgical and pharmacological manoeuvres which have been developed for the purposes of reducing gastric acid secretion, it is appropriate to first summarise the major physiological mechanisms which are responsible for stimulating acid secretion by the stomach.

4. Regulation of Gastric Acid Secretion

Establishment of the "acid" nature of gastric secretion dates back to the 1820's, from the work of Thomas Beaumonte, who demonstrated its presence in the effluent of a post-traumatic enterogastric fistula in one of his patients, Alexis St. Martin (68). At the same time, reports from Germany and France, by Tiedemann & Gmelin (69) and William Prout (70) respectively, confirmed it as being hydrochloric acid. The mechanisms by which acid release was regulated were only to become apparent at the beginning of the twentieth century, with the famous work of Pavlov, who demonstrated stimulation of gastric acid secretion secondary to the sight and smell of food (71), and that such neurally stimulated secretion was abolished by section of the vagus nerves (72). Edkins, in 1905, showed there to be a powerful stimulant to gastric secretion in extracts of canine gastric antrum when injected into dogs (73). He called this unidentified substance, gastrin. This hypothetical hormone's existence was ultimately confirmed some sixty years later, isolated from porcine antral mucosa, by Gregory and his associates (74). We now know these observations as being two of the main regulatory pathways of gastric acid secretion, namely neural and hormonal. The third is the paracrine, due to local secretion of histamine from mucosal mast cells and enterochromaffin cells. Histamine's action as a potent stimulant to gastric acid secretion was shown by Popielski in 1920 (75).

All three of the major pathways interact to produce and self-regulate acid output, and their effects have been shown to be synergistic (76). Ultimately, they act to stimulate production of acid from the gastric parietal cell, and the latter possesses specific membrane receptors for acetyl choline (the neurotransmitter released from post-synaptic parasympathetic neurones in the gastric wall), gastrin, and histamine (76). In addition, gastrin release is itself stimulated by neuronal influences; indirectly, by vagally mediated

inhibition of somatostatin release, another of the gastrointestinal peptides which acts upon both the parietal and gastrin-producing G-cells in an inhibitory fashion; and directly, by release of another peptide, bombesin (also known as gastrin releasing peptide - GRP), from post-synaptic neurones in the gastric antrum. Furthermore, histamine release is enhanced by direct action of acetyl choline and gastrin on the mast cell (76).

As acid secretion increases in response to these stimuli, the fall in intra-luminal pH leads to reflex inhibition of further acid output. This is the result of acid-stimulated release of somatostatin from cells in the mucosa of the gastric antrum and duodenum, which lie in close proximity to G-cells, and which are directly inhibited by the peptide (76). As will be discussed later, it is the loss of this inhibitory feedback mechanism, and the resultant chronic elevation of circulating levels of gastrin that attends surgically or pharmacologically induced abolition of gastric acid secretion, that may be a critical factor in the future development of colorectal and other cancers.

5. Evolution of Surgery for Peptic Ulcer

The surgical treatment of peptic ulcer disease initially stemmed from operations devised for resection of gastric cancer. However, as the understanding of the physiology of gastric secretion improved, new procedures were developed and others refined to specifically target the stimuli to gastric acid secretion.

5.1. Distal Gastric Resection (Antrectomy) - Bilroth I and Bilroth II Partial Gastrectomy, Gastroenterostomy and Pyloroplasty

5.1.1. Antrectomy

The first successful distal gastric resection for gastric cancer in man was performed by Professor Theodor Bilroth of Vienna in 1881, with restoration of continuity by gastroduodenal anastomosis (77). The procedure bears his name to the present day as the Bilroth I partial gastrectomy. The operation was devised in 1874 in dogs by two of his students, Gussenbauer and Winiwarter (78). However, the idea of such a procedure for gastric cancer pre-dates this by almost a century and is attributed to Michaelis in 1786 (79), whose student Merrem successfully performed pylorectomy in dogs in 1810 (80). Its potential application to humans was decried by Langanbeck, a leading surgeon of the time, and as a result no progress was to be made until its resurrection later that century (79). Although Bilroth is attributed with the first "successful" partial gastrectomy (his patient died four months later from recurrent disease), unsucessful attempts on humans had been made by others, namely Pean (France) in 1879 (81) and Rydygier (Poland) in 1880 (82). It was the latter who first applied it successfully to a patient with peptic ulcer disease in 1882, in order to excise a benign pyloric ulcer (83).

Bilroth subsequently restored gastrointestinal continuity after gastric resection by means of a gastrojejunal anastomosis in 1885 and of course this is now known as the Bilroth II, or Polya, partial gastrectomy. Bilroth's assistant at that operation, Viktor von Hacker, reported the technique to the German Surgical Society (84). The alternative name is derived from Eugen Polya, a distinguished Hungarian surgeon who helped popularise gastric resection for peptic ulcer disease in America in the first half of the twentieth century, with the help of other leading proponents of the method such as Finsterer and Mayo (75). Despite Bilroth being credited historically with the technique, like the Bilroth I procedure, this form of gastroenteric anastomosis was first applied by one of Bilroth's associates, Wölfler, in September 1881, to bypass an obstructing and unresectable distal gastric tumour (85). It is said that it was in fact the first assistant at that operation, Karl Nicoladoni, who suggested the maneouvre to Wolfler (75).

5.1.2. Gastroenterostomy

Gastroenterostomy without gastric resection for uncomplicated chronic duodenal ulcer was subsequently undertaken by Codivilla in 1893, on the basis that bypassing the duodenum would rest the part and allow ulcer healing (86). By the end of the nineteenth century, gastroenterostomy alone was popularised by others such as Moynihan and Mayo and became the most commonly performed procedure for benign peptic ulcer for the first half of the twentieth century (87).

5.1.3. Pyloroplasty

The first pyloroplasty was performed by Heineke in 1881, to overcome a gastric outlet obstruction secondary to a large duodenal ulcer penetrating into the pancreas (87). It was only described in the literature some years later by one of his students, Fronmüller, in

1886 (88). Soon afterwards, Mikulicz described an identical procedure to allow access to cauterise a bleeding ulcer (89). This was in 1887, and due to the close temporal relation of these descriptions, both surgeons are immortalised by the term, Heineke-Mikulicz pyloroplasty. Various modifications to this procedure have been described by others, but it is in its original form that it was most widely performed.

As already mentioned, gastroenterostomy on its own was the most widely practiced surgical treatment for peptic ulcer in the United States of America (87). This was because it was a widely held view that gastric resection, more widely practised in Europe, was accompanied by significantly higher morbidity and mortality than the former (87). With time, however, it became increasingly apparent that gastroenterostomy was accompanied by a high incidence of recurrent ulceration, with rates of 30-50% quoted in some studies (87). Clearly, gastroenterostomy was, on its own, an inadequate treatment in effecting long-term cure of the ulcer diathesis and its popularity declined in favour of gastric resection by the mid-1930s (87). Exponents of gastric resection also realised from retrospective review, and the acceptance that the antrum produced a potent acid secretagogue, as demonstrated by Edkins (73), that at least two-thirds gastrectomy was required to lead to resolution of the ulcer and avoid recurrence (79). Furthermore, a higher recurrent ulcer rate after Bilroth I gastrectomy led to its relative unpopularity compared to Bilroth II (79). This was probably due to food-stimulated release of duodenal gastrin occurring in the former, as was to be demonstrated many years later (90). Gastric resection, with its low incidence of recurrent ulceration, but significant incidence of side-effects, was thus established as the surgical mainstay of treatment for both gastric and duodenal ulcer by the middle of the century. Its position, however, was

soon to be challenged by the introduction of a new approach to reduce gastric acid secretion.

5.2. Vagotomy

5.2.1. Truncal Vagotomy

In 1944, Lester Dragstedt and Frederick Owens from Chicago reported the effect of division of the vagus nerves above the diaphragm on gastric secretion in two patients with symptomatic duodenal ulcer (91). In each case, the vagi were approached through a left thoracotomy with removal of the eighth rib, and after mobilisation of the lower ten centimetres of the oesophagus, the vagal fibres were separated from it and lengths of three to four centimetres excised. They observed a 70% reduction in the volume of nocturnal acid secretion in early post-operative measurements compared to pre-operative values (91). Furthermore, the acidity of these post-vagotomy secretions was considerably reduced compared to pre-operative levels, with pH values of 2.70-2.73 and 1.25-1.50 respectively. Similar measurements made two to three months later, confirmed that these changes were maintained. Most importantly, both patients were rendered asymptomatic and no difficulty with eating or swallowing was reported after the operation. In view of the small patient number and the brevity of the post-operative follow-up period, the authors cautiously concluded that although both the patients studied had appeared to benefit from the procedure, they could not recommend its introduction into clinical practice for treatment of duodenal ulcer.

Dragstedt had simply applied long recognised physiological knowledge in the development of his operation. Benjamin Brodie, in 1814, had demonstrated that dividing the vagi below the diaphragm significantly reduced gastric secretion induced by arsenic in

dogs (92). At the turn of the century, Pavlov and co-workers demonstrated long-standing inhibition of gastric secretion by both supra- and subdiaphragmatic vagotomy in dogs (72,93). In the early part of the 20th century, sub-diaphragmatic vagotomy was employed by a number of clinicians, for a variety of gastric maladies (94). Exner was probably the first to employ the technique in humans in 1911, for cure of gastric symptoms of tabes dorsalis (94). Bircher reported using the technique in twenty patients in 1920 (95). Two years later, Latarjet reported its use in twenty-four patients, six of whom had duodenal ulcer (96). He recognised that vagotomy, apart from denervating the secretory parietal cell mass, also led to gastric stasis and he feared that this would aggravate or induce ulceration in the stomach. He therefore employed a gastroenterostomy in all cases, and indeed attributed the success of his procedures more to the latter than to vagal transection. He hypothesised that vagotomy might be used to complement gastroenterostomy for the treatment of ulcer. Several other reports of varying success with vagotomy were to follow. Dragstedt's advocacy of complete division of all vagal fibres arose from his conclusion that many of the failed operations that had been performed previously were the result of incomplete vagotomy (94). His initial avoidance of a complementary drainage procedure on the stomach was to ensure that the latter would not be attributed as the major factor in the success of the operation, as Latarjet had thought. Despite his initial successes, he soon came to realise the need for an adequate gastric drainage procedure in conjunction with truncal vagotomy, and in order to perform both, subdiaphragmatic vagotomy from an abdominal approach led largely to the abandonment of his initial intrathoracic vagotomy (94).

5.2.2. Selective Gastric Vagotomy

Shortly after the introduction of vagotomy, refinements to the procedure were advocated. These were prompted by the recognition of unwanted extragastric side-effects accompanying the operation. Jackson (97) and Franksson (98), working in separate centres, questioned the need for complete transection of all vagal fibres, which included those running to the hepato-biliary system and small bowel, when the sole aim was to denervate the stomach alone. Thus the operation of selective gastric vagotomy was to become established in clinical practice. In fact, the technique of selective gastric denervation was probably employed by the earliest proponents of vagotomy, such as Bircher (95) and Latarjet (96), but it was to this incomplete form of vagotomy that Dragstedt attributed the generally poor results of these early operations (94). Despite the selectivity of denervation, all nerves to the stomach were divided, including the motor branches to the antrum, and hence it soon became apparent that an accompanying drainage procedure was still required, just as with truncal vagotomy. Because of this, and the fact that it was technically more demanding to perform, without any convincing evidence of physiological superiority to the truncal form, it did not become universally popular (40,99).

For the next twenty to thirty years, vagotomy, either truncal or selective, became the most popular procedure for peptic ulcer. It was combined with either pyloroplasty, gastroenterostomy or antrectomy. The preference for the accompanying drainage procedure, or incorporation of antral resection, was very much dependant on the mortality, morbidity and rate of recurrent ulceration experienced by individual surgeons for each type of operation.

5.2.3. Highly Selective Vagotomy

In an effort to reduce the unwanted sequelae of truncal or selective vagotomy, which were largely the result of the drainage procedure required to overcome gastric stasis, the next refinement to the procedure was conceived in 1957 by Griffiths and Harkins, who performed selective denervation of the vagal fibres supplying the acid-secreting parietal cell mass only, preserving the hepatic, coeliac and gastric antral fibres (100). This was performed in ten dogs with good results (100). Such highly selective vagotomy (H.S.V.), also known as proximal gastric vagotomy, parietal cell vagotomy or selective proximal vagotomy, was performed in humans by Ferguson in 1960 (101), and Holle in 1961 (102). However, in each case, they combined the vagotomy with gastric resection and pyloroplasty respectively. The first reports of highly selective vagotomy without a combined drainage procedure or gastric resection were first published in 1970 by Amdrup & Jensen (103) and Johnston & Wilkinson (104), almost three decades after Dragstedt's initial description of vagotomy in man (91). Whilst the morbidity and mortality associated with this procedure was appreciably lower than with previous forms of anti-ulcer surgery (105), it was attended by a higher ulcer recurrence rate (106). Its widespread use was hindered by the tedious and time-consuming nature of the surgery, as well as the technical expertise required to achieve good results. Ultimately, the latest modifications to the technique were introduced in the 1980's, to overcome this. This comprised posterior truncal vagotomy combined with anterior lesser curve seromyotomy (107,108). This procedure has been shown to achieve similar results in terms of ulcer cure and post-operative sequelae as highly selective vagotomy (109). It also avoids the rare, but commonly fatal, complication of lesser curve necrosis which is peculiar to highly selective vagotomy, and is due to interruption of the blood supply to the lesser curvature of the stomach during the dissection and section of the nerve branches (105). The newer

operation avoids this by preserving all the posterior lesser curve blood vessels, since this area is left undisturbed at operation. Whilst the myotomy achieves compete transection of all nerve branches, if performed properly it should avoid division of the accompanying blood vessels which run more deeply in the stomach wall than the nerves (107).

Finally, gastric surgery has not escaped the revolution of laparoscopic surgery and truncal vagotomy (with pyloric stretch), highly selective vagotomy, and the latest techniques with lesser curve myotomy, as well as simple closure of perforated ulcers in the emergency setting, have all been performed using minimal access techniques (110).

6. Medical Therapy for Peptic Ulcer

Elective surgery for peptic ulcer has witnessed a tenfold decrease over the past thirty years (111). This, in part, is due to the decline in the natural incidence of the disease (37). However, a large proportion of that decrease, especially in recent years, must be attributed to the rapid evolution of powerful anti-ulcer drugs dating from the introduction into clinical practice of the first histamine receptor antagonist, cimetidine, in 1977. Prior to that time, the mainstays of medical therapy before the histamine receptor antagonist era of the seventies, were prescribed diets, antacids and anti-cholinergic drugs. Over recent years, newer, more potent antagonists of acid secretion have been developed, including those with the same mode of action as cimetidine, and others such as the proton pump inhibitors. Omeprazole, the first of these proton pump inhibitors, is central to the experimental studies which are to be presented later. Other agents which do not affect gastric acid secretion in any way, but which are thought to stimulate the protective mechanisms of the gastroduodenal mucosa, so-called cytoprotective agents, have also been introduced over this period and are widely used. The latest development in medical therapy for peptic ulcer has been the introduction of specific antibacterial regimes in conjunction with anti-ulcer drugs to eradicate Helicobacter pylori infection in the presence of peptic ulcer. A brief summary of these drugs now follows.

6.1. Restrictive Diets

From the turn of the century until the 1970's, the prescription of bland diets for peptic ulcer sufferers was commonplace. The best known of these was the Sippy diet (112). This consisted of a prolonged period of hospitalisation, sedation and bedrest, often up to six weeks, in combination with hourly administration of small amounts of food, in the

form of milk, cream or eggs along with "Sippy powders" which were combinations of antacid salts such as sodium bicarbonate, calcium carbonate and magnesium oxide. Sedation was provided by administration of phenobarbitol. Even before the advent of modern therapeutic agents, the requirement for such restricted diets were questioned, and a number of investigators showed there to be little difference in healing rates in patients taking unrestricted diets (112). The milk-based diets, based on their acid-buffering capacity, may have done more harm than good, and indeed milk, with its high protein content, actually increases gastric acid secretion (112). The impact of such diets on the risk of cardiovascular disease also led to their condemnation.

6.2. Antacids

Antacids have been used for centuries for the treatment of dyspeptic symptoms. John Abercrombie of Edinburgh, in 1828, recommended milk for relief of ulcer symptoms (113), and ancient physicians reported the efficacy of crushed coral (calcium carbonate) in patients with dyspepsia, as long ago as the 1st century A.D. (43). Even now, their widespread availability as "over-the-counter" formulations, with patients treating their dyspeptic symptoms themselves, causes some concern that in a proportion of such cases, more sinister upper gastrointestinal lesions are escaping early diagnosis.

The commonest formulations in present day use are oxide and hydroxide salts of aluminium and magnesium, or combinations thereof. The basis of antacid therapy was, until recently, to administer enough of the compound to buffer all free acid in the stomach (41,45). As gastric secretion is a continual process, albeit with meal-stimulated peaks, this results in a regime of frequently repeated daily doses result in large total doses, up to 1,000 millimoles per day. Maximal buffering capacity of meal-stimulated acid is achieved if the antacid is taken approximately thirty minutes after food, with continued action for

up to two to three hours thereafter (114). However, recent studies have shown that low dose antacid therapy, that is, of 120millimoles per day, has comparable healing rates to the traditional regimes (41,46,114). This calls into question whether their action is purely the result of neutralisation of acid. This dose is clearly inadequate to buffer the acid produced over a twenty-four hour period, and it is suggested that cytoprotective effects such as stimulation of prostaglandin release, mucus and bicarbonate secretion, mucosal regeneration and mucosal blood flow, may be the mechanism responsible for this protection (45). Such low dose regimes will reduce the side-effects commonly seen in up to 60% of patients with the more traditional schedules, and include constipation with aluminium salts and diarrhoea with magnesium(41). Hypophosphateaemia may also occur with aluminium compounds, due to formation of insoluble precipitates in the gut, with resultant symptoms of fatigue, anorexia and skeletal pain (114). Both aluminium and magnesium are absorbed as chloride salts, and toxicity may occur in the patient with chronic renal failure (41,114). Even in health, there are concerns as to the effect of tissue deposition of aluminium in prolonged therapy, as a contributor to osteoporosis and osteomalacia, and possibly Alzheimer's disease (46).

Calcium carbonate and sodium bicarbonate have also been used for their ability to buffer gastric acid secretion, but they have fallen from favour for a number of reasons. Sodium bicarbonate is highly soluble, hence apart from having a short duration of action, it is also promptly absorbed from the small intestine and repeated dosage may result in a metabolic alkalosis, especially in the renally impaired (45,114). Calcium carbonate is not recommended due to the significant absorption of calcium that occurs and which may lead to the symptoms of hypercalcaemia, with precipitation of renal stones (45). Indeed, excessive dosing with combinations of these two agents can lead to the potentially dangerous milk-alkali syndrome, characterised by metabolic alkalosis and transient renal

insufficiency (112,114). In most cases, normality resumes with discontinuation of therapy, but in some cases, a more chronic form of the syndrome ensues. This is characterised by hypercalcaemia with bone pain, anorexia and metastatic deposition of calcium in soft tissues such as the eye. A further reason to avoid calcium-containing antacid formulations is that residual calcium left after acid neutralisation has occurred actually stimulates gastric acid secretion, possibly by increasing gastrin release and also by a more direct action on the parietal cell. This is known as the "acid-rebound" effect (45).

6.3. Anticholinergic drugs

Such agents act by blocking the M1 muscarinic acetyl choline receptors on the parietal cell to abolish vagally stimulated acid secretion. However, their absorption is low and erratic, and they reduce both basal and meal-stimulated acid secretion only by about 30 to 50% (41,114). The classical "atropine-like" agents, such as propantheline, hyoscine and butylscopolamine, act on both types of muscarinic receptors, M1 and M2, and at therapeutic doses, this results in a number of troublesome side effects such as dry mouth, blurred vision, tachycardia, constipation and urinary retention (41). Newer selective M1 receptor antagonists, pirenzepine and telenzepine, have been developed, but like their forebears, have no advantage over the new types of anti-secretory agent available, and as such, the use of anticholinergic drugs in peptic ulcer disease is largely now of historical interest only.

6.4. Carbenoxolone

For the purpose of completeness, mention must be made of this compound derived from liquorice, which was used briefly in the 1970's in the treatment of gastric, and to a lesser extent, duodenal ulcers. It has no activity against acid secretion and exerts a

cytoprotective effect, possibly by reducing prostaglandin breakdown in the mucosa (41,114). Its side effects stem from its corticosteroid-like activity, leading to salt and fluid retention and hypokalaemia, which may precipitate or exacerbate congestive heart failure in susceptible individuals. For this reason and its lack of obvious benefits over other agents, its vogue was shortlived.

6.5. H_2-Histamine Receptor Antagonists

When Sir Andrew Watt Kay, in 1953, described the augmented histamine stimulation test for the purposes of investigating gastric acid output, he observed that histamine's secretory action, and the associated facial flushing which occured with its administration, were not abolished by the administration of the standard antihistamine type drugs available at the time (115). In 1966, Ash and Schild showed that histamine acted on more than one form of receptor (116), and this prompted an intensive search for novel drugs capable of blocking these so-named H_2 receptors. By 1972, three such agents had been developed and tested on both animals and humans, by Sir James Black and his team at the Smith-Kline & French laboratories (117). Their rapid, sustained and marked inhibition of maximally stimulated acid secretion, assessed by administering pentagastrin to a Zollinger-Ellison patient with pre-existing supernormal basal levels, was profound (118). The extremely low incidence of side effects was also a prominent feature. Of these original three compounds, cimetidine was found to be the most potent with the highest safety profile and was subsequently introduced to the market in 1976. It is still one of the most commonly used agents in its class today.

Since then, the group has expanded with the subsequent introduction of ranitidine, famotidine, nizatidine and roxatidine, which on a dose equivalent basis, are more potent than cimetidine (46). Apart from the smaller dose requirements which stems from this, the

superiority of the newer agents compared with cimetidine is questionable and at best modest (41,46).

The H_2 receptor antagonists competitively inhibit such receptors on the gastric parietal cell. Because of the synergy between the three main stimulatory pathways of acid secretion, they also significantly reduce vagal and gastrin stimulated acid secretion. They therefore inhibit up to 90% of daily acid output, but do not abolish meal-stimulated peaks (114). The realisation that ulcer healing rates were positively correlated with reduction of nocturnal acid output rather than daytime or total daily output is evidence that the persistence of meal-stimulated acid output while being treated with such agents does not detract from their efficacy (45). Indeed, such observations have led to alterations in the dosing schedules of such agents, from three or four divided doses daily to single nocturnal or twice daily dosing. Ulcer healing rates on these agents are of the order of 75% and 95% at four and eight weeks respectively, for duodenal ulcer. Healing rates for gastric ulcer are slightly lower at approximately 60% and 85% at four and eight weeks respectively (45). These drugs have an excellent safety profile with less than 3% of patients reporting adverse effects (45), which include minor gastrointestinal and central nervous system disturbances, such as diarrhoea, nausea, headache and drowsiness. Gynaecomastia and impotence are rare but well known side effects of cimetidine, and are due to an anti-androgenic action of the drug with displacement of dihydrotestosterone from peripheral receptors (45).

The significant medical advance that Black's discovery produced was recognised by his peers, being awarded the Nobel prize in 1988 for his work. Despite the introduction of an even more powerful class of gastric acid inhibitor since his discovery, the place of H_2 receptor antagonists in the treatment of peptic ulcer disease seems assured at the present time.

6.6. Proton Pump Inhibitors

The final common pathway by which hydrogen ions are secreted from the parietal cell in response to vagal, gastrin and histamine mediated stimulation, occurs at a cell membrane enzyme, Hydrogen Potassium Adenosine Triphosphatase, also known as the proton pump (41). When active, it excretes a hydrogen ion in exchange for a potassium. It is abundant on the invaginated cell surface that forms the secretory canaliculus on the mucosal surface of the parietal cell. It is by the selective and irreversible inhibition of this enzyme that the latest class of acid inhibitory drugs exert their powerful action.

The first of these agents, omeprazole, became available for clinical use in the late 1980's. It is in fact an inactive pro-drug, and as a weak base, is rapidly degraded in the stomach unless administered with a protective enteric coating. Intestinal absorption is rapid and the drug distributes widely throughout the body, initially to the extracellular space (45). It ultimately diffuses through the parietal cell to reach the acidic milieu of the secretory canaliculus, and within four hours of dosing, it has been demonstrated that the majority of the administered drug comes to be concentrated in this area (41). Here, it becomes protonated, which prevents the drug from passing through the mucus surface barrier into the lumen of the stomach. High concentrations of the drug accumulate in the canaliculus as a result. Protonation also leads to a configurational change in the molecule to its active sulphenamide form, and it binds irreversibly to the H+/K+ ATPase enzyme by covalent disulphide bonds (41). As a result, enzymatic activity only returns when sufficient new enzyme is synthesised and it is for this reason that the action of the proton pump inhibitors is so marked and prolonged. Hence, complete abolition of total daily gastric acid output is achieved with once daily dosing of sufficient strength, and unlike the H2 receptor antagonists, meal stimulated acid peaks are also abolished (41). Rapid healing of ulcers ensues, with rates at two and four weeks comparable to those achieved by H2 antagonists

at four and eight weeks (114). Due to its administration as a pro-drug, and rapid and almost complete convergence at its site of action, its adverse reaction profile is minimal. Omeprazole does however interact with certain members of the drug-metabolising family of cytochrome P450 enzymes, situated predominantly in the liver (as do the H_2 antagonists) and this can lead to potentiation of effects of drugs such as phenytoin and warfarin, if administered concurrently (114). As will be discussed later, this action may also be important when considering a possible protective role in the development of experimental colonic tumours, which constitutes a large portion of the study in this thesis. Of more concern are the potential sequelae of the physiological changes which accompany the profound and complete acid suppression afforded by these agents, and this too will be discussed in more detail later in this chapter.

6.7. Cytoprotective Agents

With the growing realisation of the significance of mucosal defence mechanisms in the pathophysiology of peptic ulcer, a number of drugs which do not exert any action on acid secretion have also become established in the past twenty years.

6.7.1. Sucralfate (41,45,46,114)

This compound is a complex sugar molecule with bound aluminium hydroxide residues. In the presence of acid, the aluminium hydroxide dissociates, leaving negatively charged sulphate groups which bind electrostatically to proteins and mucins on the ulcer base. This binding is thought to inhibit penetration of aggressors such as acid, pepsin and bile salts. It is possible that it further enhances mucosal protection by stimulating prostaglandin synthesis, mucus and bicarbonate secretion, and exerting a trophic effect on gastric mucosa. Very little of the drug is absorbed, most of an oral dose subsequently

being recovered in the faeces, and as such is virtually devoid of side-effects. Similar rates of ulcer healing to those with H_2 antagonists have been shown.

6.7.2. Bismuth (41,45,46,114)

Bismuth-containing compounds, such as colloidal bismuth subcitrate and bismuth subsalicylate, have been used to treat peptic ulcer since the mid-1970's. In the presence of acid, crystals of bismuth oxychloride and bismuth citrate precipitate out of solution and bind preferentially to the base of ulcer craters, producing an impenetratable barrier to further damage from luminal acid and pepsin. They also stimulate prostaglandin synthesis and bicarbonate secretion in the mucosa. One further striking feature of the drug is its ability to eradicate Helicobacter pylori in up to 30% of cases, and ulcer recurrence rates after treatment are significantly lower than with H_2 antagonists. Part of its success in healing peptic ulcer may be due to this antibacterial action, by inhibition of its urease enzyme and death of individual organisms due to coating with bismuth salts (51). As with sucralfate, systemic absorption is minimal and reports of adverse effects are low, though blackening of the tongue and darkening of the faeces are commonly seen with treatment and patients should be warned to expect this.

6.7.3. Prostaglandin Analogues (41,45,46,114)

A number of synthetic prostaglandins have been manufactured for use in peptic ulcer disease, in an attempt to confer the protective effects of their natural derivatives upon the gastroduodenal mucosa. Of these, misoprostil has been licensed for use in most countries, including Great Britain and North America. They increase mucosal bicarbonate and mucus production, enhance mucosal blood flow and exert a small, but direct, inhibition of gastric acid secretion. Even at therapeutic doses, they do produce troublesome side

effects such as diarrhoea and abdominal pain. In view of this, and because of their absence of obvious benefits over the other commonly used anti-ulcer drugs, they are not widely used as first line therapy in peptic ulcer disease. Their commonest indication for use is as prophylaxis against NSAID-induced ulceration, and indeed are to be found combined with such agents as single formulations, for instance Arthrotec, which is misoprostil combined with diclofenac.

6.8. Therapy aimed at eradication of Helicobacter pylori (51)

In the past few years, with the recognition of the dramatic reduction in ulcer recurrence rates that accompany successful eradication of Helicobacter pylori, a multitude of drug regimes have been studied in an attempt to identify the most effective at attaining this ideal. Initially, single agent therapy, using either bismuth formulations alone, or single antibiotic agents, was tried. It soon became apparent that no significant impact on ulcer recurrence was achieved by such monotherapy, and recrudescence of infection occurred frequently. Helicobacter also soon developed resistance to many of the antibiotics to which it was initially shown to be sensitive in vitro. Attempts at dual therapy, either with bismuth or an acid secretory inhibitor in combination with an antibiotic, or double antibiotic therapy alone, were, with few exceptions, also disappointing, and development of antibiotic resistance was still a common feature. This ultimately led to triple therapy regimes, and in view of the high success rates achieved with them, they are now the recommended form of therapy to heal ulcers, eradicate Helicobacter infection, and thus significantly reduce ulcer recurrence. A number of combinations have shown to be equally efficacious, and usually consist of either bismuth, an H_2 antagonist, or a proton pump inhibitor, in combination with amoxycillin or clarithromycin and metronidazole. Such regimes achieve 80 to 100% eradication of the organism (41,51), and resultant

recurrence rates of approximately 20% as compared to around 90% or more, with single

agent acid reduction therapy (41,52,59).

7. Effect of Gastric Surgery and Drug Therapy on Gastrointestinal Physiology

A number of pathophysiological changes occur in response to ulcer treatment and are presented below. It is these changes that may be the cause of the described association between such treatment and subsequent cancer development in the gastrointestinal tract.

7.1. Inhibition of Gastric Acid Secretion

It is clear from the foregoing that the commonest and indeed, the desired, physiological change that accompanies most forms of surgical or medical treatment for peptic ulcer is a reduction in the "normal" amount of gastric acid produced. Dependant on the nature of surgery performed or degree of pharmacological acid inhibition achieved, this may result in partial acid reduction - hypochlorhydria ; or complete abolition of its secretion - achlorhydria. Clearly, this does not apply to treatment with agents that have no inhibitory effect on gastric acid production, nor to operations that do not significantly interfere with the acid-secreting parietal cell mass or its stimulatory influences.

The extent to which various surgical procedures reduce acid output have been widely studied in patients. Two parameters are usually measured, basal and maximal acid output. Basal acid output (BAO) measures the amount of acid secreted whilst the stomach is in the fasting state, whereas maximal acid output (MAO) is that occurring in response to parenteral administration of a potent secretagogue, either histamine or pentagastrin. Pentagastrin is a synthetic analogue of gastrin, consisting of the five amino acids chain peptide present at the C-terminus of the natural hormone which confers its physiological activity. Both BAO and MAO are usually expressed as milliequivalents per hour, as the acid present in the collected gastric aspirate is indirectly measured by titration with a strong alkali such as sodium hydroxide until neutral pH is reached.

As one might expect, neither gastroenterostomy nor pyloroplasty, when performed alone, significantly alter the acid output of the stomach (119,120). On the other hand, antrectomy resulted in a mean reduction of acid output of 82% from pre-operative levels in one study of forty-nine patients (121). Professor Kay's observed value in gastrectomised individuals was slightly lower at 70% (122). Broomé and colleagues observed a mean 50% reduction in maximal acid output in thirteen post-antrectomy subjects (123).

Truncal vagotomy and drainage procedures have been shown to reduce acid secretion to a similar degree to partial gastrectomy. Measured reductions of acid output for vagotomy and gastroenterostomy include a mean of 68.4% and an 80% reduction in MAO in response to histamine (121,124). Similar results are achieved with vagotomy and pyloroplasty. For example, 78% and 64% reductions occured in basal and maximal output respectively, in one study (125), and 67% and 78% decrease in these parameters in another (126). The type of vagotomy performed, whether truncal, selective or proximal, do not have quantitively different effects on the degree of acid reduction obtained (127-129). In their presentation of twenty-five patients treated by highly selective vagotomy, Johnston and Wilkinson observed maximal acid output to be reduced by 70% (104).

As one may have predicted, the combination of antrectomy with vagotomy and hence removal of the two main stimuli to gastric secretion, cause even greater acid reduction. Over 90% decreases in both BAO and MAO from pre-operative values have been demonstrated in one study and 76% and 85% respectively, in another (126). Furthermore, the magnitude of acid reduction was increased by a further 33% after the addition of vagotomy in Broomé's thirteen previously antrectomised patients (123). Not surprisingly, this form of surgery is associated with the lowest disease recurrence rate of

all the classical peptic ulcer operations, with quoted figures of 2% or less in most series (40).

Medical therapy with histamine receptor antagonists results in up to 90% reduction in total 24-hour acid output in a linear dose dependant fashion (45). Complete inhibition is not seen, and in particular, daytime meal-stimulated peaks of secretion still occur (45). The proton pump inhibitors, such as omeprazole, however, are capable of rendering the patient completely achlorhydric, even by once daily pharmacological dosage (45).

A number of pathophysiological sequelae occur as a result of iatrogenic hypochlorhydria. That which has attracted much interest, for reasons that will be discussed fully later, is the uninhibited secretion of gastrin into the circulation, due to loss of the negative feedback mechanism already described. Serum gastrin levels have been measured for all the main types of ulcer operation in concert with acid secretion, in most of the studies quoted above.

7.2. Effect on Circulating Gastrin Levels

Although both antrectomy and vagotomy result in similar degrees of hypochlorhydria, the post-operative gastrin concentrations differ markedly. Resection of the antrum is performed to abolish gastrin-stimulated acid secretion, by removing the main source of that stimulus. As a result, gastrin concentrations after distal gastrectomy either drop or remain unchanged from normal physiological values as measured pre-operatively. For instance, McGuigan observed a 77% reduction in mean fasting gastrin levels from 114 pg/ml to 26 pg/ml in four patients who underwent antrectomy in addition to vagotomy (125). Whether these patients underwent Bilroth I or Bilroth II anastomosis was not specified. In another study of seven patients examined before and after antrectomy with Bilroth I anastomosis, mean fasting gastrin values were slightly less post-operatively, at

128 and 109 pg/ml respectively (126). Circulating gastrin is not *completely* abolished by antrectomy as G-cells in the proximal duodenum continue to secrete the hormone, albeit to a lesser extent than the gastric antrum. This was demonstrated by Stern and Walsh who observed that patients who had undergone Bilroth I antrectomy, where continuity is restored by gastro-duodenal anastomosis, had meal stimulated release of gastrin due to the presence of food entering the duodenum (90). This response to food was not seen in patients with a Bilroth II anastomosis, where no food enters the defunctioned duodenum but empties further down the small bowel via the gastrojejunostomy. For the same reason, basal gastrin values after the Bilroth I procedure were modestly and insignificantly reduced compared to pre-operative levels whereas the Bilroth II patients had significant reductions in these measurements. This probably explains the differing magnitude of the reduced gastrin levels seen between the first two studies quoted above.

Vagotomy without any concomitant gastric resection causes elevation of circulating gastrin concentrations, due to the G-cells of the intact antrum being continually stimulated to secrete hormone in response to the persistently elevated luminal pH to which they are exposed. Most measurements have been performed on subjects having undergone vagotomy with pyloroplasty as the drainage procedure. McGuigan and Trudeau (125) observed an increase in mean basal values from 98 to 123 pg/ml, which was not statistically significant, but most other studies have shown the elevation to be significant, with increase from pre-operative values of 29% (126), 98% (130), 137% (90), 435% (131), and even 800% (131) in one group of patients. The nature of the vagotomy, whether truncal, selective or highly selective, all have the same effect of increasing circulating gastrin (132,133).

Gastric acid suppression by histamine receptor antagonists or proton pump inhibitors also result in elevation of gastrin levels. Twenty-four hour profiles of intragastric acidity and

plasma gastrin concentrations for all the available histamine receptor antagonists and proton pump inhibitors have been measured in humans, and all show a consistent elevation in mean gastrin levels compared to pre-treatment values (134). Gastrin concentrations have an inverse linear relationship to the degree of acid inhibition, which in turn varies directly in a dose dependant fashion to the amount, and potency, of the drug administered (134). Thus, the increase in gastrin levels is modest with the standard doses of histamine receptor blockers, for instance, approximately 25% increase with cimetidine 800 mgs nocte and ranitidine 150 mgs nocte, but in excess of 300% with Omeprazole 20 mgs once daily (134).

7.3. Effect on the Bacterial Population of the Upper Gastrointestinal Tract

Another consequence of reduced or absent gastric acid secretion is bacterial colonisation of the stomach and upper small intestine. Acid reduction by whatever means, surgical, medical, or secondary to disease such as atrophic gastritis, leads to the survival and proliferation of swallowed bacterial organisms from the oropharynx and nasopharynx, which are normally destroyed in the acidic gastric milieu (135-137). "Faecal" organisms such as faecal streptococci, faecal bacteroides, enterobacteriae and klebsiellae, normally found in the colon, are also encountered in the stomach in such conditions. Significantly increased amounts of bacteria have been recovered from gastric aspirates after even short periods of acid suppression (138-141). The potential pathological consequences of this will be discussed later.

7.4. Sequelae peculiar to Gastric Surgery - the Postgastrectomy Syndromes

A number of clinico-pathological entities occuring after ulcer surgery are well recognised. For the purpose of completeness, they are briefly summarised in the following pages.

Although not confined to gastric resection procedures alone, they are commonly referred to as the postgastrectomy syndromes. They occur in approximately 20% of all operated patients to a varying degree and fortunately most cases are mild, self-limiting and transient (111). Of those that are not, the majority will respond to simple dietary manipulation and hence only a minority of patients are sufficiently disabled to require some form of revisional surgery (111). These syndromes occur as a result of interference with the normal motor function of the stomach and alteration in its rate of emptying. In normality, two vagally mediated reflex mechanisms occur to increase the reservoir capacity of the stomach (111). The first of these is receptive relaxation of the proximal stomach in response to swallowing, and further relaxation and distension of the stomach occurs as food accumulates within it. The latter is known as accomodation. Thereafter, the ingesta is reduced into small amounts of semi-digested foodstuffs (chyme) by peristaltic waves in the stomach wall. This is then expelled in metered amounts onwards into the duodenum for further digestion. An anatomically and physiologically normal pylorus is a pre-requisite for this and it is evident that, with the exception of highly selective vagotomy, this is disrupted or bypassed by the operations devised for peptic ulcer.

7.4.1. Dumping (111,142,143)

This syndrome has been long recognised and indeed the term "dumping" was coined by Mix in 1922, who observed the rapidity with which radiological contrast passed from the stomach to the small intestine, through a gastroenterostomy stoma (144). The arrival of a large amount of hyperosmolar contents into the proximal small bowel leads to its distension with reflex increase in its activity. There also occurs the release of a variety of vasoactive peptides from the gut, for instance, serotonin and vasoactive intestinal peptide (VIP). Furthermore, an extracellular fluid shift into the lumen of the intestine with a

corresponding decrease in the circulating intravascular volume ensues. These responses result in a variety of vasomotor and gastrointestinal symptoms. These include sweating, acute weakness, flushing and palpitations in combination with nausea, abdominal cramps and profuse diarrhoea. Such symptoms usually occur within half to one hour of ingestion of a meal and affected individuals can avoid their onset by altering their eating pattern to eating smaller, more frequent, less carbohydrate-rich meals, a short period of recumbency, and liberal fluid intake after eating.

7.4.2. Post-Prandial Hypoglycaemia (Late Dumping) (111,142,143)

This problem is often referred to as "late dumping", to differentiate it from true "early" dumping, but it is best labelled as above as it is causally and temporally distinct from the latter. As a result of an excessive carbohydrate load being delivered to the small bowel, there occurs release of enteroglucagon, which in turn sensitizes the insulin-secreting ß-cells of the pancreatic islets to respond to the excessive carbohydrate load being absorbed. The insulin response is more than sufficient to redistribute it to intracellular sites, either for storage as glycogen in hepatocytes, or for metabolism in active organs, and a relative hyperinsulinaemia ensues. This results in symptomatic hypoglycaemia, manifesting clinically with acute lethargy, sweating, tremor, confusion and in some cases, collapse. The similarity of the symptoms to those occuring in the dumping syndrome have led to its alternative label; the "lateness" of their onset referring to the fact that they usually occur some three to four hours post-prandially. Unlike the dumping syndrome, gastrointestinal symptoms are not a feature. Once again, alteration in dietary habits is the mainstay of treatment, with avoidance of excessive amounts of carbohydrate-rich food, and educating affected individuals to recognise symptoms early in their onset, so that hypoglycaemia can be avoided by self-administration of a glucose-rich snack or drink.

7.4.3. Post-Vagotomy Diarrhoea (111,142,143)

For reasons similar to those in the dumping syndrome, namely the fast delivery of hyperosmolar contents to the small bowel, and the resultant intra-luminal fluid shift that occurs in response to it, this can manifest itself as an increase in the frequency of bowel habit, with the passage of loose, watery motions. As a result, this can be reported after either partial gastrectomy or vagotomy, but a distinctive form is recognised after the latter, hence the term "post-vagotomy" diarrhoea. It can be distinguished from the diarrhoea that accompanies dumping in that the latter only occurs in response to a meal and is usually accompanied by other symptoms of the syndrome. Post-vagotomy diarrhoea is unrelated to eating, is often episodic and can result in the passage of up to twenty or more loose stools in a day. Its exact atiology remains unknown but denervation of the gut and biliary tract, as occurs in truncal vagotomy, but not the more selective forms of the procedure, is thought to play a role in its pathophysiology.

7.4.4. Alkaline Reflux Gastritis (111,142,143)

The formation of a non-anatomical communication between the stomach and intestine, as is the case in gastrojejunal anastomosis, is inevitably associated with the regurgitation of enteric contents into the stomach, to a varying degree. Even loss of the normal pyloric sphincter mechanism, as occurs in pyloroplasty, leads to increased retrograde passage of bile and pancreatico-duodenal secretions into the stomach. In most cases, such reflux is asymptomatic, though the alkaline fluid is irritant to the gastric mucosa and sub-clinical gastritis occurs in most patients. The gastritis may manifest itself clinically with dyspeptic symptoms similar to those for which the patient was treated in the first place. In severe cases, distressing bile vomiting occurs. A number of such cases may be the result of surgical inattention, due either to an excessively large stoma, or one created at a non-

dependant position, too high on the greater curvature of the stomach. The large majority of cases,however, occur in the absence of any identifiable surgical cause. Symptomatic control may be attempted with administration of cytoprotective drugs such as sucralfate or agents which bind bile salts, such as cholestyramine, but a minority of troublesome cases may require some form of revisional surgery, designed to transfer the flow of bile away from the stomach. These will be described shortly, as a few patients in the clinical study have undergone such procedures.

7.4.5. Small Stomach Syndrome (111,142,143)

This can be seen after gastric resection, where the natural reservoir function of the stomach is altered, simply by a decrease in the volume of the viscus. However, it is more common after vagotomy without gastric resection, and is the result of gastric atony consequent upon denervation of the gastric musculature. This abolishes the normal physiological responses of the stomach described earlier, to distend in response to food and to empty its contents distally in a graded fashion. Clinically, such patients complain of early satiety during meals and vomiting of foodstuffs shortly after their ingestion. As with dumping, re-education of the patient's eating regime is the usual treatment, advising the intake of small, but frequent meals.

A number of other syndromes are associated with specific types of surgery.

7.4.6. Afferent Loop Syndrome and Blind Loop Syndrome (111)

This occurs in a minority of patients who possess a gastroenterostomy stoma, whether or not fashioned to an intact or antrectomised stomach. Narrowing of the afferent loop of jejunum at the stoma, usually the result of kinking or twisting, leads to the build-up of biliary, pancreatic and enteric secretions proximal to the obstruction. Intermittently, this

partial obstruction is overcome, either once the volume and pressure in the loop reach a critical level, or patient movement results in temporary unkinking of the bowel. This sequence of events is manifest clinically by intermittent episodes of worsening, crampy epigastric pain which is relieved completely by an episode of profuse bile vomiting. Treatment is usually by surgery, to overcome the site of obstruction (see later). Afferent loop syndrome may be further complicated by "blind loop" syndrome, where stasis in the obstructed afferent limb results in bacterial overgrowth with deconjugation of bile acids and resultant fat malabsorption and steatorrhoea

7.4.7. Efferent Loop Syndrome (111)

Mechanical obstruction of the distal limb can also occur at or near the gastro-enteric anastomosis. Symptoms may be similar to those of afferent loop obstruction and contrast radiology may be required to differentiate the two. Treatment is surgical but may vary, dependant on the cause of obstruction found, from division of adhesions to reconstruction of the gastroenterostomy.

7.5. Remedial Surgery for Postgastrectomy Syndromes

A brief mention should be made of the surgical maneouvres employed in an attempt to correct the above problems, as a minority of the clinical study cohort have undergone such procedures. The commonest of these is creation of a Roux-en-Y gastroenterostomy, most often used to overcome troublesome symptoms of bile reflux through a gastroenterostomy stoma. The afferent limb of the gastroenterostomy stoma is divided where it meets the stomach and then sutured end-to-side to the small bowel at a distance of 40 to 60 centimetres from the gastroenterostomy. This diverts hepato-biliary secretions away from the stomach. It is also employed for afferent loop syndrome and severe dumping. Its

success in the latter problem is due to alterations in the neuro-muscular activity of the efferent limb which occur as a result of jejunal transection during construction of the Roux loop (111,142). Contractions which normally propogate from the duodenum and proximal jejunum distally, are prevented from travelling to the efferent limb. In addition, ectopic contractions also occur and travel proximally towards the stomach. The combined result is slowed gastric emptying and propulsion of chyme in the efferent limb. Unfortunately, this may itself result in the Roux stasis syndrome with symptoms secondary to markedly reduced or absent gastric emptying which necessitate take-down of the loop. Nine of the patients in the cohort studied in this thesis also went on to have a jejunal interposition whereby a short (10cm) segment of proximal jejunum is sutured isoperistaltically between the gastric outlet and duodenum in an attempt to slow gastric emptying and relieve the symptoms of dumping.

7.6. Nutritional Sequelae of Gastric Surgery

Gastrectomised patients have long been recognised to suffer from varying degrees of malnutrition and malabsorption with resultant weight loss (143). Much of this may be simply the result of reduced dietary intake owing to symptoms induced by the surgery itself, such as early satiety. Reduction in dietary intake may also occur in the unfortunate patient who does so deliberately to avoid the onset of symptoms of one of the postgastrectomy syndromes. Decreased absorption of nutrients may result from a number of post-operative factors, including the rapid delivery of large food boluses to the small intestine, poor mixing of these with the digestive secretions of the upper gastrointestinal tract, delayed or reduced pancreatico-biliary secretion secondary to denervation by truncal vagotomy, bacterial colonisation of the proximal small bowel and increased small bowel transit. More specific nutritional defects, described below, are also seen.

7.6.1. Anaemia

Iron deficiency anaemia has long been recognised to be a common late sequel to partial gastrectomy (145-147). A significantly high incidence (43.5%) was also seen in 255 patients reviewed 15 years after vagotomy and gastroenterostomy (148). Although some cases will reflect a generally inadequate dietary intake, it is known that most dietary iron is in the ferric form, which is poorly dissociated from food in the absence of acid, and also more poorly absorbed than when in the ferrous state (149). Such conversion is normally accomodated in the acid milieu of the stomach, which is, of course, lost or markedly reduced after surgery for peptic ulcer.

Megaloblastic anaemia secondary to malabsorption of vitamin B12, can occur where there has been excessive resection of the parietal cell mass of the stomach (150). The latter is the site of production of Intrinsic Factor, a glycoprotein which binds the vitamin and allows its absorption by receptor-mediated endocytosis through the mucosa of the distal small bowel (151).

7.6.2. Osteomalacia

Reduced mineral bone mass has been demonstrated in up to 25% of gastrectomised subjects and is secondary to decreased absorption of calcium and vitamin D (152,153). Calcium is solubilised in the presence of acid to allow optimum absorption and absorption of vitamin D may suffer as a result of fat malabsorption (154).

7.7. Other documented sequelae of Peptic Ulcer Surgery

7.7.1. Cholelithiasis following Truncal Vagotomy

Truncal vagotomy not only denervates the stomach, but also transects the parasympathetic supply to the liver, biliary tree, pancreas, small bowel and proximal half of the colon. The recognition that the incidence of post-vagotomy diarrhoea is significantly less with the more specific forms of gastric vagotomy is strongly suggestive that denervation of these extra-gastric sites plays an important role in its aetiology (111). Distension and decreased contractility of the gallbladder has been observed in humans after truncal vagotomy (155-157). This in turn may be partly responsible for the reported increased incidence of gallstones following truncal vagotomy (158,159), secondary to biliary stasis. Increased lithogenicity of bile due to a reduction in bile salts is also seen after vagotomy and this may be equally, if not more, important in this phenomenon (160,161).

8. The Association between Ulcer Surgery and Development of Malignancy, with particular reference to Colorectal Cancer

The last of the recognised sequelae of peptic ulcer surgery has been one of the most widely studied post-surgical phenomena in the past forty years; the development of malignant disease in the years following surgery for benign peptic ulcer. This is at the core of the work to be presented in this thesis and a review of the many previous studies follows.

Since Balfour, in 1922 (162) and Beatson, in 1926 (163) described carcinoma in the post-surgical stomach, there have been a plethora of reports regarding this phenomenon in the worldwide medical literature. Until the 1950's this was thought to be a relatively rare occurrence when the paucity of reported cases was reviewed (164). Since then however, the number of reported cases has become more frequent. Studies began comparing the observed disease rates with expected values derived from the general population or from the numbers seen in control groups such as forensic autopsy subjects. Most of these studies reported statistically significant excess numbers of gastric cancers in patients previously having undergone surgery for peptic ulcer disease. The number of studies of a similar nature have flourished in the literature to the present day. The vast majority of studies have concentrated on gastric cancer, which is perhaps not surprising as the patients under study have initially had some form of operation on this viscus and if there are to be any ill-effects of that surgery, then the obvious place for it to occur would be in close proximity to the operation itself. As such, appropriate time and length will be given to review the literature regarding this important observation. However, as it is the possible association between peptic ulcer treatment and colorectal cancer which is the

subject of this thesis, then the somewhat comparatively smaller volume of work, on the association between gastric surgery for benign ulcer disease and subsequent colorectal cancer development, which has only accumulated in the past 15 years, will be discussed first.

8.1. Gastric Surgery and Colorectal Cancer

The first observation of a significant excess of colorectal cancers in patients previously operated on for peptic ulcer was made by McLean Ross and colleagues from Edinburgh in 1982 (165). In order to test the suggested hypothesis that people previously subjected to peptic ulcer surgery are poor life insurance risks, they examined the mortality in a group of 779 men who had undergone such surgery a minimum of 15 years previously. Eighty six per cent of the cohort had undergone gastrectomy, the exact nature of which was not specified. The remainder had vagotomy with or without a drainage procedure. The relative proportions of doudenal to gastric ulcers was not presented. A total of 16 deaths from colorectal cancer were observed in the study cohort, compared to the expected value of 8.9, calculated from mortality statistics for Scotland. This difference was significant, with a probability value of less than 0.01.

In a study of similar design and purpose to that above, Watt and colleagues from Belfast (166) examined the mortality in a group of 735 patients who had undergone vagotomy and drainage only, between 15 and 25 years previously. Seventy eight per cent of the patients were male and 22% females. As was their intention, this was the first study to look at mortality after vagotomy, as all previous series, McLean Ross's included, had dealt mostly, or exclusively, with antrectomised patients. They correctly pointed out that it was important to identify any unusually common diseases in vagotomised subjects, so they could be specifically looked for in the post-operative follow-up period. Like McLean

Ross's group of predominantly antrectomised men, a significant excess of colorectal cancer deaths were found in the vagotomy patients. Twelve were observed as compared to 5.2 expected, giving an observed to expected ratio of 2.3 with probability value less than 0.05.

Following soon after these two observations, further evidence for an association between previous peptic ulcer surgery and colorectal cancer came from a matched case control study performed by Bundred in Edinburgh (167). Two hundred and eighty nine patients presenting with colorectal carcinoma between 1979 and 1983 formed the study group. The sexes were represented approximately equally, with 151 females and 138 males. Control subjects, chosen from hospital admission records, were matched for age, sex and admission date. Twenty seven patients (9.3%) in the colorectal carcinoma group had undergone previous surgery for peptic ulcer, compared to 13 patients (4.5%) in the control group, which was a statistically significant difference (P<0.05). Within the group of 27 colorectal cancer patients identified as previously having had peptic ulcer surgery, 15 had undergone partial gastrectomy and the remaining 12, vagotomy. In the 13 control group patients, 9 had previously undergone partial gastrectomy and 4 a vagotomy. The excess in the study group was mostly accounted for by males; the mean time from surgery to diagnosis of colorectal carcinoma was 16 years (range 3 to 40 years); and no difference existed for site, Dukes' stage or histological grade of tumour compared to the population as a whole.

The evidence for a link between bowel cancer and previous gastric surgery grew, when in 1987, Caygill reported a small (1.6-fold), but significant, excess mortality from colorectal carcinoma in a group of 5,018 patients operated on some 25 or more years previously (168). The majority of patients had undergone partial gastrectomy. This increased risk only became apparent 20 years post-operatively. Indeed, within the first 20 years from

gastric surgery, the risk was slightly, but significantly, less than that of the general population with relative risk equal to 0.7, with probability value of less than 0.05. Interestingly, both of these observations occured only in patients whose original pathology was gastric ulcer rather than duodenal ulcer, with relative risks equal to 3.0 and 0.8 respectively. Furthermore, the reduced risk within 20 years of operation was only evident in men, and the excess thereafter was mainly accounted for by women.

Findings from a study from Denmark, also published in 1987, were however, to be contradictory to the evidence which had accumulated up until then (169). Toftgaard reviewed the incidence of colorectal carcinoma in a cohort of 3,919 patients followed up for 22 to 27 years post-operatively or until death, and compared it with national incidence rates. As had been the rule, with the exception of Watt's group, the majority of subjects had undergone partial gastrectomy in the form of Bilroth II (69.1%) or Bilroth I (10.8%) resection. The remainder of the group comprised : simple closure of perforated ulcer (14.5%), gastrojejunostomy alone (2.7%) and only 0.9% had a vagotomy & drainage procedure. There was a 2 to 1 prevalence of duodenal to gastric ulcer in the group. Eighty four cases of large bowel cancer were observed, differing little from the 85.9 expected. No difference existed between the sexes nor did any operation type exhibit an excess risk, though it is plain from looking at the breakdown of the group by operation type, that the conclusion drawn from this study has little relevance to vagotomised patients, as indeed the authors admitted. Furthermore, unlike Caygill's study (168), relative risk between gastric ulcer and duodenal ulcer patients had not been presented, and it was not therefore possible to determine the prevalence of each in those patients subsequently developing colorectal cancer. If the majority of such patients had been operated upon for duodenal rather than gastric ulcer, then if Caygill's observations applied

to the population under scrutiny, one would not expect to see an excessive risk for cancer of the colorectum.

Not since Watt's series in 1984, had a study looked exclusively at outcome following vagotomy, but in 1989, Ditlevsen attempted to do just that (170). He presented the mortality of 824 patients from Aarhus County in Denmark who had undergone various forms of vagotomy procedure. The series comprised : selective gastric vagotomy and drainage (384 patients), selective gastric vagotomy and antrectomy (60 patients) and parietal cell vagotomy with or without drainage (380 patients). The operative diagnosis was duodenal ulcer in 588 patients, pyloric or pre-pyloric ulcer in 208 patients, or a combination of the two (28 patients). In stark contrast to the results of Watt (166), the ratio of deaths from colorectal cancer between cases and controls was 0.3. However, despite both of these "vagotomy" studies being of similar magnitude, the follow-up period in the Danish study was short in comparison to others, including that of Watt's, being in the order of only 8 to 13 years. This may be insufficient time from surgery to see the true long-term sequelae of the procedure.

In 1990, a prospective screening study by Mullan, compared the prevalence of large bowel neoplasms in 100 asymptomatic patients who had undergone truncal vagotomy some 10 years or more previously, with that of age and sex-matched forensic autopsy subjects (171). The former agreed to undergo investigation by both barium enema and colonoscopy. Fourteen per cent of the vagotomy group, (which also represents 14 patients, by virtue of the convenient cohort size) were found to have colorectal neoplasms compared to only 3% of the control group (P = 0.01). Of these, 11 patients had adenomas, 3 (all male) had carcinomas, one of whom had 2 cancers and 3 adenomas.

Stemmerman, in a study of 432 Hawaiian Japanese men who had undergone subtotal gastrectomy for peptic ulcer, were next to show a positive association between gastric

surgery and bowel cancer (172). They demonstrated a 1.9-times relative risk for gastrectomised individuals to develop colonic carcinoma (4.9% v 2.6%, P = 0.008). The risk for cancer of the rectum, though still increased at 1.4, did not reach significance. Once adjustment was made for the possible contributory factors, cigarettes and alcohol, the increased risk of developing colonic cancer remained significant.

Also reported in 1990, by Lundegardh, was the incidence of large bowel cancer in a cohort of 6,459 patients (173). Within this group of patients, whose follow-up spanned 25 to 33 years, an overall total of 131 cases of colorectal cancer were identified, compared to an expected 150.3 cases (relative risk = 0.87). On further scrutiny, it was noted that the relative risk in such patients was lower in the first nineteen years post-operatively, for instance (x0.75, x0.58, x0.96 for each six year time band) and slightly higher thereafter (x1.02, x0.79, x1.29). Though the latter show a degree of variability, it is apparent that those patients who are in the group with longest follow-up, and hence have been longest exposed to the carcinogenic factors and/or environment postulated to occur after gastric surgery, do indeed have an increased risk of developing bowel cancer. Neither operation type (Bilroth II 73%, Bilroth I 27%), nor original pathology (duodenal ulcer 61%, gastric ulcer 27%, other 12%) were shown to confer any significant difference on outcome.

The most recent study to present mortality data following gastric surgery came from Edinburgh, by Macintyre and O'Brien in 1994 (174) and observed the outcome of 2,241 patients who had undergone surgery for duodenal ulcer, with a post-operative follow-up period of 20 to 40 years. Although slightly higher than that in the general population, the death rate from cancer of colon and rectum was not shown to be significantly different, with an observed to expected ratio of 1.25. As with most of the previous studies, the

results reflect mainly on a gastrectomised population (Bilroth II 59.9%, vagotomy +/-
drainage 29.1%) and no sub-analysis by operation was presented.

8.2. Gastric Surgery and Gastric Cancer

As already mentioned in the introduction to this section, cancer developing in stomach
following surgery for peptic ulcer disease was recognised as long ago as the 1920's
(162,163). For the first 30 years or so following these initial observations, the scarcity of
similar reported cases in the literature reinforced the opinion that such an event was a
relatively rare occurrence and therefore of little relevance clinically.

However, a number of reports from Scandinavia appeared in the 1950's and their findings
were contradictory to the views held until then. One of the first studies to examine
specifically the late outcome of patients operated upon for peptic ulcer was that by Urban
Krause, published in 1957 (146). As he pointed out, there had been a plethora of series in
the preceding 30 years examining the morbidity and mortality from such surgery and from
these were identified the sequelae which we know so well today, such as dumping,
diarrhoea, anaemia etc., and which have already been discussed earlier in this chapter.
However, the length of follow-up in these studies was relatively short, usually in the order
of 5 to 15 years, rarely longer. Krause therefore reviewed a total of 361 patients in whom
Bilroth II gastrectomy had been performed some 23 to 50 years previously. The principal
cause of death in the group was found to be from an excessive number of cases of gastric
carcinoma, with 28 deaths compared to an expected 11.3. Interestingly, he also noted
there to have been 11 deaths from carcinoma of the alimentary tract and 9 from carcinoma
"elsewhere", but no further definition of these cases or statistical analysis was presented.
One can only wonder whether he inadvertently overlooked an excessive death rate from
colorectal cancer in his group of patients.

Krause's findings were consistent with those of Helsingen and Hilstadt (175) who had presented data from 229 gastrectomised patients. They found a 3 to 1 excess of gastric cancers in their patients, but however noticed that all of the 11 cancers developed in patients operated on for gastric ulcer rather than doudenal ulcer, which led them to suggest that the increased chance of developing gastric cancer may be related to the original diagnosis rather than the surgical procedure itself.

These findings prompted a number of other workers to see if such an occurence was identifiable in other cohorts of gastrectomised patients. Liavaag (164) found that 25 patients (2.6%) from a total of 934 diagnosed as having gastric cancer over a 20 year period between 1940 and 1960 had undergone previous ulcer surgery, either gastroenterostomy alone (11 cases) or partial gastrectomy (14 cases). The average latency from surgery to diagnosis of cancer was 29 years and 16 years in these respective operative groups. However, both of these groups developed their cancer at a similar age of 62 years, which was no different from the average age in the whole group and they were unable to show any significant increase in risk in those previously operated on for peptic ulcer, even when examined by diagnostic category of gastric or duodenal ulcer. The same conclusion was reached by De Jode (176) in a similar study. He reported a 2% incidence (19 patients) of previous gastric surgery in a series of 1,000 diagnosed with gastric cancer in a 10 year period. Twelve of these 19 patients had undergone gastroenterostomy only and 7, partial gastrectomy.

Stalsberg (177) studied a total of 17,070 patients who underwent necropsy over a 10 year period, in which 630 were confirmed as having a gastric cancer. When compared to age and sex-matched control autopsy subjects, the incidence of previous surgery for peptic ulcer was 3 times more common in the gastric cancer group than in controls (P<0.0005). The pattern was identical in both sexes. No specific form of operation nor underlying

ulcer type, whether duodenal or gastric, appeared in relative abundance to any other, in the patients identified as having developed gastric cancer post-operatively. The length of time after such surgery was shown to be of significance and the excess of patients with previous surgery in the carcinoma group was 6 times that of the control group when such surgery had been performed 25 years or more before death. Domellöf (178) observed a significant excess of gastric cancers in patients having undergone both Bilroth I and Bilroth II gastrectomy, with observed to expected ratios of 4 to 1.6 and 10 to 6.6 respectively. There was no significant difference in risk when these were compared and therefore further analysis of the group was performed with both operative groups combined. As was now becoming commonly recognised, there was a demonstrable latent period between surgery and cancer development, and in this study, no significant increase in risk was seen until 12 years or more had elapsed. Furthermore, unlike Helsingen (175), they could not find any difference in risk dependant on the type of ulcer for which surgery was undertaken.

In the study of Mclean Ross (165), already discussed in the previous section on colorectal cancer, no increased risk from gastric cancer was demonstrated, with observed and expected values of 8 and 10.4 respectively. These findings were consistent with two others reported at the same time. Fischer (179) did not find any notable increase in incidence in a group of 1,000 patients who had undergone Bilroth II gastrectomy for duodenal ulcer, with the 13 cases observed being slightly, but insignificantly greater than the 10.6 expected. This was also found to be the case in a study of 338 patients who had undergone various forms of anti-ulcer surgery, mostly Bilroth II, between 1935 and 1959, for duodenal ulcer, by Schafer and colleagues (180). They reported no increased risk of malignancy in the gastric remnant, with an observed to expected ratio of 2.0 to 2.6, and although not specifying any further particular disease state, they did note that "the cohort

did not appear to be at unusual risk of death from other causes, either". Presumably colorectal cancer is included in the "other causes" category.

In 1983, a study from the Western Infirmary in Glasgow was reported by Totten and colleagues (181). In this series, 40 out of 1,092 patients diagnosed as having gastric carcinoma over a 22 year period (1956 to 1978) were found to have previously undergone surgery for duodenal ulcer. Thirteen patients had undergone truncal vagotomy with or without drainage and 27 had partial gastrectomy. No significant difference existed between the two groups as regards age at operation or duration of dyspeptic symptoms prior to surgery. Whilst the prime aim of the study was not concerned with comparison of the disease rate with unoperated subjects, their findings are worthy of note. The mean time between surgery and diagnosis of malignancy was 8.5 years for the vagotomy group compared to 24 years in the gastrectomy group (P<0.001). Indeed, 12 of the 13 vagotomised patients were diagnosed within 15 years of the original operation. This differed markedly from the gastrectomy patients, in whom all, with the exception of one, were diagnosed 15 years or later, from time of surgery. In addition, age at diagnosis of cancer was significantly lower in the vagotomy group, at 55 years, as compared to 64 years in the gastrectomy group (P<0.05). The resectability of the tumours, determined by the extent of local and distant spread, in the previously vagotomised group was found to be in the order of 48% versus 67% in gastrectomised individuals, though this difference did not reach statistical significance. The authors concluded that when gastric cancer developed in patients post-vagotomy, it did so after a shorter latent period when compared with patients who had undergone partial gastrectomy. The study also suggested that tumours developing in post-vagotomy patients were more aggressive and less likely to be amenable to resection than in their gastrectomised counterparts. Whilst the study was not designed to assess whether vagotomised patients were at a similar

increased risk of gastric cancer as was being noted for gastrectomised individuals in some earlier studies, it was one of the first to suggest that if indeed such a risk existed for vagotomy, then it may be associated with tumours of a more aggressive nature than seen after gastrectomy.

Shortly after the publication of the above observation on vagotomised patients, Watt and colleagues presented the mortality statistics of their 735 patients, all of whom had exclusively undergone vagotomy and drainage. A significantly greater number of deaths from gastric cancers than expected were seen, with 16 observed as opposed to an expected 4.8 (166).

The emphasis in other studies, however, continued to be with post-gastrectomy patients. Pickford (182), in 1984, reported a threefold excess of gastric cancers over that expected in 307 patients who had Bilroth II gastrectomy 20 to 30 years previously. The risk was significantly, but not exclusively, greater in those operated on for gastric ulcer compared to duodenal ulcer. These results were at odds with a much larger group of 3,827 patients, also published in 1984, by Tokudome, who had undergone either Bilroth I or Bilroth II gastrectomy in the preceding 11 to 33 years. In this study, a significantly *lower* number of cancers were observed than that expected over the period of follow-up, with 11 versus 52.85 respectively, P<0.01 (183). This report, however, was the exception to the rule. Viste (184) detected a twofold excess of observed to expected gastric cancers in a cohort of 3,470 Norwegian patients who had predominantly undergone Bilroth II resection. This increased risk became apparent 10 years after operation and increased with length of post-operative follow-up, such that the relative risk was 7.3 after 40 to 45 years of follow-up. No difference in risk was demonstrated between the sexes, nor was risk affected by ulcer or operation type. Caygill (185) also found a 4.5 excess of observed over expected gastric cancer deaths in their cohort of 4,466 patients. Like most series, however, the

number who had undergone vagotomy and drainage was relatively small at 534 patients (11.6%) when compared with the proportion who had had Bilroth I or Bilroth II gastrectomy, which constituted 29% and 59.1% of the total, respectively. Again, the risk only became apparent after a latent period, and in this study it was 20 years for the group as a whole. This was true for both duodenal and gastric ulcer subjects, but these groups differed in that the former exhibited a significantly lower risk prior to that time (0.43), whereas for gastric ulcer patients, their propensity to develop cancer post-operatively was apparent even before 20 years had elapsed, with a cumulative risk of 2.67 for this period. It is also notable that when the duodenal ulcer patients were analysed according to operation type, the risk for vagotomy subjects was much greater than that for gastrectomised subjects, being 7.8 and 2.8 respectively. In the gastric ulcer group, both types of gastrectomy were associated with an increased risk, though it was greater for Bilroth II than Bilroth I, at 8.6 and 4.0 respectively. Women with duodenal ulcer were consistently seen to inherit the greatest risk, irrespective of operation performed, but no difference between the sexes existed in the gastric ulcer group.

The concerns regarding post-operative incidence of, and mortality from, gastric carcinoma continued to dominate the literature, with the publication of three large post-gastrectomy patient series in 1988, by Offerhaus (186), Arnthorsson (187) and Lundegardh (188). Cohort sizes ranged from 1,795 to 6,459 patients with length of post-operative follow-up from 25 to 45 years. All three studies showed there to be an increased risk of gastric cancer post-operatively, after a latent period of 15 to 25 years. No sub-analysis by ulcer site or type of gastrectomy was presented by Offerhaus (186) or Arnthorsson (187), but Lundegardh (188) found that Bilroth I patients ran a significantly lower risk of developing gastric cancer post-operatively. Indeed, relative risk was less than one, both in "less than 20 year" and "greater than 20 year" post-operative time periods, when compared to

Bilroth II patients, which was partly at odds with the findings of Domellof (178) and Caygill (185), where both forms of gastrectomy were associated with an increased risk. In addition, those undergoing surgery for gastric ulcer had an increased risk relative to duodenal ulcer cases. This finding concurs with those of Helsingen (175) and Caygill (185).

It is clear from the foregoing, that despite the increasing number of studies, there was a lack of consistency in their findings. The minority showed no increased risk at all, and of those that did, such risks were or were not shown to vary with the original nature of the ulcer and/or type of surgery performed. In an attempt to define which, if any, specific sub-groups of post-operative ulcer patients were at significant risk of gastric cancer, Tersmette (189) performed a meta-analysis of 20 such studies combined. There was a significant overall relative risk of gastric cancer, to the order of 1.6. This increase in risk only became apparent 15 years or more after operation, relative risk for this period being 1.48, with that in the first 15 years being only 0.91. Patients whose pre-existing diagnosis was gastric ulcer were seen to be the ones who inherited such an increased risk, with a relative risk of 2.12 as opposed to duodenal ulcer patients, where it was 0.84. Analysis by sex and operation type, Bilroth I or Bilroth II gastrectomy, did not show any significant difference in risk, although it was slightly higher in females and after Bilroth II.

Thereafter, Ditlevsen's vagotomy study (170) concurred with the findings of the only previous vagotomy group. He reported an increased gastric cancer mortality, at 2.7 times greater than expected. However, in the largest study of vagotomised patients to date, by Lundegårdh (190), with 7,198 patients in total, a higher, but insignificant, incidence of gastric carcinoma was shown. There were 34 cases diagnosed compared with 25.6 expected, constituting a relative risk of 1.33. This risk was unaffected by any of the following factors: age at operation, sex, diagnosis (gastric ulcer 17%, duodenal ulcer

67%, other 16%), operation type (vagotomy and drainage 26%, vagotomy only 74%) or length of follow-up. However, the follow-up was relatively short, at 9 to 17 years, and as the authors concluded, a longer period is required before any firm conclusion on cancer risk following vagotomy can be drawn. Clearly, this large cohort of vagotomised patients, if re-examined in years to come, will provide important information on the long-term cancer risk, not only of stomach, but of colorectum and other sites, of this surgical procedure. Unlike the previous "vagotomy" study of Ditlevsen (170), that of Lundegårdh (190) excluded all patients who underwent antrectomy in addition to vagotomy, and, arguably, therefore gives a more accurate reflection of the cancer risk attendant with the latter acid-reducing procedure, with retention of the gastric antrum.

Finally, in Macintyre's cohort of 2,241 patients who had undergone surgery for duodenal ulcer only (174), between 20 to 40 years previously, no significant excess of gastric cancers was found.

8.3. Carcinoma at other sites and Morbidity and Mortality in general after Ulcer Surgery

Although it can be seen from the above that reports of gastric cancer risk, and to a lesser extent, colorectal cancer, have dominated most research in the field, some degree of perspective should be put on the relative importance of these to other diseases which occur in patients after ulcer surgery. An overall increased mortality for such patients has been consistently shown in many of these studies and much of it can be attributed to lifestyle factors, ranging from the psychological profile of peptic ulcer sufferers, to their consumption of tobacco and alcohol. For instance, in Krause's study (146), the mortality of the group as a whole was excessive with 210 observed deaths in comparison to the 163.1 expected. As with gastric cancer, deaths from tuberculosis and suicide were also seen to be unduly common (146). When deaths from gastric carcinoma and tuberculosis

were excluded, although not reaching statistical significance, the mortality was still greater

in the gastrectomy group under study (161 observed deaths compared to 143.1 expected),

suggesting that deaths from other causes were also increased in gastrectomy patients.

Cardiovascular disease was the commonest cause of death in the group, with seventy

fatalities, and as noted earlier, eleven deaths from carcinoma of the alimentary tract and

nine from carcinoma "elsewhere", but without further definition of these cases or

statistical analysis being presented.

Despite this evidence, in a symposium on peptic ulcer presented to the Edinburgh Medico-

chirurgical Society in 1963 (121), Mess'rs Small and Sircus of the Gastro-intestinal Unit

at the Western General Hospital suggested that there was no justifiable reason for marking

up such patients in terms of life insurance risk, especially if three to four years from

gastrectomy had elapsed without any significant sequelae of the operation occuring.

However, in a long term follow-up study of 1,679 such patients from the same unit (191),

they, like Krause, came to realise their longer term decreased life expectancy. This was

the result of an excessive number of deaths from lung cancer and ischaemic heart disease,

which they logically attributed to the use of tobacco and alcohol in such patients, rather

than any direct consequence of the surgery itself. Later studies confirmed such a scenario,

with Mclean Ross (165) reporting an overall excess mortality from all causes of death in

their patients under scrutiny that persisted within each age-group division (20-29 years,

30-39 years, 40-49 years, 50-59 years), with a tendency to premature death and a

decreased life expectancy of approximately 9 years. Statistically significant excess

mortality was seen in smoking-associated diseases (192) when assessed as a whole but not

individually. This far outnumbered any other causes of death, including gastric cancer,

which was no more common than in the general population. Like Krause many years

before, a statistically proven excess of deaths by suicide was evident in this group of

patients (10 observed : 3 expected, P<0.05) as was death from liver cirrhosis (7 observed : 2.2 expected, P<0.05), both strongly associated with chronic alcohol abuse.

Watt's group of 735 patients (166), all of whom had exclusively undergone vagotomy & drainage procedures in a 10 year period from 1957 to 1967, were also seen to have an excessive mortality compared to the general population with an observed 281 deaths in comparison to a calculated 184 expected, constituting a relative risk of 1.53 (P<0.0001). This was consistent for both sexes and within each sub-group divided by age. Significantly excessive death rates were noted from the following causes : lung carcinoma, gastric carcinoma, cerebrovascular accident, bronchopneumonia and as previously discussed, colorectal carcinoma. The latter accounted for 12 deaths in the group and was of similar magnitude to the 16 observed deaths from gastric cancer, but both of these are small in comparison to the 46 deaths from lung carcinoma. The authors therefore concluded that smoking-associated diseases were still the most important factor in deaths following surgery for peptic ulcer, despite the move from gastric resection to vagotomy.

Ditlevsen's group of 824 vagotomy patients (170) were also seen to be at increased risk of death compared to their age and sex-matched controls with statistically significant risks of 1.3 and 1.5 in males and females respectively. With the exception of gastric cancer, no other cancers were significantly more common, although there was a small excess of pancreatic and lung cancers in the study group, the relative risks being 2.0 and 1.5 respectively. In addition, no excessive mortality from respiratory or cardiovascular disease was evident from this study, though one must bear in mind its relatively short period of post-operative follow-up. Once again, however, deaths from suicide and liver cirrhosis were significantly raised.

The most recent study to present mortality data following gastric surgery is that of Macintyre and O'Brien (174) in which 2,241 patients who had undergone surgery for

duodenal ulcer only, were reviewed 20 to 40 years post-operatively. The death rate of the cohort was shown to be significantly excessive to that of the general population (observed : expected = 1.13), which in turn was due to a significant excess of deaths from neoplasia (observed : expected = 1.25) and digestive disease (observed : expected = 1.71). The former was also shown to be due to higher mortality from carcinoma of lung (observed : expected = 1.37) and from other smoking-associated cancers (lung, oesophagus, pancreas, rectum and bladder) (192) when assessed as a whole (observed : expected = 1.32). When examined individually however, with the exception of lung cancer, none reached statistical significance, though it is noteworthy that all have relative risk ratios greater than unity. As in most previous series, the only significant excess mortality was seen 20 years or more post-operatively, again predominantly because of smoking-related cancers and respiratory disease. Also in concordance with previous studies, the death rate from liver cirrhosis was more than twice that to be expected.

Other groups results are similar with respect to the predominance of lung cancer with increased rates of the disease also reported by Caygill (168) : times 3.9 ; Stemmerman (172) : times 2.1 and Lundegårdh (193) : times 1.5.

Pancreatic cancer was initially observed to be significantly more frequent in gastrectomised patients in the same study that first observed a similar scenario for colorectal cancer, namely that of McLean Small in 1982 (165), where 11 deaths from the disease occured in the cohort compared to an expected 3.9 (P<0.01). Caygill (168) also found there to be a highly significant excess of pancreatic cancers in her group of patients, with a relative risk of times 4. Modestly elevated rates have also been shown in the vagotomy subjects of Watt (166) : times 2; and Ditlevsen (170) : times 2, but are insignificant due to the small numbers of cases involved in computing these risks. Similar

analyses have been reported by Stemmerman (172) : times 1.5 and McIntyre (174) : times 1.2.

Oesophageal cancer is another disease that has been mentioned in a few studies, but with little evidence to suggest any increase in risk for this disease in the years after peptic ulcer surgery. Shearman (194) reported that 9% of 92 patients with squamous carcinoma of the oesophagus were found to have undergone previous surgery for peptic ulcer, which exceeded reported values of the 3 to 8% incidence for achalasia of the cardia, a condition accepted as conferring an increased risk for this cancer. However, in a similar but slightly larger scale study of 203 oesophageal cancer patients, presented by MacDonald (195), there was only a history of previous ulcer surgery in 2%. These observations were corroborated in a necropsy case-control study by Stalsberg (196) where the incidence of previous surgery in the oesophageal cancer group of 185 was a little over 1%, which was less than that in controls (3%). Since these early studies, the evidence from the larger series would tend to endorse there being no significant excess of this tumour after ulcer surgery.

Finally, mention must be made of the remainder of Caygill's study (168), where an increased risk of cancer at multiple sites was found after a latency of 15 to 20 years post-operatively. In addition to those sites already discussed, malignancy at the following sites were also increased : biliary tract (times 9.1), bladder (times 2.4), female breast (times 4.0); and the overall cancer risk was times 3.3. These finding are not, however, as alarming as they may initially appear as they tended to be offset by decreased risk ratios during the first two post-operative decades to such a degree that the overall post-operative risk was not significantly different from the general population. The two later patient series of Lundegårdh (193) and McIntyre (174), which also assessed risk at all sites, showed no overall risk for the majority of cancers, lung cancer excepted, although

small excesses of biliary tract and oesophageal cancer in specific sub-groups when divided by sex and original type of peptic ulcer, were reported by Lundegårdh (193).

It would therefore appear that the social habits and personality of the peptic ulcer sufferer probably play a large part in creating the morbidity and mortality statistics seen in the years following their surgery. However, the frequency with which diseases such as gastric and colorectal cancer, which have much less of an association with tobacco and alcohol use, have been reported in such patients, does implicate some effect of the surgery itself on influencing their development.

The hypotheses as to what mechanism(s) may lead to their development, some with strong experimental evidence to back them up, others less so, are explored in the following section. As before, as the main subject of this thesis is colorectal cancer, appropriate length will be given to work specifically dealing with that disease entity.

9. Possible causes for the association between Ulcer Surgery and Cancer Development

The physiological change that is common to both antrectomy and vagotomy, and is shared with the H_2 receptor antagonists and proton pump inhibitors, is a significant reduction, or complete abolition, of gastric acid output. Concerns as to the dangers of prolonged acid suppression stem from the recognition that individuals with type A auto-immune atrophic gastritis, better known as pernicious anaemia, and which results in achlorhydria, have an increased incidence of gastric cancer. That risk is in the magnitude of 3 to 4 times that of the general population (197-199). Small, but statistically insignificant increases in colorectal cancer incidence have been observed in such patients in two studies (199,200). As already seen, hypochlorhydria leads to a number of pathophysiological sequelae including bacterial colonisation of the stomach and upper small bowel. In addition, where acid reduction occurs in an intact stomach; for example, as occurs in vagotomy and pharmacological acid blockade, but not antral resection, there is a reflex increase in circulating levels of gastrin originating from the antrum of the stomach. Not surprisingly, therefore, much of the research that has been undertaken in an attempt to identify a causal link between ulcer surgery and subsequent tumour development has concentrated on the above phenomena.

9.1. Gastric Cancer

9.1.1. Pre-malignant histological mucosal changes

Microscopically discernable alterations in the gastric mucosa have been shown to be commonplace in the post-operative stomach. These vary from acute gastritis to chronic gastritis of varying severity and also intestinal metaplasia and dysplasia. All may co-exist

in the same stomach. For instance, Domellof (201) performed gastroscopy on 214 patients still alive 20 years or more after Bilroth II gastrectomy and performed 6 biopsies each from the region of the gastroenteric anastamosis and also from the fundic region of the stomach. This revealed that 97% of these patients had changes of chronic gastritis, of mild, moderate or severe degree. In addition, 33% of patients biopsied had intestinal metaplasia of the gastric epithelium and 70% cystic dilatation of mucosal glands. These findings are similar to those of Pickford and colleagues (182) who endoscopically examined and biopsied 54 patients 31 to 39 years after Bilroth II gastrectomy. In addition to changes of gastritis, they reported 35% of stomachs as containing mild to moderate degrees of mucosal dysplasia. Although never conclusively proven, nor universally accepted, all of these histological changes have been implicated as possible precursors to cancer development in a number of animal and human studies (201-205). Hypotheses as to why they occur include hypochlorhydria per se, withdrawal of the trophic effects of gastrin (see later), and as a consequence of bile reflux through the gastroenterostomy stoma. As regards the latter, in the above studies, 77 to 100% of patients had bile reflux at endoscopy, but this did not correlate with the severity of gastritis demonstrated at biopsy.

9.1.2. Bacterial Colonisation

The confirmation of significant bacterial colonisation of the hypochlorhydric stomach has already been presented (135). The main concern arising from this observation has been the production of potentially carcinogenic N-nitroso compounds from nitrites. The latter are formed from dietary nitrates and refluxed bile salts by the action of bacteria possessing the nitrate reductase enzyme, aptly named nitrate reducing bacteria (206). Increased numbers of such strains of organisms have been confirmed in studies showing the

generalised increase in bacterial numbers in the stomach rendered hypo/achlorhydric, whether by disease, medical or surgical means (138,139,141,143,207). N-nitroso compounds such as nitrosamines, nitrosamides and nitrosoureas are carcinogenic in experimental animals (208-210) and significantly raised concentrations of such compounds have been demonstrated in the human stomach during treatment with cimetidine (211,212), omeprazole (140) and after peptic ulcer surgery (213). It must be stated however, that comparable studies with omeprazole (141), cimetidine (214), and also ranitidine (139), as well as in vagotomised and gastrectomised patients (139,215) have been unable to demonstrate excessive amounts of N-nitroso compounds, despite most of them confirming the presence of increased numbers of nitrate reducing bacteria and nitrite concentrations in the stomach (139,141,215). Differences in the techniques used for measuring these relatively unstable substances (141,214), contamination during sampling (141), and indeed what represents "normal" levels of these compounds (141) have been cited as possible reasons for the discrepancies in the results of these studies. Delivery of such bacterially metabolised compounds distally to the mucosa of the colorectum has also been suggested as a cause for the increased incidence of tumours in this organ after ulcer surgery (166,167).

9.1.3. Gastrin and Gastric Carcinoma

The confirmation of gastrin's second major physiological function as a trophic factor for gastro-intestinal mucosa subsequently led investigators to ask whether the same trophic effects would be conferred on the growth of neoplasms arising from it. Gastrin binds to gastric adenocarcinoma cells (216) and stimulates their growth in vitro (217,218). Administration of gastrin or pentagastrin increases the incidence of chemically induced (219) and transplanted (220) gastric cancers in rats, and vagotomy enhances the growth of

such tumours in dogs (221) and rats (222). However, from the literature review earlier, it is clear that the greatest number of post-surgical gastric cancers have arisen in antrectomised stomachs, where the major source of endogenous gastrin has been removed. Although conditions such as pernicious anaemia and atrophic gastritis, both characterised by hypergastrinaemia secondary to hypo/achlorhydria, do have an established strong association with development of gastric adenocarcinoma (198-199,205), it seems more likely that the events leading to mucosal carcinogenesis are a result of the decreased acid milieu at local mucosal level, which is common to antrectomy, vagotomy and the above maladies, rather than circulating hypergastrinaemia. Despite it being unlikely that gastrin is the initiating factor in gastric carcinogenesis, it is still possible that in the presence of chronic hypergastrinaemia, gastric cancer, once established, may behave in a more aggressive fashion. The observations of Totten (181) lend support to this hypothesis. In that study, discussed in detail earlier, gastric cancers arose significantly earlier in vagotomy patients compared to those who had undergone antrectomy, and the lesions were histologically and clinically more aggressive; further advanced and less likely to be resectable.

9.1.4. Helicobacter pylori

The presence of the above organism, discussed in detail earlier in the chapter, is now recognised as a possible risk factor for the future development of gastric cancer (223), with several studies demonstrating a significant excess of cancers in H. pylori positive subjects compared to those who do not possess the organism (224-226). Furthermore, there is evidence to suggest that one specific phenotype of the organism, the so-called CagA H.pylori, named after the cytotoxic protein which it produces, may be the specific type which confers such a risk (227). The mechanism is postulated to be due to atrophy

developing in gastric mucosa chronically inflamed as a result of infestation with the organism. These observations are based on cancer developing in the intact stomach. It is unknown what influence the organism may have in the development of stomach cancer after peptic ulcer surgery, though it is interesting to note that the vast majority of duodenal ulcer sufferers, a condition long recognised to have a strong inverse relationship with gastric cancer development (227,228), are H.pylori positive (42). Differences in phenotypes of the infecting organisms as described above, may partly explain this paradox, but clearly much more research is required in this area.

9.2. Colorectal Cancer

A number of hypotheses have been postulated as to the excess of colorectal neoplasia seen after peptic ulcer treatment. By far, most interest has focused on the relationship between gastrin and colorectal tumour growth and hence this will be discussed first, beginning with a brief resumé of the salient features of the hormone itself.

9.2.1. Gastrin and Colorectal Cancer

In 1906, Edkins proposed the existence of a hormone that influenced gastric acid secretion (73). The hypothesised hormone, "gastrin", was only isolated over half a century later from the gastric antrum of the pig (75). It was shown to be a heptadecapeptide existing in two microheterogeneic forms, that is, differing only by the presence, or not, of a sulphated tyrosine in the sixth position from the C-terminus of the molecule (75). Soon afterwards came the development of useful antibodies against gastrin (229), which allowed demonstration of gastrin as also existing in various macroheterogeneic forms, that is, varying in amino-acid chain length, namely Gastrin-34, Gastrin-17 and Gastrin-14 (230). The biological activity of gastrin is confined to the constant C-terminal

tetrapeptide of all these variants which, however, is shared by the other major gut hormone, cholecystokinin (231). The latter, however, differs from gastrin in that it contains tyrosine in the seventh position, not the sixth, from the C-terminus and this is likely to account for the difference in their respective biological actions (232).

9.2.1.(i) Physiological Function of Gastrin

Gastrin is the major stimulus to secretion of acid and pepsin from the body of the stomach (233). It originates from the G-cells of the gastric antrum (234) and is released in response to a number of stimuli including presence of intraluminal peptides and amino-acids, acetyl choline and gastrin-releasing peptide from post-ganglionic neurones. Consequent upon such stimuli, gastrin is released into the circulation and induces acid secretion by direct action on the parietal cell (76,233), and indirectly by stimulating release of histamine, which in turn also causes release of acid from the parietal cell (76,233).

9.2.1.(ii) Evidence for the trophic action of Gastrin

The first indication that gastrin was involved in mucosal growth was the observation that the remaining gastric mucosa atrophied following antrectomy (235,236). Conversely, patients with Zollinger-Ellison syndrome, who have chronic and persistent elevation of circulating gastrin due to secretion of the latter by a tumour, have gastric mucosal hyperplasia (237).

The earliest experimental evidence of gastrin's trophic function was observed when both pentagastrin and gastrin were shown to stimulate protein synthesis in gastric and duodenal mucosa of rats, as measured by incorporation of ^{14}C-labelled leucine, both in-vitro (238) and in-vivo (239). This growth-promoting effect was further substantiated by

demonstration of significantly increased mitotic rates and protein content of both human and rat gastric mucosal cells cultured and maintained in-vitro, when treated with pentagastrin compared to saline-treated controls (240). Similarly, in further work from the same centre, rat duodenal mucosal cells in vitro, when treated with pentagastrin, showed a significant reduction in doubling time versus saline-treated controls (241). These same cells also demonstrated increased uptake of tritiated thymidine (^{3}H-thymidine) which occurs during active DNA synthesis and is indicative of cells in proliferative phase (241). DNA synthesis was also shown to markedly and significantly rise in the oxyntic, duodenal and ileal mucosa of rats after a single intraperitoneal injection of pentagastrin (250 micrograms/kg) with sacrifice and collection of tissue for analysis sixteen hours later (44-100%, 62-81%, 58% increase over control values, respectively) (242). Furthermore, atrophy of oxyntic and duodenal mucosa has been demonstrated in animal models where circulating levels of endogenous gastrin have been lowered from physiological values, by antrectomy or starvation with or without intravenous feeding, and that such atrophy can be prevented or reversed by administration of pentagastrin (243,244).

9.2.1.(iii) Gastrin as a growth factor for Colorectal Mucosa

In a series of experiments along similar lines to those on gastro-duodenal mucosa, tritiated thymidine incorporation and total DNA content of colonic mucosa were found to be significantly elevated in rats which had received 6 equally spaced injections of pentagastrin 250micrograms/kg over 48 hours (and sacrificed 8 hours after the final dose), compared to saline-treated controls (245). Identical results were obtained for administration of the natural porcine gastrins G-17 I, G-17 II and G-34 II in equivalent experiments, with no significant differences in the degree of stimulation by one particular form of gastrin over

another (245). This constituted the first published evidence of a trophic effect of gastrin, in a variety of physiological forms, on colorectal mucosa.

Dembinski (246) then demonstrated that endogenous antral gastrin was also trophic for colorectal mucosa, by observing that antrectomy in rats significantly reduced colonic weight and DNA synthesis in comparison to normogastrinaemic controls. Significantly reduced blood gastrin levels were confirmed in the antrectomised animals. A group of antrectomised rats then received 1 week's treatment with 6-hourly intraperitoneal injection of pentagastrin 250μg/kg, while a control group received an equivalent amount of saline. Pentagastrin treatment resulted in stimulation of colonic DNA synthesis to 75% above control values. This observation, along with the fact that antrectomy caused a greater reduction in weight and DNA synthesis of colon than any other tissue (acinar pancreas behaved similarly), prompted the authors to conclude that colonic mucosa was the most sensitive gastrointestinal tissue for gastrin, due to the marked changes caused by interference with levels of the hormone. Furthermore, in observing that both RNA and DNA content both responded similarly to the alterations in gastrin stimulus, and thus the RNA to DNA ratio did not change, they concluded that true hyperplasia of the mucosa was occuring, rather than hypertrophy, where RNA synthesis tends to increase without a concomitant rise in DNA.

Sirinek (247) also confirmed a trophic response of 5 different human colonic mucosal cell lines maintained in tissue culture, to treatment with pentagastrin 5μg/ml. Saline treated control cells showed a mean threefold increase in number over a 72 hour incubation period, but those with pentagastrin in culture increased fivefold over the same period, a mean increase of 65% (range 40 to 80%) compared to controls (P<0.05).

9.2.1.(iv) Gastrin as a growth factor for colorectal neoplasms

Somewhat inevitably, in light of these observations, questions were raised as to what influence gastrin may have on neoplasms arising from such a responsive tissue. Indeed, in Sirinek's in-vitro study (247), the authors also studied the response of 5 human colonic adenocarcinoma cell lines to the same concentration of pentagastrin in the culture medium. A stimulatory effect similar to that observed in the non-neoplastic colonic mucosal cells was evident. The pentagastrin treated carcinoma cells increased in number by approximately sevenfold as compared to saline treated controls which increased to 4.5 times their initial number ($P<0.05$).

Other workers confirmed this effect for natural gastrin in vitro. Kusyk and colleagues (248) studied the action of physiological doses of gastrin-17 on in vitro growth of the human colonic adenocarcinoma cell line, LoVo. They confirmed a significant increase in DNA synthesis in gastrin treated cells, as measured by incorporation of tritiated thymidine, and also in cell numbers, with increases of over 200% and 50% of control values, respectively. They also noted that such measurements were maximal in cells supplemented with either feotal bovine serum or "conditioned medium", the latter prepared from medium which had been used to maintain LoVo cells for the previous 48 hours. They concluded that the enhanced effect on growth by cells supplemented with the above was a result of small amounts of gastrin and other growth factors present in serum and in the "conditioned medium", suggesting the presence of autocrine growth factors released by the cancer cells themselves. Palmer Smith (249) also found significantly increased values of tritiated thymidine incorporation and cell counts in HT29 human colon cancer cells in vitro in response to a wide dose range of gastrin-17, varying from 0.4 to 400 picomoles per litre. Since these early observations, gastrin's trophic action on

colorectal cancer cells in vitro has been confirmed in a substantial number of cell lines studied (250).

The results of a number of in vivo studies also served to lend weight to gastrin being an important factor in colorectal tumour growth. One of the first of these was by McGregor (251), who observed significant increases in chemically induced colonic tumour synthesis and concentrations of DNA, RNA and protein in rats rendered endogenously hypergastrinaemic by surgical antral exclusion. Similar results were observed in animals that were administered exogenous pentagastrin, compared to antrectomised or sham operated animals in whom gastrin levels did not differ significantly. Similarly, Winsett and collleagues (252) studied the effect of varying doses of pentagastrin on growth of an implanted colonic tumour xenograft (MC-26) in Balb/c mice. Significant increases in tumour weight and DNA content were confirmed in all pentagastrin treated animals who received the hormone for 14 days at a dose of either 125, 250 or 500µg/kg 8-hourly. Maximal stimulation of tumour growth appeared to be present at the lowest dose of pentagastrin as no significant difference in the measured parameters was evident between any of the 3 different dosage groups. Thus, in a second experiment utilising this dose of pentagastrin, they measured the survival of mice bearing the colonic tumour after an initial 7 or 14 days treatment with pentagastrin or saline placebo. Compared to control animals, all pentagastrin treated animals had a significantly reduced survival as a result of excessive tumour growth. For instance, at day 35, 90% of control mice were alive as compared to 55% of those treated with pentagastrin. By day 55, all of the latter group were dead whereas 80% of the controls were still alive. In a study of similar design, Palmer Smith (249) also found that pentagastrin-treated nude mice bearing xenografts of 2 human colonic adenocarcinomas, CX1 or X56, had significantly increased tumour volumes, weights, protein and DNA content compared to control animals. Similar results were

reported by Alonso (253), who utilised Balb C mice inoculated with CT26 human adenocarcinoma of the colon, and observed a significant reduction in the survival time of animals so treated. Chu (254), observed similar effects of endogenous gastrin on a human colon carcinoma xenograft implanted in the colon of athymic Lewis rats. Those rendered hypergastrinaemic by fundectomy (excision of the entire oxyntic gland bearing area of the stomach) had increased proliferation of tumour, as measured by metaphase arrest index. The tumours in this group of animals were also more aggressive, with direct local extension of tumour outwith the bowel evident in all hypergastrinaemic rats and liver metastases in 20%, findings which were absent in sham-operated controls.

Like most hormones, gastrin's action on colorectal cancer cells was presumed to be due to its interaction with a specific receptor on the effector cell. Specific gastrin receptors had already been identified and characterised on gastric parietal cells of the rat (255) and dog (256). In the light of gastrin's apparent action on colorectal mucosal and tumour growth, the search for a similar receptor on such cells began and gastrin receptors were indeed identified in normal mammalian colonic mucosa (257). Subsequently, high affinity gastrin receptors were also identified on a substantial proportion (38 of 67, 56.7%) of human colorectal carcinomas tested in one study, suggesting that some, but not all, such tumours were under the influence of gastrin (258). Furthermore, specific pharmacological gastrin receptor antagonists have been developed and shown to inhibit the trophic effects of both natural and synthetic gastrins on susceptible tumours in vitro and in vivo (259-262), introducing the exciting possibility of hormonal manipulation in the treatment of the disease, as already occurs in breast and prostate cancer.

Most of the above information has been obtained from laboratory based experiments, either in cell culture or small mammal based studies. To date, there is little substantial clinical evidence to suggest that gastrin significantly influences the development and

growth of colorectal cancer in man. In 2 studies of patients with pernicious anaemia and its associated chronic hypergastrinaemia, the incidence of colorectal cancer was unchanged (199) and slightly, but insignificantly, higher (200) than in the general population. In a study of patients with longstanding medically controlled Zollinger-Ellison syndrome, also characterised by persistently elevated circulating gastrin levels, the number of colonic crypt cells in the proliferative S-phase of the cell cycle, measured by labelling of single stranded DNA with the thymidine analogue, 5'-Bromodeoxyuridine, was noted to be significantly higher than in control subjects (263). Despite this, the total number of cells in the colonic crypts was not significantly different in the 2 groups and thus no resultant mucosal hyperplasia ensued. In the 23 Zollinger-Ellison patients studied, 5 were found to have adenomas at colonoscopy, and 1 of these patients also had a neuroendocrine tumour at the splenic flexure. None of the control subjects had any such lesions because a normal colonoscopy was one of the criteria for admission to the control group. The authors concluded that the prevalence of adenomas in their small study group was probably no greater than that found in the general population and that the results of a study of this size could not accurately compare their relative prevalence anyway. However, their results are almost identical to those of a larger study of 97 Zollinger-Ellison patients, in whom the prevalence of asymptomatic colonic polyps found at colonoscopy was 18% (264). Finally, in a study of 7 patients who had undergone hepatic resection of colorectal liver metastases and then had augmentation of their chemotherapy with the gastrin receptor antagonist, proglumide 1200mgs/day for 2 years, only one (14%) had recurrence after a median follow-up of 39 months in comparison to 24 of 46 (52%) control individuals who had chemotherapy only (265). Despite the impressive percentage difference here, one must appreciate the very small size of the treatment group.

Further evidence of an association between hypergastrinaemia and human colorectal cancer was suggested by the studies of Palmer-Smith (266) and Seitz (267), who found significantly elevated circulating gastrin levels in patients with colorectal cancer. Charnley (268) also confirmed significantly elevated gastrin levels in their subjects, but also questioned whether the measured gastrin was being produced by the tumours themselves, as gastrin-like immunoreactivity (269) in colorectal tumour cells and secretion of a gastrin-like protein (270) from them had previously been detected by other workers. Charnley (268) therefore measured gastrin levels in draining mesenteric vein blood intra-operatively. No significant difference between portal or peripheral venous gastrin levels was found, and a further two studies (271,272), which did not show any significant elevation of pre-operative gastrin values in colorectal cancer patients, also confirmed that these measurements were not altered after the tumour had been resected, further evidence against a significant production of gastrin by the tumours. However, these findings are at odds with those of Wong (273) and Seitz (274) who both found that serum gastrin levels fell after apparent curative tumour resection. However, it is still possible that any autocrine gastrin production by the tumours is either too small in amount or is completely utilised at the local cellular level to be detected by the above methods, as both gastrin mRNA (275-278) and protein products thereof, including immature precursor gastrins (277-280) such as preprogastrin, progastrin, glycine-extended gastrin as well as the physiologically active, mature carboxyamidated form (279-281), have been identified in human colorectal carcinomas.

Gastrin's exact role in growth of normal colorectal mucosa and in colorectal carcinogenesis remains controversial however, as the results of an equally substantial number of studies have been unable to corroborate the findings reported above. Thus, in a number of in vivo studies with rats, alterations of endogenous gastrin levels induced by

surgical procedures such as fundectomy and antrectomy (282,283), treatment with proton pump inhibitors (284,285) or H2-antagonists (285) and administration of exogenous pentagastrin (286) or physiological gastrin-17 via osmotic mini-pumps (287) have not shown any significant effect on growth of normal colonic mucosa or of chemically induced colorectal tumours. Also, not all human colorectal tumours studied have been found to contain gastrin receptors (288), though this does not rule out the hormone as having a significant action on those that do possess such receptors. Finally, as already mentioned, a number of studies have not shown any elevation of gastrin levels in patients with colorectal cancer compared to controls (271,272,289,290).

9.2.2. Other potential mechanisms for Colorectal Carcinogenesis after surgical or medical treatment of Peptic Ulcer

Although the majority of work has concentrated on the potential role of gastrin in colorectal tumour growth, a number of other hypotheses have been forwarded to explain the increased incidence of the disease seen in some studies. Indeed, hypergastrinaemia cannot be implicated in those tumours seen after procedures such as antrectomy, and thus it is possible that the consequences of some factor shared by antrectomy, vagotomy and drug treatment, such as hypochlorhydria, may be responsible for their development.

Alterations of bile acid metabolism following gastric surgery have been suggested as a possible cause for the increased incidence of colorectal cancer (166,167,171). The constitution of bile reaching the distal gastrointestinal tract is known to be altered following both partial gastrectomy (291) and vagotomy (171,292). This may be the result of metabolism by the bacteria shown to colonise the stomach and upper small bowel in the presence of hypochlorhydria, with an increased ratio of secondary to primary bile acids being the predominant feature (171,292). Similar ratios have been found in the faeces of

patients with colorectal neoplasms (293,294) and in those at high risk of developing colorectal cancer (295,296). These changes also occur after cholecystectomy (297,298) which itself has been associated with an increased risk of colorectal cancer (298-301). Cholelithiasis too, has been associated with an increase risk of bowel cancer (302,303), and the lithogenicity of bile is known to increase after gastric surgery (158-161,304). Secondary bile acids have been shown to promote experimental colorectal carcinogenesis in rodents (305-307) and cytosolic receptors for such have been demonstrated in colorectal cancer cells (308).

Stemmerman (172) proposed that the nutritional sequelae that occur after gastric surgery, which have been discussed earlier, may lead to the increased incidence of bowel cancer after operation. His group of 432 gastrectomised patients were lighter and had lower serum lipid levels than their normal counterparts, suggesting that undernutrition may be the common thread. They also had reduced calcium intake, a further risk factor for colorectal cancer development.

10. Omeprazole - an Inhibitor of Colorectal Carcinogenesis?

10.1. The Evidence

Despite the concerns of prolonged acid suppression and hypergastrinaemia on colorectal

mucosa, the few experimental studies using omeprazole to evoke this state have not

shown any stimulatory action on the growth of chemically-induced or transplanted colonic

tumours in rats (284,285). Existing studies of long term acid suppresion by omeprazole in

the treatment of gastro-oesophageal reflux or peptic ulcer in humans, usually in the order

of 5 years or less (309,310), cannot be compared to the much longer periods of follow-up

in the studies of operated patients presented earlier, and are at present unlikely to yield

any useful information regarding the long-ranging effects of chronic hypochlorhydria or

hypergastrinaemia secondary to acid-inhibitory drugs.

The results of a study by Penman (311), designed to assess the effect of omeprazole-

induced hypergastrinaemia on chemically-induced colonic tumour growth in rats, actually

found that in omeprazole treated rats, despite significant hypergastrinaemia (and one

therefore presumes, achlorhydria), as confirmed by blood sampling, there was a

significantly reduced tumour incidence compared to normogastrinaemic control animals,

with 12 out of 19 animals affected (63%) in the omeprazole-treated group compared to

19 out of 20 (95%) in the control group (P<0.02). In addition, in the treatment group of

animals, the total number of tumours and the median number of tumours per rat were also

significantly less than in controls (28 versus 59, median (range) 1(0-5) versus 3(0-10)

respectively ; P = 0.02). This unique finding was unexpected, and to date, remains

unexplained. Only one other study (312), looking at the in vitro action of omeprazole on

the growth of 3 carcinomas derived from human colorectal mucosa, has suggested that the

drug may have an inhibitory effect on the growth of such tumours. The growth of 1 of

the 3 cell lines tested was inhibited by omeprazole, irrespective of whether gastrin was present in the growth medium. These two isolated reports of a potential anti-proliferative action of omeprazole on colorectal cancer are the stimulus to the laboratory based work to be presented in this thesis.

10.2. Omeprazole - Pharmacokinetics (313)

Omeprazole, (5-methoxy-2-{4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphinyl}-1H-benzimidazole) exerts its powerful acid inhibitory action by selective and non-competitive binding to the H+/K+ Adenosine Triphosphatase enzyme which is situated in the membrane of the intracellular canaliculus of the gastric parietal cell. This enzyme is presently thought to be exclusive to this cell, although a similar potassium-dependant pump has been identified in rabbit colon (314). The drug is rapidly absorbed, with maximum plasma concentrations being seen within 25 minutes or less of a standard oral dose in humans. It distributes rapidly to extravascular sites with its volume of distribution being similar to that of the extracellular fluid compartment. In rats, within half an hour of dosage, the highest concentrations of the drug, as assessed by measured radioactivity after ingestion of ^{14}C-labelled drug, are found in the stomach, liver, kidneys and duodenum. This reflects the concentration of the drug at its specific site of action and its routes of excretion via bile and urine. Its high concentration at the latter sites, occuring within half an hour of dosing also indicate its rapid first-pass metabolism through the liver and rapid elimination. The average plasma half-life of the drug in humans is approximately 60 minutes. All of the administered dose is biotransformed to inactive metabolites in the liver, as no unchanged drug is recoverable in the urine or faeces. In humans, approximately 70% of the metabolites are renally excreted, with the remainder excreted in the bile and ultimately in the faeces. The principal metabolites are hydroxy-omeprazole

and its carboxylic acid, with much lesser amounts of omeprazole sulphone and sulphide

identified in human plasma. None of the metabolites are considered to play any part in the

inhibition of acid secretion, and the prolonged effect of a single dose reflects the non-

competitive and irreversible nature of the binding to the proton pump, by formation of a

disulphide bond between the proton pump and the active sulfenamide form of the drug.

10.3. Proposed mechanisms for Omeprazole's anti-neoplastic action

Penman (311) proposed 2 hypotheses for the apparent anti-neoplastic action of

omeprazole. The first of these was to suggest that alterations in the bacterial milieu of the

colon, in response to the profound acid suppression upstream, may lead to increased

bacterial metabolism of the administered carcinogen, azoxymethane, to inactive

metabolites, with a resultant decrease in tumour development.

The second hypothesis suggested that omeprazole may interfere with some stage of the

body's metabolism of the carcinogen. Azoxymethane is itself a procarcinogen, which

requires to undergo a series of metabolic reactions before the active carcinogen, the

alkylating methyldiazonium ion, is generated (315). It is site specific with the vast

majority of tumours arising in the colorectum. This is why no tumour growth occurs at

the site of subcutaneous injection of the drug and the animal model of azoxymethane-

induced colorectal cancer is now widely utilised in the investigation of the disease. Most

known carcinogens to which the average human body is exposed, like azoxymethane,

exist as procarcinogens and require metabolism, usually hydroxylation, to their active

genotoxic form (316). Such metabolism usually occurs in the liver, primarily by a large

and heterogenous family of microsomal hepatic enzymes, the cytochrome P450s (317).

The metabolic activation of most of these carcinogenic compounds, or xenobiotics, such

as heterocyclic amines found in cooked food, is mainly undertaken by two specific types

of these enzymes, cytochromes P450-1A1 and -1A2 (318). Omeprazole has been found

to induce production and increase activity of both these specific enzymes in the liver

(319) and in other extrahepatic sites such as duodenal and colonic mucosa (320). This has

raised concerns that this may in turn lead to increased production of carcinogens from

ingested dietary xenobiotics. Somewhat paradoxically, it has been shown that induction

of cytochromes P450 1A1 and 1A2 actually leads to reduced activity of administered

carcinogens in vivo, thus increased enzymatic activity does not necessarily correlate with

increased mutagenicity (321,322). The exact number and type of cytochromes which are

involved in the conversion of azoxymethane to its active metabolite are not fully known,

with only one, cytochrome P450IIE1, having been identified as an intermediary enzyme in

its metabolism (323). However, the activity of this particular enzyme has not been shown

to be affected by omeprazole (319).

Defining where omeprazole acted in the carcinogenesis process would be beneficial in

elucidating the drug's mode of action. The process of chemical carcinogenesis can be

broadly divided into two separate stages : initiation and promotion (324). Initiation

occurs by a direct action of the carcinogen on the DNA genome of the cell, and

consequently that altered cell replicates by mitosis. Initiation is an irreversible process and

results in the development of a population of such "initiated" cells. These cells, however,

will only go on to develop cancer if they are further exposed to a promoting agent, which

in most cases is the continuing presence of the initiating carcinogen. The exact number of

steps involved in the promotion phase is not known, but the latter is usually a prolonged

process, as the latency with which lung cancer appears after many years of cigarette

smoking displays. Furthermore, unlike initiation, promotion can be considered a

reversible process. For instance, not all smokers will develop lung cancer and those who

discontinue the habit will enjoy a progressive reduction in their risk of developing the

disease with increasing time. Furthermore, areas of tissue hyperplasia and dysplasia which are regarded as early pre-malignant changes, do not all inevitably progress to become cancerous.

From the design of Penman's study (311), it cannot be identified at which of these 2 main stages of the carcinogenesis process omeprazole exerted its effect, as the drug was given throughout the duration of the study. The animals were therefore exposed to omeprazole both during carcinogen administration (initiation) and thereafter (promotion). The experimental work described in this thesis was designed to clarify the influence of omeprazole on the initiation and promotion phases of colorectal carcinogenesis and on in vitro colorectal cancer cell proliferation.

11. Summary

Peptic ulcer disease and colorectal cancer remain two of the most prevalent diseases worldwide.

With the advent of new, powerful acid inhibitory drugs, the management of all but the most resistant cases of peptic ulcer is medical. These drugs have largely superceded effective acid reducing surgery which was popular up until about 20 years ago. A legacy of such surgery has been the recognition of a decreased life expectancy and a propensity for development of malignancy, usually occuring 15 to 20 years post-operatively. Gastric cancer and lung cancer have frequently been seen as the commonest malignancies to occur in such patients, but so too has colorectal cancer. Potential explanations for the mechanism leading to its development include alterations in the bacterial milieu of the gastrointestinal tract secondary to the surgically produced hypo/achlorhydria, leading to increased production and delivery of carcinogens to the colorectal mucosa, and dietary changes in response to such surgery.

The vast majority of patients studied have undergone partial gastrectomy as opposed to vagotomy and drainage. Studies of post-vagotomy patients have either had small patient numbers or too brief a follow-up period in comparison to their gastrectomised counterparts to give a true picture of the mortality/cancer risk attendant with this procedure. The risk following vagotomy is important to establish for a number of reasons. Firstly, we are presently at a time approximately 25 years on from when vagotomy was the commonest procedure employed in the surgical treatment of peptic ulcer. If there is a latent period for vagotomy patients similar to that for gastrectomised individuals, then we may now just begin to see an increased incidence of colorectal cancer and other malignancies as a consequence of the operation. Furthermore, vagotomy,

unlike gastrectomy, leads to chronic elevation of circulating levels of the antral hormone, gastrin. Gastrin has been shown to be trophic for colorectal carcinoma both in vitro and in vivo, and blockade of gastrin receptors found on such tumours by specific antagonists leads to their reduced growth. Despite this evidence, the association between hypergastrinaemia and tumour growth remains controversial. If such an association is true, however, it is possible that persons with chronically elevated gastrin levels, such as those previously vagotomised, may have a substantially increased risk for development of colorectal cancer, and may benefit from screening for the disease in the long-term post-operative period. Finally, if patients with long term acid suppression and hypergastrinaemia, do have an increased incidence of colorectal cancer, this may have safety implications for the long-term prescription of agents such as omeprazole and other proton pump inhibitors.

In view of these concerns, Penman and colleagues designed an in vivo study to assess the effect of omeprazole induced hypergastrinaemia on the yield and growth of chemically-induced colorectal cancers in rats. Unexpectedly, and to their surprise, they found that the drug treated animals had a significantly lower yield of such tumours, compared to control animals, despite confirmation of significant elevation of gastrin levels in the former group. This finding remains unique and similar studies looking at omeprazole-induced hypergastrinaemia on colorectal carcinogenesis have shown no significant effect, inhibitory or otherwise, on tumour development. The mechanism for such an inhibitory action of omeprazole on tumour growth, if real, remains unknown. The design of their study does not allow identification of the possible time at which omeprazole exerted its action on the carcinogenesis process. One further isolated report exists for omeprazole inhibiting the growth of a human carcinoma cell line of colorectal origin in vitro, suggesting a direct action of the drug at cellular level.

The treatment of colorectal cancer remains primarily surgical, and despite increases in our knowledge of the genetic defects that occur early in its genesis, survival after treatment has changed little in the past 20 or 30 years. Identification of potential high-risk groups for the disease, such as those with a history of previous ulcer surgery, may lead to earlier diagnosis, treatment, and hopefully, improved survival. The possibility that some tumours may be influenced by the hormone, gastrin, whose action can be blocked by specific antagonist drugs, opens an exciting new avenue in potential treatment of the disease. So too, does the potential use of a widely used drug with minimal adverse reactions, for reducing colorectal tumourigenesis, if isolated reports of such an action can be substantiated. These constitute the reasons for the work contained in the following pages.

12. Statement of Aims

The aims of the studies undertaken for this thesis are therefore :

1. To identify whether patients previously treated surgically for peptic ulcer disease, predominantly by vagotomy and drainage, run an increased risk for the development of malignancy, in particular colorectal cancer, in the long-term post-operative period.

2. To investigate further, a possible inhibitory action of the gastric acid-suppressing agent, omeprazole, on growth of colorectal tumours in vivo and in vitro, despite the production of endogenous hypergastrinaemia which occurs during treatment with the drug, and which itself may promote growth of such tumours.

3. If such an action of omeprazole is confirmed, to attempt to identify at which stage of the carcinogenesis process this effect occurs, and assess whether the drug acts directly on the cancer cell, by investigation of its effect on human colorectal cancer cells in vitro.

Chapter 2 - Materials and Methods

1. Prospective Study of Cancer Incidence following Surgery for Benign Peptic Ulcer, with particular reference to Colorectal Neoplasia after Vagotomy.

1.1. Introduction

A specialist clinic dedicated to the investigation and treatment of peptic ulcer ran for approximately 20 years, from the mid 1960's to 1983, at the Western Infirmary, Glasgow. Apart from serving the population of Glasgow and the surrounding towns, its catchment of patients extended from the Western Isles in the north to Dumfries and Galloway in the south, thus covering the whole of the West of Scotland.

Each patient attending this clinic had details of their medical history, examination findings and treatment, recorded on standardised proforma. Despite the clinic being disbanded in 1983, these files were retained. Thus, there existed an ideal and untapped patient database that would address the issue of cancer incidence in the years following surgery for benign peptic ulcer disease.

1.2. Selection of Study Cohort

Four thousand, five hundred and thirty three (4,533) patients attended the clinic between 1965 and 1983. Patients who received no operative treatment and those in whom data was incomplete were excluded from further study. Similarly, a small number in whom it was felt co-incident factors may significantly alter their cancer risk or affect their endogenous gastrin status (for instance: one patient with hereditary polyposis coli, one

patient who had an extensive small bowel resection for volvulus) were also excluded. This left a total of 1,992 patients for analysis.

In contrast to previous studies of a similar nature, there were no further exclusion criteria. Therefore, patients who had more than one operative procedure, whether for recurrent ulcer or for treatment of sequelae or complications of the initial procedure such as bile vomiting, small stomach syndrome or dumping, were not excluded. Neither were those who had simple suture of a perforated ulcer, whether followed or not by a more definitive anti-ulcer procedure. Nor were patients who had underwent cholecystectomy in addition to ulcer surgery, the former also being associated with an increased risk of colorectal carcinoma (298-301). This was thought appropriate as such select sub-groups have either been totally excluded or had relatively little attention paid to them by previous workers, and therefore little, if anything, is known of the cancer risk attendant with multiple gastric procedures or gastric surgery and cholecystectomy combined.

1.3. Characteristics of the Cohort

1.3.1. Distribution by Age and Sex (Figure 1)

Patients age at operation, which was also their age at entry into the study, followed a normal distribution for the whole cohort and this was also the case when the male and female populations were examined seperately. The majority of patients were operated upon between the ages of 25 and 55. Males predominated in the series 4-fold with 1,578 males compared to 414 females, 79.2% and 20.8% of the total cohort respectively.

1.3.2. Number of Operations (Figure 2)

The 1,992 patients underwent a total of 2,628 operative procedures, giving a mean value of 1.32 procedures per patient. The median value was 1, and the range 1 to 5.

Materials and Methods - Figure 1

Peptic Ulcer Clinic Study Cohort - Age at Operation

Frequency vs 10 year Age Group

Legend: MALE (shaded), FEMALE (open), TOTAL (line)

Age Group	Male	Female	Total
<25	156	16	172
25-34	371	64	435
35-44	423	111	534
45-54	347	124	471
55-64	215	64	279
65-74	58	32	90
75-84	8	3	11
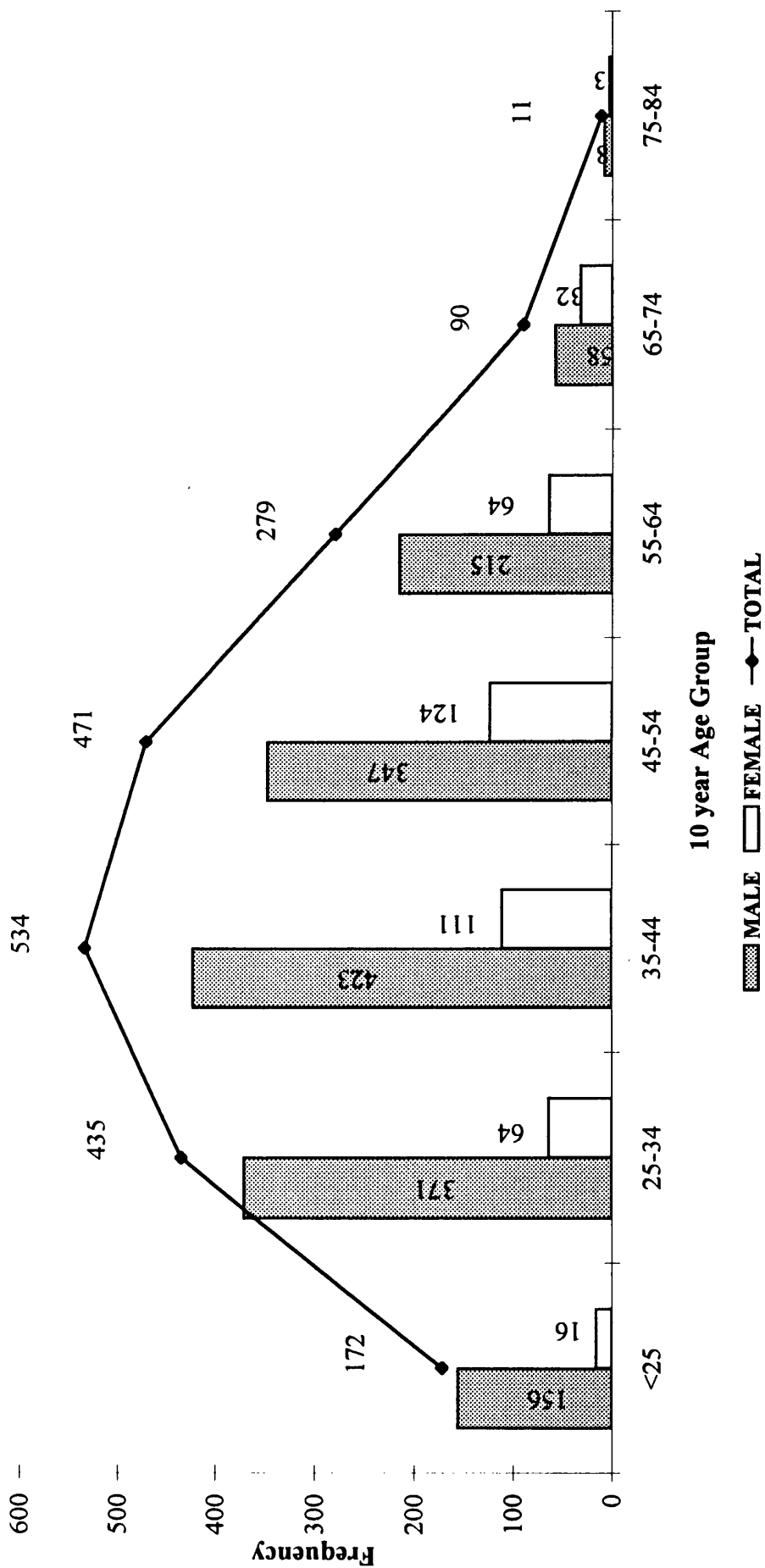
Materials and Methods - Figure 2

Peptic Ulcer Clinic Study Cohort - Number of Operations
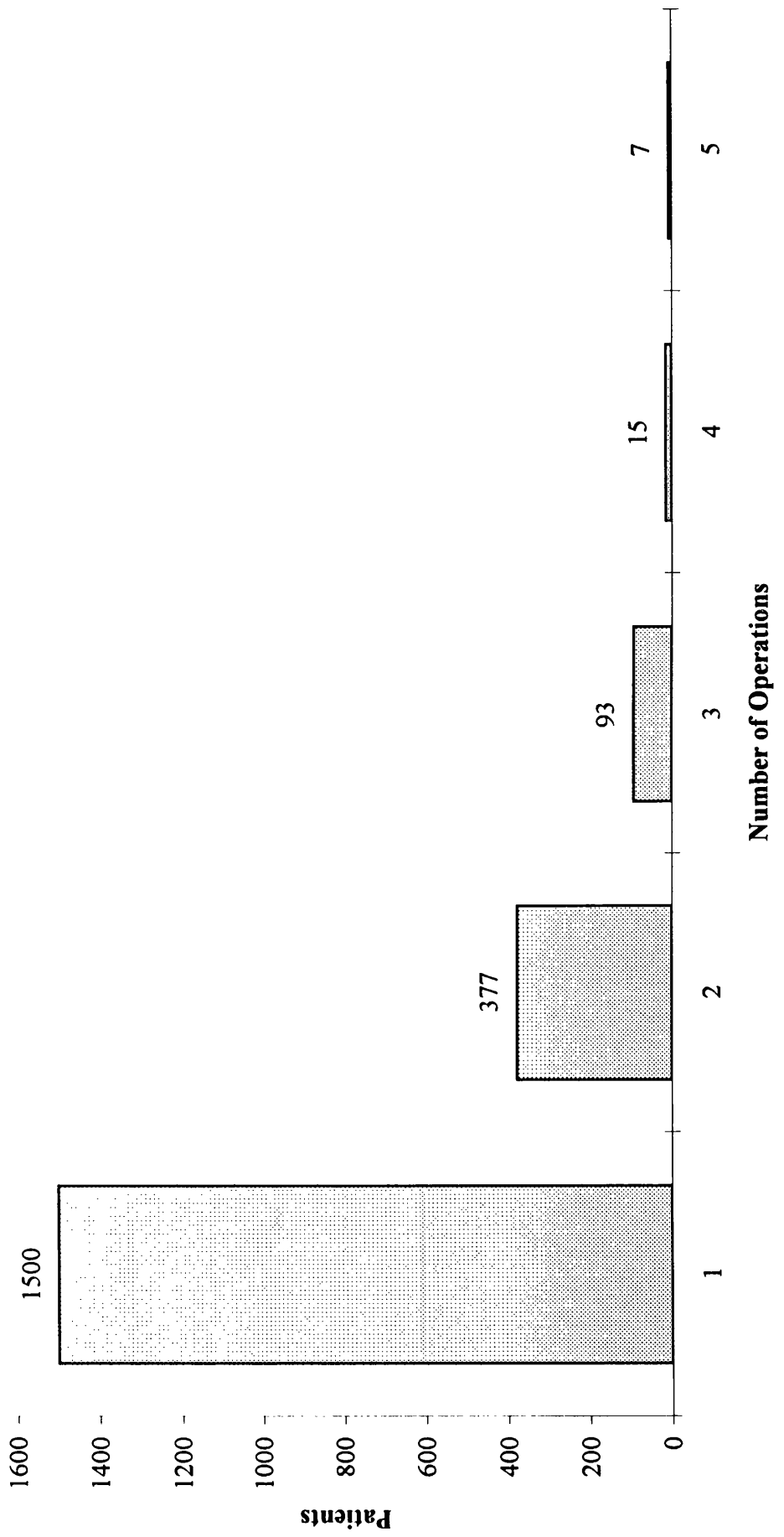
One thousand, five hundred (1,500) had only one operation and a further 371 had two, the latter usually accounted for by simple repair of a perforated ulcer preceding their elective, definitive procedure.

1.3.3. Operative Diagnosis (Table 1 and Figure 3)

The majority of both male and female patients had undergone surgery for duodenal ulcer. This diagnosis accounted for 80% of all operations performed. Gastric ulcer was more frequent in the female population but was still 5 times less common than duodenal ulcer in this group.

1.3.4. Nature of Surgery performed (Table 2 and Figure 4)

The types and frequency of operations performed is outlined below. As can be seen, for definitive surgery for peptic ulcer, vagotomy, with or without an associated drainage procedure, predominates.

1.3.5. Length of follow-up (Figure 5)

Patients underwent surgery over a period of 81 years, ranging from 1906 to 1987. Exclusions were not made if surgery was performed outwith the lifespan of the clinic as long as there was sufficiently accurate information on procedures performed. Despite this relatively broad timescale, 90% of all operations were performed within a 25 year period, from 1954 to 1979, the 5th and 95th centiles occuring in these years respectively, with the median in 1969.

The follow-up period was defined as time from first definitive ulcer operation until diagnosis of cancer, cancer-free survival till death or the end of July 1995. Definitive surgery was any surgery performed in an attempt to cure the ulcer diathesis, such as

vagotomy and drainage or partial gastrectomy, as opposed to, for instance, repair of

perforated ulcer.

Materials and Methods - Table 1

Peptic Ulcer Clinic Study Cohort - Operative Diagnosis

(values in parentheses are percentages)

Diagnosis	Males	Females	Total
Duodenal Ulcer	1724 (82.7)	364 (67.3)	2088 (79.5)
Gastric Ulcer	67 (3.2)	67 (12.4)	134 (5.1)
Co-existent Duodenal and Gastric Ulcer	38 (1.8)	10 (1.8)	48 (1.8)
Recurrent Ulcer	146 (7.0)	27 (5.0)	173 (6.6)
*Other	87 (4.1)	64 (11.6)	151 (5.7)
**No Abnormality	24 (1.2)	10 (1.8)	34 (1.3)
All Operations	2086	542	2628

* Other - a variety of diagnoses such as duodenitis, gastritis, bile reflux etc..

** No abnormality - such cases usually had exploratory doudenotomy closed as a pyloroplasty

Materials and Methods - Figure 3

Peptic Ulcer Clinic Study Cohort -
Operative Diagnosis
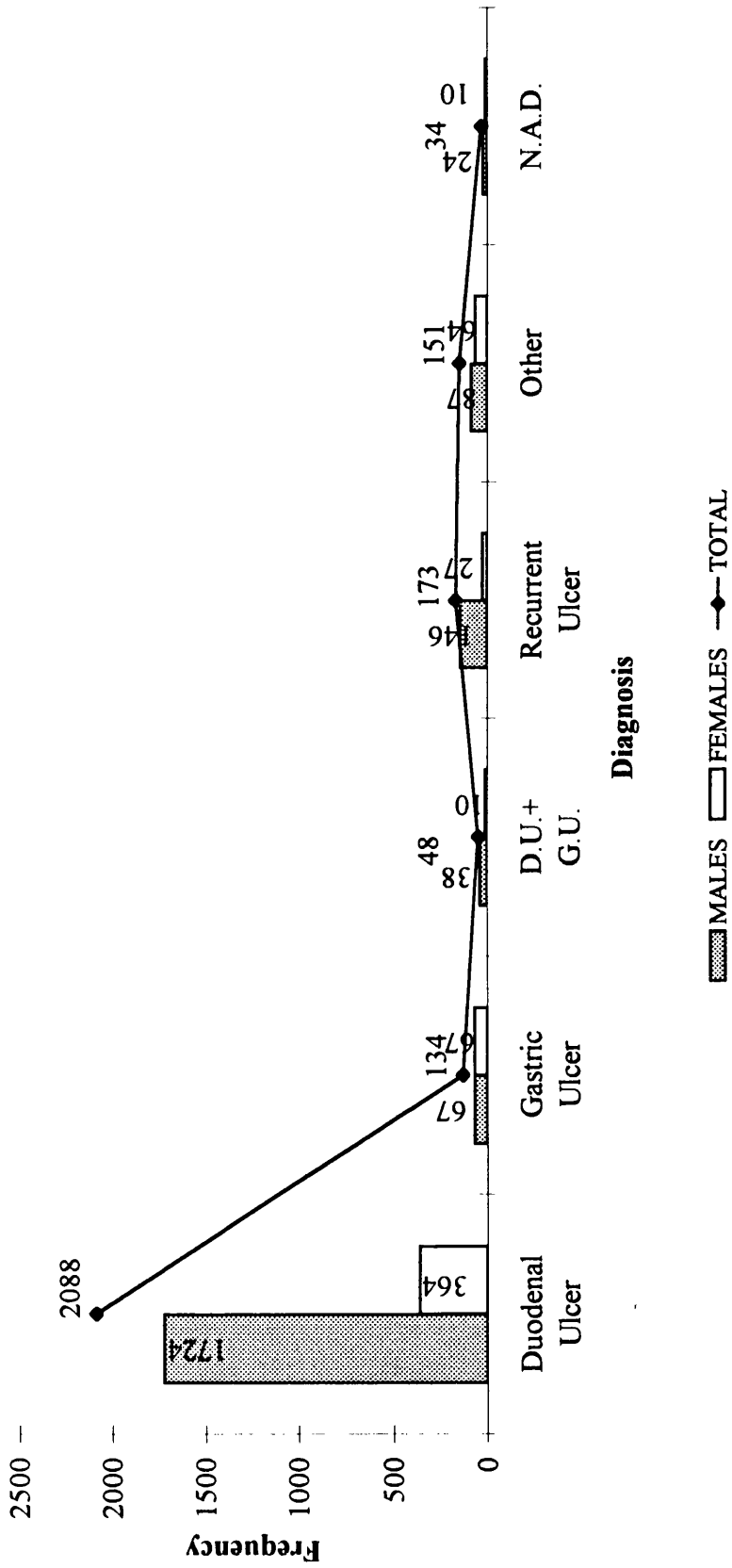
Other - refers to a variety of diagnoses. e.g. : gastritis, duodenitis, gallstones, bile vomiting

N.A.D. - No abnormality detected at operation (such cases usually had exploratory gastro-duodenotomy closed as a pyloroplasty)

Materials and Methods - Table 2

Peptic Ulcer Clinic Study Cohort - Operations Performed

(values in parentheses are percentages)

Operation type	Frequency
Vagotomy and Pyloroplasty	918 (35.0)
Vagotomy and Gastroenterostomy	700 (26.6)
Vagotomy only	130 (5.0)
Bilroth Gastrectomy	138 (5.3)
Polya Gastrectomy	163 (6.2)
Gastroenterostomy only	63 (2.4)
Pyloroplasty only	30 (1.1)
Roux-en-Y	10 (0.4)
Jejunal Interposition	9 (0.3)
Repair of Perforation only	341 (13.0)
Cholecystectomy	68 (2.6)
*Other	58 (2.2)
Total	2628

*Other - a heterogeneous group of procedures,
for instance : local excision of ulcer, segmental gastrectomy

Materials and Methods - Figure 4

Peptic Ulcer Clinic Study Cohort - Operations Performed

Frequency
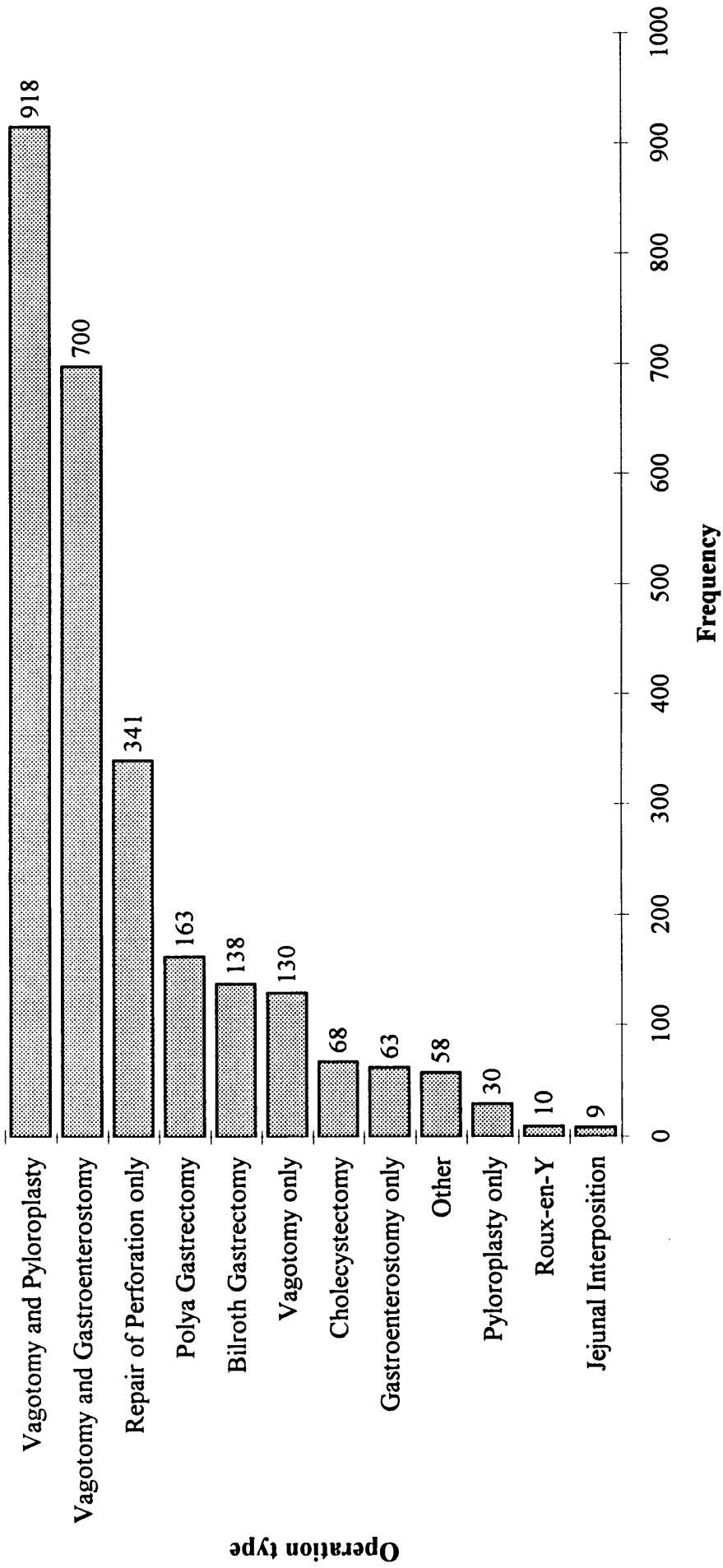
Operation type

Materials and Methods - Figure 5

Peptic Ulcer Clinic Study Cohort - Year of Operation
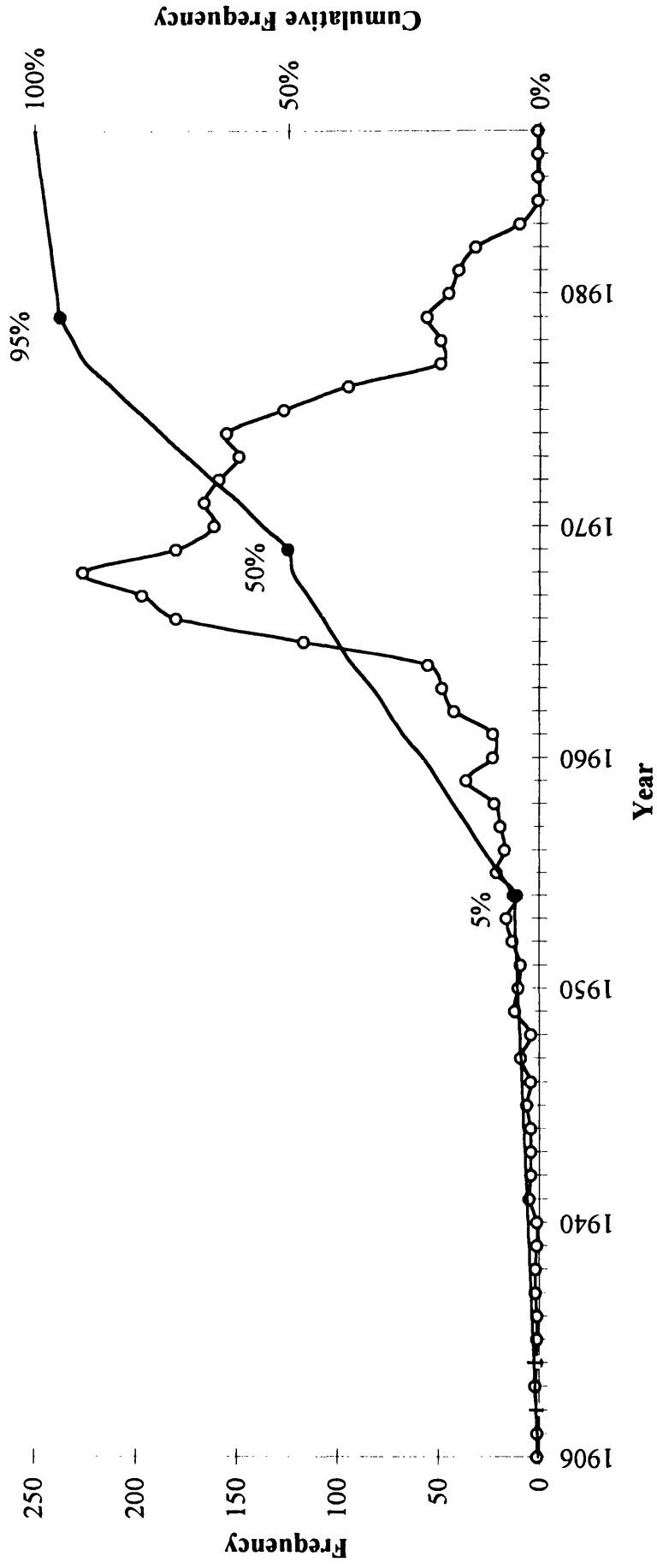
1.4 Data Collection

Patient files were individually examined and relevant information pertinent to the study noted on a form designed for that purpose (Appendix 1). This allowed the data on all patients to be collated on computer for collective analysis at a later date.

Sufficient information was also recorded to allow definite identification of patients from the records of the local Cancer Registry, including name, date of birth, hospital registration number, last recorded address and General Practitioner's name and address.

1.5. Identification of Patients subsequently developing Cancer

Once all the relevant data for the study cohort had been recorded, each patient's details were checked against the files of the West of Scotland Cancer Surveillance Unit, based at Ruchill Hospital, Glasgow. The latter agency registers cancers from six Health Boards in the West of Scotland, namely Greater Glasgow, Argyll and Clyde, Lanarkshire, Ayrshire and Arran, Dumfries and Galloway and Forth Valley. This population area mirrors closely the referral area of the peptic ulcer clinic at the Western Infirmary. Cancer registration in this region began in 1958 and became computerised in 1985. The estimated population of the region in June 1985 was 2, 777, 715 (325).

A patient identification was considered positive when at least two or more of the following criteria concurred between the files of the Peptic Ulcer Clinic and the Cancer Registry : Name, Address, Date of Birth, Hospital Unit Number. Where any doubt existed as to the identification, that patient's hospital casenotes were retrieved and perused.

1.6. Data Analysis

By summating the length of follow-up for each patient, this gave the person-years at risk for the cohort. When multiplied by the cancer incidence rates prevailing in the general population over the same calender period of the study (see study end-points above), this gave the number of cases that would be expected in that number of patients over that particular period of time. This calculated figure was compared with the observed number of cancer cases in the cohort and an observed : expected relative risk ratio was derived. Significance was assessed by application of the Poisson distribution. The "general population" referred to above, is the population of the West of Scotland, from which all the patients in the cohort originated and whose specific cancer rates were used to calculate the expected cancer numbers.

2. In vitro experiments - Clonogenic Assays

2.1 Introduction

The following experiments utilise the method of clonogenic assay, which measures the proportion of a known number of cells surviving after a period of exposure to a range of concentrations of the drug under investigation. After the period of drug exposure has ended, the cells are plated out onto petri dishes. Viable cells form colonies, a count of which gives an index of survival during the period of drug treatment. The method is widely utilised in the investigation of cytotoxic drugs.

2.2 Human Colorectal Carcinoma Cell Lines and Culture Medium employed (Appendix 2)

Three well established and widely used human colonic adenocarcinoma cell lines were used : LoVo - derived from a colorectal tumour metastasis in a supraclavicular lymph node of a 56 year old male (326); HT29 - from the ATCC (American Type Culture Collection) (327); and B.E. (328). They grow as adherent cultures in a 50/50 mixture of Ham's F10 Nutrient Mixture (Integra Biosciences, Northumbria Biologicals, U.K) and Dubecco's modified Eagles medium (Gibco BRL, LifeTechnologies Ltd, Paisley, Scotland, U.K.) containing glutamine 2mmol/L (GibcoBRL, Paisley) and 10% foetal calf serum (Globepharm Ltd, Esher, Surrey, U.K.).

2.3. Preparation of Omeprazole and Pentagastrin

Omeprazole powder was donated by the manufacturers (Astra Hassle, Molndahl, Sweden). In accordance with their instructions for use of the drug in vitro, and due to its poor solubility in water, an initial solution of 0.01mol/L was prepared by diluting 3.45 mgs of omeprazole powder in an organic solvent, buffered dimethyl sulphoxide (DMSO).

This solution was then serially diluted with culture medium, initially by a factor of 50, to achieve the strongest concentration to be tested, 0.2mmol/L. Four mLs of the latter was then diluted by a factor of 5 to achieve the second test concentration, 40μmol/L, as were all subsequent concentrations, giving a broad range of test concentrations down to 12.8nmol/L. The therapeutic range and maximal plasma concentrations of the drug as observed in animals and humans at various dosing regimes fall well within the above range (313,329), as do the concentrations used in previous in vitro studies (330,331).

Pentagastrin was obtained from I.C.I Pharmaceuticals, Wilmslow, Cheshire, U.K., as Peptavlon, which is packaged in glass ampoules each containing 500μgs Pentagastrin B.P. in 2mLs solution (sodium chloride, ammonium chloride and water). This constitutes an initial concentration of 0.3mmol/L. This was serially diluted with medium, as described above, to give a range of test concentrations from 6μmol/L down to 15.4 picomol/L.

2.4. Assay Technique

Established monolayers of each cell line were loosened from the flask surface and from one another by addition of 2mLs of 0.5% trypsin solution, which was left to bathe the cells for 1 minute. Once the cell monolayer was disrupted, 8mLs of culture medium was then added to form a suspension of the loosened cells. A sample of this was taken and counted in a Coulter counter (Coulter Electronics Ltd., Luton, Bedfordshire, England), which calculates the number of particles in a suspension by measurement of the scatter of a beam of electrons passed through it. After appropriate dilution of the suspension, cells were plated out at a density of 7.75×10^4 cells per $25cm^2$ flask in 5mLs of culture medium and allowed to attach and grow for 2 days in a humidified atmosphere of 2% CO_2 in air. The medium was then removed from each of the flasks and replaced with 4 mLs medium containing the drug under investigation, namely, omeprazole, pentagastrin or the drug

vehicle only (DMSO), at varying concentrations. One flask was replenished with drug-free medium and served as the control.

Drug exposure lasted 24 hours after which medium was removed from each of the flasks. The cells were then trypsinised as before, and re-suspended in 10mLs of medium. Following this, the resultant cell suspensions were transferred to universal containers and centrifuged for 5 minutes at 3,000 revolutions per minute. The supernatant was removed and the cell pellets re-suspended in 10 mLs fresh medium and cells from the untreated control flask only, were counted. This cell suspension was diluted to achieve a suspension of 10^3 cells per mL and 1mL of this suspension was then plated onto 6cm diameter petri dishes and a further 5mLs of medium added. Therefore, the control plates contained 10^3 cells per dish. The suspensions from all the drug treated flasks were then diluted and plated exactly as for the control flask. Therefore, if the drug had no effect on these cells during the period of exposure, one would expect to be plating a similar number of cells and ultimately expect similar numbers of cell colonies at the end of the experiment. Four dishes were set up from each flask. These were then returned to the incubator for 10 days to allow colonies to develop from surviving cells.

At the end of this period, growth medium was discarded, colonies were fixed in methanol and stained with 0.1% crystal violet solution. Colonies were counted by means of an Artek colony counter. Three counts of each plate were taken.

For omeprazole, 2 such identical assays were performed on each of the cell lines, LoVo and B.E., and 3 on HT29, giving a maximum of 8 and 12 colony counts respectively, at each concentration tested. A single assay was performed with the drug vehicle for omeprazole; dimethyl sulphoxide; to ensure that the concentrations of this compound, present in the omeprazole medium, would have no effect on the cells. Finally, 2 assays

addressed the potential growth promoting action of pentagastrin on colorectal cancer cells.

2.5 Statistical Analysis

The clonogenic assays were examined by non-parametric one way analysis of variance using the Kruskal-Wallis test. Where the latter suggested significant differences between treatment groups (control/drug concentrations) within an assay, individual pairs of groups were examined by the Mann-Whitney U test to identify them. Any potential trend in colony count as omeprazole concentration increased was assessed by the Wilcoxon-type non-parametric method devised by Cuzick (332) and described by Altman (333). Significance was taken at $P = 0.05$ or less.

3. In vivo Study - Effect of Omeprazole treatment (and the resultant hypergastrinaemia so induced) on the Initiation and Promotion Phases of chemically induced Colorectal Carcinogenesis in Rats.

3.1. Introduction

In order to ensure that comparisons could be made with the original work which was the stimulus to this study (311), it was designed to adhere as closely to the original experiment as possible, in terms of animal species, laboratory conditions, drug preparations and dosages.

3.2. Animal Species and Care

Seventy five, 5-week old, female Sprague Dawley rats (Harlan OLAC Ltd, UK) were housed and maintained under standard animal house conditions with a 12 hour light-dark cycle, with free access to water and rat chow. Animals were weighed on a weekly basis and examined daily for any signs of ill health which could potentially lead to early sacrifice and withdrawal from the study. Particular attention was paid to any signs suggestive of colonic tumour development, for instance: diarrhoea or abdominal distension.

3.3. Drug Preparation (Appendix 3), Administration and Handling

Dependant on treatment group and phase, each rat received a single daily dose of either omeprazole suspension or inert drug vehicle. Omeprazole was given in a dose of 40μmol/kg/day at a volume of 5mL/kg/day and inert vehicle at the same volume per kilogram of body weight. Pure omeprazole powder (Astra Hassle) was suspended in a vehicle of 0.25% methylcellulose, buffered with 0.2% sodium bicarbonate and adjusted to pH 9 with sodium hydroxide. This was kept refrigerated at 4°C for up to 5 days when in

use, but stored at a temperature of -20°C until required. Inert drug-free vehicle was treated similarly.

The proximate carcinogen, azoxymethane (Sigma Chemicals, Dorset, UK) was diluted with sterile normal saline to a concentration of 100mgs/mL and stored in a locked container at 4°C. Immediately prior to administration, it was further diluted with normal saline to 10 mgs/mL. In accordance with "Control of Substances Hazardous to Health" (COSHH) guidelines, to avoid exposure to the chemical, all work with azoxymethane was performed inside a unidirectional airflow hood whilst wearing a mask with organic chemical filter and disposable gown and double gloves. Non-essential staff were excluded from the vicinity of the procedure room and the area used to house the animals. All contaminated materials including disposable overclothes and animal waste and bedding were double-bagged and destroyed by incineration.

3.4. Study Design (Table 3 and Figure 6)

After a settling-in period of 1 week, the 75 rats were randomly allocated into 3 equally sized treatment groups of 25, as shown below. The duration of the experiment was 24 weeks.

Omeprazole and placebo dosage were given by once daily oral gavage. Each animal was gently restrained in one hand in the proper fashion and a semi-rigid plastic catheter was passed perorally down the oesophagus into the stomach, at which point the appropriate amount of drug was instilled from an attached syringe. This technique ensured that an exact dose was administered and received by the animal. It has clear advantages over other forms of oral administration, such as addition of drug to drinking water or food, where ingestion of a specified amount varies and cannot be guaranteed.

Materials and Methods - Figure 6

In vivo study - Drug and Carcinogen Administration

Vehicle

Vehicle

Vehicle

Omeprazole

Omeprazole

Vehicle

Azoxymethane

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Week of Study

Group A ∙ ∙ ∙ Group B ▬ ▬ Group C ▬■▬ All Groups
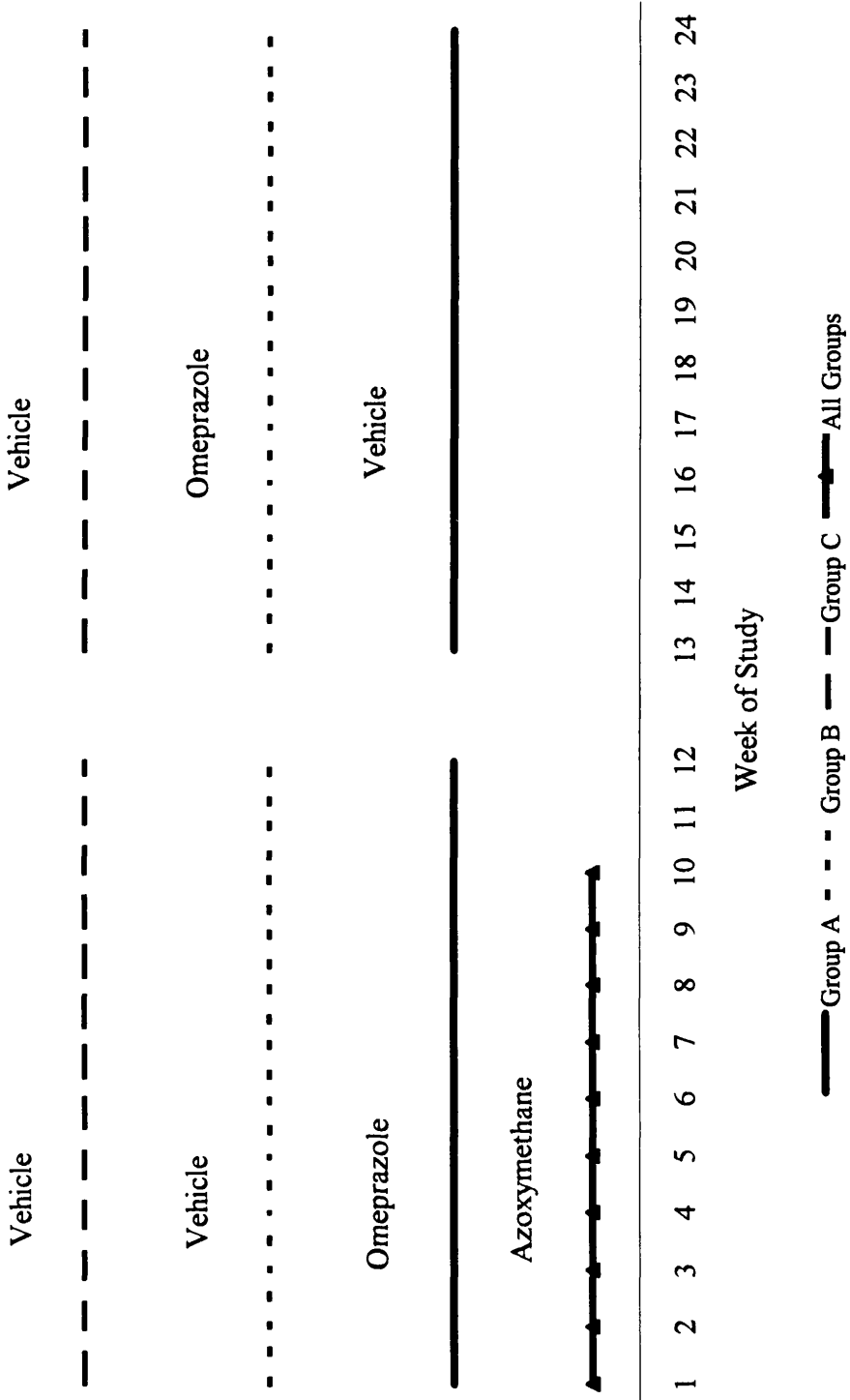
Table 3

Treatment Phase

	Week 1 - 12	Week 13 -24
Group A :	Omeprazole 40μmol/kg/day	Inert drug vehicle 5mL/kg/day
Group B :	Inert drug vehicle 5mL/kg/day	Omeprazole 40μmol/kg/day
Group C :	Inert drug vehicle 5mL/kg/day	Inert drug vehicle 5mL/kg/day

All animals received azoxymethane by once-weekly subcutaneous injection, in a dose of 10mg/kg/week for 10 weeks (weeks 1 to 10 inclusive) resulting in a total dose per rat of 100mg/kg.

Group A subjects received omeprazole during the initiation phase of carcinogenesis; that is: in conjunction with administration of carcinogen; and crossed over to placebo treatment in the promotion phase, after cessation of exposure to carcinogen. Conversely, Group B animals only commenced active treatment with omeprazole after carcinogen exposure had ceased. The treatment cross-over point was delayed for 2 weeks after the final azoxymethane injection to ensure complete metabolism and clearance of the carcinogen from the animals prior to change in treatment. Group C received placebo for the duration of the study and acted as the control group.

3.5. Assessment of Endogenous Gastrin Response to Treatment

To assess endogenous gastrin levels in response to omeprazole treatment, samples of tail vein blood, up to a maximum of 1mL, were taken from each rat at 3 time points during the study. The first sample was collected in the week prior to commencement of treatment (week 0), to obtain baseline fasting values of plasma gastrin. A second sample was taken in week 6, representative of the first treatment phase of the study, and the final

sample was collected in week 24, representative of the second treatment phase of the study. All blood sampling was performed between 1200 - 1600 hours which, in the case of the latter two sampling points, was within 2 to 4 hours of drug or placebo administration. Blood was collected into Eppendorf tubes containing 0.1 mL lithium-heparin solution, centrifuged and the plasma separated and pipetted into Nunc containers and stored at -70°C for analysis at a later date. As in Penman's study, plasma gastrin was analysed by radioimmunoassay with R98 antibody (334).

3.6. Post-Mortem Analysis

At the end of the study, all rats were killed by cervical dislocation, and post-mortem examination performed. The entire colorectum from caecum to anus was removed, opened longtitudinally, pinned flat on a cork board, cleaned and examined by naked eye. All visible mucosal lesions were noted, and their volume (product of height x length x breadth) and distance from anal verge measured. Each tumour was assigned an identification code, then excised, fixed in 10% formalin and thereafter processed to paraffin, stained with haematoxylin and eosin and examined by light microscopy. The latter was performed by a pathologist who had no knowledge from which treatment group the specimens had originated.

Lesions were classified as adenocarcinomas or adenomas and in the case of adenocarcinomas, depth of invasion of the bowel wall was classified by stage as either Dukes A or Dukes B, as described in Chapter 1.

Only macroscopic abnormalities were taken for analysis. Therefore, neither visibly or palpably normal mesocolon, was routinely submitted for microscopic examination and thus it is accepted that some of the lesions classified as Dukes stage B may in fact be

Dukes C due to the presence of microscopic metastases in the draining mesenteric lymph nodes, hence Dukes B lesions are referred to as Dukes B+.

3.7. Statistical Analysis

Tumour incidence, Dukes stage and carcinoma to adenoma ratio were analysed by Fishers exact test. Total tumour yield, tumour volume and distribution were assessed by the Mann Whitney U test. Plasma gastrin values in the different phases of the study were compared using Student's paired T-test, and animal weight and growth rate by Student's unpaired T-test. Correlation between tumour development and final animal weight and growth rate was examined by calculation of Spearman's rank correlation co-efficient(r_s). Significance in all tests was assumed at $P = 0.05$ or less.

Chapter 3 - Results

1. Cancer Incidence following Surgery for Benign Peptic Ulcer

From the cohort of 1,992 patients operated on for peptic ulcer, a total of 352 cancers arising in 339 of these patients were identified. Eleven patients developed 2 malignancies and one patient had 3. The latter unfortunate man had a skin cancer diagnosed 9 years after his ulcer operation and more significantly, developed colorectal cancer 15 years post-operatively, before succumbing to a gastric neoplasm within a year of this. Figure 1 indicates the frequency of the most commonly encountered neoplasms. The observed and expected numbers of the commonest neoplasms, with the observed: expected relative risk ratios (O:E) are summarised in Table 1. The results from analysis by specific operation type are summarised in Table 2. The category labelled "Other" in the results presented is a heterogenous group of diagnoses which includes various types of haematological and reticulo-endothelial malignancies; benign and in-situ neoplasms; secondary carcinomas with primary origin unspecified; and isolated cancers at a variety of sites, including cervix (1), ovary (4), testis (3), gall bladder (2), liver (1) etc ... In view of either the small numbers of cases involved, or difficulty in accurately categorising many of these diagnoses, analysis of these was not performed.

Overall, the incidence of malignant disease within the cohort was not excessive compared to the population from which the patients originated. Indeed, less malignancies than expected were seen (observed 352 : expected 412 ; RR = 0.85 ; P = 0.99). Carcinoma of the lung was the most frequently observed cancer in the cohort and was slightly more prevalent than in the population from which the patients originated, though just outwith

statistical significance (observed 119 : expected 106 ; RR = 1.13 ; P = 0.09). A

significant excess for this disease was however evident in the vagotomised group of

patients (observed 95 : expected 79 ; RR = 1.20 ; P = 0.04).

Cancers of the large bowel were the next most frequently observed neoplasm but were

less than expected (observed 30 : expected 45 ; RR = 0.67 ; P = 0.9). This applied

regardless of operation type (vagotomy with or without drainage : observed 25 : expected

33 ; RR = 0.76 ; P = 0.94 ; partial gastrectomy : observed 3 : expected 8 ; RR = 0.38 ; P

= 0.99). The latent period between ulcer operation to diagnosis of colorectal cancer

ranged from 5 to 29 years. The median period was 17 years and the median age at which

cancer was diagnosed in these patients was 65 years (range 54 - 89years).

Carcinoma of the stomach accounted for 27 cancers in the cohort and this was only

slightly greater than the expected 20 (RR = 1.17 ; P = 0.23). These presented from as

early as 2 years up to 31 years post-operatively.   The median value for the latent period

was 11 years and median age at diagnosis was 63 years (range 38 - 89years).

When the patients are subdivided according to operative procedure performed, it is clear

that vagotomised patients do run a significantly greater risk of gastric cancer (observed 24

: expected 16 ; RR = 1.50 ; P = 0.04). Patients undergoing partial gastrectomy would

appear to have a decreased risk, of the order of 50 %, compared to the population at

large, though the numbers are small (observed 2 : expected 4 ; RR = 0.5 ; P = 0.91).

When analysing the cohort as a whole, only laryngeal cancer was found to be significantly

excessive with 12 observed cases against an expected 6 (RR = 0.5 ; P = 0.04), and this

was found to be due to a significant excess in the vagotomised group of patients

(observed 10 : expected 4 ; RR = 2.5 ; P = 0.01). With the exception of this and the

commonest cancers discussed above, none of the other cancers were any more common

than would be expected and this persisted even when they were analysed for each specific operation sub-group (see below).

As previously mentioned, some sub-groups were also examined, which, by and large, have been ignored or excluded from previous studies. Thus, 89 patients underwent simple closure of a perforated peptic ulcer and no further operation. For these patients, no significant differences between observed or expected cases of cancer were apparent, either overall, or for any individual cancers. A similar scenario was evident for patients having undergone gastroenterostomy only (24 patients), pyloroplasty only (22 patients - such patients were found to have no significant pathology at operation and had exploratory gastroduodenotomy closed as a Heinecke-Mickulicz pyloroplasty) and those who also had a history of cholecystectomy in addition to their ulcer surgery (68 patients). These groups are numerically small but their inclusion should not invalidate the results of the cohort as a whole, as no significant variations in risk are apparent within these select groups. Therefore, included within the major subgroups of vagotomised and gastrectomised patients (1,857 in total), 299 had preceding closure of perforated ulcer, a gastroenterostomy or pyloroplasty only. Also included are the 68 patients mentioned above who had cholecystectomy in addition to their ulcer operation.

In addition, the small subgroups of patients who had undergone "multiple procedures" , that is, more than one definitive type of ulcer surgery were studied. Examples of this were patients who had vagotomy and drainage followed by a partial gastrectomy, usually for recurrent ulcer (143 patients) and/or biliary diversion procedures (19 patients). Such patients did not appear to have any significant difference in risk with respect to the population at large. However, as the aim of the study was to accurately assess and compare the long term sequelae of specific operation types, this group of patients were excluded from analysis of the major vagotomy and partial gastrectomy operation

subgroups, for two reasons. Firstly, and quite simply, they were unable to be assigned to one particular ulcer operation category. Furthermore, and more importantly, such patients would have variations in potentially significant factors such as circulating gastrin levels and entero-gastric reflux as a result of the different operations performed, within the follow-up period. For instance, a patient may have significant hypergastrinaemia following a vagotomy and drainage procedure, but then be rendered normo or even hypogastrinaemic after having a subsequent antrectomy some years later. That same patient may then undergo a Roux-en-Y biliary diversion for bile reflux some months or years following the antrectomy and therefore also have significant alteration the degree of gastro-enteric reflux. As such, it would be very difficult to hypothesise what particular pathophysiological change might be responsible for subsequent cancer development, were it to occur in such an individual.

Results - Figure 1

Peptic Ulcer Clinic Study Cohort - Frequency of Cancers in Follow-up Period
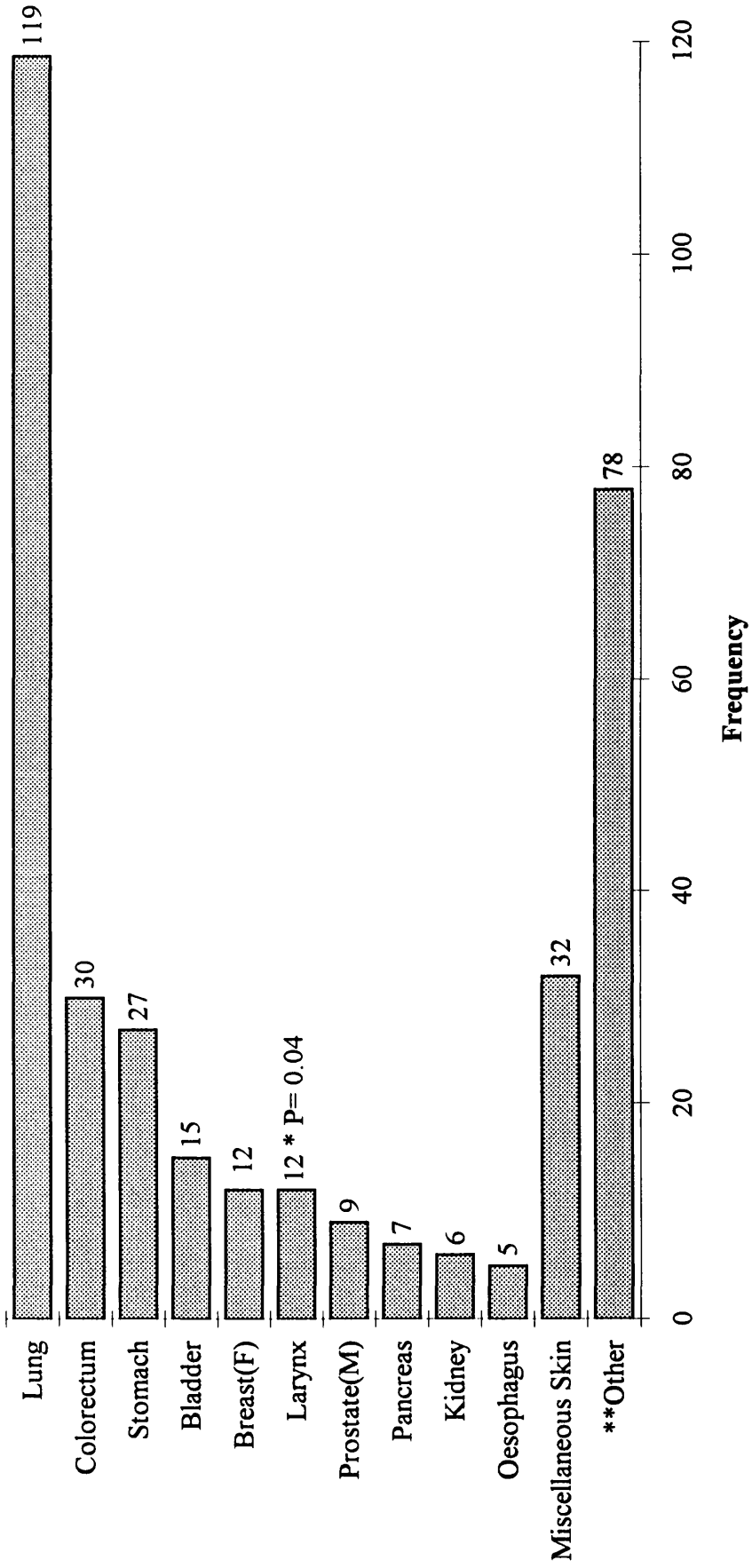
* P = 0.04, Expected Value = 5.2 ; all other results not significantly different from expected.   ** Other - see text.

Results - Table 1
Cancer Incidence in Study Cohort

*ICD Number	Cancer	Observed Frequency	% total cohort	Expected Frequency	O:E ratio	***P value
162	Lung	119	5.97%	106	1.13	0.09
153, 154	Colorectum	30	1.51%	45	0.67	0.9
151	Stomach	27	1.36%	23	1.17	0.23
188	Bladder	15	0.75%	25	0.6	0.99
174	Breast(F)	12	0.60%	15	0.8	0.8
161	Larynx	12	0.60%	6	2	**0.01**
185	Prostate(M)	9	0.45%	31	0.29	0.99
157	Pancreas	7	0.35%	10	0.7	0.86
189	Kidney	6	0.30%	7	0.86	0.72
150	Oesophagus	5	0.25%	11	0.46	0.98
172,173	Miscellaneous Skin	32	1.61%	49	0.65	0.99
-	**Other	78	3.91%	-	-	-
		352	17.66%	412	0.85	0.99

*ICD - International Classification of Disease ; **Other - see text ; ***One way analysis - Poisson Distribution (significant results in bold)

Results - Table 2
Incident Cancers by Operation Category

Total Cohort(1992)

	Observed	Expected	Relative Risk	*P value
All Cancers	352	412	0.85	0.99
Stomach	27	23	1.17	0.23
Colorectal	30	45	0.67	0.9
Lung	119	106	1.12	0.09
Bladder	15	25	0.6	0.99
Larynx	12	5.52	2.17	**0.01**
Breast(F)	12	14.8	0.81	0.8
Prostate(M)	9	30.7	0.29	0.99
Pancreas	7	9.9	0.71	0.86
Kidney	6	7.25	0.83	0.72
Oesophagus	5	10.9	0.46	0.98
Skin	32	48.6	0.66	0.99
**Other	78	-	-	-

Vagotomy+/- Drainage(1556)

	Observed	Expected	Relative Risk	
All Cancers	267	306	0.87	0.98
Stomach	24	16	1.5	**0.04**
Colorectal	25	33	0.76	0.94
Lung	95	79	1.2	**0.04**
Bladder	11	21	0.53	0.99
Larynx	10	4.34	2.32	**0.01**
Breast(F)	10	11.6	0.86	0.72
Prostate(M)	8	22.7	0.35	0.99
Pancreas	7	7.4	0.95	0.61
Kidney	4	5.5	0.73	0.8
Oesophagus	5	8.2	0.61	0.91
Skin	24	40.2	0.6	0.99
**Other	44	-	-	-

Partial Gastrectomy(301)

	Observed	Expected	Relative Risk	
All Cancers	49	79	0.62	0.99
Stomach	2	4	0.5	0.91
Colorectal	3	8	0.38	0.99
Lung	14	18	0.78	0.86
Bladder	3	5	0.64	0.85
Larynx	1	0.8	1.25	0.55
Breast(F)	2	3.3	0.66	0.84
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	2	1.1	1.8	0.3
Oesophagus	0	-	-	-
Skin	6	9.6	0.63	0.92
**Other	16	-	-	-

*P value - One way analysis, Poisson distribution
**Other - see text

Results – Table 2 (continued)
Incident Cancers by Operation Category

Cholecystectomy(68)

	Observed	Expected	Relative Risk	
All Cancers	6	16	0.38	0.99
Stomach	0	1	-	-
Colorectal	0	2	-	-
Lung	1	3	0.33	0.95
Bladder	0	-	-	-
Larynx	0	-	-	-
Breast(F)	0	-	-	-
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	0	-	-	-
Oesophagus	0	-	-	-
Skin	0	-	-	-
**Other	5	-	-	-

Repair of Perforated ulcer Only(89)

	Observed	Expected	Relative Risk	
All Cancers	15	19	0.78	0.85
Stomach	0	1	-	-
Colorectal	1	2	0.5	0.99
Lung	7	5	1.4	0.24
Bladder	1	5	0.2	0.99
Larynx	0	-	-	-
Breast(F)	0	-	-	-
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	0	-	-	-
Oesophagus	0	-	-	-
Skin	2	9.3	0.22	0.99
**Other	4	-	-	-

Gastroenterostomy only(24)

	Observed	Expected	Relative Risk	
All Cancers	6	8	0.75	0.81
Stomach	1	1	1	0.99
Colorectal	1	1	1	0.99
Lung	2	2	1	0.59
Bladder	0	-	-	-
Larynx	0	-	-	-
Breast(F)	0	-	-	-
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	0	-	-	-
Oesophagus	0	-	-	-
Skin	0	-	-	-
**Other	2	-	-	-

*P value - One way analysis, Poisson distribution
**Other - see text

Results - Table 2 (continued)
Incident Cancers by Operation Category

	Pyloroplasty only(22)			
	Observed	Expected	Relative Risk	*P value
All Cancers	2	3	0.67	0.81
Stomach	0	0	-	-
Colorectal	0	0	-	-
Lung	1	1	1	0.63
Bladder	0	-	-	-
Larynx	0	-	-	-
Breast(F)	0	-	-	-
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	0	-	-	-
Oesophagus	0	-	-	-
Skin	0	-	-	-
**Other	1	-	-	-
	Multiple Procedures(143)			
	Observed	Expected	Relative Risk	
All Cancers	20	30	0.67	0.97
Stomach	0	2	-	-
Colorectal	1	3	0.33	0.9
Lung	5	8	0.63	0.9
Bladder	1	2	0.56	0.83
Larynx	0	-	-	-
Breast(F)	2	0.9	2.2	0.23
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	0	-	-	-
Oesophagus	0	-	-	-
Skin	3	3.6	0.83	0.7
**Other	8	-	-	-
	Jejunal Interposition/Roux-en-Y(19)			
	Observed	Expected	Relative Risk	
All Cancers	4	6	0.67	0.84
Stomach	0	0	-	-
Colorectal	0	1	-	-
Lung	2	2	1	0.59
Bladder	0	-	-	-
Larynx	0	-	-	-
Breast(F)	0	-	-	-
Prostate(M)	0	-	-	-
Pancreas	0	-	-	-
Kidney	0	-	-	-
Oesophagus	0	-	-	-
Skin	0	-	-	-
**Other	2	-	-	-

*P value - One way analysis, Poisson distribution
**Other - see text

2. Clonogenic Assays

The results for each assay are presented in tabular fashion (Tables 3 - 7) and also graphically (Figures 2 - 6).

2.1. Cell line LoVo and Omeprazole (Table 3 & Figure 2)

Multivariate analysis by the Kruskal Wallis test confirmed there to be significant differences in colony counts between the various treatment groups in the assay (P = 0.03). Separate pairs of groups were then analysed by the Mann Whitney U-test. This confirmed that treatment with the highest concentration of omeprazole (0.2mmol/L) resulted in significantly fewer surviving cells, as reflected in the lower colony counts, when compared to both control (P = 0.006) and all cell groups treated with lesser concentrations of omeprazole (P = 0.004 to 0.006). Cell survival after exposure to all lesser doses of omeprazole did not differ from control cultures (P = 0.07 to 1.00). The Wilcoxon-type test for trend was positive (P = 0.03) and inspection of Figure 1 does indeed show a modest reduction in median colony count as omeprazole concentration increases, most obvious from 0.32µmol/L upwards.

2.2. Cell line B.E. and Omeprazole (Table 4 & Figure 3)

The results for cell line B.E. are similar to those for LoVo. Multivariate analysis showed there to be significant variation within the assay groups (Kruskal Wallis, P = 0.02), and after separate analysis of pairs of groups, this was found to be due to significantly reduced colony counts after exposure to the highest omeprazole concentration compared to control (Mann Whitney, P = 0.04) and all lesser tested drug doses (Mann Whitney, P = 0.0007 to 0.04). As with LoVo cells, none of the other drug doses resulted in any

Results - Table 3

Cell Line LoVo - Clonogenic Assay with Omeprazole

Test Concentration

*Plate	Control	12.8nmol/L	64nmol/L	0.32µmol/L	1.6µmol/L	8µmol/L	40µmol/L	0.2mmol/L
1	133.6	126.6	117	123	97.6	178.3	24.6	Plate Infected
2	115.6	111.6	155	149.6	122.6	132.3	35	Plate Infected
3	125.6	149.3	152	178.6	128.6	125.3	21.6	Plate Infected
4	Plate Infected	Plate Infected	164.3	Plate Infected	159.6	140.6	47	Plate Infected
5	180.3	220.3	330.6	246.3	398.6	146.6	245.3	58.6
6	380.3	293.6	215.6	354.3	373.6	114.3	302	52.3
7	236	184	181	242.6	265.3	108	206.6	62.3
8	276.6	348.3	474.6	253.6	222	97.6	296.6	48
MEDIAN	180.3	184	172.7	242.6	190.8	128.8	126.8	**55.5

*Mean of 3 counts/plate ; **P<0.05 compared to controls *and* those at all other tested concentrations (Mann Whitney U-test)

Results - Figure 2

LoVo & Omeprazole
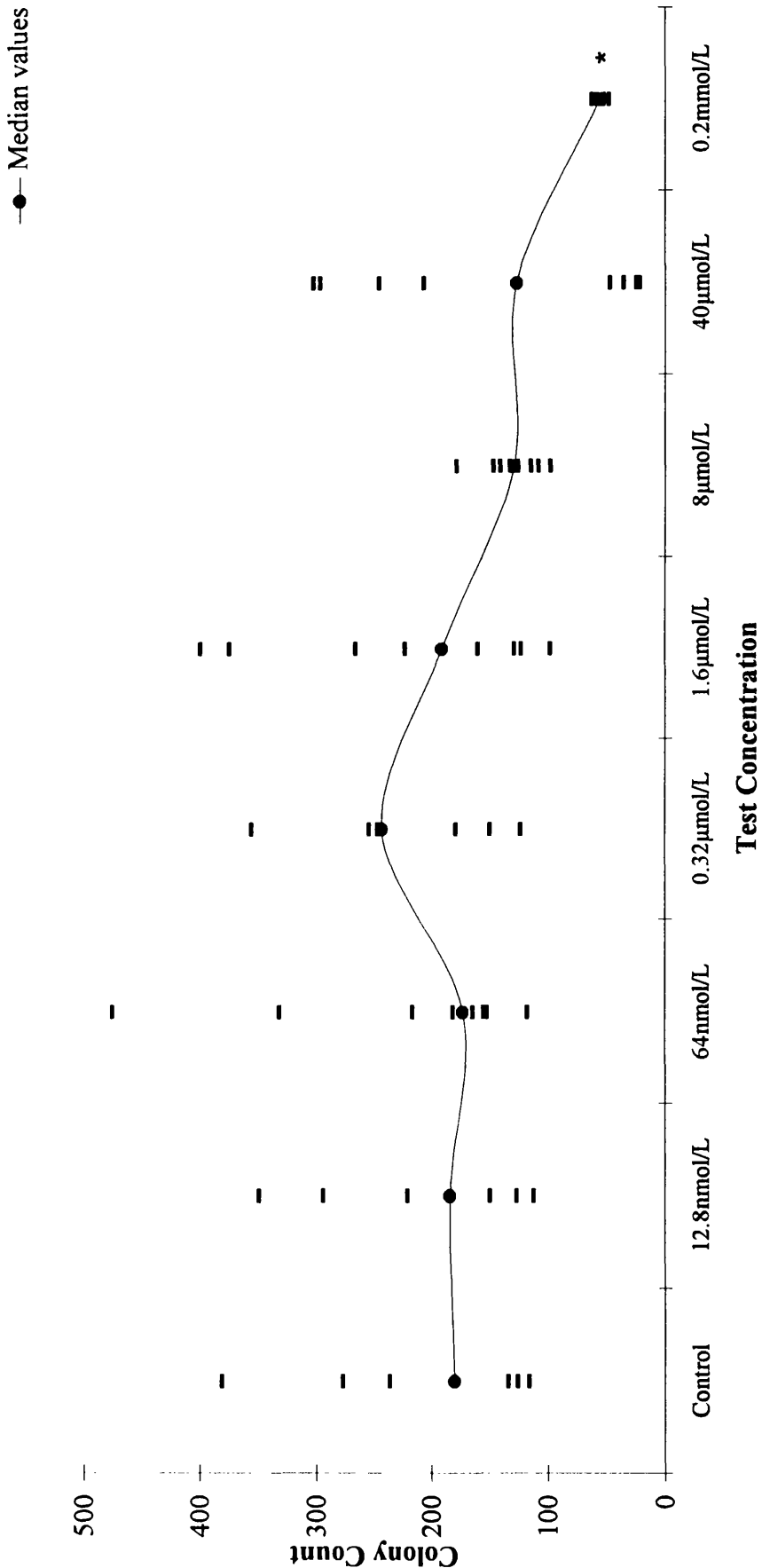
*P<0.05 compared to control and all other test concentrations(Mann Whitney U-test)

Results - Table 4

Cell Line B.E - Clonogenic Assay with Omeprazole

Test Concentration

	Control	12.8nmol/L	64nmol/L	0.32µmol/L	1.6µmol/L	8µmol/L	40µmol/L	0.2mmol/L
1	120.3	63.6	103.6	106	Plate Infected	146.6	134	21
2	101	84.6	95	118	116.6	148.6	141.3	17.3
3	103.6	109.6	103.3	112.3	126.3	127.3	131	18.3
4	118	80.6	84.6	97.6	134	139.6	134	24.3
Plate 5	181.3	234.3	169.3	209.3	194.3	203	220.3	120.6
6	208.3	214.6	179	207	206	215.6	210.3	130
7	195.6	209.6	169	211.3	206	201.6	239.6	Plate Infected
8	197.6	192.6	195	205.3	204.3	211.3	217	Plate Infected
MEDIAN	150.8	151.1	136.3	161.7	194.3	175.1	175.8	*22.7

*P<0.05 compared to controls *and* those at all other tested concentrations (Mann Whitney U-test)

Results - Figure 3

B.E. & Omeprazole
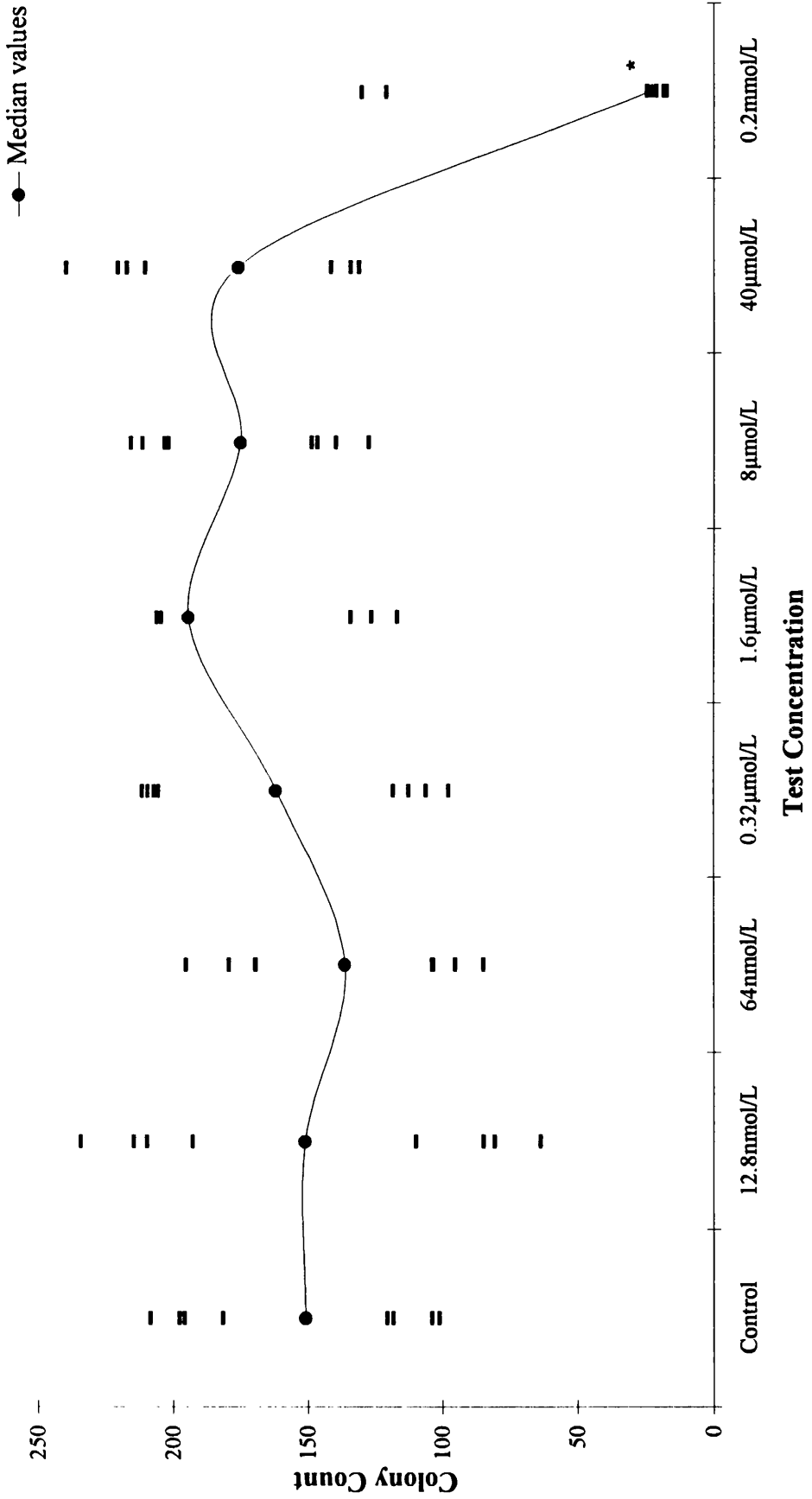
*P<0.05 compared to control and all other test concentrations(Mann Whitney U-test)

significant variation in survival compared to control cells (Mann Whitney, $P = 0.11$ to 0.96). No significant trend in cell survival existed as omeprazole concentration increased ($P = 0.94$).

2.3. Cell line HT29 and Omeprazole (Table 5 & Figure 4)

Perusal of the plotted results for cell line HT29 show that, like the other two cell lines, the lowest colony count is obtained at 0.2mmol/L omeprazole. However, the colony counts obtained after exposure to this concentration of omeprazole, and those at all lesser concentrations, did not differ significantly from control counts or each other (Kruskal Wallis, $P = 0.07$). Neither was there any significant trend in results as drug concentration increased ($P = 0.09$)

2.4. Assay to assess any potential effect of drug vehicle on cell survival

(Table 6 & Figure 5)

All of the above assays utilised cells which were grown in culture medium only, as controls. To ensure that this was legitimate and that the "inert" drug vehicle, dimethyl sulphoxide (DMSO), would not by itself have any confounding effect on the results, a single assay was performed, using cell line B.E., exposing groups of cells to the range of concentrations of DMSO present at each of the tested concentrations of omeprazole.

Multivariate analysis of the results again confirmed statistically significant variations to exist within the assay treatment groups (Kruskal Wallis, $P = 0.01$). When sub-analysis was performed this showed that both the cells treated with omeprazole 0.2mmol/L and those exposed to the equivalent concentration of DMSO only, had significantly reduced counts compared to drug and vehicle-free control cells (Mann Whitney, $P = 0.007$ and $P = 0.02$, respectively) but were themselves similar in magnitude (Mann Whitney, $P = 0.55$).

Results - Table 5

Cell Line HT29 - Clonogenic Assay with Omeprazole

Test Concentration

Plate	Control	12.8nmol/L	64nmol/L	0.32μmol/L	1.6μmol/L	8μmol/L	40μmol/L	0.2mmol/L
1	333	139	185.3	170.6	115.3	Plate Infected	198	27.3
2	385.3	121.6	178.6	156	125.3	190.6	172	10
3	Plate Infected	131	170	146	107	177	167.6	14.3
4	Plate Infected	148	166.6	174.3	113	166.3	169.3	17
5	373	562	585	559.3	511.6	552.6	537.3	373
6	379.3	534	630.3	612.6	538	505.3	565.3	447.3
7	357.6	246.3	Plate Infected	Plate Infected	505	501.3	575	364.3
8	158	571.3	Plate Infected	Plate Infected	531	499	560	409.6
9	190.3	241.3	246.6	218.6	127.6	231.3	185	62.3
10	190.6	220.3	225	194.6	146.3	231.6	183.6	53.3
11	176.6	269.6	274.3	175.3	148.3	217	181.3	63.6
12	Plate Infected	202.3	325.6	209.6	143	217.6	Plate Infected	33
MEDIAN	333	230.8	235.8	185.0	144.7	231.3	185	57.8

Non-parametric one way analysis of variance(Kruskal Wallis) showed no significant difference between any of the groups

Results - Figure 4

HT29 & Omeprazole
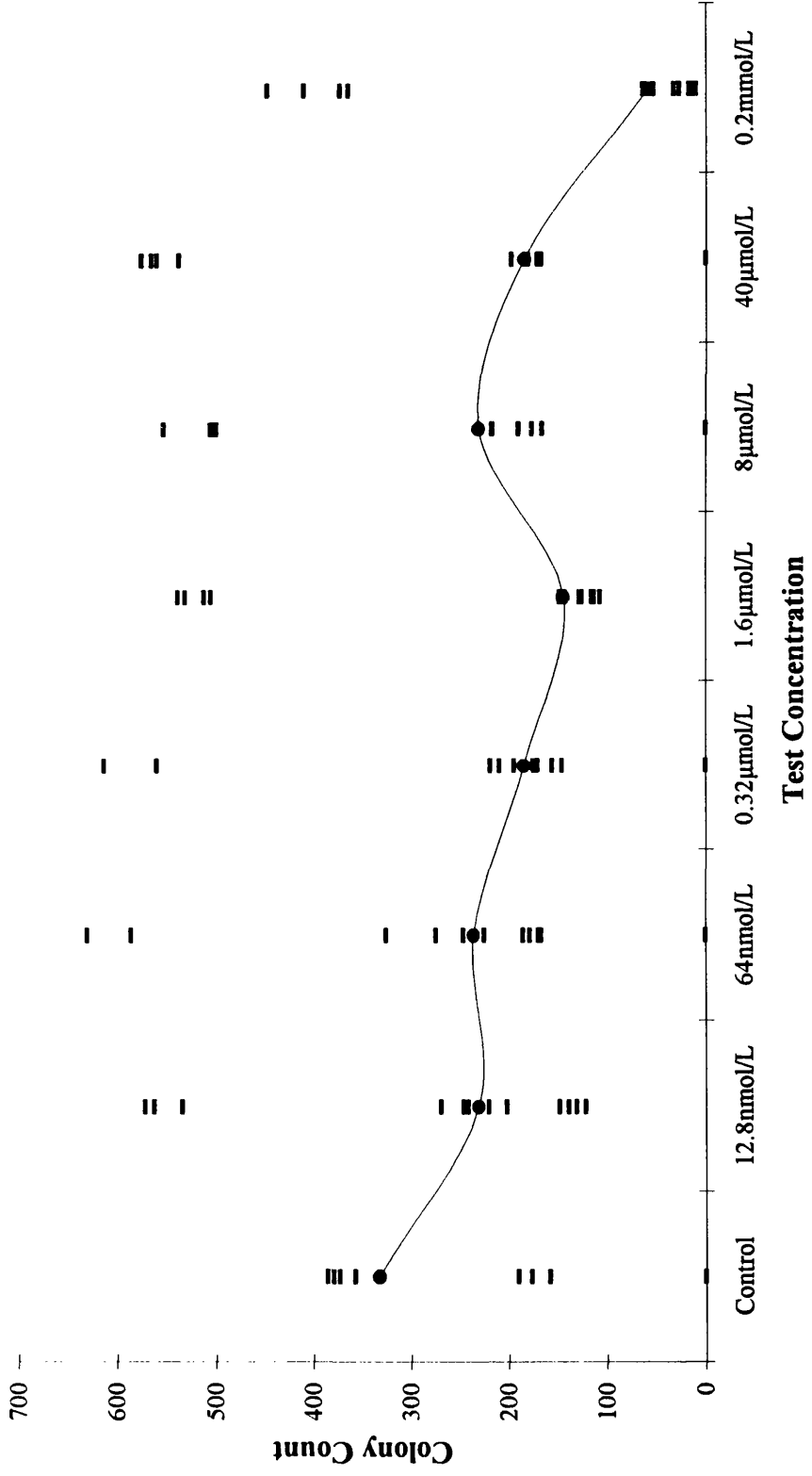
Non-parametric one way analysis of variance(Kruskal Wallis) showed no significant difference between any of the groups

Results - Table 6

Assay to assess effect of drug vehicle, Dimethyl Sulphoxide(DMSO) - Cell line B.E.

Test Concentration

	Control	12.8nmol/L	64nmol/L	0.32µmol/L	1.6µmol/L	8µmol/L	40µmol/L	0.2mmol/L
1	120.3	63.6	103.6	106	Plate Infected	146.6	134	21
2	101	84.6	95	118	116.6	148.6	141.3	17.3
3	103.6	109.6	103.3	112.3	126.3	127.3	131	18.3
4	118	80.6	84.6	97.6	134	139.6	134	24.3
Plate 5	181.3	234.3	169.3	209.3	194.3	203	220.3	120.6
6	208.3	214.6	179	207	206	215.6	210.3	130
7	195.6	209.6	169	211.3	206	201.6	239.6	Plate Infected
8	197.6	192.6	195	205.3	204.3	211.3	217	Plate Infected
MEDIAN		151.1	136.3	161.7	194.3	175.1	175.8	*22.7

Corresponding Concentration of Drug Vehicle

	Control	12.8nmol/L	64nmol/L	0.32µmol/L	1.6µmol/L	8µmol/L	40µmol/L	0.2mmol/L
1	183.6	188.6	185.6	178	162.3	196	218.6	97.4
2	189.3	185.6	182	184.6	158	222	186.3	114.6
Plate 3	203.3	206.3	161	187.6	173.3	179	196.6	91.7
4	187.6	Plate Infected	171	180.6	158.6	215.6	Plate Infected	Plate Infected
MEDIAN	185.6	188.6	176.5	182.6	160.5	205.8	196.6	*97.4

*P<0.05 compared to Control

153

Results - Figure 5

Assay to assess effect of drug vehicle, Dimethyl Sulphoxide (DMSO)

– MAX
● MEDIAN
– MIN

Test Concentration/Equivalent Vehicle Concentration
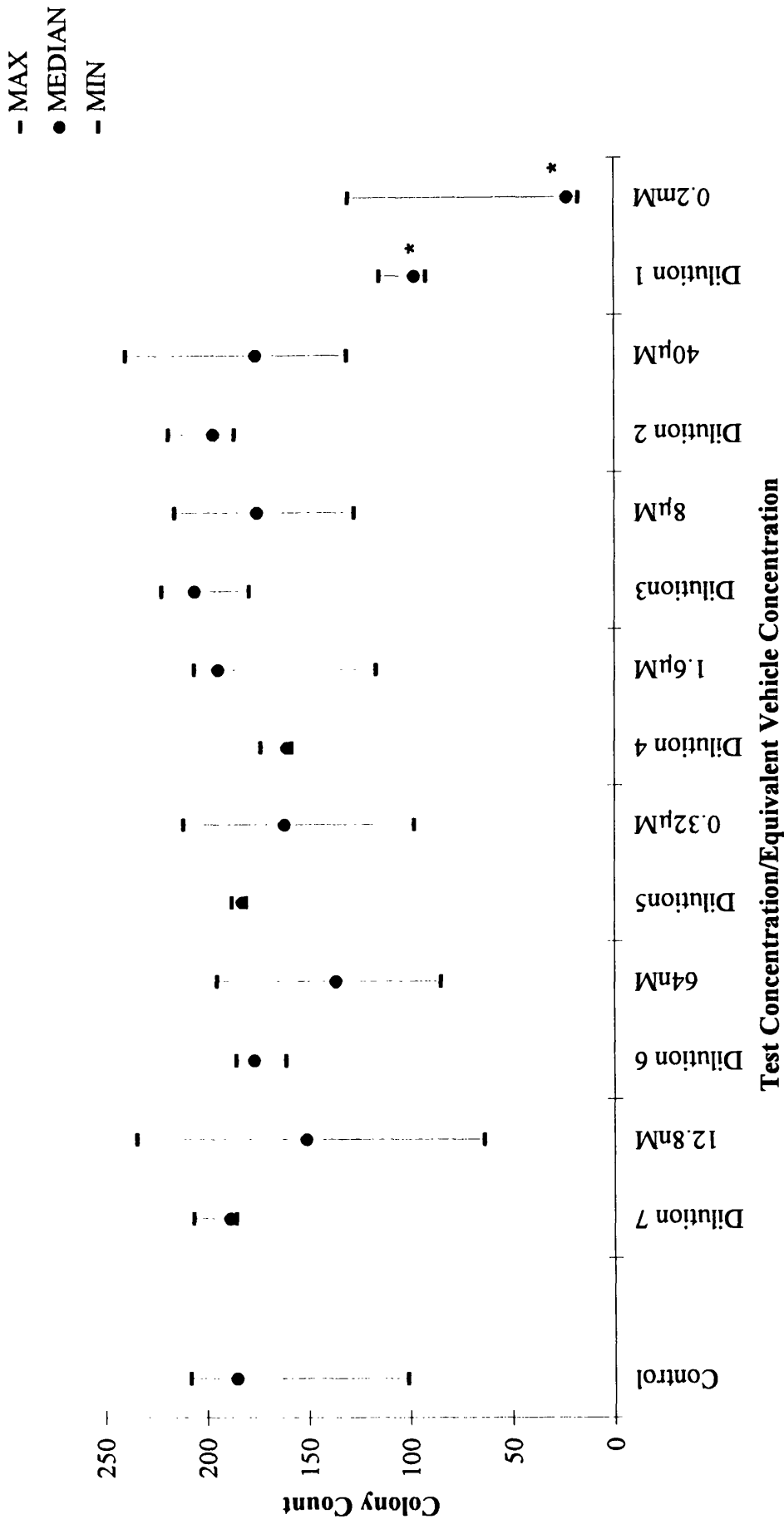
*p<0.05 compared to Control Count

As before, at all lesser concentrations of both drug and vehicle, no significant differences existed compared to Control counts (Mann Whitney, P = 0.06 to 0.91) and like omeprazole 0.2mmol/L and its corresponding "vehicle only" concentration, no significant differences in colony counts existed between drug exposed/vehicle only exposed cells at all the other test concentrations (Mann Whitney, P = 0.21 to 1.00).

2.5. Cell line HT29 and Pentagastrin (Table 7 & Figure 6)

Although the median colony counts of pentagastrin treated cells at all concentrations were modestly elevated in comparison to control, this was not statistically significant (Kruskal Wallis, P = 0.55). Nor was there any significant trend with drug concentration (P = 0.6).

Results - Table 7

Cell Line HT29 - Clonogenic Assay with Pentagastrin

Test Concentration

	Control	15.4pmol/L	76.8pmol/L	0.38nmol/L	1.92nmol/L	9.6nmol/L	48nmol/L	0.24µmol/L	1.2µmol/L	6µmol/L
1	228.3	131.6	121.3	155.6	615	104	476.6	149.6	472	230
2	311	118	123	265.3	560.7	82.3	502.6	153	498.3	236
3	375.3	203.6	168	170.3	582	131.6	575	Plate Infected	489.6	221.6
4	270.3	189.6	188.3	303.3	584.3	169.6	Plate Infected	Plate Infected	507.6	Plate Infected
*Plate 5	55.3	574.3	399	472.3	111	534	158	555.3	257.6	560.6
6	138.6	557.3	369	526.6	123.3	516.3	194	545.6	246.3	421.6
7	87.6	574	384.3	532.6	152	503.6	219.6	503.3	234.3	391
8	105.6	616.6	385.3	505	143	546.6	122	528	229.6	444.3
MEDIAN	**183.5**	**380.5**	**278.7**	**387.8**	**356.4**	**336.6**	**219.6**	**515.7**	**364.8**	**391**

Non-parametric one way analysis of variance(Kruskal Wallis)showed no significant difference between any of the groups

Results - Figure 6

HT29 & Pentagastrin
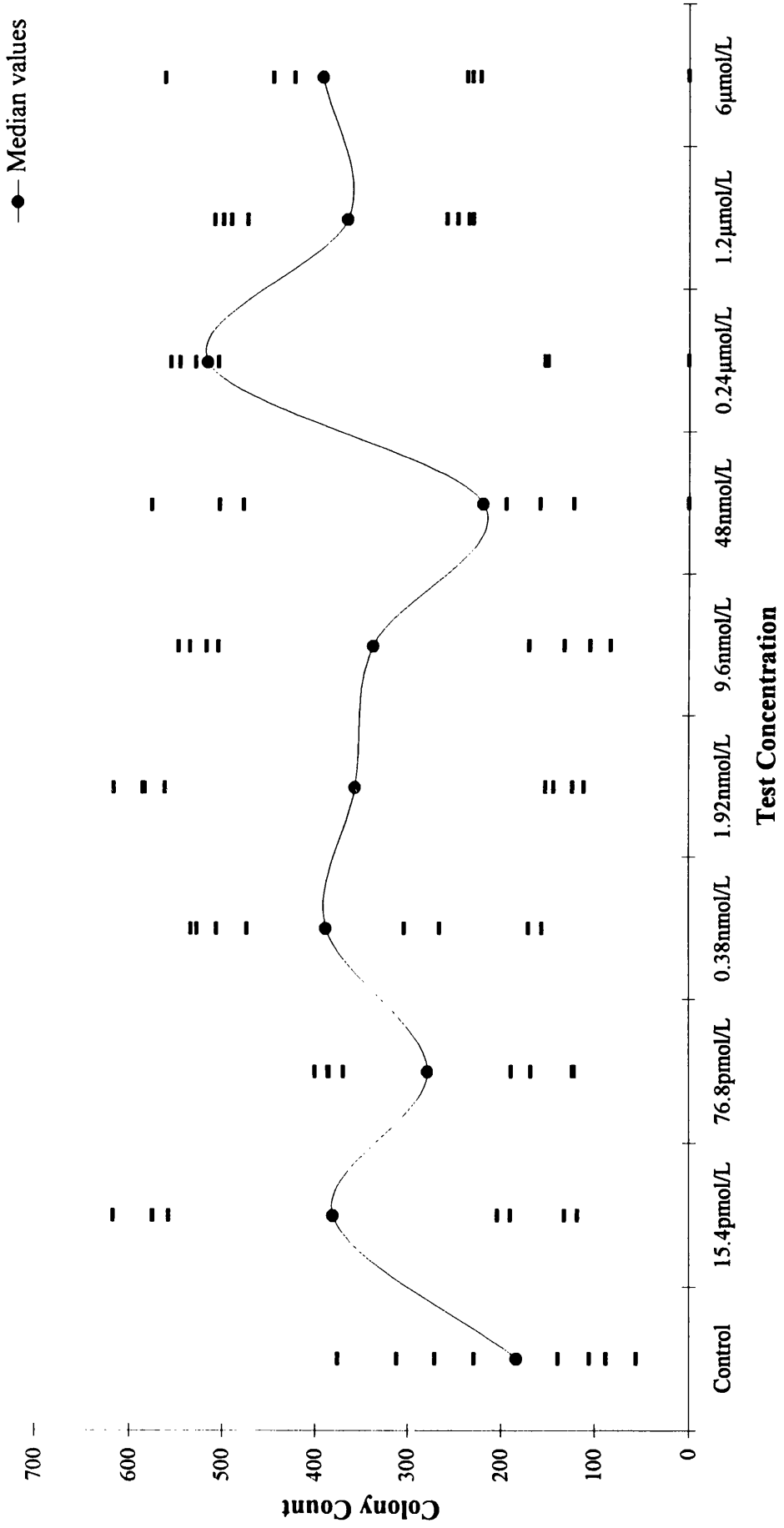
Non-parametric one way analysis of variance (Kruskal Wallis) showed no significant difference between any of the groups

3. In vivo Study - Effect of Omeprazole treatment (and the resultant hypergastrinaemia) on the Initiation and Promotion Phases of chemically induced Colorectal Carcinogenesis in Rats.

3.1. Endogenous Gastrin Response to Treatment (Figure 7A - C and Appendix 1)

As described in the previous chapter, each rat had blood taken for measurement of endogenous circulating gastrin levels at 3 time points in the study. The first of these were taken in the week before any dosing with omeprazole, inert drug vehicle or azoxymethane began (week 0) and represent "normal" baseline values. The next set of samples were taken at week 6, the mid-point of the first phase of the study, at which time group A were being dosed daily with omeprazole and groups B and C were receiving inert drug vehicle. Finally, blood samples were collected in the last week of the study, when group B were receiving omeprazole and groups A and C, the drug vehicle. This was to confirm that omeprazole treatment caused the expected elevation of the hormone. This was indeed the case, as can be seen from inspection of Figure 6A-C. Omeprazole treatment caused significant hypergastrinaemia compared to baseline values and those in the placebo phases of the study in both groups A and B (P < 0.0001 for all tests, Student's paired T-test).

With one exception, baseline gastrin values and those measured during administration of placebo drug vehicle were very similar (Students paired T-test: Group B, P = 0.12; Group C, P = 0.61 and 0.94 for phase 1 and phase 2 of the study respectively). However, this was not the case in group A where baseline measurements were significantly increased over vehicle values (P < 0.0001). Inspection of the plotted values also indicates the group at this stage to have significantly elevated baseline levels over those for groups B and C (P < 0.0001 for both, Students unpaired T-test). This was unexpected but probably represents random, food-stimulated gastrin levels due to these animals inadvertently not

Results - Figure 7a-c
Plasma Gastrin Variations

a) Group A

Omeprazole v Baseline, P<0.001 ; Omeprazole v Vehicle, P<0.001 ; Baseline v Vehicle, P<0.001* *see text

b) Group B

Omeprazole v Baseline,P<0.001 ; Omeprazole v Vehicle, P<0.001 ; Baseline v Vehicle, P=0.12

c) Group C (Control)

Baseline v Vehicle - phase 1, P=0.61 ;Baseline v Vehicle - phase 2, P=0.94 ; Vehicle 1 v Vehicle 2, p=0.80
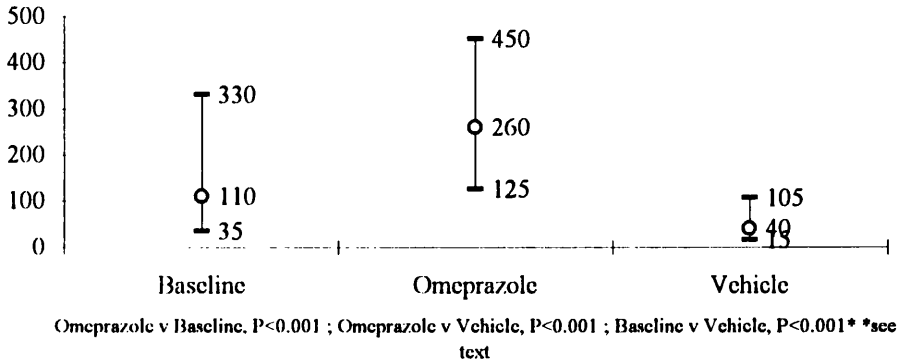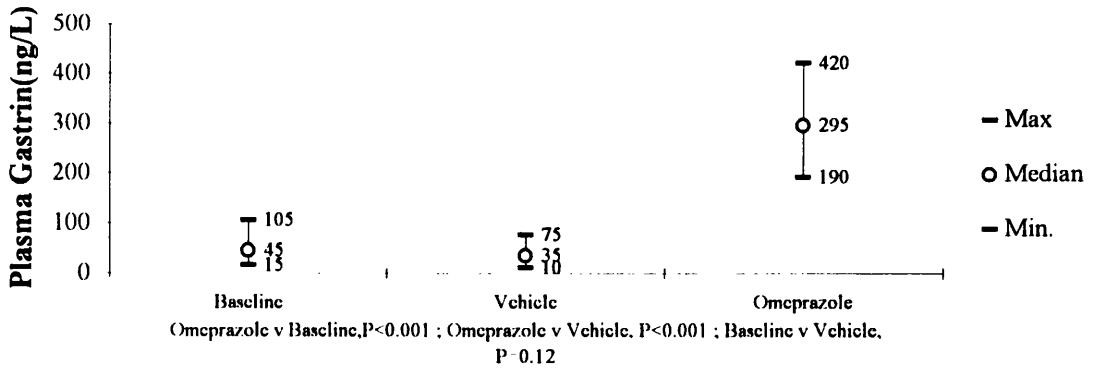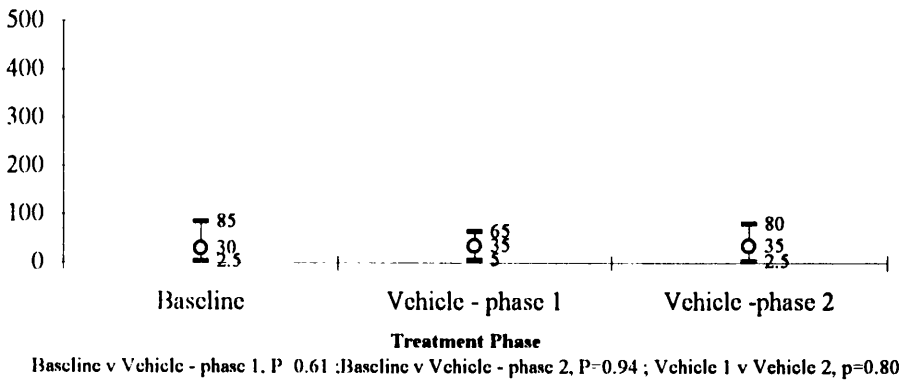
Statistical Analysis by Student's paired T-test

being fasted for the 2 to 4 hours prior to blood sampling, as the study design protocol dictated. They still had significantly lower gastrin levels compared to those in the active treatment arm and as such, it is believed that this observation will not significantly affect the study end-point.

3.2. Disease incidence, tumour yield, type and stage, distribution and volume & animal growth rates

Each of the above is discussed individually below but the results are summarised in Table 8. A complete summary of these measured outcomes for each individual animal subject is presented in Appendices 2 to 4. The weekly weights, final weights and growth rates for each animal are tabulated in Appendices 5 to 7.

3.2.1. Disease Incidence

Disease incidence in each of the three groups was very similar. Of the 25 Group A animals, who received omeprazole in the initiation phase of carcinogenesis, 16 had macroscopic tumours at the end of the study. In Group B, where omeprazole was administered during the promotion phase of carcinogenesis, 18 of 25 animals developed disease. Finally, in the control group C, 17 of 25 animals were found to have tumours. As can be predicted from these values, no significant difference for disease incidence existed between any of the 3 groups (Group A versus Group B, $P = 0.38$; A versus C, $P = 0.50$; B versus C, $P = 0.50$; Fisher's exact test).

3.2.2. Total tumour yield

In Group A, a total of 18 lesions developed in the 16 affected animals. For Group B, the total was slightly higher with 24 tumours in the 18 rats to have developed disease.

Finally, the greatest yield of tumours was evident in Group C, with 27 developing in the 17 affected rats. However, these differences did not reach statistical significance.(Group A versus Group B, P = 0.63; A versus C, P = 0.68; B versus C, P = 0.85; Mann Whitney U test)

Indeed, the slightly greater number of tumours in Groups B and C are largely accounted for by a minority of rats found to have multiple lesions (2 animals in group B had 3 tumours, as did 2 in group C, along with 1 rat with 4 lesions) but the median number of tumours per rat in each group was 1.

3.2.3. Tumour type and stage

Carcinomas were the commonest lesion in all 3 treatment groups, and the relative proportions of carcinomas to adenomas in each group were similar and did not differ significantly (Group A versus Group B, P = 0.28; A versus C, P = 0.22; B versus C, P = 0.58; Fisher's exact test).

The stage of carcinomas was similar in all three groups. Of the 17 carcinomas in group A, 15 were Dukes A and 2 Dukes B+. In group B, there were 15 Dukes A and 5 Dukes B+ tumours and finally, in group C, 19 Dukes A and 3 Dukes B+. These ratios were not significantly different when compared by Fisher's exact test (Group A versus Group B, P = 0.28; A versus C, P = 0.63; B versus C, P = 0.29). It should be reiterated that as grossly normal mesocolon and its contained lymph nodes was not routinely submitted for histology, it is possible that some of the tumours labelled Dukes B are in fact Dukes C by virtue of microscopic metastases in the draining nodes, hence the reason Dukes B lesions have therefore been classified as Dukes B+, as seen in Table 8. Only one animal, in group A, was noted to have a single visibly and palpably enlarged mesocolic lymph node at post mortem, which was retained and examined, microscopy confirming it as containing

metastatic adenocarcinoma, thus confirming the bowel lesion as a Dukes C stage tumour.

There was no evidence of more distant disease, for instance, liver metastases or peritoneal

deposits in any of the animal subjects.

3.2.4. Tumour distribution in the colorectum (Figure 8A & 8B)

All three groups had a similar distribution of lesions within the colorectum (Group A

versus Group B, P = 0.62; A versus C, P = 0.85; B versus C, P = 0.59, Mann Whitney U

test), with the majority of tumours being found in the mid-portion of the bowel,

approximately halfway between the caecum and anus.

Figure 8A - Tumour Distribution in the Colorectum

Group A ●
Group B ⬚
Group C O
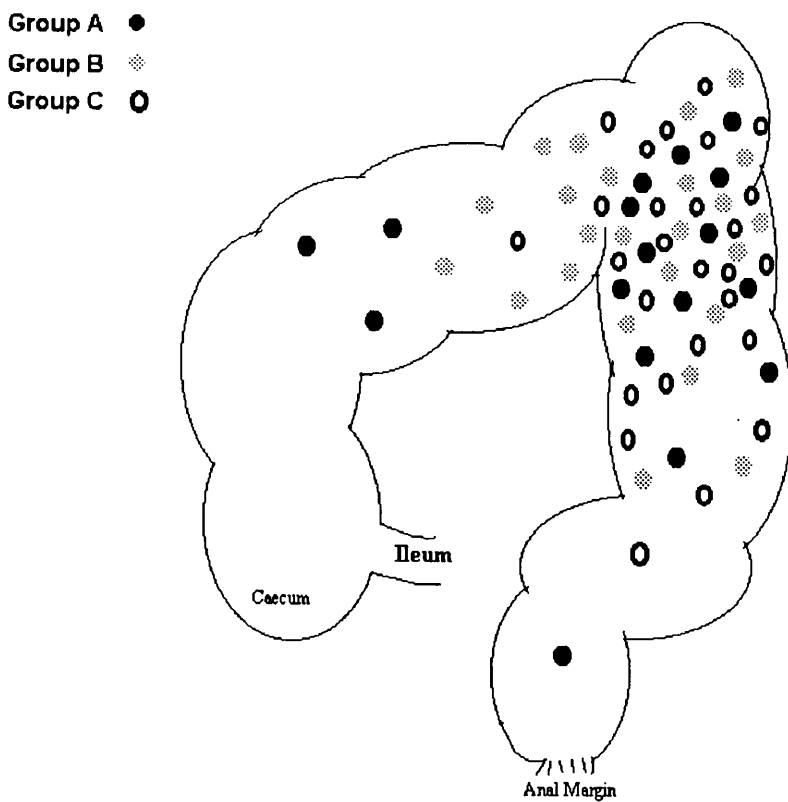
Results - Figure 8b

Tumour Distribution (expressed as % of total colon length)

Distance from Anal Verge (as % of total colon length)
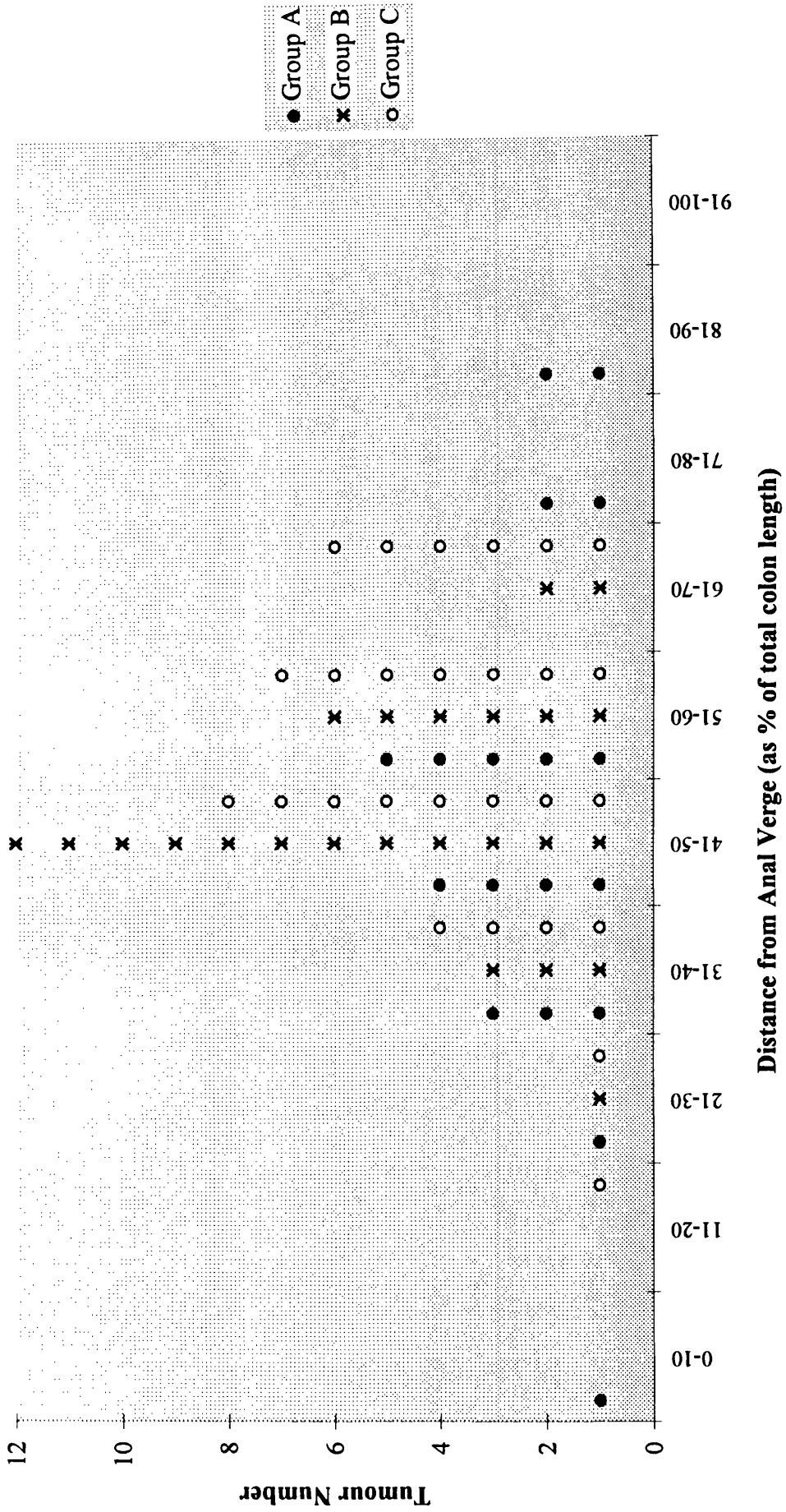
No statistical differences in distribution between any of the groups (Mann Whitney U test)

Results - Table 8
In vivo experiment results

		Group A	Group B	Group C
Disease Incidence (%)		16 (64)	18 (72)	17 (68)
Total Tumour Yield		18	24	27
N° Tumours per rat	*Mean*	0.72	0.96	1.08
	Median (Range))	1(0 - 2)	1(0 - 3)	1(0 - 4)
Adenomas		1 (6%)	4 (17%)	5 (19%)
Carcinomas		17 (94%)	20 (83%)	22 (81%)
	Dukes A	15	15	19
	Dukes B+	2	5	3
Tumour Volume(mm^3)	*Mean*	159	148	90
	Median(range)	95(2-864)	116(18-648)	65(2-288)
Tumour distribution*	*Mean*	50.5	46.6	48.8
	Median(range)	49.5(8-83)	46(24-61)	50(18-67)
Animals	*Mean Growth Rate (grams/week)*	3.06	3.2	3.38
	Mean Final Weight (grams)	297.52	291.92	301.44

No statistically significant differences exist between the groups for any of the recorded parameters.

*distance from anal verge expressed as % of total colon length.

3.2.5. Tumour volume

Both Group A and Group B animals had slightly larger lesions than their Group C counterparts, mean values being 159 mm^3, 148 mm^3 and 90mm^3 respectively, but these were not significantly different (Group A versus Group B, P = 0.80; A versus C, P = 0.61; B versus C, P = 0.25, Mann Whitney U test).

3.2.6. Animal Growth Rate (Appendices 5 to 7)

The animals in all 3 treatment groups gained weight at a similar rate of approximately 3 grams per week and ultimately had similar weights at the end of the study, with no significant variation in growth rate (Group A versus Group B, P = 0.57; A versus C, P = 0.17; B versus C, P = 0.41, Student's unpaired T-test) or final weight (Group A versus Group B, P = 0.42; A versus C, P = 0.58; B versus C, P = 0.16, Student's unpaired T-test).

Chapter 4 - Discussion and Conclusions

1. Cancer Incidence after peptic ulcer surgery

1.1 Summary of Findings

1.1.1. Overall cancer risk in the cohort

In the study of patients treated surgically for peptic ulcer some 26 years previously, there was no overall increased cancer risk conferred upon the cohort as a result of the operation. It must be remembered that patients did undergo a variety of operative procedures, some of which will result in markedly different ensuing pathophysiological changes to the patient, as discussed in Chapter 1. Therefore, each category of operation was analysed seperately. This sub-divided the study population into a large group of patients, almost 80% of the total cohort, who had underwent vagotomy, almost always accompanied by a drainage procedure, either gastroenterostomy or pyloroplasty, as their one and only definitive anti-ulcer procedure. The next largest group of patients, accounting for 15% of the study population, had previously undergone partial gastrectomy as their definitive anti-ulcer procedure. Thereafter, a number of small select groups accounted for the remainder. In none of the operative groups, vagotomy or otherwise, was their any propensity for cancer development as a whole, in the years after surgery.

1.1.2. Incidence of site-specific cancers in the whole cohort

When site-specific cancer incidence rates were studied, the incidence of laryngeal cancer was found to be slightly more than twice as common as one would expect in the general

population over a similar period of time, a statistically significant finding. No other form of cancer was found to be significantly raised over expected rates, though bronchial carcinoma and gastric cancer were slightly commoner than expected, both falling just short of statistical significance. No other cancers, and in particular colorectal cancer, were any more frequent than expected.

1.1.3. Site-specific cancer incidence after specific forms of gastric surgery

Analysis of individual cancer incidence rates within each operative category gives a more concise measure of any risk attendant with a specific operation type. Indeed, within the main group of vagotomised subjects, a statistically significant excess of laryngeal, bronchial and gastric neoplasms was apparent within the follow-up period. As observed in the study population as a whole, colorectal cancer was no commoner than expected in the vagotomy patients. Similar analyses in the gastrectomy group and all other operative groups, showed no increase in risk for this or any other form of cancer.

Clearly, the findings of this study reflect mainly on the operation of vagotomy. However, it does mean that caution should be exercised in interpreting the findings for the other groups, such as gastrectomy (301 patients) in whom the number of subjects is small, and as such, any conclusions to be drawn are less valid. This, however does not apply to the vagotomised patients, who totalled 1,556 in number and who had a substantial period of follow-up. Potential explanations for the significant findings in this group must be sought.

1.2. Bronchial and Laryngeal Cancer

Carcinoma of the bronchus has been found to be excessive in a large proportion of previous studies of a similar nature to the present one (165,166,168,170,172,174,191,193). All have concluded that this has little to do with any

consequence of the surgery per se, but reflects the excessive use of tobacco by the subjects under scrutiny. It is difficult to argue with this conclusion, when one appreciates the tobacco consumption by the present study population. As illustrated in Figure 1, 77% of the cohort were, at the time of surgery, habitual tobacco consumers, mostly in the form of cigarettes, now accepted as one of the major risk factor for the disease (192). Clearly, one accepts that such information has been collected at only one time point in the life of each patient, and that some patients may have reduced their risk by discontinuing the habit at a later stage, but the findings of a recent study would suggest that this is likely to be a small minority (338). Ekbom and colleagues (338) demonstrated that although the incidence of lung cancer was significantly higher in peptic ulcer patients as a whole, the risk for the disease was significantly higher still, in those who had previously undergone surgery for their ulcer diathesis, which in the case of that particular study, was vagotomy. Whilst not rejecting outright the possible influences of physiological and dietary sequelae as a result of the operative procedure itself leading to this outcome, they hypothesised that the findings reflected the continuation of smoking in the operated subjects, as a result of decreased anti-smoking counselling in the years after surgical "cure" of their ulcer, as opposed to those unoperated, and presumably, symptomatic, patients who were in more frequent contact with doctors and therefore more likely to have been given repeated anti-smoking advice. Thus, the information in Figure 1 is felt to be a fair reflection of the smoking habits of the cohort throughout the follow-up period of the study. The excess of laryngeal cancers, also strongly associated with smoking (192), is also undoubtedly due to the high prevalence of tobacco use in the group under study.

Discussion - Figure 1

Peptic Ulcer Clinic Study Cohort - Smoking Status
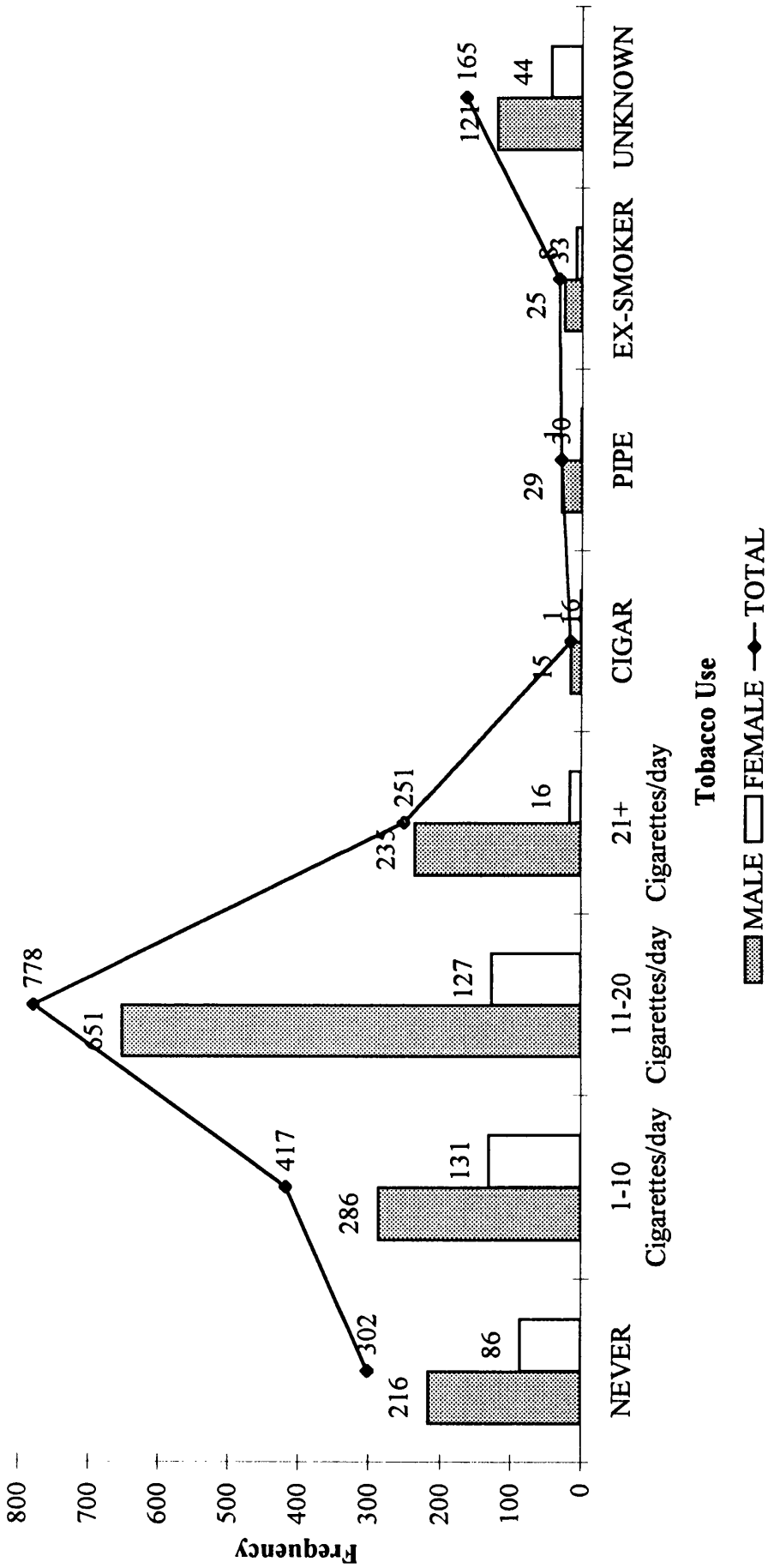
1.3. Gastric Carcinoma

Smoking has also become increasingly recognised as an independent risk factor for gastric

cancer (339,340) but the association is not as strong as that for lung or laryngeal cancer

(192,341). There was a 50% increase over expected values for gastric cancer in

vagotomised patients in this study. This is in concordance with the findings of many of

the previous studies reviewed in Chapter 1, and although the majority of these have dealt

with gastrectomised patients (175,176,178,179,182,184-189), a similar outcome has also

been reported in the smaller numbers of vagotomised subjects studied to date

(166,170,185).

The fact that this group of vagotomy patients would appear to suffer the same outcome as

their gastrectomised counterparts, suggests that some factor common to both forms of

surgery is the underlying mechanism to the appearance of the disease in the post-operative

stomach. This factor may well prove to be the prolonged hypochlorhydria which ensues

after both vagotomy and partial gastrectomy. What the study was not designed to answer

is what sequelae of such acid inhibition are responsible for malignant change in the

stomach, such as mucosal dysplasia, bacterial colonisation and formation of intraluminal

carcinogens, all of which have been discussed earlier.

Although one may assume that the majority of the vagotomy patients will have had

chronic, modest elevation of circulating gastrin levels, the fact that a similar risk for the

development of gastric cancer has already been shown in gastrectomy patients, whose

gastrin levels are either unchanged or reduced, would tend to exclude the hormone as

being of major importance in the development of the disease.

One unknown variable is what part, if any, is played by Helicobacter pylori in the

development of cancer in the post-operative stomach. The latter organism is now

recognised as a risk factor for the development of gastric cancer, at least in the intact

stomach (222). Bearing in mind that the vast majority of the patients treated by vagotomy in the present study suffered from duodenal ulcer, of which approximately 95% of cases are known to be helicobacter pylori positive (42), then it is not unreasonable to assume that a large proportion of these patients will have been carriers of the organism. The exact number will remain unknown, as will the effects of the surgery on the post-operative helicobacter status of the individual, but hypochlorhydria per se is not a hostile environment for the organism, and one could also assume that in the years following their surgery, such individuals will continue to carry the organism in such an hypoacidic milieu. Indeed, a recent study showed that 83% of patients previously vagotomised for peptic ulcer were still carriers of the organism, when tested a mean of 10 years later (342). Persistence of the organism after partial gastrectomy was found to be lower at around 50% (342). This observation, in concert with the other factors cited above, may combine to produce an increased potential for malignant change in the gastric mucosa post-operatively

1.4. Colorectal Cancer

The colorectal cancer risk after peptic ulcer surgery remains a matter of debate. A select number of studies showed there to be an increased incidence of the disease following both vagotomy (166,171) and gastrectomy (165,168,172). The former, by virtue of increasing circulating gastrin levels, may confer a substantial risk for the disease, given the sizeable body of evidence, which shows gastrin to be a trophic factor for such neoplasms (see Chapter 1). Based on the findings of the present study, however, this hypothesis cannot be supported as no excess cases of the disease occured in the substantial period of follow-up after vagotomy. This is also the case for all other select operative sub-groups studied,

including gastrectomy, though as already mentioned, in the context of the present study, conclusions for these numerically small patient groups are less valid.

1.5. Summary

In summary, the group of patients studied showed no overall excess risk for development of neoplasia in the years after surgery for peptic ulcer. Only laryngeal cancer was significantly more frequent in the study cohort. Small but insignificant increases in gastric and bronchial cancer were also evident. When formal analysis by different operation type was performed, this showed a significant excess of cancer at these three sites in the largest group under study, namely vagotomy and drainage, but still no increased risk for cancer as a whole. Colorectal cancer was not any more frequent than expected in the general population, either after vagotomy or any other type of operation.

2. In vitro and in vivo studies

The question of whether elevation of gastrin levels may lead to increased colorectal tumour development, was one of the reasons central to the study of a group of predominantly vagotomised individuals, the findings of which have been discussed above. This was also the stimulus to the experimental work of Penman and colleagues (311), who questioned whether elevation of endogenous gastrin levels secondary to administration of the potent acid inhibitory agent, omeprazole, would lead to an increased yield of carcinogen-induced colorectal tumours in rats so treated. Their findings were completely unexpected, in that the drug appeared to exert an inhibitory effect on tumour development despite significant endogenous hypergastrinaemia occuring with omeprazole treatment. The aims of the studies performed for this thesis were to try and identify at which stage in the carcinogenesis process this unexplained action of omeprazole had occurred, and hopefully give some idea as to how the drug had acted. Only one other study had suggested that omeprazole may have such an anti-neoplastic action on colonic tumour cells and this was from Tobi (312), who found that in one of 3 cell lines of colonic tumour origin treated by omeprazole in vitro, there was a significant inhibition of tumour cell growth. Although not postulated as a possible mode of such an action for the drug by Penman and his colleagues (311), the results of this in vitro study suggested that some form of direct action of the drug on the tumour cell might account for their findings. To this end, the clonogenic assays were performed. The three cell lines, all derived from classical human colonic adenocarcinomas, were exposed to a wide range of concentrations of omeprazole in cell culture, spanning, and exceeding, the normal pharmacological concentrations seen in humans during both normal and supra-normal dosing with the drug (313.329).

2.1. Effect of omeprazole on survival of colorectal cancer cells in vitro

For all 3 cell lines, omeprazole did not exert any effect, inhibitory or otherwise, on the growth or survival of the tumour cells. Although it initially appeared that treatment with the highest test concentration, 0.2mmol/L, significantly reduced the numbers of surviving cells, this was probably due to a direct toxic action of the organic solvent, DMSO, in which omeprazole was initially dissolved. At this concentration of omeprazole, DMSO was present in the cell culture medium at a concentration of 1 in 50, and when cells were treated with DMSO at this concentration, in the absence of omeprazole, similar reductions in colony count to those obtained with omeprazole 0.2mmol/L were obtained (see Results - Table 6 and Figure 5). All lesser concentrations of DMSO were shown not to significantly affect cell growth compared to those in DMSO-free medium. Even if the reduced cell survival seen after exposure to omeprazole 0.2mmol/L were due to a specific action of the drug, it should be appreciated that this concentration is approximately twenty times greater than recorded maximal plasma concentrations after a supra-therapeutic 90mg oral dose in humans (313). Furthermore, it is over 100 times greater than that after a standard 20mg oral dose, and close to maximal levels found in mice during carcinogenicity studies (313). Any potential clinical use of the drug at this level would probably not be feasible. It therefore seems unlikely that the reduction in tumour yield seen in Penman's original experiment was consequent upon any direct action of omeprazole on tumour cells. In addition, the only cell line of the 3 employed in Tobi's study to exhibit an inhibitory response was a tumour with carcinoid features and as such, one should exercise caution in inferring a similar response by cells from a classical adenocarcinoma of colorectal mucosa, a disease with markedly differing clinical and prognostic parameters from carcinoid tumours. This point has already been emphasised by Hurwitz and colleagues (342).

2.2. Effect of pentagastrin on human colonic tumour growth in vitro

Using an identical assay technique to that above, the opportunity to re-examine the effect of pentagastrin on colonic tumour cell growth was taken, as several previous workers have confirmed the trophic action of both natural and synthetic gastrin on human colonic tumour growth in vitro (247-250). Whilst a modest increase in colony numbers can be observed at all concentrations of pentagastrin compared to control values (see Results - Figure 6), the differences were not significant. This is similar to the findings of Sirinek and colleagues (247) who observed a 29% increase in HT29 cell numbers to pentagastrin treatment compared to controls, which was not significant. They postulated that this might be the result of acquired changes in this maintained cell line over time, rendering it less responsive to hormonal stimulation, as the other four freshly prepared colonic carcinoma cell cultures utilised in their study, did show a significant growth response to pentagastrin, with a mean increase in cell numbers of 59% compared to control cells. Palmer Smith (249) also used cell line HT29 and found it to respond significantly to gastrin-17 at a dose of 400 picomoles per litre, though the mean increase in cell count is similar to that of Sirinek's, at 28%. There are marked differences in the design, methodology and statistical analysis of the results between the studies, and these are likely to account for the apparent inconsistency in the statistical significance of the similar growth responses seen.

2.3. In vivo study

The in-vivo investigation failed to show any significant differences in either tumour incidence, number, size or stage in omeprazole treated rats compared to untreated controls, either during the initiation or promotion stages of carcinogenesis.

Hypothetical explanations for the reduced colorectal tumour yield seen in Penman's study included interference with carcinogen metabolism by omeprazole, as previously discussed. Were omeprazole to have such an action on carcinogen metabolism, one would not expect to see any effect on tumour development if the drug is not administered concurrently with carcinogen. However, in the present experiment (as in Penman's study), rats in Group A received omeprazole concurrently with azoxymethane, and therefore if some significant action of omeprazole on carcinogen metabolism exists, one would expect to see a reduction in tumour development in this group. However, disease incidence in Group A was virtually identical to that of both the control group C (16/25 versus 17/25 respectively, P = 0.5) and the "promotion" group B (16/25 versus 18/25, P = 0.4). Although the tumour yield was higher in control group C than either of the omeprazole treated groups, this did not reach significance and was largely accounted for by one animal in Group C which was found to have a total of 4 separate neoplasms (3 carcinomas & 1 adenoma). Indeed, the median number of tumours per animal in all three groups was identical. One possible confounding variable which may have caused the reduced tumour incidence in omeprazole-treated rats in Penman's study, cited both by the authors and others (311,342), was the possible anti-tumorigenic effect that is observed in experimental rodents with reduced caloric intake and growth rate (343,344), which was evident in omeprazole treated rats in that study, which were on average 12% lighter than the control group by the end of the study (P<0.005) (311). In the present investigation, the average weekly growth rate and final animal weights in all three treatment groups were very

similar and this could possibly explain the discrepancy in outcome between the two studies. However, in the present study, there was no appreciable relationship between tumour development and either of these parameters (Figure 2A and B), as assessed by Spearman's rank correlation co-efficient (rs = 0.097, P = 0.59 and rs = 0.13, P = 0.26 respectively) to suggest that this would account for any such effect on the results.

Results for animals in group B, treated with omeprazole in the promotion phase of carcinogenesis, were also similar to controls. This was not particularly unexpected as there is little existing evidence to suggest that omeprazole exerts any significant effect on colorectal tumours once established and is in accordance with findings of those studies (albeit not specifically designed to assess any specific action of omeprazole per se on tumour growth) where omeprazole treatment has commenced after the phase of tumour initiation has been completed (284,285).

These latter two studies also concluded that omeprazole-induced hypergastrinaemia did not enhance colonic tumour growth (284,285). Our results concur in this respect. Both Group A and Group B animals, which were rendered significantly hypergastrinaemic whilst receiving omeprazole (Results - Figure 2A & B), both developed similarly sized neoplasms that were not significantly larger than those in control rats (Group A v C, P = 0.60; B v C, P = 0.25; A v B, P = 0.80, Mann Whitney U).

The suggestion that drugs such as omeprazole, widely used in upper gastrointestinal acid related disorders with excellent tolerability and low incidence of adverse effects, could have a place in the management of such a common and often fatal disease as colorectal neoplasia is an exciting one and the potential for use is wide. For instance, would high risk groups such as members of hereditary cancer families, patients with previously excised benign colonic polyps and long term ulcerative colitis sufferers benefit from prophylactic treatment? Could it have a place as an adjuvant therapy in patients following

Discussion - Figure 2

a) Relationship between Growth Rate and Tumour Development
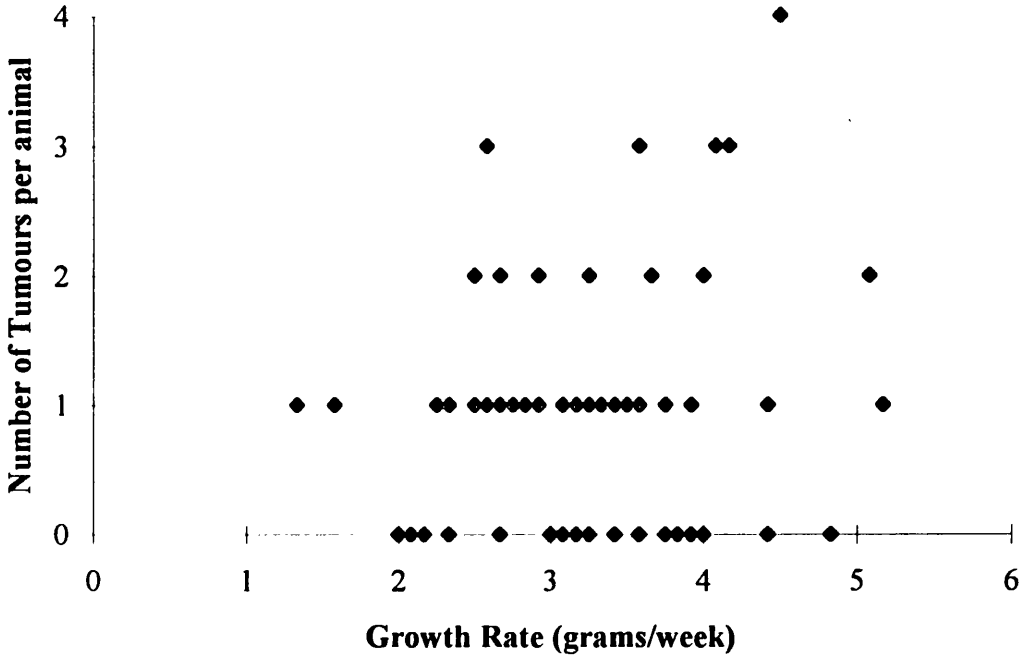
b) Relationship between Final Animal Weight and Tumour Development
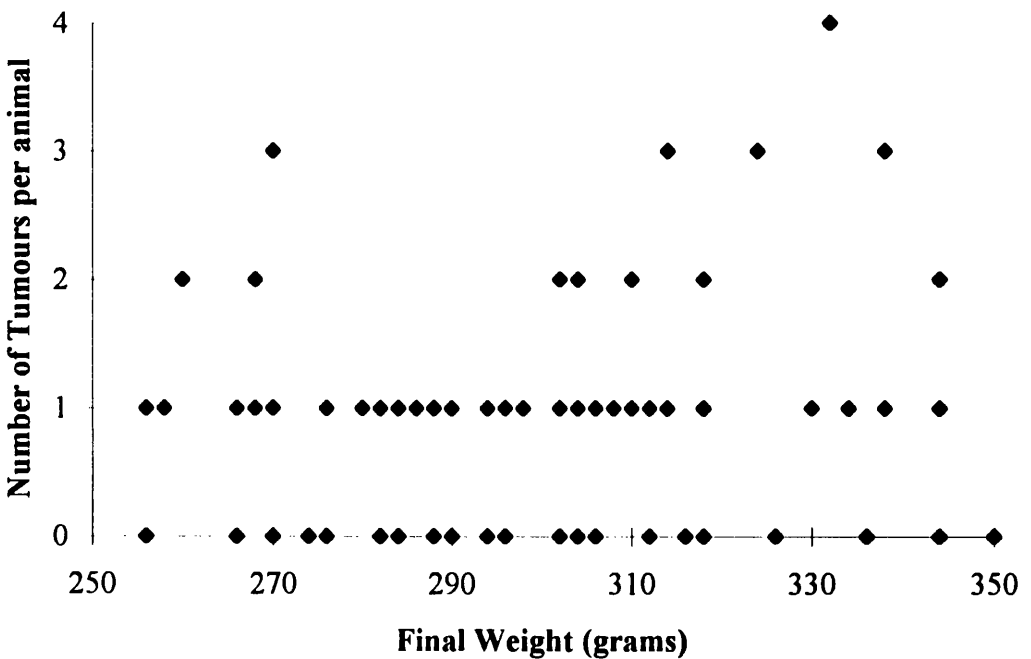
Spearman's Rank Correlation Co-efficient r_s :

a) $r_s = 0.13$, $P = 0.26$ b) $r_s = 0.097$, $P = 0.59$

tumour excision? Thinking such as this has been controversially described as "loose and heretical" by Rehfeld in a recent editorial on the subject (344), but the evidence, though sparse and confined to laboratory based studies, was certainly worthy of further investigation, as Rehfeld later agreed (345). However, the results of these present studies do not support those potentially exciting earlier findings.

It is also noteworthy that omeprazole-induced hypergastrinaemia did not significantly affect tumour number, growth or aggressiveness compared to normogastrinaemic controls. With the results of the previous similar studies of Graffner (284), Pinson (285), and even those of Penman (311), such findings should allay concerns regarding long term usage of such drugs, at least from the colorectal cancer perspective. However, it seems that the controversy on the subject of endogenous gastrin on colorectal tumour growth is not yet ended as a recent study by Chu and colleagues (254) showed significant enhancement of growth, invasion and metastatic potential of a human colonic carcinoma transplanted into athymic rats rendered hypergastrinaemic by gastric fundectomy. The tumour used in this study was shown to express gastrin receptor mRNA and to be responsive to a specific gastrin receptor antagonist. It could be argued that the chemically induced tumours utilised in the present study and others may lack the appropriate receptors which would allow gastrin to exert its trophic action. However, a recent report showed high expression of gastrin receptors on azoxymethane-induced colonic tumours (337). Therefore, quite why elevation of antroduodenal gastrin by surgery, and not by drug administration, should lead to enhanced tumour growth is unclear but the findings of the present study do not support any action of the drug per se, to account for "neutralisation" of the hormone's action on colorectal neoplasia. Indeed, this may suggest that factors other than gastrin, which are invoked following acid reducing gastric surgery but not necessarily by acid suppression itself, are responsible. For example, bile

composition has been shown to be significantly altered following vagotomy in humans (171,291) and bile acids have been implicated as having a potential role to play in colorectal carcinogenesis (304-307). Once again however, the findings of the clinical study would tend to contradict even this hypothesis.

Thus, it is possible that studies such as the present one, where elevation of endogenous gastrin levels is achieved by administration of omeprazole and similar drugs are invalidated for the simple reason that neither the drug nor the hormone (at least of antroduodenal origin) interact significantly in the genesis or growth of large bowel cancer.

3. Summary

A number of common conclusions may be drawn from the combined findings of the three main lines of investigation, spanning in vitro, through in vivo, to clinical study, undertaken in this thesis.

The studies were all performed primarily to examine the effects of treatment for peptic ulcer, both old and new, on the development and growth of colorectal cancer. A small number of previously performed studies had suggested an increased incidence of the disease in patients treated operatively for their ulcer. In addition, a substantial volume of experimental evidence had accumulated to suggest that the antral hormone, gastrin, may be an important trophic factor in colorectal tumour growth. Recognising that chronic elevation of this hormone occurs as a result of the operation of vagotomy for peptic ulcer, it seemed appropriate to study such a group of patients to see whether such a phenomenon could be observed. However, the rate of colorectal cancer in a sizeable cohort of such patients followed up for approximately 25 years was not significantly different from that which was expected in the general population over the same period of time. As such, no sequelae of the surgery, whether hypergastrinaemia or otherwise, have been seen to exert any carcinogenic effect on the colonic mucosa over a sustained period of time.

Furthermore, in the in-vivo study, rats rendered hypergastrinaemic by treatment with omeprazole did not exhibit any exagerrated response in either colonic tumour number, stage or size as a result. It is accepted that the 24-week timespan of this laboratory based study and the much longer follow-up period of vagotomised patients in the clinical study are temporally incomparable, but the results of both are against any significant influence of elevated endogenous gastrin levels on the genesis or growth of colorectal tumours.

The absence of an obvious promoting action of omeprazole-induced hypergastrinaemia on colorectal tumour growth in this study, as well as the absence of any excess of such tumours in the group of vagotomised patients should consolidate the reassurances already given by others as to the relative unimportance of elevated antral gastrin levels in the development of the disease (344,346). This conclusion, however, should not be interpreted as meaning that gastrin is not a growth factor for some tumours, as has been shown (247-250) However, any such action is possibly more likely due to autocrine production and local release of the hormone and its precursors by some colorectal tumours (277-281), to act on appropriate cell surface receptors also synthesised by the tumour cells (258). The concentrations of such self-produced hormone within the immediate extracellular environment may be more important than circulating blood levels in promotion of tumour growth.

At the same time, although no obvious tumour-promoting action of elevated circulating gastrin levels was seen, this was not due to any confounding effect of omeprazole itself, as omeprazole did not exert any cytotoxic action on human colorectal tumour cells in vitro. Furthermore, animals treated with omeprazole either during the initiation or promotion phases of colorectal carcinogenesis, both had similar tumour numbers, of similar stage and size, to normogastrinaemic controls. These findings do not support any anti-neoplastic action of this drug on colorectal tumour growth as suggested by the studies of Penman (311) and Tobi (312), but are consistent with those of Graffner (284) and Pinson (285).

The other notable findings of the work undertaken here are the statistically significant, though modest, increased risks for bronchial, laryngeal and gastric cancer in vagotomised patients. Noting that the great majority of these patients were habitual smokers probably explains the observations for bronchial and laryngeal cancer, and is probably partly contributory to the development of gastric cancer in the cohort. In the case of the latter,

it is more likely that the prolonged acid suppression in such patients is more important in its aetiology, as has been noted in previous groups of patients treated by vagotomy or antrectomy and in patients with pernicious anaemia. What effect long-term acid suppression by medical means such as omeprazole will have in years to come remains to be seen, but the findings of the present study may indicate a potential hazard for patients so treated, and may warrant endoscopic surveillance of such groups. Despite this, it is likely that these patients will succumb to diseases strongly associated with smoking such as lung cancer, as found here, and other non-malignant conditions such as ischaemic heart disease and chronic respiratory disease, rather than gastric cancer, unless there is a major change in the lifestyle of today and tomorrow's patients with dyspeptic disorders.

4. Scope for further study

A plethora of information has been collated for the 1,992 patients reviewed in the clinical study and further analysis of this database may provide an important insight into why persons who have been submitted to ulcer surgery have come to be associated with such poor health in the years after it. Although the present study was confined to cancer incidence, computerised linkage to the national mortality statistics held by the Registrar General's Office, would allow disease-specific mortality rates for the cohort, not only for cancer, but all diseases, to be compared to those of the general population.

In a manner similar to that for smoking, alcohol consumption and socio-economic status have also been recorded for each patient in the study and any correlation to outcome for each of these factors could be determined.

Data has also been collected on the results of post-operative "Hollander" insulin tests, used to assess the completeness of vagotomy in those so treated, and a study of the completeness of vagotomy in both the gastric and colorectal cancer patients identified would be of interest. An inadequate vagotomy would be synonymous with persistence of vagally stimulated acid secretion, and also continuation of negative feedback control of circulating gastrin, both factors which, as already explained, may be influential in the development of these diseases. Comparison of disease incidence between complete and incompletely vagotomised subjects would possibly give a more precise indicator of the influence of that procedure on colonic or gastric tumour development.

Further experimental studies, similar to those performed here, using newer forms of proton pump inhibitors, such as lansoprazole and pantoprazole, might be considered. However, the absence of any demonstrable action of either omeprazole, nor the hypergastrinaemia produced by it, on colorectal tumour growth in vitro or in vivo, would

suggest that similar results would be obtained with these newer compounds, which elevate endogenous gastrin levels to a similar degree as omeprazole (347) and which interact similarly (348-350), or in the case of pantoprazole, to a much lesser degree (349,350), with members of the cytochrome P450 family of enzymes than omeprazole. It was the interaction of the latter with such enzymes that was postulated by Penman to be responsible for its anti-neoplastic action, an hypothesis which the findings of this study refute.

5. Conclusions

1. Previous peptic ulcer surgery, particularly vagotomy, <u>does not</u> confer an increased risk for development of colorectal cancer in the years following such surgery.

2. Hypergastrinaemia induced by present-day medical treatment for peptic ulcer disease, in the form of the proton pump inhibitor, omeprazole, <u>does not</u> enhance the development or growth of chemically-induced colorectal neoplasms in Sprague-Dawley rats.

3. Omeprazole <u>does not</u> inhibit the growth of such chemically-induced colorectal nepolasms in-vivo nor does it possess any obvious cytotoxic action on three human colorectal carcinoma cell lines studied in-vitro.

4. The risk of gastric cancer is increased by 50% over that of the general population in patients previously treated by vagotomy for peptic ulcer.

5. Despite the modest increase in risk for gastric cancer, lung cancer was the commonest neoplasm in the study cohort in the years after surgery, and with laryngeal cancer was significantly more frequent in the study cohort than the general population. Diseases such as these with a strong association with smoking are more likely to cause morbidity and mortality in such patients, rather than any long-term deleterious effects of the surgery on the gastric mucosa.

6. The modest, but significant increased risk for gastric cancer seen after vagotomy, may have implications for the safety of long-term acid supppression by present-day means such as omeprazole and related drugs.

Appendices

Materials and Methods - Appendix 1

Peptic Ulcer Clinic Study - Patient Data Collection Form

SEX :	MALE : 1 FEMALE : 2
PATIENT N° :	
DIAGNOSIS :	DOUDENAL ULCER - 1 , GASTRIC ULCER - 2 , BOTH DU & GU - 3 , RECURRENT ULCER - 4
	OTHER - 5 , NO ABNORMALITY - 6
OPERATIVE PROCEDURE :	VAGOTOMY AND PYLOROPLASTY - 1 , VAGOTOMY AND GASTROENTEROSTOMY - 2
	BILROTH I GASTRECTOMY - 3 , POLYA GASTRECTOMY - 4
	GASTROENTEROSTOMY ONLY - 5 , PYLOROPLASTY ONLY - 6 , VAGOTOMY ONLY - 7
	JEJUNAL INTERPOSITION - 8 , ROUX-EN-Y - 9 , REPAIR OF PERFORATED ULCER ONLY- 10
	CHOLECYSTECTOMY - 11, OTHER - 12
IF OTHER, SPECIFY :	
DATE OF OPERATION :	
AGE AT OPERATION :	
AGE GROUP :	(<15years) - 1 , (15-19) - 2 , (20-24) - 3 , (25-29) - 4 , (30-34) - 5 , (35-39) - 6 , (40-44) - 7
	(45-49) - 8 , (50-54) - 9 , (55-59) - 10 , (60-64) - 11 , (65-69) - 12 , (70-74) - 13 , (75-79) - 14
	(80-84) - 15 , (>85years) - 16
POST-OPERATIVE INSULIN TEST	
RESULT :	NEGATIVE - 1 , POSITIVE - 2 , NONE - 3, INCONSISTENT - 4 , NOT APPLICABLE -5
SOCIO-ECONOMIC STATUS :	1 2 3 4 5 NOT SPECIFIED - 6
SMOKING STATUS AT OPERATION :	ZERO - 1 , 1-10 - 2 , 11-20 - 3 , 21+ - 4 , CIGAR - 5 , PIPE - 6 , NOT SPECIFIED - 7
	EX-SMOKER - 8
ALCOHOL INTAKE :	ZERO - 0 , OCCASIONAL - 1 , OFTEN - 2 , HEAVY/HABITUAL - 3 , NOT SPECIFIED - 4
REGISTRATION WITH CANCER REGISTRY :	YES - 1 , NO - 2
IF YES, DATE OF DIAGNOSIS OF CANCER :	
TIME FROM GASTRIC SURGERY TO DIAGNOSIS OF MALIGNANCY :	
TYPE OF MALIGNANCY :	COLON - 1 , RECTUM - 2 , STOMACH - 3 , LUNG - 4 , OTHER - 5
IF OTHER, SPECIFY :	
DIED ? :	YES - 1 , NO - 2
IF YES, DATE OF DEATH :	
UNDERLYING CAUSE OF DEATH :	
TIME FROM GASTRIC SURGERY TO DEATH :	

Materials and Methods - Appendix 2

Preparation of Culture Medium used in Clonogenic Assays

The culture medium employed is a fifty/fifty mixture of Ham's F10 Nutrient Mixture and Dulbecco's modified Eagles containing glutamine 2mmol/L and 10% foetal calf serum.

"F10/DMEM" was prepared in the following proportions:

- 400 mLs distilled water

- 22.5 mLs F10-Hams Nutrient Mixture (Integra Biosciences,Northumbria Biologicals, U.K.)

- 22.5 mLs Dulbecco's Minimum Essential Medium(DMEM)

                         (Gibco BRL,LifeTechnologies Ltd,Paisley,Scotland, U.K.)

- 5 mLs Sodium Bicarbonate 7.5%

- 5 mLs 1 Molar Sodium Hydroxide

This constituted the stock medium, and prior to use was added:

- 50 mLs Foetal calf serum (Globepharm Ltd, Esher, Surrey, U.K.)

- 5 mLs L-glutamine 200mmol/L (GibcoBRL, Paisley)

Materials and Methods - Appendix 3

Reconstitution of Omeprazole

Molecular Formula   : $C_{17}H_{19}N_3O_3S$

Molecular Weight   : 345.42 grams

Dosage   : 40µmol/kg/day

Dose Volume   : 5mLs/kg

Conversion   : 1µmol = 0.345mgs

Concentration of Suspension   : 2.76mgs/mL.

Pure Omeprazole powder was suspended in 0.25% methylcellulose solution, buffered with 0.2% sodium bicarbonate and the pH adjusted to 9 with sodium hydroxide to give the final concentration shown above. This suspension is stable for one year in a deep freezer (-18°C to -22°C), one week in a refrigarator and one day at room temperature.

Results - Appendix 1
In Vivo Study - Plasma Gastrin Values (ng/L)

Group A			Group B			Group C		
	Treatment Phase			Treatment Phase			Treatment Phase	
Baseline	Omeprazole	Vehicle	Baseline	Vehicle	Omeprazole	Baseline	Vehicle	Vehicle
160	270	30	105	30	330	20	10	2.5
45	215	35	55	25	355	2.5	5	35
50	450	35	15	15	250	10	25	70
70	125	30	50	25	210	20	15	5
120	260	25	30	10	220	50	40	45
50	140	15	60	15	360	60	55	25
155	260	25	30	45	360	70	55	35
80	220	50	15	30	290	10	10	45
50	335	55	45	40	270	25	35	35
140	230	40	55	30	270	30	35	35
90	225	50	45	70	230	25	30	80
35	300	35	55	55	355	30	20	35
235	260	40	50	75	355	20	15	35
215	270	35	30	65	295	40	35	20
85	150	50	35	30	190	2.5	50	35
260	330	15	75	25	280	50	45	40
110	225	65	90	35	310	70	45	55
240	250	30	30	40	380	50	50	35
50	310	70	40	45	380	60	35	30
95	290	50	50	40	310	85	65	40
255	265	105	30	25	420	55	55	30
120	290	50	40	25	235	50	45	35
250	235	25	35	40	275	5	5	15
330	180	45	30	50	420	2.5	15	20
85	225	40	45	35	290	25	35	20

Results - Appendix 2 - In vivo Study - Group A - Tumour Number, Size, Distribution and Stage

Subject Identification Code	Number of Tumours	Distance from Anal Verge(% of colon length)	Tumour Volume(mms3)	Carcinoma/Adenoma
A11	0			
A12	1	27	168	Carcinoma : Dukes A
A13	1	35	8	Carcinoma : Dukes A
A14	0			
A15	1	76	75	Carcinoma : Dukes A
A21	1	48	100	Carcinoma : Dukes A
A22	0			
A23	0			
A24	2	54	147	Carcinoma : Dukes A
A24		83	120	Carcinoma : Dukes B+
A25	1	52	400	Carcinoma : Dukes A
A31	1	46	90	Carcinoma : Dukes A
A32	1	51	192	Carcinoma : Dukes A
A33	0			
A34	1	53	80	Adenoma
A35	2	81	864	Carcinoma : Dukes A
A35		46	32	Carcinoma : Dukes B+
A41	0			
A42	0			
A43	1	39	100	Carcinoma : Dukes A
A44	1	8	75	Carcinoma : Dukes A
A45	0			
A51	1	71	152	Carcinoma : Dukes A
A52	1	40	50	Carcinoma : Dukes A
A53	1	47	2	Carcinoma : Dukes A
A54	1	52	75	Carcinoma : Dukes A
A55	0			
	Total = 18	Mean = 50.5	Mean = 152	Carcinomas : 17
	Median = 1, Range 0-2	Median = 49.5, Range 8-83	Median = 95, Range 2-864	Dukes A : 15
				Dukes B+ : 2
				Adenomas : 1

Results - Appendix 3 - In vivo Study - Group B - Tumour Number, Size, Distribution and Stage

Subject Identification Code	Number of Tumours	Distance from Anal Verge(% of colon length)	Tumour Volume(mms3)	Carcinoma/Adenoma
B61	1	58	112	Carcinoma : Dukes B+
B62	1	61	120	Adenoma
B63	1	50	25	Carcinoma : Dukes A
B64	1	33	256	Carcinoma : Dukes B+
B65	0			
B71	3	44	320	Carcinoma : Dukes B+
B71		53	231	Carcinoma : Dukes A
B71		48	75	Carcinoma : Dukes A
B72	0			
B73	1	46	144	Carcinoma : Dukes B+
B74	1	45	240	Carcinoma : Dukes A
B75	1	42	60	Carcinoma : Dukes A
B81	3	46	48	Adenoma
B81		36	18	Carcinoma : Dukes A
B81		51	108	Carcinoma : Dukes A
B82	2	44	32	Carcinoma : Dukes A
B82		52	648	Adenoma
B83	1	42	320	Carcinoma : Dukes A
B84	0			
B85	0			
B91	1	56	126	Carcinoma : Dukes A
B92	1	61	18	Carcinoma : Dukes A
B93	1	38	240	Carcinoma : Dukes A
B94	2	24	18	Carcinoma : Dukes A
B94		51	32	Adenoma
B95	1	49	128	Carcinoma : Dukes A
B101	1	44	180	Carcinoma : Dukes B+
B102	0			
B103	0			
B104	0			
B105		44	48	Carcinoma : Dukes A
	Total = 24	Mean = 46.6	Mean = 148	Carcinomas : 20
	Median = 1, Range 0-3	Median=46, Range 24-61	Median =116, Range 18-648	Dukes A : 15
				Dukes B+ : 5
				Adenomas : 4

Results – Appendix 4 – In vivo Study – Group C – Tumour Number, Size, Distribution and Stage

Subject Identification Code	Number of Tumours	Distance from Anal Verge(% of colon length)	Tumour Volume(mms3)	Carcinoma/Adenoma
C111	3	18	4	Adenoma
C111		43	30	Adenoma
C111		55	50	Carcinoma : Dukes A
C112	2	40	100	Carcinoma : Dukes A
C112		57	140	Carcinoma : Dukes A
C113	2	45	8	Carcinoma : Dukes A
C113		43	60	Adenoma
C114	2	52	27	Adenoma
C114		62	147	Carcinoma : Dukes A
C115	1	66	147	Carcinoma : Dukes A
C121	3	59	288	Carcinoma : Dukes A
C121		32	50	Carcinoma : Dukes A
C121		41	32	Carcinoma : Dukes A
C122	1	38	2.25	Carcinoma : Dukes A
C123	4	65	8	Carcinoma : Dukes A
C123		46	70	Carcinoma : Dukes B+
C123		61	60	Carcinoma : Dukes A
C123		67	128	Adenoma
C124	1	46	108	Carcinoma : Dukes A
C125	1	29	50	Carcinoma : Dukes B+
C131	1	44	120	Carcinoma : Dukes A
C132	0			
C133	0			
C134	1	57	174	Carcinoma : Dukes B+
C135	1	56	100	Carcinoma : Dukes A
C141	1	51	225	Carcinoma : Dukes A
C142	0			
C143	0			
C144	1	50	180	Carcinoma : Dukes A
C145	0			
C151	0			
C152	0			
C153	0			
C154	1	62	245	Carcinoma : Dukes A
C155	1	33	32	Carcinoma : Dukes A
	Total = 27	Mean = 48.8	Mean = 96	Carcinomas : 22
	Median = 1, Range 0-4	Median = 50, Range 18-67	Median = 70, Range 2.25-288	Dukes A : 19
				Dukes B+ : 3
				Adenomas : 5

Results – Appendix 5 – In vivo Study – Group A – Weekly Weights (grams)

Week of Study

Subject Identification Code	1	2	3	4	5	6	7	8	9	10	11	12	13
A11	230	246	238	250	258	242	248	260	256	258	270	274	275
A12	220	244	246	258	260	268	270	274	290	290	290	304	306
A13	196	198	194	208	212	214	220	220	228	228	230	234	235
A14	220	224	240	228	254	256	260	268	272	292	298	288	288
A15	238	238	220	238	230	232	238	238	238	240	248	252	253
A21	230	238	236	250	252	256	264	278	272	288	298	286	286
A22	208	214	216	226	230	240	236	238	238	242	248	250	252
A23	198	248	252	274	286	276	282	286	298	304	308	310	310
A24	208	208	204	220	218	228	248	256	246	258	266	262	264
A25	236	238	214	232	232	232	228	226	232	232	240	244	244
A31	200	206	206	218	246	252	254	258	260	262	268	270	272
A32	228	236	232	244	248	248	260	260	258	260	268	272	274
A33	230	244	242	246	254	254	258	256	260	266	278	278	278
A34	230	234	230	244	224	224	230	232	232	238	244	250	251
A35	204	210	210	218	220	218	222	224	230	232	242	242	244
A41	232	218	220	228	238	240	250	254	252	258	254	258	259
A42	204	218	232	236	234	238	240	238	240	252	252	256	255
A43	214	224	224	230	238	240	246	252	258	266	266	270	273
A44	226	236	232	242	248	252	270	266	264	264	276	276	277
A45	208	238	240	244	248	248	254	254	260	260	268	274	275
A51	228	240	248	258	260	264	272	280	280	280	290	288	290
A52	220	228	234	242	246	254	264	262	266	270	274	280	284
A53	238	232	238	256	248	250	254	258	262	282	282	278	279
A54	228	238	236	244	240	248	256	258	270	260	270	274	275
A55	226	236	242	246	250	252	260	258	258	264	268	270	272

Results – Appendix 5 (continued) – In vivo Study – Group A – Weekly Weights (grams)

Week of Study

14	15	16	17	18	19	20	21	22	23	24	Growth rate (grams/week)
284	285	288	302	302	298	310	320	324	330	336	4.42
306	307	314	316	320	326	328	336	336	340	344	5.17
242	240	246	248	250	254	256	258	256	256	256	2.5
292	290	294	294	294	306	306	310	312	310	316	4
258	259	260	262	262	260	268	268	270	274	276	1.58
292	290	296	306	316	308	312	314	312	294	306	3.17
254	250	254	258	256	258	256	260	260	268	256	2
320	318	324	334	326	330	338	342	340	348	350	2.17
270	271	272	288	288	294	296	294	300	302	304	4
248	247	250	258	256	260	268	274	272	276	268	1.33
272	274	278	280	284	292	294	296	296	290	294	3.92
274	276	278	286	284	296	298	308	302	300	302	3.08
284	283	288	286	292	296	296	292	296	300	302	3
252	253	258	266	264	274	274	280	278	282	284	2.25
244	245	252	258	258	260	266	270	270	268	268	2.67
262	263	278	276	270	278	288	280	274	280	282	2.08
256	258	256	260	256	264	266	264	266	266	270	2.67
264	266	270	274	274	284	284	282	290	288	288	3.08
284	279	288	292	290	292	296	304	310	304	312	3.58
280	280	286	292	290	300	302	302	300	288	294	3.58
302	297	304	304	292	316	316	322	326	320	318	3.75
286	290	292	294	294	300	302	308	310	306	310	3.75
284	282	288	294	294	296	306	318	318	308	308	2.92
272	273	274	280	278	290	292	296	304	304	304	3.17
274	274	276	278	274	286	286	286	284	290	290	2.67
										Mean 297.52	Mean 3.06

Results – Appendix 6 – In vivo Study – Group B – Weekly Weights (grams)

Week of Study

Subject Identification Code	1	2	3	4	5	6	7	8	9	10	11	12	13
B61	206	212	216	220	230	228	232	238	236	242	246	250	254
B62	214	210	212	218	226	228	236	236	238	240	246	256	259
B63	202	208	214	224	234	234	248	250	250	256	260	260	262
B64	200	198	202	212	216	220	220	228	230	232	236	238	239
B65	196	226	226	228	238	242	248	248	254	256	258	268	260
B71	208	216	214	224	226	228	230	230	234	234	244	250	252
B72	218	226	228	234	238	242	248	250	250	252	260	266	269
B73	204	208	208	216	224	224	238	238	238	238	242	242	244
B74	208	210	216	228	230	238	238	240	244	248	252	256	258
B75	202	230	236	256	264	260	262	280	276	282	294	290	294
B81	224	232	240	250	252	260	272	274	280	274	290	296	296
B82	222	238	240	252	260	268	276	288	282	298	302	300	301
B83	228	213	220	232	234	240	242	252	262	256	260	264	266
B84	202	210	214	220	228	232	238	240	248	250	254	262	264
B85	224	234	234	244	248	256	270	264	266	268	278	284	284
B91	212	214	222	224	234	228	242	246	240	244	252	252	254
B92	212	220	222	234	238	244	246	250	252	258	266	266	265
B93	206	210	210	216	224	226	230	234	238	232	244	244	246
B94	222	230	232	240	250	254	268	270	268	268	274	282	280
B95	220	240	248	246	250	252	254	256	254	258	266	272	270
B101	236	252	250	262	266	274	276	272	282	284	292	300	304
B102	236	240	250	252	262	262	268	272	276	282	290	288	289
B103	212	226	222	236	240	244	248	268	272	264	270	280	282
B104	206	220	222	222	226	234	232	244	236	242	248	254	255
B105	218	232	232	242	244	254	252	256	258	262	268	268	270

Results – Appendix 6 (continued) – In vivo Study – Group B – Weekly Weights (grams)

14	15	16	17	18	19	20	21	22	23	24	Growth rate (grams/week)
250	252	258	258	260	258	262	262	262	268	270	2.67
260	261	262	264	264	268	268	268	268	268	270	2.33
258	258	268	268	272	278	282	290	288	288	286	3.5
238	239	254	256	254	266	262	262	268	262	266	2.75
270	269	274	280	282	284	284	282	284	288	288	3.83
248	246	258	258	258	254	256	260	262	262	270	2.58
262	264	270	274	274	276	278	280	284	278	282	2.67
244	246	252	252	254	256	260	262	266	266	266	2.58
254	256	258	260	260	270	268	270	270	268	270	2.58
290	292	302	322	318	318	332	328	330	340	334	5.17
296	294	300	306	304	310	310	316	320	320	324	4.17
300	300	304	320	318	338	334	344	336	344	344	5.08
256	259	270	274	272	280	284	298	284	282	282	2.25
248	252	268	278	272	276	272	272	274	274	276	3.08
286	284	288	304	310	308	306	302	300	300	306	3.42
252	253	260	262	264	280	284	280	282	274	296	3.5
266	264	274	274	274	286	282	288	286	284	286	3.08
244	245	250	256	250	260	256	258	264	270	258	1.33
288	285	290	298	294	292	306	310	310	308	310	3.66
276	275	276	286	288	298	294	298	304	296	296	3.17
306	303	304	312	306	314	316	316	318	326	330	3.92
294	292	304	308	304	296	314	318	318	314	312	3.17
274	278	278	284	284	292	290	290	292	292	296	3.08
244	249	248	254	254	274	264	264	268	272	284	3.25
272	271	272	286	282	286	284	294	306	292	296	3.25
										Mean 291.92	Mean 3.2

Results – Appendix 7 – In vivo Study – Group C – Weekly Weights (grams)

Week of Study

Subject Identification Code	1	2	3	4	5	6	7	8	9	10	11	12	13
C111	252	256	264	272	276	284	288	290	296	296	308	310	314
C112	232	238	244	248	254	258	266	264	268	272	274	280	282
C113	200	208	208	220	224	226	230	230	230	238	246	252	256
C114	240	252	254	266	262	264	270	272	276	278	290	296	300
C115	216	-	216	230	232	236	242	242	244	246	252	262	266
C121	216	228	234	234	240	248	252	254	264	268	264	284	286
C122	232	244	250	260	266	266	274	276	276	274	282	300	302
C123	224	232	238	252	252	266	272	272	282	286	292	300	307
C124	216	222	230	240	242	252	252	264	266	268	272	284	286
C125	226	238	240	250	256	262	260	268	268	268	274	282	287
C131	202	214	214	220	228	224	232	234	240	244	250	252	255
C132	220	224	226	238	240	244	244	250	250	252	256	266	269
C133	194	208	206	214	220	220	226	230	230	238	242	244	247
C134	212	218	218	232	234	238	244	256	264	254	266	270	275
C135	210	218	220	238	240	246	242	250	258	256	266	266	268
C141	234	246	252	258	270	274	276	282	282	292	292	296	298
C142	218	236	234	238	236	240	246	244	246	254	264	274	279
C143	228	230	250	252	258	262	270	280	290	278	286	296	301
C144	220	228	234	234	240	244	246	246	254	254	262	270	274
C145	222	244	230	248	248	260	268	268	278	278	288	290	295
C151	232	246	256	262	272	272	294	298	290	290	300	306	309
C152	214	224	230	236	248	250	254	260	262	260	272	280	283
C153	210	224	222	234	238	246	250	254	256	260	268	264	268
C154	214	222	232	238	244	244	248	258	256	260	262	258	260
C155	224	234	240	246	252	256	256	266	266	272	280	280	281

Results – Appendix 7 (continued) – In vivo Study – Group C – Weekly Weights (grams)

14	15	16	17	18	19	20	21	22	23	24	Growth rate (grams/week)
312	314	314	322	320	324	332	338	342	340	338	3.58
282	283	286	288	288	296	310	312	302	304	302	2.92
248	250	252	254	252	256	258	260	258	258	260	2.5
288	296	296	304	300	314	312	314	314	314	318	3.25
256	250	266	266	268	266	272	278	280	276	280	2.67
278	280	286	296	302	302	302	306	314	308	314	4.08
296	296	308	308	308	320	324	328	328	326	338	4.42
294	290	304	312	308	312	314	326	324	330	332	4.5
278	278	290	294	294	304	308	308	308	306	306	3.75
282	280	288	292	290	296	302	312	308	304	308	3.42
248	246	252	256	256	266	266	266	268	270	270	2.83
264	256	266	270	268	270	272	272	276	274	276	2.33
246	247	254	254	252	256	258	264	264	262	266	3
270	273	280	298	286	290	288	304	300	294	298	3.58
264	265	272	274	274	284	284	288	288	284	290	3.33
296	298	302	308	306	316	314	316	316	314	314	3.33
270	256	270	270	270	274	278	284	286	286	290	3
300	301	294	302	312	318	322	340	346	348	344	4.83
266	266	272	280	280	294	290	298	314	306	296	3.17
290	292	300	308	310	312	314	328	336	320	318	4
310	312	310	316	310	320	318	322	328	322	326	3.92
278	274	284	286	284	292	294	296	296	306	304	3.75
264	267	270	270	270	272	274	274	274	274	274	2.67
258	259	260	266	262	268	272	272	272	270	270	2.33
282	283	284	284	284	286	290	292	300	314	304	3.33
										Mean 301.44	**Mean** 3.38

Bibliography

1. World Health Organisation, Geneva 1995. The World Health Report 1995 - Bridging the gaps. Chapter 1. The state of world health.

2. Boyle P. Progress in preventing death from colorectal cancer. Br J Cancer 1995;72(3):528-530.

3. Scottish Health Statistics 1995. ISD Vol 37 Section 3 - Morbidity - Scottish Cancer Registration Statistics.

4. Sandler R.S. Epidemiology and Risk Factors for Colorectal Cancer. In: C.R.Boland, editor. Colorectal Neoplasia, Part I. The scientific basis for current management. Gastroenterol Clin North Am 1996;25(4):717-735.

5. Burt R.W. Familial risk and colorectal cancer. In : C.R.Boland, editor. Colorectal Neoplasia, Part I. The scientific basis for current management. Gastroenterol Clin North Am 1996;25(4):793-803.

6. Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34(5):424-425.

7. Cawkwell P, Quirke P. A new class of colorectal cancer gene. Gut 1995;36(5):641-643.

8. Nicolaides NC, Papadopoulous N, Liu B, Wei Y-F, Carter KC, Ruben SM et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994;371:75-80.

9. Carethers JM. The cellular and molecular pathogenesis of colorectal cancer. In : C.R.Boland, editor. Colorectal Neoplasia, Part I. The scientific basis for current management. Gastroenterol Clin North Am 1996;25(4):737-754.

10. Haenszel W, Kurihara M. Studies of Japanese migrants. I. Mortality from cancer and other chronic diseases among Japanese in the United States. J Natl Cancer Inst 1968; 40:43-68.

11. Staszewski J, McCall MG, Stenhouse NS. Cancer mortality in 1962-66 among Polish migrants to Australia. Br J Cancer 1971;25:599-610.

12. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975;15:617-631.

13. Howell MA. Diet as an etiological factor in the development of cancer of the colon and rectum. J Chronic Dis 1975;28:67-80.

14. Doll R. The geographical distribution of cancer. Br J Cancer 1969;23:1-8.

15. Burkitt DP. Epidemiology of cancer of the colon and rectum. Cancer 1971;28:3-13.

16. Ferraroni M, La Vecchia C, D'Avanso B, Negri E, Frances-Chi S and Decarli A. Selected micronutrient intake and the risk of colorectal cancer. Br J Cancer 1994;70:1150-1155.

17. Thun MJ, Namboodiri MM, Health CW Jr.. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991;325:1593-1596.

18. Labayle D, Fischer D, Vielh P, Drouhin F, Parienti A, Bories C et al. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology 1991;101:307-311.

19. Skinner SA, Penny AG, O'Brien PE. Sulindac inhibits the rate of growth and appearance of colon tumours in rats. Arch Surg 1991;126:1094-1096.

20. Dukes CE. The classification of cancer of the rectum. J Pathol Bacteriol 1932;35:323.

21. Dukes CE. The surgical pathology of rectal cancer. (President's address) Proc R Soc Med 1944;37:131.

22. Giles GR. The colon,rectum and anal canal. In: Cuschieri A., Giles GR, Moossa AR, editors. Essential Surgical Practice 2nd ed. Oxford: Butterworth Heinemann Ltd; 1988. p.1193-1231.

23. Pihl E, Hughes ES, McDermott FT, Milne BJ, Korner JM, Price AB. Carcinoma of the colon. Cancer specific long term survival. A series of 615 patients treated by one surgeon. Ann Surg 1980;192(1):114-117.

24. Hermanek P. Colorectal carcinoma: histopathological diagnosis and staging. International Practice and Research. Colorectal Cancer. Baillières Clin Gastroenterol 1989;3(3):511-529.

25. Astler VB, Coller FA. Prognostic significance of direct extension of carcinoma of colon and rectum. Ann Surg 1954;139:846-852.

26. Kirklin JW, Dockerty MB, Waugh JM. Role of peritoneal reflection in prognosis of carcinoma of rectum and sigmoid colon. Surg Gynecol Obstet 1949;88:326.

27. Turnbull RB Jr., Kyle K, Watson FR, Spratt J. Cancer of the colon: the influence of the no-touch isolation technique on survival rates. Ann Surg 1967;166:420-427.

28. Fisher ER, Sass R, Palekar A, Fisher B, Wolmark N. Dukes' classification re-visited. Findings from the National Surgical Adjuvant Breast and Bowel Projects (Protocol R-01) Cancer 1989;64:2354-2360.

29. Nathanson SD, Schultz L, Tilley B, Kambouris A. Carcinomas of the colon and rectum. A comparison of staging classifications. Am Surg 1986;52:428-433.

30. Deans GT, Parks TG, Rowlands BJ, Spence RA. Prognostic factors in colorectal cancer. Br J Surg 1992;79:608-613.

31. Hermanek P, Sobin LH, editors. Union Internationale Contre le Cancer. TNM classification of Malignant Tumours. 4th ed. 1987. p. 197.

32. Eckhauser FE, Knol JA. Surgery for primary and metastatic colorectal cancer. Colorectal Neoplasia, Part II. Diagnosis and Treatment. Gastroenterol Clin North Am 1997;26(1):103-128.

33. Cheng TO. Glimpses of the past from the recently unearthed ancient corpses in China. Ann Int Med 1984;101:714-715.

34. Jordan PH. Surgery for Peptic Ulcer Disease. Historical Overview. Curr Probl Surg 1991;28(4):271-272.

35. * Lenepneau I. Cas de perforation de duodenum de lien d'une ancienne cicatrice de cet intestina. Gaz Hop 1838;35:137.

36. Wastell C. Chronic Duodenal Ulcer. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 3rd ed. Boston: Little, Brown and Co; 1977. p. 235-256.

37. Katz J. The Course of Peptic Ulcer Disease. Med Clin North Am 1991;75(4):831-840.

38. Sonnenberg A. Temporal trends and geographical variations of peptic ulcer disease. Aliment Pharmacol Ther 1995;9 Suppl 2:3-12.

39. Peterson WL. Bleeding Peptic Ulcer. Epidemiology and Nonsurgical Management. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):155-170.

40. Sachdeva AK, Zaren HA, Sigel B. Surgical Treatment of Peptic Ulcer Disease. Med Clin North Am 1991;75:999-1012.

41. Isenberg JI, McQuaid KR, Laine L, Rubin W. Acid-Peptic Disorders. In: Yamada T, editor. Textbook of Gastroenterology - Volume 1. Philadelphia: JB Lippincott Company; 1991. p. 1241-1339.

42. Mertz HR, Walsh JH. Peptic Ulcer Pathophysiology. Med Clin North Am 1991;75:799-814.

43. Quigley EM, Turnberg LA. pH of the microclimate lining human gastric and duodenal mucosa in vivo studies in control subjects and in duodenal ulcer patients. Gastroenterology 1987;92:1876-1884.

44. Sanders MJ, Ayalon A, Roll M, Soll AH. The apical surface of canine chief cell monolayers resists H^+. Nature 1985;313:52-54.

45. McQuaid KR, Isenberg JI. Medical Therapy of Peptic Ulcer Disease. Gastric Surgery. Surg Clin North Am 1992;72(2):285-316.

46. Freston JW. Overview of Medical Therapy of Peptic Ulcer Disease. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):121-140.

47. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;i:1273.

48. Salomon H. Veber das Spirillum des Saugetiermagens und sein Verhalten zu den Belegzellen. Zentralbl Bakteriol 1896;19:433.

49. Doenges JL. Spirochaetes in the gastric glands of Macacus rhesus and humans without definite history of related disease. Proc Soc Exp Biol Med 1938;38:536.

50. Freedberg AS, Barron LE. The presence of spirochetes in human gastric mucosa. Am J Dig Dis 1940;7:443.

51. Heatley RV. H. pylori: the clinical problem. In: The Helicobacter pylori Handbook. 1st ed. Blackwell Science; 1995. p. 10-20.

52. Clearfield HR. Helicobacter Pylori: Aggressor or Innocent Bystander? Med Clin North Am 1991;75(4):815-829.

53. Sarosiek J, Slomiany A, Slomiany BL. Evidence for weakening of gastric mucus integrity by Campylobacter pylori. Scand J Gastroenterol 1988;23:585-590.

54. Figura N, Guglielmetti P, Rossolini A, Barberi A, Cusi G, Musmanno RA et al. Cytotoxin production by Campylobacter pylori strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. J Clin Microbiol 1989;27:225-226.

55. Taylor DE, Eaton M, Chang N, Salama SM. Construction of a Helicobacter pylori genome map and demonstration of diversity at the genome level. J Bacteriol 1992;174:6800-6806.

56. Levi S, Beardshall K, Haddad G, Playford R, Ghosh P, Calam J. Campylobacter pylori and duodenal ulcer: the gastrin link. Lancet 1989;1:116-118.

57. Lichtenberger LM, Dial EJ, Romero JJ, Lechago J, Jarboe LA, Wolfe MM. Role of luminal ammonia in the development of gastropathy and hypergastrinaemia in the rat. Gastroenterology 1995;108:320-329.

58. El-Omar EM, Penman IA, Ardill JES, Chittajallu RS, Howie C, McColl KEL. Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. Gastroenterology 1995;109:681-691.

59. Tytgat GNJ, Rauws EAJ. Campylobacter pylori and its role in Peptic Ulcer Disease. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):183-196.

60. Graham DY. The relationship between Nonsteroidal Anti-inflammatory Drug Use and Peptic Ulcer Disease. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):171-182.

61. Graham DY, Agrawal NM, Roth SH. Prevention of NSAID-induced gastric ulcer with misoprostil : Multicentre, double-blind, placebo-controlled trial. Lancet 1988;2:1277-1280.

62. Polacek MA, Ellison EH. Parietal cell mass and gastric acid secretion in the Zollinger-Ellison syndrome. Surgery 1966;60(3):606-614.

63. Zollinger RM, Ellison EH. Primary peptic ulceration of the jejunum associated with islet cell tumors of the pancreas. Ann Surg 1955;142:709-728.

64. Gregory RA, Grossman MI, Tracy HJ, Bentley PH. Nature of the Gastric Secretagogue in Zollinger-Ellison Tumours. Lancet 1967;2:543-544.

65. Del Valle J, Yamada T. Zollinger-Ellison Syndrome. In: Yamada T, editor. Textbook of Gastroenterology - Volume 1. Philadelphia: JB Lippincott Company; 1991. p. 1340-1352.

66. Berg CL, Wolfe MM. Zollinger-Ellison Syndrome. Med Clin North Am 1991;75(4):903-921.

67. Vinayek R, Frucht H, Victor Chiang HC, Maton PN, Gardner JD, Jensen RT. Zollinger-Ellison Syndrome. Recent Advances in the Management of the Gastrinoma. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):197-217.

68. Beaumont W. Experiments and observations on the gastric juice and the physiology of digestion. Plattsburgh F.P. Allan 1833:9-23.

69. † Tiedemann F, Gmelin L. Recherches expérimentales, physiologiques et chimiques sur la digestion. Paris: Baillière 1827. p. xviii-xxi.

70. Prout W. On the nature of the acid and saline matters usually existing in the stomachs of animals. Philosophical Transactions of the Royal Society of London 1824;1:45-49.

71. † Pavlov IP. The work of the digestive glands. London: Charles Griffin & Co. Ltd. 1910.

72. † Pavlov IP, Schumova-Simanovskaja E. Innervation der Magendrüsen beim Hunde. Zentralbl. Physiol. 1889;3:113.

73. Edkins JS. The chemical mechanism of gastric secretion. J Physiol 1906;34:133-144.

74. Gregory RA, Tracy HJ. The constitution and properties of two gastrins isolated from hog antral mucosa. I. The isolation of two gastrins from hog antral mucosa. II. The properties of two gastrins isolated from hog antral mucosa. Gut 1964;5:103-117.

75. Wangensteen OH, Wangensteen SD. History of Gastric Surgery: Glimpses into its early and more recent past. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 3rd ed. Boston: Little, Brown and Co; 1977. p. 235-256.

76. Schubert ML, Shamburek MD. Control of Acid Secretion. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):1-25.

77. *† Bilroth T. Offenes Schreiben an Herrn Dr. L. Wittelshöfer. Wien Med Wochenschr. 1881;31:161.

78. *† Gussenbauer C, Winiwarter A. Die partielle Magenresection. Eine experimentelle operative Studie. Arch Klin Chir 1876;19:347.

79. Jordan PH. Surgery for Peptic Ulcer Disease. Gastric Resection without Vagotomy. Curr Probl Surg 1991;28(4):276-280.

80. *† Merrem DCT. Animadversiones quaedam chirurgicae experimentis in animalibus factis illustratae. Giessen: Tasché & Müller. 1810.

81. *† Péan JE. De l'ablation des tumeurs de l'estomac par la gastrectomie. Gaz. Hôp [Paris] 1879;52:473.

82. *† Rydygier L. Extirpation des carcinomatosen Pylorus; tod nach zwölf Stunden. Dtsch Z Chir 1881;14:252.

83. *† Rydygier L. Die erste Magenresection beim Magengeschwür. Berl Klin Wochenschr 1882;19:39.

84. * von Hacker V. Zur Casuistik und Statistik der Magenresektionen und Gastroenterostomieen. Arch Klin Chir 1885;32:616.

85. *† Wölfler A. Gastroenterostomie Zentralbl Chir 1881;8:705.

86. † Codivilla A. Considerations on 40 cases of gastric surgery. Bull Med Sci 1898;9:651.

87. Jordan PH. Surgery for Peptic Ulcer Disease. Gastric Drainage Operations. Curr Probl Surg 1991;28(4):272-275.

88. * Fronmüller F. Operation der Pylorusstenose. (Erlangen dissertation). Fürth: Schröder, 1886.

89. *† Mikulicz-Radecki J. Zur operativen Behandlung des stenosirenden Magengeschwürs. Dtsch Gesellsch Chir 1887;16:337.

90. Stern DH, Walsh JH. Gastrin release in postoperative ulcer patients: Evidence for release of duodenal gastrin. Gastroenterology 1973;64(3):363-369.

91. Dragstedt LR, Owens FM. Supra-diaphragmatic section of the Vagus Nerves in Treatment of Doudenal Ulcer. Proc Soc Exp Biol Med 1943;53:152-154.

92. * Brodie BC. Experiments and observations on the influence of the nerves of the eighth pair on the secretions of the stomach. Philos Trans R Soc Lond (Bio) 1814;104:102.

93. * Jurgens NP. Sur la sécrétion stomacale chez les chiens ayant subi la section sousdiaphragmatique des nerfs pneumogastrique. Archs Sci Biol (St. Petersb.)1892;1:323.

94. Jordan PH. Surgery for Peptic Ulcer Disease. Vagotomy. Curr Probl Surg 1991;28(4):280-285.

95. * Bircher E. Die Resektion von Aesten der N. Vagus zur Behandlung gastrischer Affektionen. Schwiez Med Wochenschr 1920;50:519.

96. * Latarjet A. Résection des nerfs de l'estomac. Technique opératoire. Résultats cliniques. Bull Acad Natl Méd (Paris) 1922;87:681.

97. Jackson RG. Anatomic study of the vagus nerves, with a technique of transabdominal selective gastric vagus resection. Arch Surg 1948;143:80.

98. Franksson C. Selective abdominal vagotomy. Arch Chir Scand 1948;96:409.

99. Griffith CA. Selective Gastric Vagotomy. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 3rd ed. Boston: Little, Brown and Co; 1977. p. 275-305.

100. Griffiths CA, Harkins HN. Partial gastric vagotomy: An experimental study. Gastroenterology 1957;32:96-102.

101. Ferguson DJ, Billings H, Swenson D, Hoover G. Segmental gastrectomy with innervated antrum for duodenal ulcer: Results at one to five years. Surgery 1960;47:548-556.

102. Holle F, Hart W. Neue Wege der chirurgie des gastro-duodenal ulcus. Med Klin 1967;62:441-450.

103. Amdrup E, Jensen HE. Selective vagotomy of the parietal cell mass preserving innervation of the undrained antrum: A preliminary report of results in patients with duodenal ulcer. Gastroenterology 1970;50:522-527.

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

105. Johnston D. Operative mortality and postoperative morbidity of highly selective vagotomy. BMJ 1975;iv:545-547.

106. Wastell C. Proximal Gastric Vagotomy. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 4th ed. Boston: Little, Brown and Co; 1986. p. 365-394.

107. Taylor TV. Lesser curve myotomy. An experimental study. Ann Surg 1980;191(4):414-418.

108. Taylor TV, Gunn AA, MacLeod DAD, MacLennan I. Anterior lesser curve seromyotomy with posterior truncal vagotomy in the treatment of chronic duodenal ulcer. Lancet 1982;ii:846-848.

109. Taylor TV, Gunn AA, MacLeod DAD, Van Vroonhoven ThJMV, Bornman PC, Terblanche J et al. Mortality and morbidity after anterior lesser curve seromyotomy with posterior truncal vagotomy for duodenal ulcer. Br J Surg 1985;72:950-951.

110. Cuschieri A. Laparoscopic Vagotomy. Gimmick or Reality. Gastric Surgery. Surg Clin North Am 1992;72(2):357-367.

111. Eagon JC, Miedima BW, Kelly KA. Postgastrectomy syndromes. Gastric Surgery. Surg Clin North Am 1992;72(2):445-465.

112. Marotta RB, Floch MH. Diet and Nutrition in Ulcer Disease. Med Clin North Am 1991;75(4):967-979.

113. Abercrombie J. Diseases of the stomach. 1828; Edinburgh.

114. Rubin W. Medical Treatment of Peptic Ulcer Disease. Med Clin North Am 1991;75(4):981-998.

115. Kay AW. Effect of large doses of histamine on gastric secretion of Hcl: An augmented histamine test. BMJ 1953;2:77.

116. Ash ASF, Schild HO. Receptors mediating some actions of histamine. Br J Pharmacol Chemother 1966;27: 427.

117. Black JW, Duncan WAM, Durant CJ, Ganellin CR, Parsons EM. Definition and antagonism of histamine H_2 receptors. Nature 1972;236:385.

118. Wyllie JH. Special Comment: Histamine H$_2$-receptor Blockade in man. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 3rd ed. Boston: Little, Brown and Co; 1977. p. 124-127.

119. Tankel HI, Gillespie IE, Clark DH, Kay AW, McArthur J. A clinical and statistical study of the effect of gastrojejunostomy on human gastric secretion. Gut 1960;1:223-229.

120. Gelb AM, Baronofsky ID, Janowitz HD. The effect of vagotomy and pyloroplasty on the maximal acid response to histamine. Gut 1961;2:240-245.

121. Sircus W, Small WP. The problem of peptic ulcer. Scott Med J 1964;9(11):453-468.

122. Kay AW. Gastrointestinal surgery and human physiology. J R Coll Surg Edinb 1962;7:275.

123. Broomé A, Bergström H, Olbe L. Maximal acid response to histamine in duodenal ulcer patients subjected to resection of the antrum and duodenal bulb followed by vagotomy. Gastroenterology 1967;52:952.

124. Gillespie IE, Clark DH, Kay AW, Tankel HI. Effect of antrectomy, vagotomy with gastrojejunostomy, and antrectomy with vagotomy on the spontaneous and maximal gastric acid output in man. Gastroenterology 1960;38:361-367.

125. McGuigan JE, Trudeau BM. Serum gastrin levels before and after vagotomy and pyloroplasty or vagotomy and antrectomy. N Engl J Med 1972;286(4):184-188.

126. Becker HD, Reeder DD, Thompson JC. Effect of truncal vagotomy with pyloroplasty or with antrectomy on food-stimulated gastrin values in patients with duodenal ulcer. Surgery 1973;74(4):580-586.

127. Anonymous. The effect of vagotomy on gastric secretion elicited by pentagastrin in man. A multicentre study. Lancet 1967;2(515):534-536.

128. Mason MC, Giles GR, Graham NG, Clark CG, Goligher JC. An early assessment of selective and truncal vagotomy. Br J Surg 1968;55(9):677-680.

129. Jepson K, Lari J, Humphrey CS, Smith RB, Wilkinson AR, Johnston D. A comparison of the effects of truncal, selective and highly selective vagotomy on maximal acid output in response to pentagastrin. Ann Surg 1973;178(6):769-772.

130. Saik RP, Wiesenfeld N, Peskin GW. Human gastrin response after ulcer surgery. J Surg Res 1977;22:352-356.

131. Korman MG, Hansky J, Scott PR. Serum gastrin in duodenal ulcer. Part III Influence of vagotomy and pylorectomy. Gut 1972;13:39-42.

132. Thompson JC, Fender HR, Watson LC, Villar HV. The effects on gastrin and gastric secretion of five current operations for duodenal ulcer. Ann Surg 1976;183(5):599-608.

133. Jaffe BM, Clendinnen BG, Clarke RJ, Williams JA. Effect of selective and proximal gastric vagotomy on serum gastrin. Gastroenterology 1974;66(5):944-953.

134. Pounder R, Smith J. Drug-induced changes of plasma gastrin concentration. Peptic Ulcer Disease. Gastroenterol Clin North Am 1990;19(1):141-153.

135. Allan Gray JD, Shiner M. Influence of gastric pH on gastric and jejunal flora. Gut 1967;8:574-581.

136. Hill M. Normal and pathoogical microbial flora of the upper gastrointestinal tract. Scand J Gastroenterol 1985;20 Suppl 111:1-6.

137. Stockbruegger RW. Bacterial overgrowth as a consequence of reduced gastric acidity. Scand J Gastroenterol 1985;20 Suppl 111:7-16.

138. Snepar R, Poporad GA, Romano JM, Kobasa WD, Kaye D. Effect of cimetidine and antacid on gastric microbial flora. Infect Immun 1982;36(2):518-524.

139. Meyrick Thomas J, Misiewicz JJ, Cook AR, Hill MJ, Smith PLR, Walters CL et al. Effect of one year's treatment with ranitidine and of truncal vagotomy on gastric contents. Gut 1987;28:726-738.

140. Sharma BK, Santana IA, Wood EC, Walt RP, Pereira M, Noone P et al. Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole. BMJ 1984;289:717-719.

141. Verdu E, Viani F, Armstrong D, Fraser R, Siegrist HH, Pignatelli B et al. Effect of omeprazole on intragastric bacterial counts, nitrate, nitrites, and N-nitroso compounds. Gut 1994;35:455-460.

142. Herrington Jr. JL, Sawyers JL. Remedial operations. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 3rd ed. Boston: Little, Brown and Co; 1977. p. 537-569.

143. Debas HT, Orloff SL. Surgery for peptic ulcer disease. In: Yamada T, editor. Textbook of Gastroenterology - Volume 1. Philadelphia: JB Lippincott Company; 1991. p. 1379-1398.

144. Mix CL. "Dumping stomach" following gastrojejunostomy. Surg Clin North Am 1922;2:617-622.

145. Owren PA. The pathogenesis and treatment of iron deficiency anaemia after partial gastrectomy. Acta Chir Scand 1953;104:206.

146. Krause U. Late prognosis after partial gastrectomy for ulcer. A follow-up study of 361 patients operated upon from 1905 to 1933. Acta Chir Scand 1958;114:341-354.

147. Baird IM, Blackburn EK, Wilson GM. The pathogenesis of anaemia after partial gastrectomy. Q J Med 1959;28:21.

148. Wheldon EJ, Venables CW, Johnston IDA. Late metabolic sequelae of vagotomy and gastroenterostomy. Lancet 1970; 1: 437-440.

149. Schron CM. Vitamins and minerals. In: Yamada T, editor. Textbook of Gastroenterology - Volume 1. Philadelphia: JB Lippincott Company; 1991. p. 392-410.

150. Rygvold O. Hypovitaminosis B_{12} following partial gastrectomy by Bilroth II method. Scand J Gastroenterol Suppl 1974;29:57.

151. Daugherty DF, Lucey MR, Yamada T. Gastric Secretion In: Yamada T, editor. Textbook of Gastroenterology - Volume 1. Philadelphia: JB Lippincott Company; 1991. p. 233-264.

152. Sitrin M, Meredith S, Rosenberg IH. Vitamin D deficiency and bone disease in gastrointestinal disorders. Arch Intern Med 1978;138:886.

153. Eddy RL. Metabolic bone disease after gastrectomy. Am J Med 1971;50:442.

154. Imawari M, Kozawa K, Akanuma Y, Koizumi S, Itakura H, Kosaka K. Serum 25-hydroxyvitamin D and vitamin D-binding protein levels and mineral metabolism after partial and total gastrectomy. Gastroenterology 1980;79:255.

155. Johnson FE, Boyden EA. The effect of double vagotomy on the motor activity of the human gall bladder. Surgery 1952;32:591.

156. Parkin GJS, Smith RB, Johnston D. Gallbladder volume and contractility after truncal, selective and highly selective (parietal cell) vagotomy in man. Ann Surg 1973;178:581.

157. Rudick J, Hutchison JSF. Effects of vagal nerve section on the biliary system. Lancet 1964;1:579.

158. Clave RA, Gaspar MR. Incidence of gall bladder disease after vagotomy. Am J Surg 1969;118:169.

159. Nielsen JR. Development of cholelithiasis following vagotomy. Surgery 1964;56:909.

160. Cowie A, Clark GC. The lithogenic effect of vagotomy. Bull Soc Int Chir 1972;31:7.

161. Tompkins RK, Kraft AR, Zimmerman E, Lichtenstein JE, Zollinger RM. Clinical and biochemical evidence of increased gallstone formation after complete vagotomy. Surgery 1972;71:196.

162. Balfour DC. Factors influencing the life expectancy of patients operated on for gastric ulcers. Ann Surg 1922;76:405-408.

163. Beatson, Sir George T. Carcinoma of the stomach after gastroduodenostomy. BMJ 1926;1:15.

164. Liavaag K. Cancer development in gastric stump after partial gastrectomy for peptic ulcer. Ann Surg 1962;155:103-106.

165. McLean Ross AH, Smith MA, Anderson JR, Small WP. Late mortality after surgery for peptic ulcer. N Engl J Med. 1982;307:519-522.

166. Watt PCH, Patterson CC, Kennedy TL. Late mortality after vagotomy and drainage for duodenal ulcer. BMJ 1984;288:1335-1338.

167. Bundred NJ, Whitfield BCS, Stanton E, Prescott RJ, Davies GC, Kingsnorth AN. Gastric surgery and the risk of subsequent colorectal cancer. Br J Surg 1985;72:618-619.

168. Caygill CPJ, Hill MJ, Hall CN, Kirkham JS, Northfield TC. Increased risk of cancer at multiple sites after gastric surgery for peptic ulcer. Gut 1987;28:924-928.

169. Toftgaard C. Risk of colorectal cancer after surgery for benign peptic ulceration. Br J Surg 1987;74:573-575.

170. Ditlevsen S. Survival after vagotomy: Results of the Aarhus County Vagotomy Trial. World J Surg 1989;13:776-781.

171. Mullan FJ, Wilson HK, Majury CW, Mills JOM, Cromie AJ, Campbell GR et al. Bile acids and the increased risk of colorectal tumours after truncal vagotomy. Br J Surg 1990;77:1085-1090.

172. Stemmermann GN, Nomura AMY, Chyou P-H. Cancer incidence following subtotal gastrectomy. Gastroenterology 1991;101:711-715.

173. Lundegårdh G, Adami HO, Helmick C, Zack M. The risk of large bowel cancer after partial gastrectomy for benign ulcer disease. Ann Surg 1990;212:714-719.

174. Macintyre IMC, O'Brien F. Death from malignant disease after surgery for duodenal ulcer. Gut 1994;35:451-454.

175. Helsingen N, Hillestad L. Cancer development in the gastric stump after partial gastrectomy for ulcer. Ann Surg 1956;143:173-179.

176. De Jode LR. Gastric carcinoma following gastroenterostomy and partial gastrectomy. Br J Surg 1961;48:512-514.

177. Stalsberg H, Taksdal S. Stomach cancer following gastric surgery for benign conditions. Lancet 1971;2:1175-1177.

178. Domellöf L, Janunger K-G. The risk for gastric carcinoma after partial gastrectomy. Am J Surg 1977;134:581-584.

179. Fischer AB, Græm N, Jensen OM. Risk of gastric cancer after Billroth II resection for duodenal ulcer. Br J Surg 1983;70:552-554.

180. Schafer LW, Larson DE, Melton III LJ, Higgins JA, Ilstrup DM. The risk of gastric carcinoma after surgical treatment for benign ulcer disease. A population-based study in Olmsted County, Minnesota. N Engl J Med 1983;309:1210-1213.

181. Totten J, Burns HJG, Watt Kay A. Time of onset of carcinoma of the stomach following surgical treatment of duodenal ulcer. Surg Gynecol Obstet 1983;157:431-433.

182. Pickford IR, Craven JL, Hall R, Thomas G, Stone WD. Endoscopic examination of the gastric remnant 31-39 years after subtotal gastrectomy for peptic ulcer. Gut 1984;25:393-397.

183. Tokudome S, Kono S, Ikeda M, Kuratsune M, Sano C, Inokuchi K et al. A prospective study on primary gastric stump cancer following partial gastrectomy for benign gastroduodenal diseases. Cancer Res 1984;44:2208-2212.

184. Viste A, Opheim P, Thunold J, Eide GE, Bjørnestad E, Skarstein A et al. Risk of carcinoma following gastric operations for benign disease. A historical cohort study of 3470 patients. Lancet 1986;ii:502-505.

185. Caygill CPJ, Kirkham JS, Hill MJ, Northfield TC. Mortality from gastric cancer following gastric surgery for peptic ulcer. Lancet 1986;i:929-931.

186. Offerhaus GJA, Tersmette AC, Huibregtse K, Van De Stadt J, Tersmette KWF, Stijnen TH et al. Mortality caused by stomach cancer after remote partial gastrectomy for benign conditions: 40 years of follow up of an Amsterdam cohort of 2633 postgastrectomy patients. Gut 1988;29:1588-1590.

187. Arnthorsson H, Tulinius H, Egilsson V, Sigvaldason H, Magnusson B, Thorarinsson H. Gastric cancer after gastrectomy. Int J Cancer 1988;42:365-367.

188. Lundegårdh G, Adami H-O, Helmick C, Zack M, Meirik O. Stomach cancer after partial gastrectomy for benign ulcer disease. N Engl J Med 1988;319:195-200.

189. Tersmette AC, Offerhaus GJA, Tersmette KWF, Giardiello FM, Moore GW, Tytgat GNJ et al. Meta-analysis of the risk of gastric stump cancer: detection of high risk

patient subsets for stomach cancer after remote partial gastrectomy for benign conditions. Cancer Res 1990;50:6486-6489.

190. Lundegårdh G, Ekbom A, McLaughlin JK, Nyrén O. Gastric cancer risk after vagotomy. Gut 1994;35:946-949.

191. Din NA, Small WP. Death after partial gastrectomy for peptic ulcer- a long term study [Abstract]. Gut 1974;15:335.

192. Doll R, Peto R. Mortality in relation to smoking : 20 years' observations on male British doctors. BMJ 1976; 2: 1525-1536.

193. Lundegårdh G, Adami H.-O, Helmick Ch, Zack M. Risk of cancer following partial gastrectomy for benign ulcer disease. Br J Surg 1994; 81: 1164-1167.

194. Shearman DJC, Finlayson NDC, Arnott SJ, Pearson JG. Carcinoma of the oesophagus after gastric surgery. Lancet 1970;i:581-582.

195. Macdonald JB, Waissbluth JG, Langman MJS. Carcinoma of the oesophagus and gastric surgery. Lancet 1971;i:19-20.

196. Stalsberg H. Carcinoma of the oesophagus after gastric surgery [Letter]. Lancet 1972; : 381.

197. Mosbech J, Videbaek A. Mortality from and risk of gastric carcinoma among patients with pernicious anaemia. BMJ 1950;ii:390-394.

198. Blackburn EK, Callender ST, Dacie JV. Possible association between pernicious anaemia and leukaemia: a prospective study of 1,625 patients with a note on the very high incidence of stomach cancer. Int J Cancer 1963;3:163-170.

199. Brinton LA, Gridley G, Hrubec Z, Hoover R, Fraumeni JF. Cancer risk following pernicious anaemia. Br J Cancer 1989;59:810-813.

200. Talley J, Chute CG, Larson DE, Epstein R, Lydick EG, Melton LJ. Risk for colorectal adenocarcinoma in pernicious anaemia. A population-based cohort study. Ann Intern Med 1989;111:738-742.

201. Domellöf L, Eriksson S, Janunger K-G. Carcinoma and possible precancerous changes of the gastric stump after Bilroth II resection. Gastroenterology 1977;73:462-468.

202. Morson BC. Carcinoma arising from areas of intestinal metaplasia in the gastric mucosa. Br J Cancer 1955;9:377.

203. Korn ER. Intestinal metaplasia of the gastric mucosa. Am J Gastroenterol 1974;61:270-275.

204. Siurala M, Varis K, Wiljasulo M. Studies of patients with atrophic gastritis; a 10-15 years follow-up. Scand J Gastroenterol 1966;1:40-48.

205. Walker IR, Strickland RG, Ungar B, Mackay IR. Simple atrophic gastritis and gastric carcinoma. Gut 1971;12:906-911.

206. Axon ATR. Potential hazards of hypochlorhydria in the treatment of peptic ulcer. Scand J Gastroenterol 1986; 21 Suppl 122:17-21.

207. Ruddell WSJ, Axon ATR, Findlay JM, Bartholomew BA, Hill MJ. Effect of cimetidine on the gastric bacterial flora. Lancet 1980;i:672-674.

208. Magee PN, Barnes JM. Carcinogenic nitroso compounds. Adv Cancer Res 1967;10:163-246.

209. Tannenbaum SR. N-nitroso compounds: a perspective on human exposure. Lancet 1983;i:629-632.

210. Lijinsky W. Nitrosamines and nitrosamides in the aetiology of gastrointestinal cancer. Cancer 1977;40:2446-2449.

211. Reed PI, Smith PLR, Haines K, House FR, Walters CL. Effect of cimetidine on gastric juice N-nitrosamine concentration. Lancet 1981;ii:553-556.

212. Stockbruegger RW, Cotton PB, Eugenides N, Bartholomew BA, Hill MJ, Walters CL. Intragastric nitrites, nitrosamines and bacterial overgrowth during cimetidine treatment. Gut 1982;23:1048-1054.

213. Reed PI, Smith PLR, Haines K, House FR, Walters CL. Gastric juice N-nitrosamines in health and gastroduodenal disease. Lancet 1981;ii:550-552.

214. Milton-Thompson GJ, Lightfoot NF, Ahmet Z, Hunt RH, Barnard J, Bavin PMG et al. Intragastric acidity, bacteria, nitrite, and N-nitroso compounds before, during, and after cimetidine treatment. Lancet 1982;i:1091-1095.

215. Keighley MRB, Youngs D, Poxon V, Morris D, Muscroft TJ, Burdon DW et al. Intragastric N-nitrosation is unlikely to be responsible for gastric carcinoma developing after operations for peptic ulcer. Gut 1984;25:238-245.

216. Weinstock J, Baldwin GS. Binding of gastrin(17) to human gastric carcinoma cell lines. Cancer Res 1988;48(4):932-937.

217. Watson SA, Durrant LG, Morris DL. Growth promoting action of gastrin on human colonic and gastric tumour cells cultured in vitro. Br J Surg 1988;75:342-345.

218. Aspegren K, Eriksson S, Liedberg G, Tropé C. In vitro responsiveness of human gastric carcinoma to pentagastrin. Scand J Gastoenterol 1977;12:253-256.

219. Tahara E, Haizuki S. Effect of gastro-entero-pancreatic endocrine hormones on the histogenesis of gastric cancer in rats induced by N-methyl-N'-nitro-N-nitrosoguanidine, with special reference to development of scirrhous gastric cancer. Gann 1975;66:421-426.

220. Ohkura H, Kazuko H, Maruyama K, Kitaoka H, Watanabe S, Kameya T. Gatrin-enhanced tumour growth of a xenotransplantable human gastric carcinoma in nude mice. Jpn J Clin Oncol 1980;10:255-264.

221. Fujita M, Takami M, Usagane M, Nampei S, Taguchi T. Enhancement of gastric carcinogenesis in dogs given N-methyl-N'-nitro-N-nitrosoguanidine following vagotomy. Cancer Res 1979;39:811-816.

222. Morgenstern L. Vagotomy, gastroenterostomy and experimental gastric cancer. Arch Surg 1968;96:920-923.

223. IARC Working Group on the evaluation of carcinogenic risks to humans. Helicobacter pylori. In: Schistosomes, liver flukes, and Helicobacter pylori: views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon: IARC 1994. p. 177-240.

224. Parsonnet J, Freidman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127-1131.

225. Nomura AMY, Stemmerman GN, Chyou P, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma in a population of Japanese-Americans in Hawaii. N Engl J Med 1991;325:1132-1136.

226. Parsonnet J, Freidman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut 1997;40:297-301.

227. Fischer A, Clagett T, McConald JR. Co-existent duodenal ulcer and gastric malignancy. Surgery 1947;21:168-174.

228. Ryan EP, Beal JM. The development of carcinoma of the stomach in patients with duodenal ulcer. Surgery 1957;42:271-275.

229. McGugan JI. Immunochemical studies with synthetic human gastrin. Gastroenterology 1968;54:1005-1011.

230. Berson SA, Yalow RS. Nature of immunoreactive gastrin extracted from tissues of the gastrointestinal tract. Gastroenterology 1971;60:215-222.

231. Mutt V, Jorpes E. Structure of porcine cholecystokinin-pancreozymin. Eur J Biochem 1968;6:156-162.

232. Dimaline R, Dockray GJ. Evolution of the gastrointestinal endocrine system (with special reference to gastrin and CCK). Baillieres Clin Endocrinol Metab The Gut as an Endocrine Organ. 1994;8(1):1-24.

233. Kent Lloyd KC. Gut hormones in gastric function. Baillieres Clin Endocrinol Metab The Gut as an Endocrine Organ. 1994;8(1):111-136.

234. McGuigan JE. Gastric mucosal intracellular localisation of gastrin by immunoflourescence. Gastroenterology 1968;55:315-327.

235. Lees F, Grandjean LC. The gastric and jejunal mucosae in healthy patients with partial gastrectomy. Arch Intern Med 1958;101:943-951.

236. Gjurldsen ST, Myren J, Fretheim B. Alterations of gastric mucosa following a graded partial gastrectomy. Scand J Gastroenterol 1968;3:465-470.

237. Polacek MA, Ellison EH. Parietal cell mass and gastric acid secretion in the Zollinger-Ellison syndrome. Surgery 1966;60(3):606-614.

238. Johnson LR, Aures D, Yuen L. Pentagastrin-induced stimulation of protein synthesis in the gastrointestinal tract. Am J Physiol 1969;217(1):251-254.

239. Johnson LR, Aures D, Håkanson R. Effect of gastrin on the in vivo incorporation of ^{14}C-leucine into protein of the digestive tract. Proc Soc Exp Biol Med 1969;132:996-998.

240. Miller LR, Jacobson ED, Johnson LR. Effect of pentagastrin on gastric mucosal cells grown in tissue culture. Gastroenterology 1973;64:254-267.

241. Lichtenberger L, Miller LR, Erwin DN, Johnson LR. Effect of pentagastrin on adult rat duodenal cells in culture. Gastroenterology 1973;65:242-251.

242. Johnson LR, Guthrie PD. Secretin inhibition of gastrin-stimulated deoxyribonucleic acid synthesis. Gastroenterology 1974;67:601-606.

243. Johnson LR, Chandler AM. RNA and DNA of gastric and duodenal mucosa in antrectomised and gastrin-treated rats. Am J Physiol 1973;224(4):937-940.

244. Johnson LR, Lichtenberger LM, Copeland EM, Dudrick SJ, Castro GA. Action of gastrin on gastrointestinal structure and function. Gastroenterology 1975;68:1184-1192.

245. Johnson LR. New aspects of the trophic action of gastrointestinal hormones. Gastroenterology 1977;72:788-792.

246. Dembinski AB, Johnson LR. Growth of pancreas and gastrointestinal mucosa in antrectomised and gastrin-treated rats. Endocrinology 1979;105:769-773.

247. Sirinek KR, Levine BA, Moyer MP. Pentagastrin stimulated in vitro growth of normal and malignant human colon epithelial cells. Am J Surg 1985;149:35-39.

248. Kusyk CJ, McNiel NO, Johnson LR. Stimulation of growth of a colon cancer cell line by gastrin. Am J Physiol 1986;251:G597-G601.

249. Palmer Smith J, Solomon TE. Effects of gastrin, proglumide, and somatostatin on growth of human colon cancer. Gastroenterology 1988;95:1541-1548.

250. Watson SA, Durrant LG, Crosbie JD, Morris DL. The in vitro growth response of primary human colorectal and gastric cancer cells to gastrin. Int J Cancer 1989;43(4):692-696.

251. McGregor DB, Jones RD, Karlin DA, Romsdahl MM. Trophic effects of gastrin on colorectal neoplasms in the rat. Ann Surg 1982;195:219-223.

252. Winsett OE, Townsend Jr. CM, Glass EJ, Thompson JC. Gastrin stimulates growth of colon cancer. Surgery 1986;99(3):302-307.

253. Alonso M, Galera MJ, Reyes G, Calabuig R, Viñals A, Rius X. Effects of pentagastrin and of the somatostatin analog (SMS 201-995) on growth of CT26 in vivo adenocarcinoma of the colon. Surg Gynecol Obstet 1992;175:441-444.

254. Chu M, Nielsen FC, Franzén L, Rehfeld JF, Holst JJ, Borch K. Effect of endogenous hypergastrinaemia on gastrin receptor expressing human colon carcinoma transplanted to athymic rats. Gastroenterology 1995;109:1415-1420.

255. Brown J, Gallagher ND. A specific gastrin receptor site in the rat stomach. Biochim Biophys Acta 1978;538:42-49.

256. Soll AH, Amirian DA, Thomas LP, Reedy TT, Elasloff JD. Gastrin receptors on isolated canine parietal cells. J Clin Invest 1984;73:1434-1447.

257. Singh P, Rae-Venter B, Townsend Jr. CM, Khalil T, Thompson JC. Gastrin receptors in normal and malignant gastrointestinal mucosa: age-associated changes. Am J Physiol 1985;249:G761-G769.

258. Upp Jr. JR, Singh P, Townsend Jr. CM, Thompson JC. Clinical significance of gastrin receptors in human colon cancers. Cancer Res 1989;49:488-492.

259. Presti ME, Gardner JD. Receptor antagonists for gastrointestinal peptides. Am J Physiol 1993;264:G399-G406.

260. Beauchamp DR, Townsend CM, Singh P, Glass EJ, Thompson JC. Proglumide, a gastrin receptor antagonist, inhibits growth of colon cancer and enhances survival in mice. Ann Surg 1985;202:303-309.

261. Hoosein NM, Kiener PA, Curry RC, Rovati LC, McGilbra DK, Brattain MG. Antiproliferative effects of gastrin receptor antagonists and antibodies to gastrin on human colon carcinoma cell lines. Cancer Res 1988;48:7179-7183.

262. Watson S, Durrant L, Elston P, Morris D. Inhibitory effects of the gastrin receptor antagonist (L-365,260) on gastrointestinal tumour cells. Cancer 1991;68:1255-1260.

263. Sobhani I, Lehy T, Laurent-Puig P, Cadiot G, Ruszniewski P, Mignon M. Chronic endogenous hypergastrinaemia in humans: Evidence for a mitogenic effect on the colonic mucosa. Gastroenterology 1993;105:22-30.

264. Orbuch M, Venzon DJ, Lubensky IA, Weber HC, Gibril F, Jensen RT. Prolonged hpergastrinaemia does not increase the frequency of colonic neoplasia in patients with Zollinger-Ellison syndrome. Dig Dis Sci 1996;41(3):604-613.

265. Kameyama M, Nakamori S, Imaoka S, Yasuda T, Nakano H, Ohigashi H et al. Adjuvant chemo-endocrine chemotherapy with gastrin antagonist after resection of liver metastasis in colorectal cancer. Gan To Kagaku Ryoho 1994;21(13):2169-2171.

266. Palmer Smith J, Wood JG, Solomon TE. Elevated gastrin levels in patients with colorectal cancer and adenomatous polyps. Gastroenterology 1985;92:1646.

267. Seitz JK, Giovannini M, Gauthier A. Elevated gastrin levels in patients with colorectal cancer. J Clin Gastroenterol 1989;11:362-363.

268. Charnley RM, Thomas WM, Stanley J, Morris DL. Serum gastrin concentrations in colorectal cancer patients. Ann R Coll Surg Engl 1992;74:138-141.

269. Watson SA, Durrant LG, Wencyk PM, Watson AL, Morris DL. Intracellular gastrin in human gastrointestinal tumour cells. J Natl Cancer Inst 1991;83:866-871.

270. Hoosein NM, Kiener PA, Curry RC, Brattain MG. Evidence for autocrine growth stimulation of cultured colon tumour cells by a gastrin/cholecystokinin-like peptide. Exp Cell Res 1990;186:15-21.

271. Yapp R, Modlin IM, Kumar RR, Binder HJ, Dubrow R. Gastrin and colorectal cancer. Evidence against an association. Dig Dis Sci 1992;37(4):481-484.

272. Penman ID, El-Omar E, Ardill JE, McGregor JR, Galloway DJ, O'Dwyer PJ et al. Plasma gastrin concentrations are normal in patients with colorectal neoplasia and unaltered following tumour resection. Gastroenterology 1994;106:1263-1270.

273. Wong K, Beardshall K, Waters CM, Calam J, Poston GJ. Postprandial hypergastrinaemia in patients with colorectal cancer. Gut 1991;32:1352-1354.

274. Seitz JF, Giovannini M, Monges G, Sauvan R, Wartelle C, Martin P. Serum gastrin levels in colorectal cancers. Evolution after treatment. Gastroenterol Clin Biol 1992;16(5):385-387.

275. Baldwin GS, Zhang QX. Measurement of gastrin and transforming growth factor alpha messenger RNA levels in colonic carcinoma cell lines by quantitive polymerase chain reaction. Cancer Res 1992;52(8):2261-2267.

276. Finley GG, Koski RA, Melhem MF, Pipas JM, Meisler I. Expression of the gastrin gene in the normal human colon and colorectal adenocarcinoma. Cancer Res 1993;53:2919-2926.

277. Van Solinge WW, Nielsen FC, Friis-Hansen L, Falkmer UG, Rehfeld JF. Expression but incomplete maturation of progastrin in colorectal carcinomas. Gastroenterology 1993;104(4):1099-1107.

278. Singh P, Xu Z, Dai B, Rajaraman S, Rubin N, Dhruva B. Incomplete processing of progastrin expressed by human colon cancer cells: role of noncarboxyamidated gastrins. Am J Physiol 1994;266:G459-G468.

279. Kochman ML, DelValle J, Dickinson CJ, Boland CR. Posttranslational processing of gastrin in neoplastic human colonic tissues. Biochem Biophys Res Commun 1992;189:1165-1169.

280. Nemeth J, Taylor B, Pauwels S, Varro A, Dockray GJ. Identification of progastrin derived peptides in colorectal carcinoma extracts. Gut 1993;34:90-95.

281. Ciccotosto GD, McLeish A, Hardy KJ, Shulkes A. Expression, processing and secretion of gastrin in patients with colorectal carcinoma. Gastroenterology 1995;109:1142-1153.

282. Oscarson J, Håkanson R, Liedberg R, Lundqvist G, Sundler F, Thorell J. Variated serum gastrin concentration. Trophic effects on the gastrointestinal tract of the rat. Acta Physiol Scand 1979;Suppl 475:1-27.

283. Oscarson JEA, Veen HF, Ross JS, Malt RA. Dimethylhydrazine-induced colonic neoplasia: dissociation from endogenous gastrin levels. Surgery 1982;91:525-530.

284. Graffner H, Singh G, Chaudry I, Milsom JW. Omeprazole-induced hypergastrinaemia does not influence growth of colon carcinoma. Dig Dis Sci 1992;37(4):485-489.

285. Pinson DM, Havu N, Sztern MI, Mattsson H, Looney GE, Kimler BF, Hurwitz A. Drug-induced hypergastrinaemia: absence of trophic effects on colonic carcinoma in rats. Gastroenterology 1995;108:1068-1074.

286. Tatsuta M, Yamamura H, Noguchi S, Iishi H, Taniguchi H. Gastrin has no promoting effect on chemically induced colonic tumours in Wistar rats. Eur J Cancer Clin Oncol 1985;21(6):741-744.

287. Ryberg B, Axelson J, Håkanson R, Sundler F, Mattsson H. Trophic effects of continuous infusion of [Leu^{15}]-Gastrin in the rat. Gastroenterology 1990;98:33-38.

288. Baldwin GS, Whitehead RH. Gut hormones , growth and malignancy. Baillieres Clin Endocrinol Metab The Gut as an Endocrine Organ. 1994;8(1):185-214.

289. Suzuki H, Matsumoto K, Terashima H. Serum levels of gastrin in patients with colorectal neoplasia. Dis Colon Rectum 1988;31:716-717.

290. Kikendall JW, Glass AR, Sobin LH, Bowen PE. Serum gastrin is not higher in subjects with colonic neoplasia. Am J Gastroenterol 1992;87(10):1394-1397.

291. Domellof L, Reddy BS, Weisburger JH. Microflora and deconjugation of bile acids in alkaline reflux after partial gastrectomy. Am J Surg 1980;140:291-295.

292. Allan JG, Gerskowitch VP, Russell RI. The role of bile acids in the pathogenesis of post-vagotomy diarrhoea. Br J Surg 1974;61:516-518.

293. Moorehead RJ, Campbell GR, Donaldson JD, McKelvey STD. Relationship between duodenal bile acids and colorectal neoplasia. Gut 1987;28:1454-1459.

294. Hill MJ. Bile, bacteria and bowel cancer. Gut 1983;24:871-875.

295. Owen RW, Dodo M, Thompson MH, Hill MJ. The faecal ratio of lithocholic acid to deoxycholic acid may be an important aetiological factor in colorectal cancer. Eur J Cancer Clin Oncol 1983;19:1307.

296. Owen RW, Henley PJ, Day D, Thompson MH, Hill MJ. Faecal steroids and colorectal cancer: bile acid profiles in low and high risk groups. Br J Surg 1985;72 Suppl:S137.

297. Hepner GW, Hoffman AF, Maagelada JR, Szceranik PA, Klein PD. Increased bacterial degradation of bile acids in cholecystectomised patients. Gastroenterology 1974;66:556-564.

298. Vernick LJ, Kuller LH. Cholecystectomy and right sided colon cancer: an epidemiological study. Lancet 1981;ii:381-383.

299. Turnbull PRG, Smith AH, Isbister WH. Cholecystectomy and cancer of the large bowel. Br J Surg 1981;68:551-553.

300. Linos DA, O'Fallen WM, Beart RW, Beard CM, Dockerty MB, Kurland LT. Cholecystectomy and cancer of the colon. Lancet 1981;ii:379-381.

301. Moorehead RJ, McKelvey STD. Cholecystectomy and colorectal cancer. Br J Surg 1989;76:250-253.

302. Bundred NJ, Whitfield BCS, Stanton E, Prescott RJ, Davies GC, Kingsnorth AN. Cholecystectomy, cholelithiasis and colorectal carcinoma. J R Coll Surg Edinb 1985;30:115-117.

303. Gafa M, Sarli L, Sansebastiano G, Longinotti E, Carreras F, Pietra N et al. Prevention of colorectal cancer: role of association between gallstones and colorectal cancer. Dis Colon Rectum 1987;30:692-696.

304. Mazzanti R, Bechi P, Arena U, Arcangeli G, Gentilini P. Late complications of partial gastrectomy. [abstract] Gut 1986;27:A1238.

305. Chomchai C, Bhadrachari N, Nigro ND. The effect of bile on the induction of experimental intestinal tumours in rats. Dis Colon Rectum 1974;17:310-312.

306. Narisawa T, Magadia NE, Weisburgher JH, Wynder EL. Promoting effect of bile acid on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. J Natl Cancer Inst 1974;55:1093-1097.

307. Reddy BS, Watanabe K, Weisburgher JH, Wynder EL. Promoting effect of bile acids in colon carcinogenesis in germ-free and conventional F-344 rats. Cancer Res 1977;37:3238-3243.

308. Summerton J, Flynn M, Cooke T, Taylor I. Bile acid receptors in colorectal cancer. Br J Surg; 1983;70:549-551.

309. Lamberts R, Creutzfeld W, Struber HG, Brunner G, Solcia E. Long-term omeprazole therapy in peptic ulcer disease: gastrin, endocrine cell growth, and gastritis. Gastroenterology 1993;104(5):1356-1370.

310. Klinkenberg-Knol EC, Festen HP, Jansen JB, Lamers CB, Nelis F, Snel P et al. Long-term treatment with omeprazole for refractory reflux oesophagitis: efficacy and safety. Ann Intern Med 1994;121(3):161-167.

311. Penman ID, El-Omar E, McGregor JR, Hillan KJ, O'Dwyer PJ, McColl KEL. Omeprazole inhibits colorectal carcinogenesis induced by azoxymethane in rats. Gut 1993;34:1559-1565.

312. Tobi M, Chintalapani S, Goo R, Maliakkal B, Reddy J, Lundqvist M et al. Omeprazole inhibits growth of cancer cell line of colonic origin. Dig Dis Sci 1995;40(7):1526-1530.

313. Regårdh CG, Gabrielsson M, Hoffman KJ, Löfberg I, Skånberg I. Pharmacokinetics and metabolism of omeprazole in animals and man - an overview. Scand J Gastroenterol 1985;20 Suppl 108:79-94.

314. Kaunitz JD, Sachs G. Identification of a vanadate sensitive potassium-dependant proton pump from rabbit colon. J Biol Chem 1986;26:14005-14010.

315. LaMont JT, O'Gorman TA. Experimental colon cancer. Gastroenterology 1978;75:1157-1169.

316. Snyderwine EG, Schut HAJ, Adamson RH, Thorgeirsson UP, Thorgeirsson SS. Metabolic activation and genotoxicity of heterocyclic arylamines. Cancer Res 1992;52:2099-2102.

317. Guengerich FP. Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer Res 1988;48:2946-2954.

318. Turesky RJ, Lang NP, Butler MA, Teitel CH, Kadlubar FF. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. Carcinogenesis 1991;10:1839-1845.

319. Diaz D, Fabre I, Daujat M, Saint Aubert B, Bories P, Michel H, Maurel P. Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. Gastroenterology 1990;99:737-747.

320. McDonnell WM, Scheiman JM, Traber PG. Induction of cytochrome P450IA genes(CYP1A) by omeprazole in the human alimentary tract. Gastroenterology 1992;103:1509-1516.

321. Wattenberg LW, Leong JL. Inhibition of the carcinogenic action of benzo[a]pyrene by flavones. Cancer Res 1970;30:1922-1925.

322. Anderson LM, Seetharam S. Protection against tumorigenesis by 3-methylcholanthrene in mice by b-naphthoflavone as a function of inducibility of methylcholanthrene metabolism. Cancer Res 1985;45:6384-6389.

323. Sohn OS, Ishizaki H, Yang CS, Fiala ES. Metabolism of azoxymethane, methylazoxymethanol and N-nitrosodimethylamine by cytochrome P450IIE1. Carcinogenesis 1991;12(1):127-131.

324. Anderson JR. Tumours: II. The aetiology of cancer. In: Anderson JR, editor. Muir's Textbook of Pathology. 12th ed. London: Edward Arnold; 1985. p. 13.1-13.38.

325. Gillis CR, Hole DJ, Graham A, Lamont DW, Ramage S. UK, Scotland, West. In: Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay J, Powell J, editors. Cancer Incidence in Five Continents - Volume VI. Lyon: IARC 1992. p. 826.

326. Drewinko B, Romsdahl MM, Yang LY, Ahearn MJ, Trujillo JM. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res. 1976;36:467.

327. Erickson LC, Laurent G, Sharkey NA, Kohn KW. DNA cross-linking and monoadduct repair in nitrosurea-treated human tumour cells. Nature 1980;288:727-729.

328. Siegel D, Gibson NW, Preusch PC, Ross D. Metabolism of mytomycin C by DT-Diaphorase: role in mytomycin C-induced DNA damage and cytotoxicity in human colon carcinoma cells. Cancer Res. 1990;50:7483.

329. Mihaly GW, Prichard PJ, Smallwood RA, Yeomans ND, Louis WJ. Simultaneous high-performance liquid chromatographic analysis of omeprazole and its sulphone and sulphide metabolites in human plasma and urine. J Chromatogr 1983;278:311-319.

330. Romano M, Razandi M, Ivey KJ. Cimetidine and omeprazole directly protect human gastric epithelial cells in vitro [abstract]. Gastroenterology 1987;92:1599.

331. Francavilla A, Panella C, Polimeno L, Di Leo A, Makowka L. Barone M et al. Effect of cimetidine, ranitidine, famotidine and omeprazole on hepatocyte proliferation in vitro. J Hepatol 1989;8:32-41.

332. Cuzick J. A Wilcoxon-type test for trend. Stat Med 1985;4:87-90.

333. Altman DG. Practical statistics for medical research. 1st ed. London: Chapman & Hall; 1991.

334. Ardill JES. Radioimmunoassay of GI hormones. Clin Endocrinol Metab 1979;8:265-280.

335. Justin TA, Watson SA, Morris T, Robinson G, Sykes R, Hardcastle JD. Gastrin receptor expression in chemically induced colorectal carcinogenesis [abstract]. Gastroenterology 1994;35 Suppl 3:S64.

336. Ekbom A, Lundegårdh G, McLaughlin JK, Nyrén O. Relation of vagotomy to subsequent risk of lung cancer: population based cohort study. BMJ 1998;316:518-519.

337. Tredaniel J, Boffetta P, Buiatti E, Saracci R, Hirsch A. Tobacco smoking and gastric cancer: review and meta-analysis. Int J Cancer 1997;72:565-573.

338. Hansson LE, Baron J, Nyren O, Bergstrom R, Wolk A, Adami HO. Tobacco, alcohol and the risk of gastric cancer. A population-based case-control study in Sweden. Int J Cancer 1994;57:26-31.

339. Engeland A, Andersen A, Haldorsen T, Tretli S. Smoking habits and risk of cancers other than lung cancer: 28 years follow-up of 26,000 Norwegian men and women. Cancer Cause Control 1996;7:497-506.

340. Danesh J, Appleby P, Peto R. How often does surgery for peptic ulceration eradicate Helicobacter pylori? Systematic review of 36 studies. BMJ 1998;316:746-747.

341. Hurwitz A, Kimler BF, Pinson DM. Remodelling the hypergastrinaemia model [Letter reply] Gastroenterology 1995;109:1407-1408.

342. Albanes D. Total calories, body weight, and tumor incidence in mice. Cancer Res 1987;47:1987-1992.

343. Kritchevsky D. Influence of caloric restriction and exercise on tumorogenesis in rats. Proc Soc Exp Biol Med 1990;193:35-38.

344. Rehfeld JF. Gastrin and colorectal cancer: a never-ending dispute? Gastroenterology 1995;108:1307-1309.

345. Rehfeld JF, Nielsen FC. Remodelling the hypergastrinaemia model [Letter reply] Gastroenterology 1995;109:1408.

346. Creutzfeldt W, Lamberts R. Is hypergastrinaemia dangerous to man? Scand J Gastroenterol 1991;26(Suppl 180):179-191.

347. Bruley des Varannes S, Levy P, Lartigue S, Dellatolas F, Lemaire M, Galmiche JP. Comparison of lansoprazole with omeprazole on 24-hour intragastric pH, acid secretion and serum gastrin in healthy vounteers. Aliment Pharmacol Ther 1994;8:309-314.

348. Curi-Pedrosa R, Daujat M, Pichard L, Ourlin JC, Clair P, Gervot L et al. Omeprazole and lansoprazole are mixed inducers of CYP1A and CYP3A in human hepatocytes in primary culture. J Pharmacol Exp Ther 1994;269:384-392.

349. Tucker TG. The interaction of proton pump inhibitors with cytochromes P450. Aliment Pharmacol Ther 1994;8(Suppl 1):33-38.

350. Arnold R. Safety of proton pump inhibitors - an overview. Aliment Pharmacol Ther 1994;8(Suppl 1):65-70.

* References cited in :

Wangensteen OH, Wangensteen SD. History of Gastric Surgery: Glimpses into its early and more recent past. In: Nyhus LM, Wastell C, editors. Surgery of the Stomach and Duodenum. 3rd ed. Boston: Little, Brown and Co; 1977. p. 235-256.

† References cited in :

Herrington JL Jr.. Historical Aspects of Gastric Surgery. In: Scott HW Jr., Sawers JL, editors. Surgery of the Stomach, Duodenum and Small Intestine. 2nd ed. Boston: Blackwell Scientific Publications; 1992. p. 1-28.

Presentations and

Publications from Thesis

1. Letter

Duncan J.R., McGregor J.R.,O'Dwyer P.J.

Death from malignant disease after surgery for duodenal ulcer.

Gut 1995; **36**: 475.

2. Abstract

Duncan J.R., Penman I.D., Plumb J., O'Dwyer P.J., McGregor J.R.

Omeprazole is not cytotoxic to colorectal cancer cells.

European Journal of Surgical Oncology BASO Suppl. 1996

Presented to :

British Association of Surgical Oncology

51st BASO Scientific Meeting,

Royal College of Surgeons of England, London.

23rd November 1995.

3. Presentation

Duncan J.R., Penman I.D., Oein K., O'Dwyer P.J., McGregor J.R.

Omeprazole -induced hypergastrinaemia and colorectal carcinogenesis.

Scott. Med. J. December 1996; 41(6): 188.

4. Presentation

Duncan J.R., Hole D., O'Dwyer P.J., McGregor J.R.

Colorectal cancer risk is not increased following surgery for benign peptic ulcer.

Scott. Med. J. December 1996; 41(6): 188.

Both Presented to :

Joint Meeting of the Caledonian Society of Gastroenterology & the Scottish Society of Coloproctology

Western Infirmary, Glasgow

7th June 1996

5. Abstract

Duncan J.R., Hole D., O'Dwyer P.J., McGregor J.R.

Colorectal cancer risk is not increased following surgery for benign peptic ulcer.

Br. J. Surg. May 1997; 84 Suppl. 1: 58.

Presented as a Poster at :

Association of Surgeons of Great Britain and Ireland,

Bournemouth, April 1997.

6. Abstract

Duncan J.R., Hole D., O'Dwyer P.J., McGregor J.R.

Cancer incidence after peptic ulcer surgery.

Endoscopy 1997; 29(7): E36.

Presented as a Poster at :

6th United European Gastroenterology Week,

Birmingham, U.K., 18th - 23rd October 1997.

7. Presentation

Omeprazole-induced hypergastrinaemia and colorectal carcinogenesis and cancer risk following surgery for peptic ulcer.

Presented to :

Tenovus-Scotland - Meet the Researchers

Royal College of Physicians and Surgeons, Glasgow.

6th February 1996.

8. Presentation

The legacy of peptic ulcer surgery

Presented to :

Southern General Clinical Society

Southern General Hospital, Glasgow.

16th June 1998.