# A STUDY OF THE UPPER GASTROINTESTINAL COMPLICATIONS OF RENAL TRANSPLANTATION

Robert Paul Teenan.

A thesis submitted to the University of Glasgow

for the degree of Doctor of Medicine

from

The University Department of Surgery,

Western Infirmary Glasgow.

May 1990



ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

TABLE OF CONTENTS


| | PAGE |
|---|---|
| Title Page | 1 |
| Table of Contents | 2 |
| List of Tables | 9 |
| List of Figures | 11 |
| Aknowledgements | 12 |
| Declaration | 13 |
| Summary | 14 |

**PART I: INTRODUCTION AND METHODS**

**CHAPTER 1**

**PEPTIC ULCER DISEASE IN RENAL TRANSPLANT RECIPIENTS**

| | |
|---|---|
| 1. Historical Review | 19 |
| 2. The Role of Acid Secretion | 23 |
| 3. The Role of Corticosteroids | 30 |

**CHAPTER 2**

**INFECTIVE AGENTS IN THE UPPER GASTROINTESTINAL TRACT**

| | |
|---|---|
| 1. Helicobacter pylori | 36 |
| Microbiology | 36 |
| Role in Gastritis and Non Ulcer Dyspepsia | 37 |
| Role in Peptic Ulceration | 40 |
| Pathogenesis and Ultrastructural Changes | 43 |
| Immunology | 45 |

PAGE

Epidemiology 47

Helicobacter Pylori in Immunocompromised Patients 49

2. Cytomegalovirus 50

Introduction 50

Epidemiology 51

Clinical Features 51

Viral Latency 51

Cytomegalovirus in Transplant Recipients 51

Cytomegalovirus in the Gastrointestinal Tract 52

Pathogenesis 55

Conclusions 56

3. Herpes simplex 57

Infection and Epidemiology 57

Viral Latency 58

Herpes Simplex in Transplant Recipients 58

Herpes Simplex in the Gastrointestinal Tract 59

4. Immunosuppression in Transplant Recipients 61

Introduction 61

Cyclosporine 62

PAGE

**CHAPTER 3**

**METHODS**

1. Summary 64

2. Introduction

Immunohistochemistry 64

Urease Slide Test 67

Histology of Helicobacter pylori 68

3. Clinical Methods

Transplant Recipients 68

Control Tissue 70

Processing of Biopsy Specimens 70

Urease Slide Test 71

CMV Antibody Titres 71

Interpretation of Histological Sections 71

Mucosal T Lymphocyte Subsets 72

Histological Assessment of the Gastric Mucosa 72

Histological Assessment of the Duodenal Mucosa 73

Cresyl Violet Staining Technique 73

Immunohistochemistry Staining Techniques 74

Statistical Analysis 75

PAGE

**PART II: RESULTS**

**CHAPTER 4**

**CLINICAL RESULTS**

1. Results

Patient Recruitment 78

Non Attenders 78

Study Group 80

Comparison of Groups 80

Analysis of Symptoms and H2 Receptor Antagonist Use 80

Endoscopic Findings 85

Histological Assessment of the Gastric Mucosa 85

Histological Assessment of the Duodenal Mucosa 85

Summary 86

2. Discussion

Peptic Ulceration 89

Duodenitis 90

Gastritis 92

Oesophagitis 93

Duodenal Polyps 93

Symptomatic Dyspepsia 94

Relationship to Renal Function and Immunosuppression 96

Conclusions 97

PAGE

**CHAPTER 5**
**HELICOBACTER PYLORI**

1. Results

Prevalence of Helicobacter pylori 100

Comparison of Detection Methods 100

Relationship to Endoscopic and Histological Abnormalities 103

Relationship to Atrophy and Metaplasia 103

Relationship to Renal Function, Immunosuppression and Age 106

Relationship to Time Elapsed Since Transplantation 106

Summary 106

2. Discussion

Prevalence 109

Detection of Helicobacter Pylori 111

Distribution and Relationship to GI Pathology 111

Relationship to Dyspepsia 113

Relationship to Renal Function and Immunosuppression 114

Conclusions 115

**CHAPTER 6**

**CYTOMEGALOVIRUS AND HERPES SIMPLEX**

1. Cytomegalovirus Results

Prevalence of Cytomegalovirus 118

Distribution Within the Gatroduodenal Mucosa 120

Relationship to Pathology and Dyspepsia 120

Relationship of Gastroduodenal Cytomegalovirus to Systemic CMV Infection 123

PAGE

Relationship to Renal Function and Immunosuppression 123

Relationship to Age and Time Elapsed Since Transplantation 123

2. Herpes Simplex Virus 124

3. Discussion: Cytomegalovirus

Prevalence 128

Association with Pathology and Dyspepsia 128

Distribution Within the Gastroduodenal Mucosa 130

Comparison of Detection Methods 131

Relationship of Gastroduodenal Cytomegalovirus to Systemic CMV infection 132

Relationship to Immunosuppression and Renal Function 133

Conclusions 133

4. Discussion: Herpes simplex. 134

**CHAPTER 7**

**MUCOSAL T LYMPHOCYTE SUBSETS**

1. Results

Introduction 138

Transplant Recipients and Controls 138

Relationship to Immunosuppression and Renal Function 138

Influence of Gastritis and Duodenitis 140

Influence of Helicobacter Pylori 140

Influence of Cytomegalovirus 143

PAGE

2. Discussion

T Lymphocyte Subsets in Normal Gastric Mucosa 149

T Lymphocyte Subsets in Normal Duodenal Mucosa 149

Mucosal T Lymphocyte Subsets in Transplant Recipients 150

Relationship to Immunosuppression and Renal Function 151

The Influence of Helicobacter pylori 151

The Influence of Cytomegalovirus 152

Conclusions 153

**PART III : CONCLUSIONS**

**CHAPTER 8**

**CONCLUSIONS**

The Prevalence of Upper Gastrointestinal Pathology and Dyspepsia 157

The Role of Helicobacter Pylori 159

The Role of Cytomegalovirus 160

The Role of Herpes simplex 163

Mucosal T Lymphocyte Subsets 163

Final Conclusions 164

**PART IV : APPENDICES**

Appendix 1 Study Group 166

Appendix 2 Non Attenders 174

Appendix 3 T Lymphocyte subsets 175

**REFERENCES** 179

## LIST OF TABLES


| | | PAGE |
|---|---|---|
| **TABLE 1** | Details of Patients Excluded | 79 |
| **TABLE 2** | Comparison of Non Attenders and Study Group | 82 |
| **TABLE 3** | Comparison of Symptomatic and Asymptomatic Patients | 83 |
| **TABLE 4** | Details of Pre and Post Transplant Symptoms | 84 |
| **TABLE 5** | Endoscopic Abnormalities in the Study Group | 87 |
| **TABLE 6** | Histological Abnormalities in the Gastric Mucosa | 88 |
| **TABLE 7** | Histological Abnormalities in the Duodenal Mucosa | 88 |
| **TABLE 8** | Prevalence and Distribution of Helicobacter Pylori | 101 |
| **TABLE 9** | Association of Helicobacter Pylori with Upper Gastrointestinal Symptoms | 102 |
| **TABLE 10** | Relationship of Helicobacter Pylori to Endoscopic and Histological Abnormalities | 104 |
| **TABLE 11** | Relationship of Helicobacter Pylori to Atrophy and Metaplasia | 105 |
| **TABLE 12** | Relationship of Helicobacter Pylori to Renal Function, Immunosuppression and Age | 108 |
| **TABLE 13** | The prevalence of Cytomegalovirus in the Gastroduodenal Mucosa of Transplant Recipients and Control Subjects | 119 |
| **TABLE 14** | Distribution of Cytomegalovirus in the Gastroduodenal Mucosa | 121 |
| **TABLE 15** | Relationship of Cytomegalovirus to Gastroduodenal Pathology | 122 |
| **TABLE 16** | Relationship of Cytomegalovirus to Symptomatic Dyspepsia | 125 |
| **TABLE 17** | Relationship of Gastroduodenal Cytomegalovirus to Systemic CMV Infection | 126 |
| **TABLE 18** | Relationship of Cytomegalovirus to Renal Function and Immunosuppression | 127 |

PAGE

TABLE 19 T Lymphocyte Subsets in Transplant Recipients and Controls 139

TABLE 20 T Lymphocyte Subsets, Renal Function and Immunosupression 141

TABLE 21 T Lymphocyte Subsets, Gastritis and Duodenitis 142

TABLE 22 T Lymphocyte Subsets and Helicobacter Pylori (a) 144

TABLE 23 T Lymphocyte Subsets and Helicobacter pylori (b) 145

TABLE 24 T Lymphocyte Subsets and Cytomegalovirus 146

TABLE 25 T Lymphocyte Subsets and Duodenal Cytomegalovirus 147

TABLE 26 Gastric T Lymphocyte Subsets and CMV Serology 148

TABLE 27 Duodenal T Lymphocyte Subsets and CMV Serology 148

## LIST OF FIGURES


| | | AFTER PAGE |
|---|---|---|
| FIGURE 1 | Transmission electron micrograph of H pylori. | 36 |
| FIGURE 2 | Scanning electron micrograph of H pylori. | 36 |
| FIGURE 3 | Single antibody staining technique | 65 |
| FIGURE 4 | Double antibody staining technique | 65 |
| FIGURE 5 | Peroxidase anti peroxidase technique | 65 |
| FIGURE 6 | Avidin biotin staining technique | 66 |
| FIGURE 7 | Urease slide test | 67 |
| FIGURE 8 | H&E section of H pylori | 68 |
| FIGURE 9 | Cresyl fast violet section of H pylori | 68 |
| FIGURE 10 | Normal gastric mucosa | 72 |
| FIGURE 11 | Gastritis (Grade 3) | 72 |
| FIGURE 12 | Gastritis (Grade 5) | 72 |
| FIGURE 13 | Normal duodenal mucosa | 73 |
| FIGURE 14 | Duodenitis ( Grade 2) | 73 |
| FIGURE 15 | Cytomegalovirus in duodenal epithelium (H&E) x40 | 118 |
| FIGURE 16 | Cytomegalovirus in duodenal epithelium (Immunohistochemistry) x25 | 118 |
| FIGURE 17 | Cytomegalovirus in duodenal epithelium (Immunohistochemistry) x40 | 118 |
| FIGURE 18 | Duodenal Leu 3 and creatinine | 140 |
| FIGURE 19 | Duodenal Leu 3:Leu 2 creatinine | 140 |
| FIGURE 20 | Duodenal Leu 3:Leu 2 and urea | 140 |

**AKNOWLEDGEMENTS**

In a thesis such as this, involving many disciplines, many people are necessarily involved. Two people in particular, however, require special consideration. Firstly, I must extend my thanks to Dr Margaret Burgoyne for her invaluable assistance in interpreting the histological material contained in this thesis, and also extend my thanks to my adviser Mr WR Murray for his tolerance during the clinical phase of the work and for his advice during the writing of this thesis.

I am also indebted to Dr I Brown and Dr E McCruden from the Departments of Pathology and Virology, to Dr JD Briggs, the staff and most importantly, the patients of the Renal Unit. Finally I am grateful to the Trustees of the Kinnear Brown Trust who provided funding for the project and to Miss Yvonne Galbraith who typed the manuscript.

## DECLARATION

I declare that the the composition of this thesis is entirely my own work and has not previously been submitted for consideration for a higher degree.

The work was performed while I was a Research Registrar in the University Department of Surgery in the Western Infirmary, Glasgow and was continued while a Surgical Registrar in Gartnavel General Hospital, Glasgow. The design of the study described in this thesis was my own work and I was solely responsible for performing the clinical work of this study

The histological staining was performed by the laboratory staff in the Department of Pathology. All of the histological material was assessed independently by myself and by Dr M Burgoyne.

The analysis and interpretation of the data is entirely my responsibility.

## SUMMARY

The purpose of the work contained in this thesis is to investigate the aetiology of upper gastrointestinal disease in renal transplant recipients. It has been recognised for almost thirty years that transplant recipients suffer from a high prevalence of peptic ulceration and a high incidence of the complications of peptic ulceration. The aetiology of this problem remains unclear despite many studies which have attempted to define a cause.

These studies have concentrated primarily on the role of gastric acid secretion and the contribution of factors such as hypercalcaemia hypergastrinaemia and corticosteroids. Some increase in gastric acid secretion has indeed been demonstrated although these differences have not been consistent and are not markedly different from the pattern of gastric acid secretion in patients on haemodialysis. Similarly the importance of corticosteroids remains unclear.

In this thesis the specific aetiological factors studied were Helicobacter pylori, Cytomegalovirus and Herpes Simplex virus. Helicobacter pylori has been the cause of much interest in the field of peptic ulceration over the past eight years and its role in the aetiology of peptic ulceration and gastritis is a source of continuing debate, although the organism has not been previously investigated in transplant recipients. Cytomegalovirus has been implicated in case reports and uncontrolled series as a cause of peptic ulceration in transplant recipients. However the prevalence of the virus in the gastrointestinal tract of normal individuals is unknown and, because of this, its role as a pathogen in transplant recipients is still to be defined. Lastly Herpes simplex has been suggested as a cause of peptic ulceration in the general population although this is

based on indirect evidence and there are no reports of the isolation of the virus from peptic ulcers. Herpes simplex has been identified in association with oesophagitis in both immunocompetent and immunosuppressed individuals but has not been reported in the gastroduodenal mucosa except on rare occassions.

The study described in this thesis was performed on an unselected group of renal transplant recipients and on control tissue from non transplant patients. The study group underwent upper gastrointestinal endoscopy at between two and four months after transplantation. All endoscopic abnormalities were documented, and biopsy material was obtained from the gastroduodenal mucosa and stored for subsequent laboratory analysis.

The biopsy material was examined histologically to assess the degree of gastritis and duodenitis and to detect the presence of Helicobacter pylori. The presence of Cytomegalovirus and Herpes Simplex was determined by immunohistochemistry. T lymphocyte subpopulations were assessed in the gastroduodenal mucosa of transplant recipients and control patients by immunohistochemistry in an attempt to elucidate the local immunological response to infection particularly with Helicobacter pylori.

Symptomatic dyspepsia was identified in 60% of the study group. Peptic ulceration was present in 12% and a striking feature was the high prevalence of mucosal inflammatory lesions without ulceration. Duodenitis was identified in 48% and gastritis in 30%. In total 72% of the study group had one or more abnormality of the upper GI tract.

Helicobacter pylori was identified in 48% and was strongly associated with gastritis, with gastric ulceration and with symptomatic dyspepsia. There was a tendency for Helicobacter infection to be associated with a higher serum urea and creatinine and with a higher prednisolone dose

although these differences did not achieve statistical significance. Infection with Helicobacter pylori was independent of age and time elapsed since transplantation.

Cytomegalovirus was identified in 48% of the study group, but was only present in 11% of the biopsies from the control group. Infection was significantly associated with duodenitis, but no association could be found with other pathological processes or with symptomatic dyspepsia. Cytomegalovirus was not related to renal function or immunosuppression and was independent of age and time elapsed since transplantation.

Herpes simplex could not be identified in any of the biopsy material from either the study group or the control group and could not implicated in any disease process in the upper gastrointestinal tract.

Analysis of mucosal T lymphocyte subsets revealed a tendency towards an increase in the the Leu2 subset associated with Helicobacter pylori infection, but this did not achieve statistical significance.

# PART I

# INTRODUCTION AND METHODS

# CHAPTER 1

## A. PEPTIC ULCERATION IN TRANSPLANT RECIPIENTS

## B. THE ROLE OF ACID SECRETION

## C. THE ROLE OF CORTICOSTERODS

## PEPTIC ULCER DISEASE IN RENAL TRANSPLANT RECIPIENTS

Peptic ulceration and related pathologies such as gastritis and duodenitis are known to occur with increased frequency in transplant recipients. Some studies have reported the prevalence of peptic ulcer to be as high as 22% (1). In the early days of renal transplantation a high prevalence of peptic ulceration and a high incidence of the complications of peptic ulceration was recognised. In 1969 Moore & Hume reported on 14 peptic ulcers in 113 transplant recipients, a prevalence of 12% (2). Twelve of these patients presented with upper GI haemorrhage resulting in a fatal outcome in seven (58%). Two years later Libertino et al reported on 6 peptic ulcer haemorrhages, occurring in 184 transplant recipients with a fatal outcome in 5 (83%), confirming the high mortality in this group of patients (3). In the same year Hadjiyanakis reported 16 peptic ulcers in 139 transplant recipients with haemorrhage in 6 patients all of whom died (4).

The magnitude of the problem led many transplant centres to consider routine vagotomy prior to transplantation in all patients. Other centres adopted a more selective policy, advocating vagotomy only in those with a previous history of peptic ulceration or those demonstrated to have a peptic ulcer prior to transplantation (5). Other groups advocated gastric ulcer surgery in patients with gastric acid hypersecretion (6).

During the following decade upper gastrointestinal endoscopy became widely available as an investigative technique and, in addition to peptic ulcers, some of these patients were demonstrated to suffer from gastritis and duodenitis (7,8). There were also

continued reports of peptic ulcer haemorrhage occurring in between 7 and 25% of patients although with an improved mortality, perhaps reflecting an improvement in the clinical condition of transplant recipients, or improvements in the management of peptic ulcer haemorrhage (5,7).

More recently there have been suggestions that the problem is decreasing in importance. Knechtle et al demonstrated a fall in the prevalence of peptic ulcer from 10.3% in the period 1965-1974 to 5.6% in the period 1975-1984, accompanied by a fall in mortality from 40% to 23% (9). The same group also reported a decreasing incidence of peptic ulcer perforation over the same period of time (10). In 1984 Cohen reported only 8 peptic ulcers in a population of 573 renal transplant recipients (1.3%) (11). This apparent decrease has not been uniformly reported however and one recent publication from 1989 has reported a prevalence of 24% in an unselected group of transplant recipients (12). If the improved diagnostic accuracy of endoscopy is considered, however, the prevalence of peptic ulceration would appear to be decreasing and certainly the incidence of complications is decreasing.

The development of peptic ulceration and related conditions appears to occur fairly rapidly following transplantation. Many of the reports discussed above were retrospective and precise details of the timescale are not available. Petersen et al reported GI haemorrhage at a mean of 60 days post-transplantation (13), and Knechtle reported 25% of perforations occuring within 30 days of transplantation, although in this report the range was wide (4 days to 10 years) (10). Prospective studies have demonstrated a

remarkably rapid development of peptic ulceration often within days of transplantation. Timoney et al demonstrated 5 peptic ulcers by endoscopy at a mean of 13 days post-transplant (range 8-22 days) (12). Schiessel et al excluded patients with gastroduodenal lesions and performed endoscopy on the remaining patients after 3 days and again at 4 weeks (7). At the 3 day endoscopy they found 9 ulcers or erosions in 55 patients and at 4 weeks identified lesions in a further 5 patients. It is perhaps difficult to explain the development of these lesions within such a short period of time, although it is possible that operative stress and high dose immunosuppression are important factors. It is also possible that the aetiology of ulceration in the immediate post-transplant period is different from the aetiological factors producing ulceration several months after transplantation.

A further possible explanation is that many of these lesions were present prior to transplantation and are indicative of the high prevalence of upper gastrointestinal lesions which are known to occur in patients with renal failure (14,15). Alijani et al reported upper GI lesions in 10 of 13 patients (76%) who were endoscoped immediately prior to transplantation (16). This, however, would not explain the findings reported above by Schiessel where patients with known upper gastrointestinal disease were excluded from the study (7).

Elucidating the aetiology of peptic ulceration is complicated by the many variables present in the transplant population when compared with normal controls, or the same patients prior to transplantation. The possible aetiological factors proposed have fluctuated in popularity along with changing theories as to the

pathogenesis of peptic ulceration in the general population. In the 1960's and 1970's attention was focussed primarily on gastric acid secretion (1,5) and factors which might affect gastric acid secretion such as hypercalcaemia and steroid administration. This latter factor has also been implicated because of its effects on the gastric mucosal barrier (17). More recently viruses, particularly cytomegalovirus and Herpes simplex, have been implicated (8,11,18,19) and it would perhaps now be relevant to consider Helicobacter pylori as a further possible aetiological agent. The importance of these factors, acid secretion and steroid administration will be discussed now and infective agents will be discussed in the following chapter.

## THE ROLE OF ACID SECRETION

Early in the history of renal transplantation it was established that gastric acid secretion was increased in transplant recipients when compared with the normal population. This was obviously in keeping with accepted theories as to the pathogenesis of peptic ulceration in the general population and the knowledge that corticosteroids increase the parietal cell mass and thus increase gastric acid secretion (20,21).

Canavan et al studied gastric acid secretion in 10 patients prior to transplantation and repeated the studies in the same patients 3 months after transplantation (5). A standard pentagastrin test demonstrated elevated basal acid output (BAO) and maximal acid output (MAO) in haemodialysis patients but with no significant rise after transplantation. Maximal acid concentration was also higher and did not change significantly after transplantation.

A similar study was performed by Chisholm (1). Twenty five patients underwent stimulated gastric secretion studies either by histamine infusion or pentagastrin before and after transplantation. This study demonstrated a non-significant rise in BAO following transplantation in men and women and a significant rise in peak acid output (PAO) in men only, 6 weeks after transplantation. The authors however could find no correlation between this rise and an increased risk of developing peptic ulceration and they concluded that gastric secretion studies should not be used to select patients for prophylactic vagotomy.

More recent work by Doherty studied PAO in dialysis and transplant patients (22). This study reported elevated PAO in 42% of

36 dialysis patients and in 29% of 38 transplant recipients. The same paper reported a progressive fall in PAO with time following transplantation, which was independent of age but not steroid dosage.

It seems clear from the above reports that increased acid secretion in transplant recipients has to be interpreted in the light of gastric acid secretion in chronic renal failure and during haemodialysis. It is known that patients with chronic renal failure and patients undergoing haemodialysis have an increased risk of developing peptic ulceration. Shepherd demonstrated 9 ulcers in a group of 15 haemodialysis patients(60%) (14), and Ventkatesweran found 6 ulcers in 13 haemodialysis patients (46%) (15). This obviously represents a much higher prevalence of peptic ulcer than in any of the reports relating to transplant recipients. These high percentages have not been borne out by other studies however. Chisholm found only one definite duodenal ulcer and one equivocal abnormality in 35 haemodialysis patients (5.7%) (1), and Gordon found 5 duodenal ulcers in 55 patients (9%) (6). The latter study included chronic renal failure patients prior to dialysis and may not be strictly comparable to the other three reports.

The data on acid secretion in chronic renal failure are also conflicting. Both of the reports by Canavan (5), and Chisholm (1), demonstrated an elevated BAO and MAO in pre-transplant patients on haemodialysis, compared with normal control values. These findings are supported by those of Shepherd and Ventkatesweran (14,15). The latter study also demonstrated no significant difference in gastric acid secretion between dialysed and non-dialysed patients with chronic renal failure.

Gordon et al studied 56 patients on haemodialysis. They found an elevated MAO in women but not in men and could identify no significant change in gastric acid secretion before and after dialysis (6). These results however include 6 males who were achlohydric. This finding of achlorhydria was also noted by McConnell in 10 of 25 patients with chronic renal failure (23). The authors also demonstrated a gradual return to normal over a period of months following the commencement of haemodialysis. In some of these patients they obtained histology of the gastric mucosa consistent with atrophic gastritis, although parietal cell antibodies were negative.

On balance it would seem that dialysis patients have a gastric acid secretion pattern similar to that of transplant recipients and have an increased risk of peptic ulceration. There is however, some evidence to suggest that the risk of ulceration is even greater in these patients following transplantation. Schiessel found 14 patients with ulcers or erosions in 55 patients who had a normal upper GI endoscopy prior to transplantation and Walter et al identified upper GI bleeding in 12 of 47 transplant recipients who had no ulceration prior to transplantation (7,24).

Indirect evidence on the role of gastric acid secretion can be gained from studies on the prevention of peptic ulceration by acid inhibition. Jones et al, in 1978, reported a benefit from cimetidine administration in decreasing the risk of GI bleeding (25). The source of the bleeding however was not identified and the study employed a historical control group. Similarly Garvin in 1982 reported a decrease in upper GI tract complications when cimetidine

was administered routinely (26). This however was a retrospective analysis and other variables such as the immunosuppressive regime were not considered and neither of these reports conclusively prove that acid inhibition decreases the risk of GI bleeding and peptic ulceration.

Schiessel reported the results of a randomised trial of cimetidine versus placebo in renal transplant recipients and failed to demonstrate, by regular endoscopy, any decrease in the risk of peptic ulceration (7). Walter et al performed a similar randomised study in 97 transplant recipients (24). They demonstrated a highly significant reduction in GI bleeding in the cimetidine treated group. This study however was criticised by Doherty who pointed out that the source of bleeding in these patients was not identified and that the study excluded patients with peptic ulceration prior to transplantation (27).

The aetiology of abnormal gastric acid secretion is also the subject of some debate. The hypochlorhydria in patients with chronic renal failure, particularly in those prior to dialysis is reported to be due to atrophic gastritis (6,23). No satisfactory explanation for this phenomenon has been advanced however. Gordon et al found that all of the achlorhydric patients had negative parietal cell antibody titres, suggesting that this is not the autoimmune atrophic gastritis seen in the normal population (6). Another explanation, advanced by Doherty, is that hydrolysis of urea in the gastric mucosa to ammonia would neutralise gastric acid (22), but this does not explain the gastritis, which by itself would explain the hypochlorhydria (28).

In the group with hypersecretion it seems likely that the elevated serum gastrin, which is known to occur in renal failure (29) and which is unaffected by haemodialysis, is responsible. It is therefore reasonable to suggest that haemodialysis may improve the factors responsible for gastritis while not affecting the serum gastrin, explaining the higher gastric acid secretion in haemodialysis patients. Following transplantation the serum gastrin will return to normal (30) and one would expect gastric acid secretion to return to normal. Many studies, however, have demonstrated increased gastric acid secretion several months after transplantation (1,5). A possible explanation for this is the trophic affect of gastrin on the parietal cell mass (31), resulting in a gradual decrease in gastric acid secretion following removal of the gastrin stimulus. As described by Doherty this does indeed occur over a period of several months (22).

Another factor which has been proposed as a possible aetiological agent is hypercalcaemia, which has long been recognised as a stimulus to gastric acid secretion (32). The role of hypercalcaemia in stimulating acid secretion in dialysis and transplant patients has only been superficially investigated, although neither Chisholm nor Gordon could find a correlation between gastric acid secretion and serum calcium (1,6).

Recent work by Timoney et al examined the role of histamine in stimulating gastric acid secretion in transplant recipients (12) based on reports that duodenal ulcer in non-transplant patients was associated with low levels of histamine in the gastric mucosa (33,34). They reported a low level of mucosal histamine in 25

transplant recipients compared with normal controls and these were similar to levels in non-transplant patients with duodenal ulcer. They could not, however, demonstrate a significant difference between mucosal histamine levels in transplant patients with duodenal ulcer and those without duodenal ulcer. They also found an elevated serum histamine in transplant patients with duodenal ulcer when compared to those without. The significance of these findings, however, are unclear and the reason for depleted mucosal histamine and the high level of circulating histamine have not been explained. It has been suggested in other reports that gastrin may be responsible for elevation of serum histamine (34) and hypergastrinaemia, although present in renal failure, will return to normal after transplantation (30). Other possibilities are that high circulating histamine may be related to diminished renal function since the kidney is high in histamine methyl transferase. Timoney, however, could find no correlation between histamine levels and serum creatinine, and the transplanted kidney should restore histamine methyl transferase activity.

The last aetiological factors to be considered are corticosteroids which may increase the parietal cell mass (20,21) or stimulate histamine release (12). There is however conflicting evidence to incriminate steroids as a cause of gastric hypersecretion in renal transplant recipients since neither Canavan nor Chisholm found any correlation between acid secretion and steroid dosage (1,5), although this was not in accord with the conclusions of Doherty (17,27,40). The subject of steroids in the pathogenenesis of peptic ulceration will be discussed in more detail later in this

chapter.

In conclusion, the relationship of well documented gastric hypersecretion to peptic ulcer development is difficult to evaluate. It is true that the gastric secretory tests show an elevated MAO and PAO following transplantation, but most studies have shown that the level is no greater than in the same patients prior to transplantation. There also appears to be no relationship between acid hypersecretion and the subsequent development of peptic ulceration. The situation may be further confused by the different methods used to stimulate gastric acid secretion by the inclusion of pre-transplant patients both on and off dialysis and by the different time intervals between transplantation and assessment of acid secretion.

The evidence that reducing acid secretion by the administration of H2 antagonists is also weak; many of the studies were retrospective or used historical controls, and excluded patients with known pathology. Gastric acid hypersecretion, therefore, may be one factor in the high prevalence of peptic ulceration, it seems unlikely that it is the only one.

## THE ROLE OF CORTICOSTEROIDS

It has been recognised for many years that corticosteroids cause peptic ulceration and this has become a widely held belief within the medical profession over the past 30 years (35,36). It is considered that the presence of an active ulcer or a history of dyspepsia or peptic ulceration are relative contraindications to corticosteroid administration.

A critical review of the literature, however, suggests that the situation is not as clear cut as may be apparent. In 1976 Conn and Blitzer conducted a meta analysis of 26 double blind studies examining the complications of steroid administration totalling 3558 patients (37). They were unable to demonstrate a significant difference in the occurrence of peptic ulcer in the steroid treated groups (1.4%) as compared to the control groups (1%). They were also unable to demonstrate any increase in the risk of haemorrhage or perforation. The same study also analysed 16 controlled non-double blind studies with similar conclusions. The only exception was a significantly increased risk of ulceration in those receiving more than 1g of prednisolone as a total dose (5.3%).

In a more recent report Messer et al performed a similar analysis and reported a highly significant association between peptic ulceration and steroid administration with 0.2% in control groups and 1.5% in the steroid treated groups (38).

In response to this Conn and Poynard re-analysed the methodology and the reports by Messer and came to surprisingly different conclusions (39). In the 71 studies analysed by Messer, Conn considered that 28 had to be excluded because of the

administration of concurrent medication, the recent use of steroids, concurrent use of antacids, inclusion of uncontrolled groups and protocol violation prior to randomisation. They then re-calculated the data with these studies excluded and found no association between peptic ulcer and steroid administration.

It is obvious that the causative role of steroids in peptic ulceration is unclear. It is also obvious that a prospective study of steroid administration purely to look at the complications is not feasible. This therefore means that analyses of therapeutic studies have to be undertaken, a practice which is not ideal for several reasons. Firstly it cannot be assumed that these therapeutic studies have reliably looked for or have documented peptic ulceration and secondly the underlying disease process or administration of other drugs may be associated with a high risk of peptic ulceration.

In transplant recipients no studies have been performed to assess this risk and information must be gleaned from reports of peptic ulceration in groups receiving different doses of corticosteroids. Chisholm found no correlation between steroid dose and either peptic ulceration or symptomatic dyspepsia (1), a finding similar to that of Timoney who could determine no relationship between the dose of steroids and the risk of developing peptic ulcer (12). Canavan and Briggs studied gastric acid secretion before and after transplantation and concluded that steroid administration did not increase BAO or MAO (5). This conclusion however did not make allowance for the known increase in acid secretion during haemodialysis (6,14) and it is difficult to separate the effects of steroid administration from those of improved renal function,

surgical stress and immunosuppressive therapy.

These findings however are at variance with those of Doherty in 1979 who found a positive correlation between steroid dosage and PAO and who concluded that most peptic ulcers occurring post-transplant were steroid induced exacerbations of pre-existing disease (40).

Other authors have shown a decrease in peptic ulceration accompanying decreasing corticosteroid dosage. Knechtle et al demonstrated a fall in peptic ulceration from 10.3% to 5.6% in two concurrent decades, associated with a reduction in steroid dosage over this time period (9). The same period however also encompassed the change from azathioprine to cyclosporine and was accompanied by aggressive pre-transplant treatment of ulcers reported in the second decade. Other variables such as pre-transplant health of the patients may also be important. It is therefore difficult to be certain that the improvement was due solely to a reduction in the dose of corticosteroids.

The possible mechanism of action of steroids in ulceration may be due either to increased acid secretion or to possible effects on the gastroduodenal mucosal barrier. There is certainly a wealth of experimental evidence to suggest that increased acid secretion does occur with corticosteroid administration due to its trophic effect on the parietal cell mass (20,21) although, as discussed previously, this phenomenon could also be explained by the trophic effect of gastrin (31). Canavan and Briggs could not demonstrate any relationship between acid output and the dose of steroid administered (5). Doherty, however, did find a weak correlation between PAO and prednisolone dosage, although this was not independant of time

elapsed since transplantation (22).

Corticosteroids have also been reported to have a direct effect on the gastroduodenal mucosa by decreasing the rate of turnover of the epithelium. Max & Menguy demonstrated a decrease in the mitotic rate of gastric mucosal cells with ACTH administration (41) which will inhibit the healing response of the gastric mucosa (42) and may promote the action of acid and pepsin on small mucosal lesions (43).

It is difficult to ascertain the true importance of corticosteroid administration. There is obvious experimental evidence to show its effect on acid secretion and on the gastric mucosal barrier but this does not appear to be borne out by its effect on peptic ulceration in the normal population. The evidence of its effect on transplant recipients would tend to suggest that the dose of corticosteroids does not appear to increase the risk of peptic ulceration although this is disputed by some authors (40). Certainly it appears to have a minimal effect on gastric acid secretion although this has to be interpreted in the knowledge that other mechanisms such as a general improvement in patient health and resolution of hypergastrinaemia will have a tendency to decrease acid secretion. It is also, of course, impossible to separate the effect on acid secretion and/or the gastric mucosal barrier from the immunosuppressive effect of corticosteroids and the immunosuppressive effect of concomitantly administered drugs which will be discussed in the following chapter.

A further factor to be taken into account is the difference in prevalence of peptic ulceration between transplant recipients and non

transplant patients on corticosteroids. In most reported series the prevalence of peptic ulcer in transplant recipients has been between 10% and 25%, whereas even in the high dose steroid group reported by Conn and Blitzer the prevalence was only 5.3% (37), and in most reports is around 1% (38,39)

This group of patients obviously have many other variables as discussed above which may contribute to the increased prevalence of peptic ulceration. The balance of evidence would suggest that the role of corticosteroids is likely to be a small one and does not account for the high prevalence of peptic ulcer and the high incidence of complications witnessed in this group (37).

# CHAPTER 2

## INFECTIOUS AGENTS IN PEPTIC ULCERATION

### A. HELICOBACTER PYLORI

### B. CYTOMEGALOVIRUS

### C. HERPES SIMPLEX VIRUS

### D. IMMUNOSUPPRESSION IN TRANSPLANT RECIPIENTS

## HELICOBACTER PYLORI

Reports of curved or spiral bacteria in the upper GI tract were recognised as early as 1939 (44), but it was not until the work of Warren and Marshall in the early part of the last decade that their possible significance as a pathogen was appreciated (45). The organism was initially described as a campylobacter like organism (CLO), was named campylobacter pyloridis in 1984 (46) and subsequently renamed Campylobacter pylori (47). Last year a further change was made, creating a new genus, and the organism is now known as Helicobacter pylori (48). Since these initial reports many groups have studied the organism and it has been implicated in the pathogenesis of gastritis, duodenal ulceration and gastric ulceration.

### Microbiology

The organism is a curved or spiral microaerophilic gram negative bacillus (49). One of the most striking features are the enzymes produced by the organism, which include extracellular catalase and superoxide dismutase which may confer resistance to the oxidative enzymes of macrophages (50). A further important enzyme is a pre-formed urease which is present in high concentrations and will hydrolyse urea to ammonia (51). The resulting alkaline microenvironment may protect the organism from the acidic environment of the upper gastrointestinal tract. The bacterium appears to have a specific affinity for gastric antral type mucosa and is highly motile in the viscus environment of the gastric mucus layer (52) (Figs 1&2).

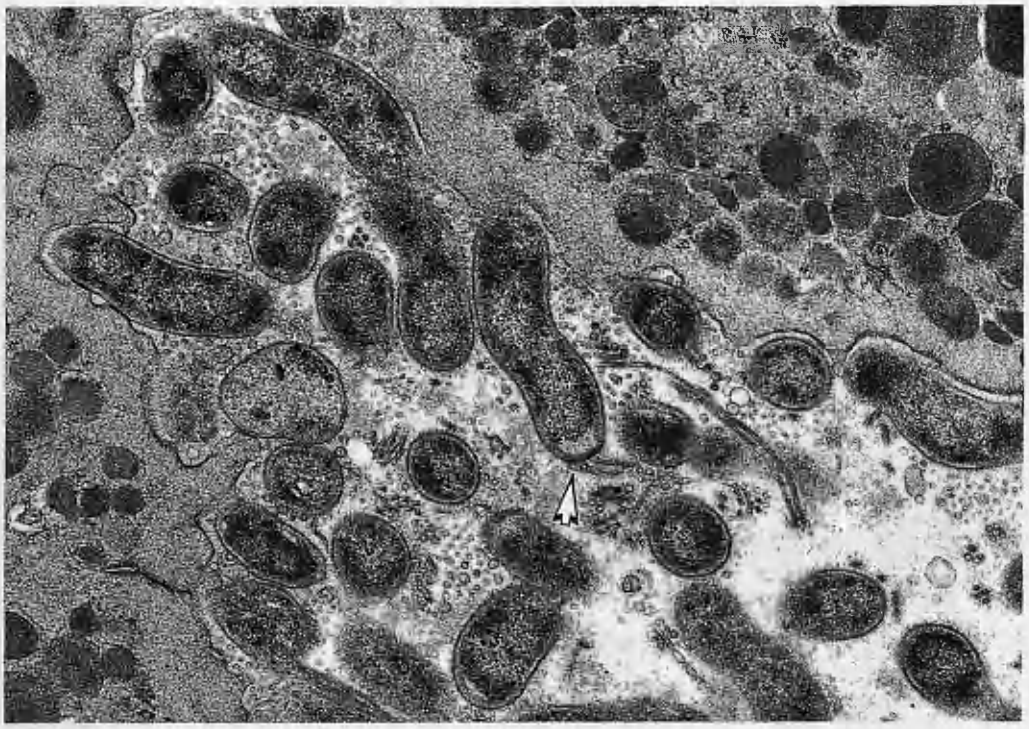


**Figure 1:** Transmission electron micrograph of H pylori (arrowed).

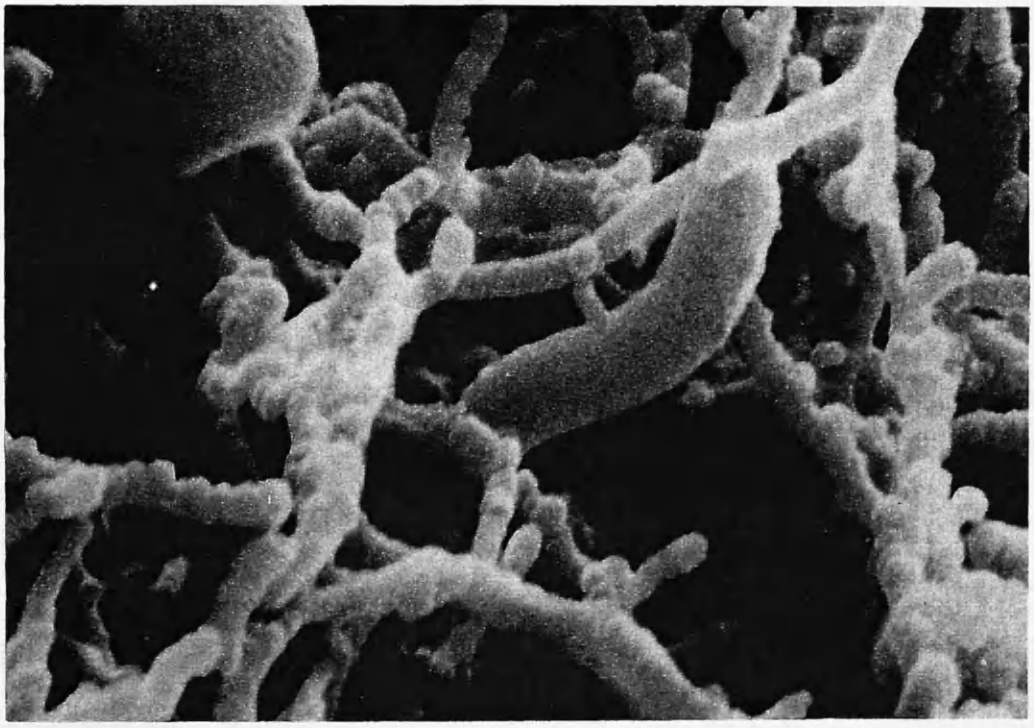


**Figure 2:** Scanning electron micrograph of H pylori within the strands the gastric mucus layer.

## Gastritis and non-ulcer dyspepsia

### Definitions

In order to understand the relationship of H pylori to gastritis and non-ulcer dyspepsia it is first necessary to define the terms. Gastritis is usually viewed as one of three types. Type A gastritis affects the body of the stomach and is associated with destruction of parietal cells, the presence of parietal cell antibodies, intrinsic factor depletion, pernicious anaemia and achlorhydria as described by Strickland and McKay (53). The same authors described an anatomically, histologically and aetiologically distinct type of gastritis, termed Type B gastritis. In contrast to Type A Type B affects the mucus secreting cells of the gastric antrum without involvement of the body of the stomach and is not associated with autoantibodies or pernicious anaemia. Although achlorhydria has been reported in Type B gastritis it is not common and usually only accompanies the more severe form of the disease (54). A third type of gastritis recognised more recently is associated with alkaline reflux and resultant intestinal metaplasia (55).

The relationship between non-ulcer dyspepsia and gastritis is a complex one. Non-ulcer dyspepsia can be broadly defined as the presence of dyspepsia in the absence of ulceration. It is possible to subdivide these patients into those with gastritis and those without gastritis (56). It has however been reported by Talley that gastritis correlates poorly with symptoms of non-ulcer dyspepsia (57) and may be reflected in the scepticism with which some clinicians view gastritis as a cause of dyspepsia (49). A further factor to be considered is the poor correlation between endoscopic appearances and

histological evidence of gastritis (58) and it is important therefore to obtain histology of the gastric mucosa before assigning a patient to the non-gastritis group (59).

Gastritis

Since Warren and Marshall's first report in 1984 the association of H pylori with gastritis has been confirmed by many authors. The organism has been identified in 89-97% of patients with type B gastritis (60,61,62) and the organism was not identified in patients with a histologically normal gastric mucosa. In some instances H pylori has been identified in an apparently normal mucosa in the body of the stomach, but is invariably associated with gastritis in the antrum (49). It has also been demonstrated that the organism is only identified in gastric type mucosa and is absent from areas of intestinal metaplasia (49).

Studies of H pylori in other types of gastritis have shown a much lower prevalence. O'Connor reported H pylori in only 21% of patients with Type A gastritis but in 85% of patients with duodenal ulcer and Type B gastritis (63). At present no association between H pylori and reflux gastritis has been demonstrated (64). In spite of the strong association between Type B gastritis and H pylori,however, many clinicians remain sceptical of its importance as a pathogen (65). Evidence to support a primary pathogenic role comes from work demonstrating primary infection in previously normal individuals, improvement in gastritis following eradication of the bacteria and ultrastructural changes in the gastric mucosa associated with Helicobacter infection. The first two will now be discussed and the

latter will be dealt with under a separate heading.

In 1978, before the recognition of the possible pathogenicity of H pylori, 17 healthy volunteers undergoing gastric pH monitoring developed achlorhydria and symptomatic dyspepsia with histological evidence of gastritis. The volunteers were followed up and improvement in gastric secretion and in histological gastritis was demonstrated after a period of 4 weeks (66). In the light of Warren and Marshall's work the biopsy specimens were re-examined and Helicobacter was identified (49). A similar phenomenon was reported by Gledhill in 1985 (67). In neither of the above reports was the electrode sterilised between subjects, the implication being that the organism was transmitted via the pH electrode.

In two reports investigators have ingested the organism and have developed gastritis. In 1985 Marshall ingested organisms while on Cimetidine (68). He subsequently developed a brief upper gastrointestinal upset and histological evidence of gastritis and Helicobacter infection. In 1987 Morris and Nicholson repeated the experiment, initially without Cimetidine, and did not develop gastritis. When they ingested the organisms with Cimetidine they too developed gastrointestinal symptoms and gastritis (69).

Further evidence to support a primary pathogenic role can be elicited from reports of eradication of the organism. Marshall reported resolution of gastritis in 8 out of 9 patients in whom H pylori was eradicated but not in patients in whom the organism persisted (70). McNulty demonstrated resolution of gastritis in 12 of 13 patients in whom H pylori was eradicated compared to only 4 of 32 in whom the organism was not eradicated (71). These findings have

been confirmed by other authors (72,73).

Non-Ulcer Dyspepsia

The role of Helicobacter in non-ulcer dyspepsia is closely related to its role in Type B gastritis. As discussed above it is possible to subdivide these patients into two groups depending upon the presence or absence of histological gastritis. Wyatt studied 141 patients with non-ulcer dyspepsia and identified 83 patients with histological gastritis (61). H pylori was found in 89% of these patients and was not visualised in any of the remaining 58 patients with a histologically normal mucosa. These findings have been confirmed by other authors (74,75). It seems therefore, that Helicobacter is only of significance in patients with histological gastritis and not in those with a normal gastric mucosa.

**Peptic Ulceration**

Duodenal Ulcer

Following upon the reports of H pylori in relationship to gastritis Marshall and Warren reported a high prevalence of the organism in the gastric antrum of patients with duodenal ulceration (76). Since this time H pylori has been identified in 80-100% of patients with duodenal ulceration (77,78,79). There has been some dissent, however, and neither Rollasson nor Girdwood could find an association between H pylori and peptic ulceration (80,81), and some authors have disputed its pathogenic role (82).

It was certainly recognised for many years that Type B gastritis was associated with duodenal ulceration. In 1967, prior to

the current interest in H pylori, Schrager reported antral gastritis in 95% of patients with duodenal ulceration, although the significance of this finding was unclear (83).

Evidence to support a pathogenic role can be gained from reports on ulcer healing and relapse following eradication of H pylori. Much of the doubt surrounding this issue is based on the widely recognised success of $H_2$ receptor antagonists in ulcer healing. These agents will heal more than 90% of duodenal ulcers and and they have no demonstrable effect on Helicobacter colonisation (78,84). If, however, $H_2$ antagonist therapy is stopped the relapse rate appears to be higher compared to patients in whom H pylori has been eradicated.

In 1987 Coghlan demonstrated an increased relapse at one year in patients who remained H pylori positive; 79% compared to 17% in H pylori negative patients (72), findings similar to those reported by Marshall (70). This is in keeping with pre H pylori evidence which demonstrated that colloidal bismuth, an agent with an antibacterial effect on H pylori, is associated with a lower rate of ulcer relapse than H2 receptor antagonists (85,86). Interpretation of this evidence, however, is open to debate. Colloidal bismuth has been shown to have a protective effect on the gastric mucosa by binding to glycoproteins and it may inhibit $H^+$ back diffusion (87). It is therefore possible that colloidal bismuth exerts its anti-ulcer effect independently of its anti-bacterial properties. Similar criticisms have been levelled at antibiotics used to treat H pylori infection since some have been shown, experimentally, to have a protective effect on the gastroduodenal mucosa which is independent

of their antibacterial action (82).

A further doubt exists as to why an organism found in the gastric antrum can give rise to duodenal ulceration, since the organism does not colonise the intestinal epithelium of the duodenum. Two possible explanations have been advanced to explain this.

High duodenal acidity, as seen in patients with duodenal ulcer (88), induces gastric metaplasia in the duodenum (89) and H pylori has been identified in these areas of metaplastic epithelium (90,91,92). It is therefore possible that areas of gastric metaplasia can become colonised by H pylori from organisms already present in the gastric antrum. Once colonisation has occurred the resultant high urease activity can induce $H^+$ back diffusion and epithelial cell injury (52).

A second possible pathogenetic process relates to the effect of antral H pylori on gastrin secretion. Levy et al demonstrated elevated serum gastrin in duodenal ulcer patients colonised by H pylori compared with those who were H pylori negative (93). This led the authors to postulate that the alkaline environment due to local urease activity gives rise to an inappropriately high gastrin secretion and subsequent increase in gastric acid output.

It is also possible that both of these mechanisms work together to produce duodenal ulceration, and Helicobacter pylori is one factor in a the multifactorial aetiology of peptic ulcer disease.

Gastric Ulcer

The role of H pylori in the pathogenesis of gastric ulcer is less certain than its role in duodenal ulcer. The association is

less marked, with the organism being identified in between 53 and 77% of patients with gastric ulceration (94,95). It is difficult to seperate gastric ulcer from gastritis since the two conditions tend to co-exist and it has been suggested that H pylori is more strongly associated with gastritis than with gastric ulceration (96).

Another possible explanation of the poor association may be the relationship of some gastric ulcers to exogenous influences such as non-steroidal anti-inflammatory agents, a factor which has not been taken into account in published reports.

Analysis of the evidence on healing fails to clarify the situation. Hui demonstrated improvement in gastritis and ulcer healing with coloidal bismuth, but no change in H pylori colonisation (95). More recently Humphreys demonstrated no association between H pylori eradication and ulcer healing (97). Tytgat confirmed that bismuth was no more effective in preventing the relapse of gastric ulceration than were H2 receptor antagonists (98), although other reports from the same author have demonstrated superior healing with bismuth (99,100).

**Pathogenesis and Ultrastructural Changes**

As discussed previously Helicobacter is only seen in relationship to gastric mucosa, being absent from areas of intestinal metaplasia and, when found in the duodenum, is identified only in areas of gastric metaplasia (101). The organism has been identified in normal body mucosa but always accompanied by histological gastritis in the antrum (49). With this exception the presence of Helicobacter is associated with gastritis in 97-100% of cases

(60,61).

Ultrastructural studies performed by Goodwin and Marshall in 1986 give strong support to a primary pathogenic role in gastritis (49). Electron microscopy studies have revealed characteristic changes in the gastric epithelium which resolve following eradication of the bacteria and which recur with reactivation of infection. The changes witnessed affected the mucus secreting cells and included ragged protrusions of the luminal cell surface, decrease or complete loss of surface microvilli and depletion of intracellular mucin granules with cellular oedema. The continuity of the epithelium was intact with no identifiable breaches in the mucosa. The changes were also witnessed in the superficial neck cells of the gastric pits but the glands themselves were normal. An infiltrate of neutrophils and lymphocytes was also identified.

The bacteria were seen to be on the cell surface and areas of adhesion were identified between the bacterial cell wall and the epithelial cell surface. The bacteria have been identified closely associated with the tight junction complexes between epithelial cells, allowing the bacteria access to the nutrients in this region. The organisms however, have not been identified in the lamina propria of normal individuals. Goodwin and Marshall did not observe intracellular organisms (49), although this has been observed as in uncommon occurrence by other authors (102,103). Goodwin & Marshall did, however, report phagocytosis by macrophages as a common occurrence (49).

The authors gave support to a pathogenic role by highlighting the similar ultrastructural abnormalities identified in

enteropathogenic E coli infection, including adherence pedicles, loss of microvilli and non-invasive bacteria restricted to the luminal surface (49).

**Immune Response to H Pylori**

Gastric colonisation with H pylori produces a typical non-specific inflammatory infiltrate of histiocytes and neutrophils, containing remnants of bacteria, presumably the result of phagocytosis (49). Infection is also accompanied by an antibody response which can be detected serologically and locally in the gastric mucosa. Circulating antibodies to Helicobacter antigens have been detected by many authors (104,105,106).

The local antibody response has been investigated by Wyatt and Rathbone. They demonstrated IgM and IgA in gastric aspirates of patients colonised with Helicobacter (106). The same authors studied antibody distribution in the gastric mucosa by immunoperoxidase staining of IgG, IgA and IgM (61). They identified positive staining of bacteria with all 3 classes of immunoglobulin, and demonstrated IgG or IgM in 86% of patients with Helicobacter associated active gastritis. They also demonstrated IgA which appeared to correlate less well with the activity of the gastritis, being demonstrated in all patients with active gastritis and in 60% of those with inactive chronic gastritis. There was no positive staining in negative control specimens. The authors also noted that bacteria in the depths of the gastric pits were not coated with antibody.

The precise nature of the antigens is unclear. They appear to react with various proteins on the outer membrane of the organism. An interesting report by Barer, in 1988, demonstrated abolition of the urease cytopathic effect by the addition of H pylori positive serum to H pylori urease, but not when added to urease from other sources, suggesting that some of the antibody is directed against the bacterial enzyme (107).Studies of antibody titres in therapeutic trials have demonstrated a fall in antibody titres following eradication of Helicobacter (105).

The above data once more lend support to the pathogenic role in gastritis with IgG and IgM secretion in the active phase and decrease in antibody titres following eradication of the organism and perhaps, in the future, serology may be a useful method of monitoring the response to treatment.

**Cell Mediated Immunity**

The cell mediated response to H pylori infection has been less extensively investigated. Yrios studied the effect of C jejuni on athymic mice (108). Passive immunisation of athymic mice did not protect them from the effects of C jejuni when compared to euthymic mice, suggesting that an intact T cell response may be an important part of normal defence mechanisms. It is of course difficult to be sure of the significance of these findings in human infection with H pylori.

Recent work by Rathbone et al, as yet only published in abstract form, has studied T cell subsets in Helicobacter gastritis (109). They demonstrated a relative reduction in T suppressor/

cytotoxic cells in both the epithelium and lamina propria of patients with H pylori associated gastritis, compared with normal antral biopsies. They also demonstrated an increase in T helper cells in the biopsies with an increased percentage expressing the CD7 marker, indicative of T cell blastogenesis, suggesting a primary immune response, presumably to H pylori

In the context of infection in transplant recipients the T cell response is likely to be the most important factor. At present the current information makes it difficult to assess the importance of the T cell response in H pylori infection and the resulting increase in infection which might be expected in patients with a deficient T cell response.

**Epidemiology of H Pylori**

At present the only known source of H pylori is the human gastroduodenal mucosa and the organism has not been isolated from other sites (50). Similar organisms have been identified in the gastric mucosa of non-human primates although this is unlikely to be of importance in the general population.

Most information on the prevalence of H pylori in dyspeptic patients has come from endoscopic biopsies. This investigative technique has obvious limitations in studying a healthy population although one endoscopic study of healthy volunteers did demonstrate H pylori in 20% (110). In population studies, however, alternative non-invasive methods have to be employed. Two such techniques are H pylori serology and $^{13}C$ urea breath testing.

Graham studied asymptomatic patients using $^{13}C$ breath testing and found that the prevalence was age dependent rising from 5% in those under 44 to 75% in those over the age of 65 (111).

Serological studies have demonstrated a high prevalence of H pylori antibodies in up to 32% and have confirmed an increase in prevalence with age rising from 10% in those under 25 to 50% in the over 55 age group (104,112). As discussed previously, however, this method does not necessarily detect active infection and may also cross react with antibody to several campylobacter species and thus overestimate the prevalence of H pylori infection (111).

All of these studies suggest that the prevalence of H pylori is around 20-30% but does show a marked increase with age. This is in keeping with the increased prevalence of gastritis which is known to occur with age (113). It is also interesting to look at the relevance of H pylori in an apparently asymptomatic population. Marshall, in an unselected group of blood donors found, that 50% of those who were H pylori positive had symptomatic dyspepsia (114), suggesting that some of the healthy volunteers in the other reports may not be completely asymptomatic.

The mode of transmission of Helicobacter pylori is unclear and although there are well documented instances of transmission by gastric pH electrodes this is not relevant in the population at large.

It seems likely that person to person transmission is the most important source of infection. A high prevalence was demonstrated in upper gastrointestinal endoscopists (115), and several authors have demonstrated an increased prevalence in families of affected

individuals and in patients in long stay psychiatric institutions (116,117). One study, as yet published only as an abstract, has demonstrated an increased prevalence of H pylori antibodies in people working with livestock (118). This is the only publication which has suggested transmission from an animal source, and the evidence at present favours person to person transmission, although the precise method of transmission is yet to be elucidated.

**H Pylori in Immunocompromised Patients**

There is little guidance in the literature on the importance of H pylori in immunocompromised patients. One case report describes gastritis in a patient with AIDS which responded to treatment with colloidal bismuth and amoxycillin (119). The clinical and histological features, however, were different from those in normal individuals. The patient had an acute severe illness with bacteria seen to invade the lamina propria, a feature not commonly identified in immunocompetent individuals. No information exists on H pylori infection in transplant recipients and the lack of data on cell mediated immunity to H pylori makes it difficult to predict the likely pattern of infection in these patients. One recent report has shown a very low prevalence of H pylori in haemodialysis patients (2.5%) although why this should be is unclear (90).

## CYTOMEGALOVIRUS

### Introduction

Cytomegalovirus is a member of the herpes family of viruses. In common with other herpesviruses it is characterised by a cycle of primary infection, latency and reactivation. Infection with CMV produces enlargement of the cell (cytomegalia) which gives the virus its name, along with prominent intranuclear and, at a later stage, cytoplasmic inclusions.

### Epidemiology

The prevalence of positive antibody titres to CMV, indicative of prior exposure to the virus, shows marked geographical and social variations. Data summarised by Krech shows titres of 40-80% in Western countries rising to 100% in the Far East and Africa (120). The peak incidence of infection occurs within the first two years of life, reaching a plateau by 50 years of age (120).

Transmission of the virus in the peri-natal period may be transplacental or via breast milk and in endemic areas respiratory transmission may be significant (121). In adults the routes of infection are less clear although sexual transmission is thought to be important (122). Other modes of transmission such as blood transfusion and allograft transplantation are well established, although of minor importance in the general population.

**Clinical Features**

Primary infection in healthy adults is most commonly asymptomatic. When symptoms do occur they usually take the form of a mild mononucleosis type of illness with lymphadenopathy, myalgia, a mild disturbance of hepatic transaminases and atypical lymphocytosis (123,124). The disease is usually self limiting, lasting two to three weeks. During the acute infective episode virus can be readily isolated from the throat and urine.

**Viral Latency**

Once the acute infection has resolved the virus establishes latency within the host cells. Identification of the sites of latency has been difficult although the virus has been demonstrated in renal tubular epithelium and salivary glands (125). The latent virus has also been identified in lymphocytes, mainly the T helper subset (126). This is in keeping with the intermittent excretion of virus in urine and saliva and transmission by blood transfusion (127).

Reactivation of latent virus is of little importance in healthy individuals, although can be a source of considerable morbidity in immunosuppressed and immunocompromised patients.

**Cytomegalovirus in Transplant Patients**

Primary infection with CMV and reactivation of latent virus can give rise to a much more severe illness in transplant recipients than is seen in the general population. Cytomegalovirus infection was

recognised as a complication in the early days of renal transplantation (128,129). The prevalence of infection varies with the definition but can be in excess of 70% based on seroconversion or a rise in antibody titres (130,131) although many of these infections are subclinical.

Cytomegalovirus has also been implicated as a factor in allograft rejection (132), with increased expression of class II MHC antigens in the allograft.This may be mediated by gamma interferon released as a result of CMV infection (133).

In the past infection was classed as reactivation if it occurred in a seropositive recipient and primary infection when it occurred in a seronegative recipient. Epidemiological evidence demonstrated an increased risk of infection in recipients of an organ from a seropositive donor suggesting that the donor organ was the major source of virus (134,135). This has been confirmed by demonstration of identical viral strains in matched recipient pairs (136). Further work with matched recipient pairs in seropositive recipients has suggested that the majority of infections are due to viral strains from the donor organ and are therefore primary infections and not reactivation of endogenous virus (137,138).

**Cytomegalovirus in the gastrointestinal tract**

In rare instances CMV has been described associated with ulceration and bleeding in the gastrointestinal tract in immunocompetent individuals. These amount to a few case reports,

usually in patients following multiple trauma (139,140,141). There have, however, been many more such reports in transplant recipients and in patients who are immunosuppressed or immunodeficient for other reasons.

Transplant Recipients

In the 1970's reports began to appear in the literature linking CMV to ulceration and haemorrhage in the upper GI tract. Millard in 1973 reported 3 cases of cytomegalic inclusions in the presence of erosive gastritis (142), and Diethelm in 1976 reported a case of haemorrhagic gastritis, again with typical cytomegalic inclusions (143). More comprehensive reviews were reported by Franzin and by Cohen in 1981 and 1985 respectively (8,11).

The first paper reported a retrospective analysis of 20 asymptomatic renal transplant recipients between one and 24 months after transplantation. The authors identified cytomegalic inclusions in 9 of the 20. In 8 of these patients the duodenum was involved and duodenitis was identified endoscopically in 6, of whom only 2 had symptomatic dyspepsia. Only 2 of the 9 patients had similar histological changes in the gastric mucosa, although 7 patients had endoscopic evidence of gastritis. The study also identified a significant association between primary infection and cytomegalic inclusions in the gastroduodenal mucosa, suggesting that primary infection of a seronegative patient carried a greater risk of infection in the upper GI tract (8).

Cohen et al reported on 11 surgical resections or autopsy specimens from renal transplant recipients. They found cytomegalic inclusions in 5 of 8 patients with peptic ulcers. All of the patients had suffered a GI bleed within the first year of transplantaion and 3 of the patients with CMV inclusions died as a result of GI haemorrhage. Serology was not available on the patients in this study and no comment could be made on the association with primary or secondary infection (11).

A more recent paper by Alexander et al reported results of a prospective study in patients before and after liver transplantation (144). The authors studied endoscopic biopsies histologically and by viral culture and also looked at smears of mucosal brushings. All patients were seropositive and infection was presumed to be re-activation. Cytomegalovirus was isolated in 33% of patients following transplantation, compared with 2% prior to transplantation. Interestingly inclusions were identified in only 25% of patients with positive cultures suggesting that the reports by Franzin and Cohen may have significantly underestimated the prevalence of infection. Alexander also found an association between cytomegalic inclusions and symptomatic dyspepsia.

Acquired Immune Deficiency Syndrome

The large and expanding population of patients with AIDS are susceptible to opportunistic infection and recent reports from the United States have identified CMV as a possible pathogen.

Inclusions have been identified in the stomach, gallbladder, small bowel and colon often with fatal results (145,146,147,148).

Mobley reported an interesting finding of multiple polypoidal lesions in the small bowel due to CMV associated submucosal lymphoid hyperplasia (149).

Clearly the severity and magnitude of the disease in patients with AIDS is much greater than in transplant recipients. This may be due to a more profound immunosuppression or may be due to the high CMV seropositivity of individuals in the high risk groups for the development of AIDS.

**Pathogenesis**

In the GI tract cytomegalic inclusions have been identified in surface and in glandular epithelium, in fibroblasts, smooth muscle cells, and vascular endothelium (8,11,143). Hinnant reported that only 10% of the infected cells were epithelial and the remainder were mesenchymal, including smooth muscle and vascular endothelial cells (147). This feature was also observed by Cohen who noted striking inclusions in vascular endothelium in ulcer bases, with evidence of thrombosis and focal epithelial necrosis (11). This is in keeping with reports of endothelial infection in other sites such as the kidney and retina.

It is clear that either epithelial infection or small vessel thrombosis and ischaemia could result in a breach of the gastroduodenal mucosa, subjecting the underlying tissue to acid

pepsin digestion.

**Conclusions**

Cytomegalovirus is a well established pathogen in transplant recipients and evidence from case reports and small series have suggested an aetiological role in the upper gastrointestinal complications of transplantation. It is, however, difficult to prove a causal role in view of the many other aetiological variables and in view of the lack of information on gastrointestinal CMV in the general population. Information must be collected prospectively with a group of control patients, along with information on donor and recipient serological status, concurrent drug administration and detailed histological assessment of the distribution of infected cells.

## HERPES SIMPLEX VIRUS

Herpes simplex virus (HSV) is the major virus of the herpes group and, as with CMV, is characterised by infection, latency and reactivation. Unlike CMV however, all of these events occur frequently in the general population. The virus can be divided serologically into two distinct types, termed HSV1 and HSV2 (150). More recently restriction endonuclease analysis has identified different strains within these two subtypes (151,152).

### Infection and Epidemiology

The two commonest forms of herpes simplex infection are oral and genital, producing the characteristic vesicular lesion on the skin and mucous membranes. The lesions are usually self limiting and resolve after two to three weeks (153). More severe forms of infection in healthy adults include aseptic meningitis and encephalitis, the latter associated with a mortality of 70% and a high incidence of neurological deficit in survivors (154).

The importance of each subtype of HSV varies with the site of involvement. HSV1 is responsible for over 90% of oral infections and HSV2 for a similar proportion of genital infection. In other sites the distinction is less obvious (155).

Prior exposure to HSV is widespread in the community as judged by serological studies. The prevalence of antibody to HSV1 tends to be falling and in developed countries is reported at around 40% (156). Transmission is by close personal contact and peak incidence of infection occurs in the second year of life.

The distribution and transmission of HSV2 is quite different.

Antibodies are not commonly detected until after puberty (157). Transmission is primarily sexual and there has been a progressive increase in HSV 2 infection over the past 20, years presumably related to changing sexual practices (158).

**Latency and Reactivation**

The mechanisms of the establishment of latency and reactivation are unclear despite early recognition of the phenomenon and its importance in transmision of the disease. Primary infection leads to migration of the virus along somatic or autonomic axons, establishing latency in neural ganglia. Following reactivation viable virus is shed from the secondary lesions (157). It is likely that most people who have been exposed will harbour latent virus, although only a minority will ever develop secondary infection (159,160). During latency it is assumed that the viral genome is innactive, and no virus derived peptides have been identified in infected ganglia (161).

The triggering factors for reactivation include nerve trauma, ultraviolet light exposure and intercurrent illnesses, although the precise triggering mechanisms remain unknown (153).

**Herpes Simplex in Transplant Recipients**

Early in the history of transplantation an increase in HSV infection was recognised in the post-transplant period. In 1973 Lopez reported HSV in 21% of renal transplant recipients (162). Most studies have reported infection with HSV in between 45% and 55% of kidney, heart and liver recipients (128,131,163). In these studies

infection is usually defined by positive culture and the prevalence of clinical infection is generally lower at around 20-50% (131,164).

The distribution of infective lesions is primarily nasopharyngeal with very few genital infections, and the disease is usually mild with no adverse effect on patient or graft survival (165). In rare instances disseminated disease has been reported producing encephalitis, hepatitis, pneumonitis and widespread cutaneous lesions (166,167,168,169). The clinical consequences of HSV infection in the transplant population, however, are much less than the consequences of CMV infection.

In the majority of transplant recipients infection is thought to be due to reactivation of latent virus with viral excretion occurring in up to 85% of seropositive recipients (170). In rare instances infection has been reported in seronegative recipients and it is possible that the virus can be acquired from the donor organ (171,172).

**Herpes Simplex in the GI Tract**

Herpes simplex infection in the GI tract is unusual in otherwise healthy individuals, although herpetic eosophagitis has been reported in such patients (173,174). Herpetic oesophagitis is, however, a well recognised problem in immunodeficient patients (175). The infection may spread from oropharyngeal infection or via the vagus nerve from latent virus in the vagal nucleus (176). Clinical features of the infection include odynophagia and dysphagia, and endoscopy may reveal multiple shallow ulcers (176).

If spread via vagal fibres is indeed a pathway for oesophageal

infection it would seem likely that spread to involve other parts of the upper GI tract would also occur. It was certainly suggested almost thirty years ago that HSV may be an aetiological agent in peptic ulceration (177). Herpes simplex has been identified in vagal ganglia, and patients with duodenal ulceration have been demonstrated to have higher HSV antibody titres than normal control subjects. In clinical practice, however, this does not appear to be the case. Buss et al found only one case of HSV associated gastritis in 50 patients with oesopahagitis (175) and there are only a few reports of infection affecting the liver and pancreas (176,178). One recent study examined the effects of acyclovir in preventing duodenal ulcer relapse (179). The rationale behind this study was that acyclovir is known to reduce the frequency of reactivation of Herpes simplex, and it was hoped that the drug would reduce the frequency of duodenal ulcer relapse. This, however, did not occur and the authors concluded that Herpes simplex was not implicated in the pathogenesis of peptic ulceration. It would, however, be difficult to draw such a definite conclusion from such an indirect study method.

Large bowel infection appears to occur more commonly and anorectal infection is widely recognised in homosexual men (180,181). More widespread colonic involvement has also been seen in immunocompromised patients including transplant recipients (182).

With the exception of oeosphagitis HSV has not been reported as a major pathogen in the upper GI tract. The histological diagnosis however is difficult and neither immunohistochemistry nor in situ hybridization techniques have been used to determine the relationship of the virus to ulceration, gastritis or duodenitis, lesions not normally associated with herpetic infection.

## IMMUNOSUPPRESSION IN TRANSPLANT RECIPIENTS

In the early days of renal transplantation it became obvious that, although an operation could be technically successful, long-term graft function was not achieved. As early as 1914 it was recognised that pharmaceutical methods of controlling rejection were necessary. This remained a problem until the mid 1950's when modest success was achieved by using corticosteroids. Renal transplantation, however, was not widely performed and did not become so until the more widespread use of immunosuppression became a practical possibility.

In the late 1950's whole body irradiation was used along with cytotoxic agents, particularly 6 mercaptopurine. By 1962 a derivative of 6MP, BW 57-322, was used by Calne and was demonstrated to confer greater graft survival and was associated with less toxicity than 6MP (183). This compound became known as azathioprine and was to become the mainstay of immunosuppression for almost 20 years. By 1963 azathioprine was used routinely with prednisolone and allowed the expansion of renal transplantation with long-term graft survival (184).

This combination, however, was not without complications. Infection was a common and almost inevitable problem, occurring in up to 80% of patients (185). Azathioprine produced a widespread immuno and myelosuppression with inhibition of humoral and cell mediated immunity and the inflammatory response, predisposing the patients to a wide range of bacterial, viral and fungal infection (186).

Around 10 years ago 3 important advances were made in the immunological management of transplant recipients. These were the

identification of the HLA DR antigen, allowing better tissue matching, a realisation that lower doses of corticosteroids could be used successfully, and the introduction of cyclosporine into clinical practice. As discussed in Chapter 1 the prevalence of GI complications appeared to decrease over this time period and was presumed to be due to decreasing corticosteroid dose (9). The other two factors, however, are also likely to be important because of their effect on viral and bacterial infection in the upper GI tract.

**Cyclosporine**

Unlike azathioprine cyclosporine exerts a much more specific effect on the immune system and is neither cytotoxic nor myelosuppressive. Cyclosporine inhibits the production of interleukin 2 (IL-2) and inhibits the responsiveness of cytotoxic T cells to IL-2. An indirect effect of its action on T cell proliferation is to inhibit the production of T cell lymphokines, thereby suppressing macrophage function. A further consequence of this is a decreased production of macrophage derived IL-1 and subsequent suppression of T helper lymphocyte activation. T suppressor cells,however,are unaffected. The net result, therefore, is an inhibition of cytotoxic and helper T cells and macrophages with sparing of suppressor T cell function (187). There is also evidence to suggest that there may be a mild inhibitory effect on humoral immunity (187), but less than experienced with azathioprine. The greater specificity of cyclosporine, therefore, results in a decreased risk of bacterial and fungal infections, although viral infections which are primarily T cell mediated are unaffected (187).

# CHAPTER 3

# METHODS

**SUMMARY**

The study was performed on renal transplant recipients obtaining endoscopic biopsies of the gastroduodenal mucosa. The biopsy material was then split, half being fixed in formalin and half frozen in liquid nitrogen. A retrospective group of normal gastric and duodenal biopsies was obtained, age and sex matched and, in addition, a prospective age and sex matched group of patients was utilised to obtain fresh tissue which was subsequently frozen. The formalin fixed tissue was analysed by immunohistochemistry using anti-CMV and anti-HSV antibody. The material was also examined for the presence of Helicobacter pylori and assessed for the grade of gastritis and duodenitis. The frozen tissue was used for the study of T cell subsets.

**INTRODUCTION**

The clinical methods used are straightforward and will be dealt with in detail later in this chapter, along with details of the laboratory methods. This introduction will, therefore, outline the background to the laboratory methods.

**Immunohistochemistry**

Immunohistochemistry was utilised in this study for the detection of CMV and HSV and for analysis of mucosal T lymphocyte subsets. This is a technique which allows the identification of a tissue consituent by means of a specific antigen - antibody reaction tagged by a visible label (191). The technique was first described in 1941 (192) but it has been the wide availability and ease of

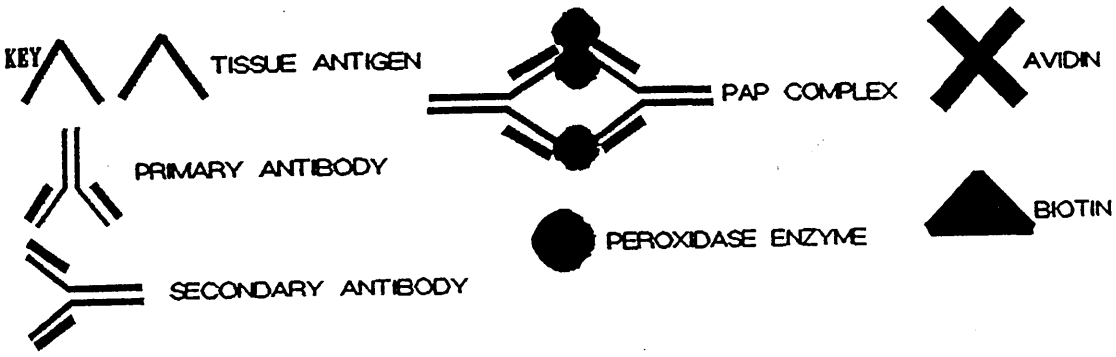
production of specific monoclonal antibodies which has prompted a rapid growth in the applications of this method over the past 10 years.

As described by Coons (192) the label used to visualise antibody localisation was Fluoresceine which necessitated viewing under ultraviolet light. It is now, however, possible to use labelling techniques which can be viewed by standard light microscopy, the common labels relying on enzymes such as peroxidase or alkaline phosphatase to produce a colour change when reacted with the appropriate substrate.

Initial descriptions of the technique utilised a single or direct method, where the primary antibody carried the label (Fig 3). A development of this technique has been the use of an indirect method whereby the primary antibody will bind to the appropriate antigen and the secondary antibody, carrying the label, will then bind to the primary antibody (Fig 4). This technique has two major advantages over the direct method.

Firstly a wide range of primary antibodies can be visualised by using a single labelled secondary antibody and, secondly, the staining can be amplified since the primary antibody will bind several molecules of labelled secondary antibody (191).

Further developments have aimed to increase the sensitivity of the staining by improving the linkage technique. One such method is the peroxidase anti peroxidase technique which utilises a third layer antibody conjugated to peroxidase (Fig 5). The most recent developement and the one used in this study utilises an avidin, biotin linkage.



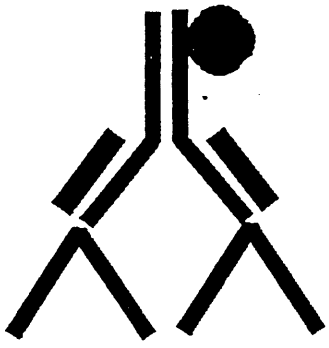



FIG 3. Direct antibody technique showing labelled antibody bound to tissue antigens.

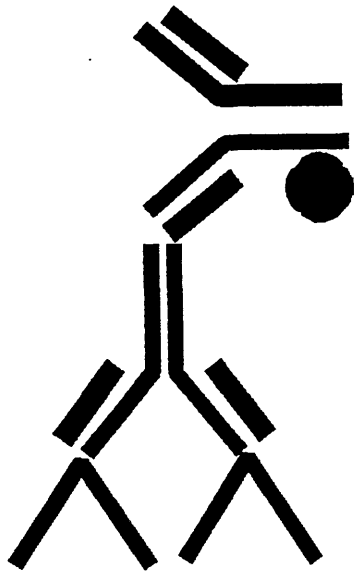


FIG.4 Indirect antibody technique illustrating labelled secondary antibody bound to the primary antibody.

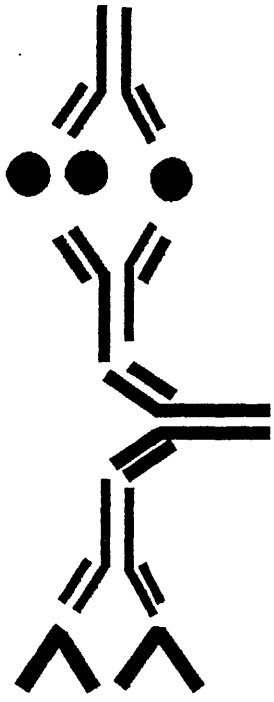


FIG.5 Peroxidase antiperoxidase technique. The third layer consists of a peroxidase antibody complex which binds to the secondary antibody.

Avidin is a glycoprotein of egg white origin which will bind to four molecules of the vitamin biotin. In this technique the secondary antibody is conjugated with biotin and the third layer consists of an avidin, biotin peroxidase complex which will bind to the antibody biotin conjugate. This amplifies the staining intensity since each avidin biotin complex will contain three molecules of peroxidase and each secondary antibody can bind several molecules of biotin (Fig 6). The technique has been demonstrated to be superior to standard indirect or peroxidase anti peroxidase methods (193).

The major disadvantages of immunohistochemical techniques are failure of the antibody to bind to the appropriate antigen producing a false negative reaction and inappropriate background staining producing a false positive reaction.

The commonest reason for a false negative reaction is destruction of the antigen by the fixation process (194). This varies with each antigen and antibody. In our work anti HSV and anti CMV can readily be used in fixed tissue (195,196) whereas the monoclonal antibodies used for T cell subset analysis must be used on unfixed tissue. In some instances binding can be increased by enzymatic digestion with trypsin or pronase prior to application of the primary antibody (197)

False positive reactions can be caused by endogenous label within the tisue specimen. This occurs commonly with peroxidase but can be overcome by pretreating with hydrogen peroxide (191).

In practice most false positive reactions occur because of non-specific antibody binding. The major step to overcoming this problem is the use of affinity purified monoclonal antibody (191). There

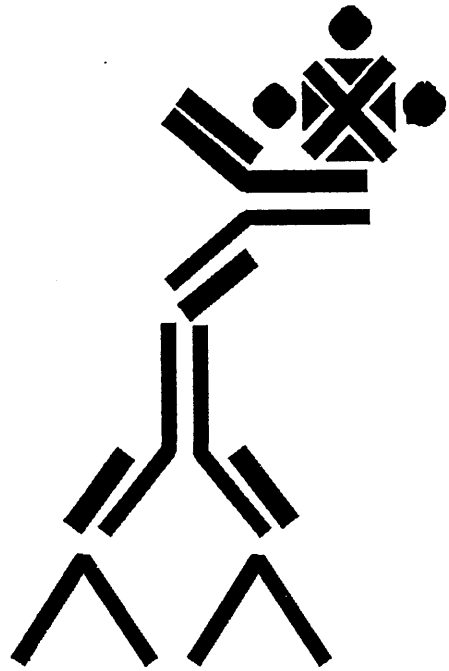


FIG 6 Avidin Biotin Technique.

will still be, however, non-specific staining due to antibody adhering electrostatically to collagen. The simplest method of overcoming this problem is to block these sites prior to application of the primary antibody with, for example, bovine serum albumin.

Based on the reasons given above the method chosen by us for this analysis is a double antibody peroxidase avidin biotin technique on fixed tissue for CMV and HSV and a double antibody alkaline phosphatase method on unfixed tissue for T cell subsets. Details of the staining techniques are at the end of this chapter.

**Helicobacter Pylori**

The presence of Helicobacter pylori was assessed in both gastric and duodenal biopsies. It is well recognised that the distribution of H pylori is patchy in the gastroduodenal mucosa (188) and, therefore, multiple biopsies were obtained. The organism was identified on histological sections and using a proprietary urease slide test.

**Urease Slide Test**

This is a rapid diagnostic test for Helicobacter pylori, developed by Marshall (60). The principle involves hydrolysis of urea by urease to ammonia. The slide test consists of a gel pellet containing urea and phenol red, buffered to an acid pH. At a pH level of less than 6.0 phenol red exists in a yellow form. In the presence of Helicobacter pylori the production of ammonia will raise the pH and induce a colour change from yellow to red (Fig 7). The proprietory slide test used in this study (CLOtest, Delta West Ltd,

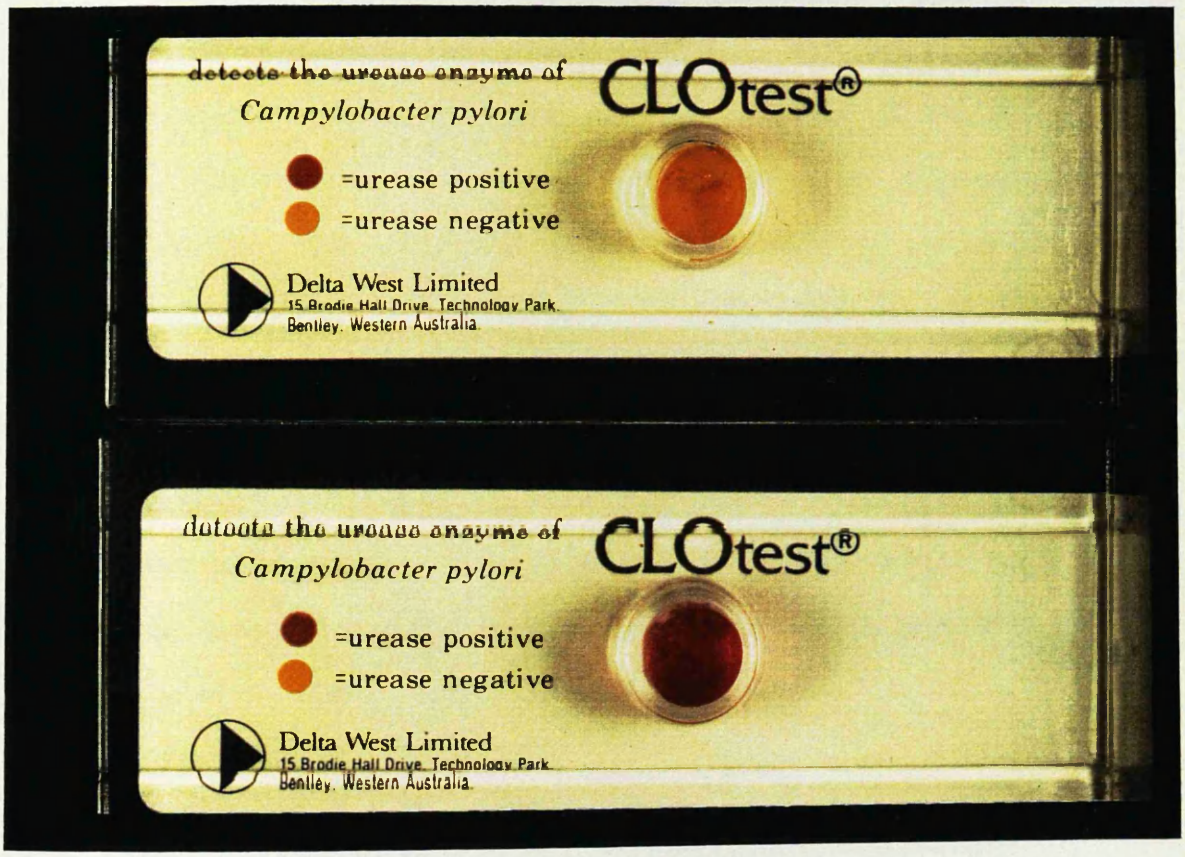



**Figure 7:** Urease slide tests showing a negative result (above) and positive result (below).

Bentley, Western Australia) is the same as that evaluated by Marshall who found a good correlation between CLOtest, culture and histological findings (60). Marshall analysed the slides for a colour change at 20 minutes, 2 hours and 24 hours, and found no false positive CLOtests if a colour change up to 24 hours was included. In the same study only one false positive was identified out of 79 patients.

**Histological Examination**

The demonstration of H pylori can be achieved on routine H&E sections (Fig 8), but is more reliably detected by the utilisation of special stains. The original papers on the subject favoured the Warthin starry silver stain (49). As with other silver stains, however, this is a complex procedure to perform and has been reported to give variable results (189,190). Our laboratory has used a cresyl fast violet stain for several years with results comparable to those for silver stains (190). One pitfall of the cresyl violet stain is uptake by intestinal mucus, however, once recognised, confusion with Helicobacter is easily avoided (Fig 9).

**CLINICAL METHODS**

Transplant Recipients

All renal transplant recipients of eighteen years and over were invited to attend for upper gastrointestinal endoscopy. The patients were approached directly or by telephone backed up by an explanatory letter. Full informed consent was obtained and ethical committee approval had been obtained. Endoscopy was timed to take place at

**Figure 8:** H & E section of the gastric mucosa. H pylori can be seen faintly in the mucus of the gastric pit. (x40)

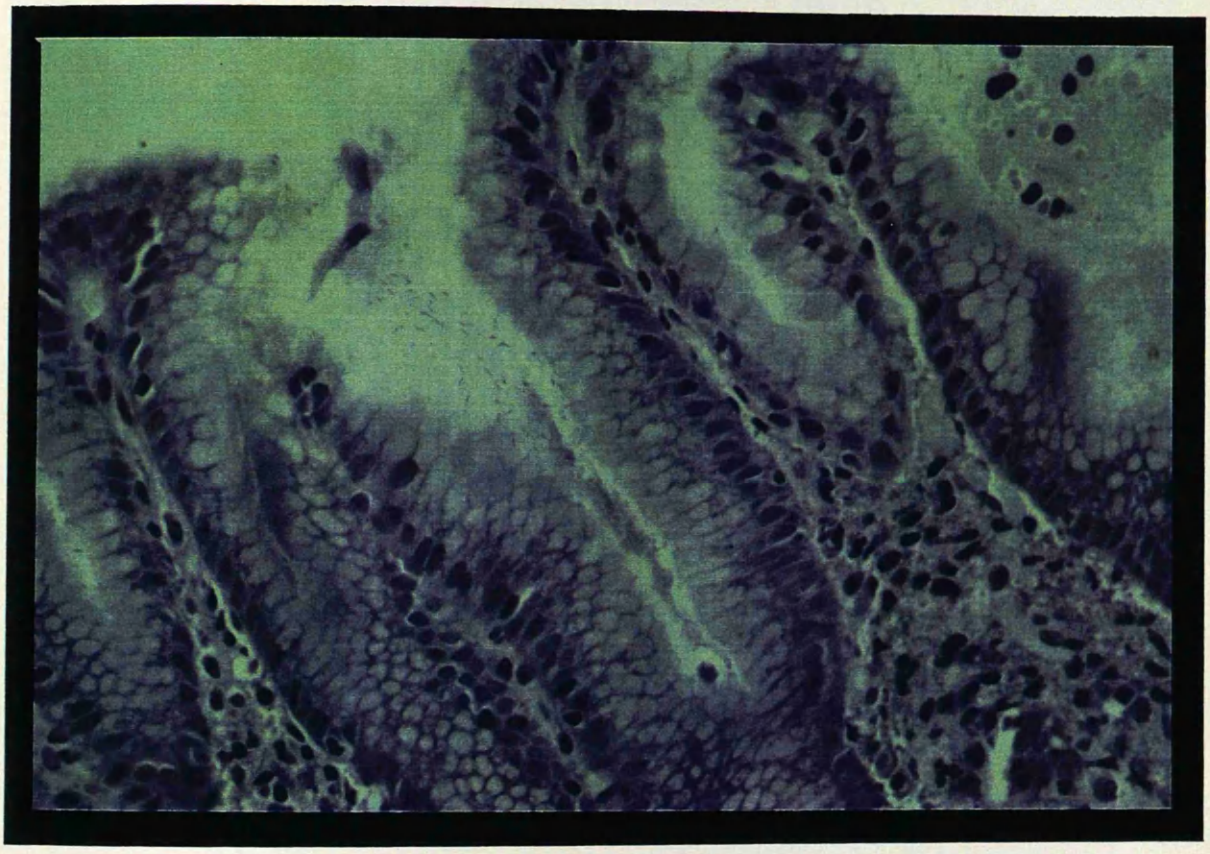


**Figure 9:** Section of gastric mucosa stained with cresyl fast violet (Figure 8). H pylori can be identified clearly as small rodlike structures on the luminal surface.(x40)

between 2 and 4 months after transplantation. Patients who had developed complications in the allograft or who, for other reasons, were deemed too ill to participate were excluded. Prior to endoscopy a questionaire was completed obtaining details of dyspeptic symptoms both before and after transplantation, along with details of current medication. The questionnaire was also completed for those patients who declined to participate in the study.

CMV status was assessed by obtaining venous blood for antibody titres prior to endoscopy. The pre-transplant CMV status of the donor and recipient were available for most of the patients as part of the routine management.

Endoscopy was performed with an Olympus 1T10 end viewing upper GI endoscope. The patients were sedated with 10mg of Diazemuls intravenously and were also given 20mg of Hyoscine butylbromide to decrease peristaltic activity during the examination. The upper gastrointestinal tract was examined as far as the second part of the duodenum. All abnormal lesions were biopsied and random biopies were obtained from the gastric antrum and from the first part of the duodenum where these were normal. At least six specimens were obtained from each site. All endoscopic findings were recorded on the patient proforma.

Immunosuppressive Regime

All of the patients were on haemodialysis or peritoneal dialysis prior to transplantation. Allografts were matched for ABO and for HLA DR. Where possible a match was also obtained for HLA A and HLA B although this was variable. The patients were commenced

on cyclosporine 16mg/kg eight hours before transplantation and were given 1G of methylprednisolone intraoperatively. In the postoperative period immunosuppresion was continued with cyclosporine and prednisolone, and information on serum cyclosporine and prednisolone dosage was available. It is also the practice of the unit to use azathioprine and OKT 3 in some instances but only three of the patients in the study group received OKT3 and none received azathioprine.

Control Tissue

Two groups of control subjects were obtained. The first group consisted of retrospective age and sex matched subjects, comprising normal gastric and duodenal biopsies drawn from formalin fixed paraffin mounted tissue in the Department of Pathology. This tissue was used for analysis of CMV and HSV. The other control tissue was obtained prospectively from patients undergoing routine diagnostic endoscopy. The examination was performed as described above and six random biopsies were obtained from both the gastric antrum and duodenum and were frozen in liquid nitrogen. The patients were age and sex matched and were matched for Helicobacter status.

Processing of Biopsy Specimens

In the early part of the study period all biopsy specimens were fixed in formalin. These were suitable for analysis of CMV, HSV, Helicobacter pylori and for grading of gastric and duodenal inflammatory changes. In the latter part of the study the specimens were split, half being fixed in formalin and half frozen immediately

in liquid nitrogen and stored at -70°C and subsequently used for analysis of T cell subsets. In the second control group all specimens were frozen and stored for T cell subset analysis and in both the transplant recipients and the second control group an additional antral biopsy was used for urease slide test analysis.

Urease Slide Test

A proprietory slide test was utilised (Clotest, Delta West Ltd, Bentley, Western Australia). A colour change up to 24 hours was deemed to be positive (60).

Antibody Titres

CMV status was assessed in the study group by complement fixation test and by IgM titres.

**Interpretation of Histological Sections**

All histological sections were examined independently by myself and by Dr M Burgoyne. The sections were identified by number only and the examiners were blind to the clinical information and to the results of the other histological sections for each patient. Where there was a difference of opinion the sections were reviewed and a consensus was reached. Analysis was for the presence of positive staining for Cytomegalovirus and Herpes simplex by immunohistochemistry and positive staining for Helicobacter pylori. In addition to the presence of staining the distribution within the section was also noted. Gastritis and duodenitis were scored as described below.

**Mucosal T Lymphocyte Subsets**

Mucosal T lymphocyte subsets were assessed on frozen tissue from the study group and from control subjects. The estimation was performed on gastric and duodenal biopsies by counting the number of Leu3 and Leu2 positive cells. The counting was performed manually by two assessors independently for the full area of the biopsy. The area of the biopsy was then measured on an Optomax image analyser and the total T cell counts were expressed per $mm^2$. The ratio of Leu3 to Leu2 was then calculated.

**Histological Assessment of Gastric and Duodenal Mucosa**

Gastric Mucosa

The system of scoring for gastritis, atrophy and intestinal metaplasia was used as described by Watt et al (201). Each section was scored from 0 to 5 as summarised below (Figs 10-12).

Gastritis

1) Minimal infiltrate of inflammatory cells in the superficial layer of the lamina propria.

3) Heavy infiltrate of inflammatory cells throughout the lamina propria.

5) Heavy infiltrate of acute and chronic inflammatory cells (active chronic superficial gastritis).

Atrophy

1) Minimal loss of specialised glands.

3) Loss of half of the specialised glands.

5) Almost complete loss of specialised glands.

Metaplasia

1) One or two gastric pits affected.

3) Half of the gastric pits affected.

5) Almost all of the gastric pits affected.

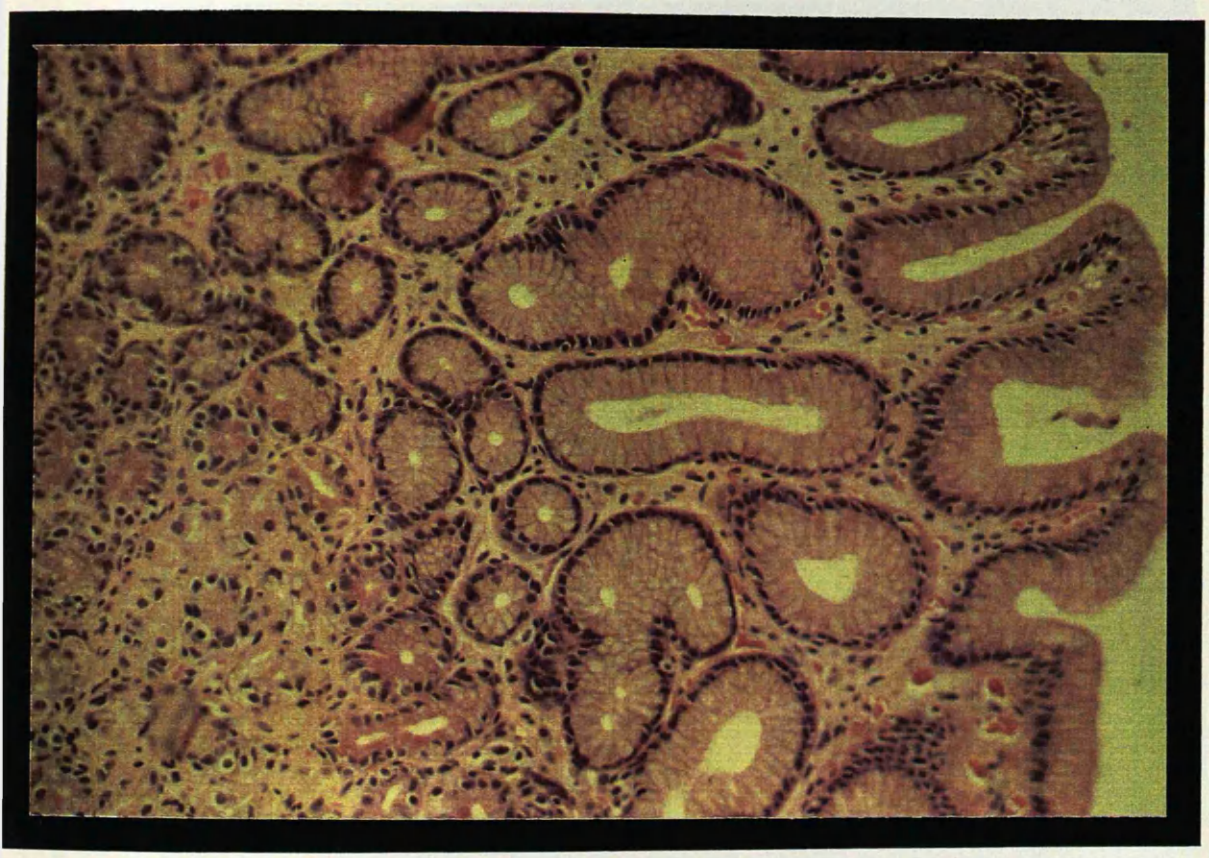


**Figure 10:** H & E section of normal gastric mucosa.(x25)

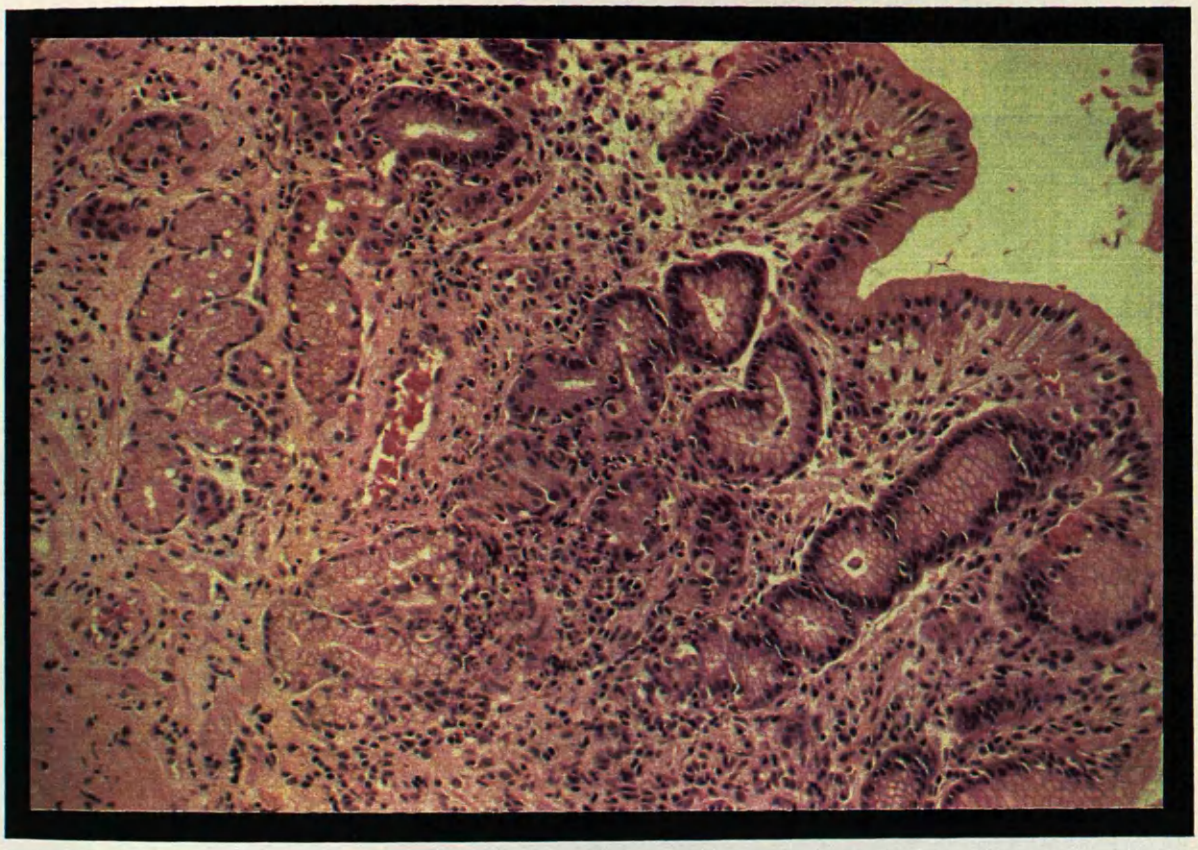


**Figure 11:** H & E section of moderate gastritis (Grade 3).(x25)

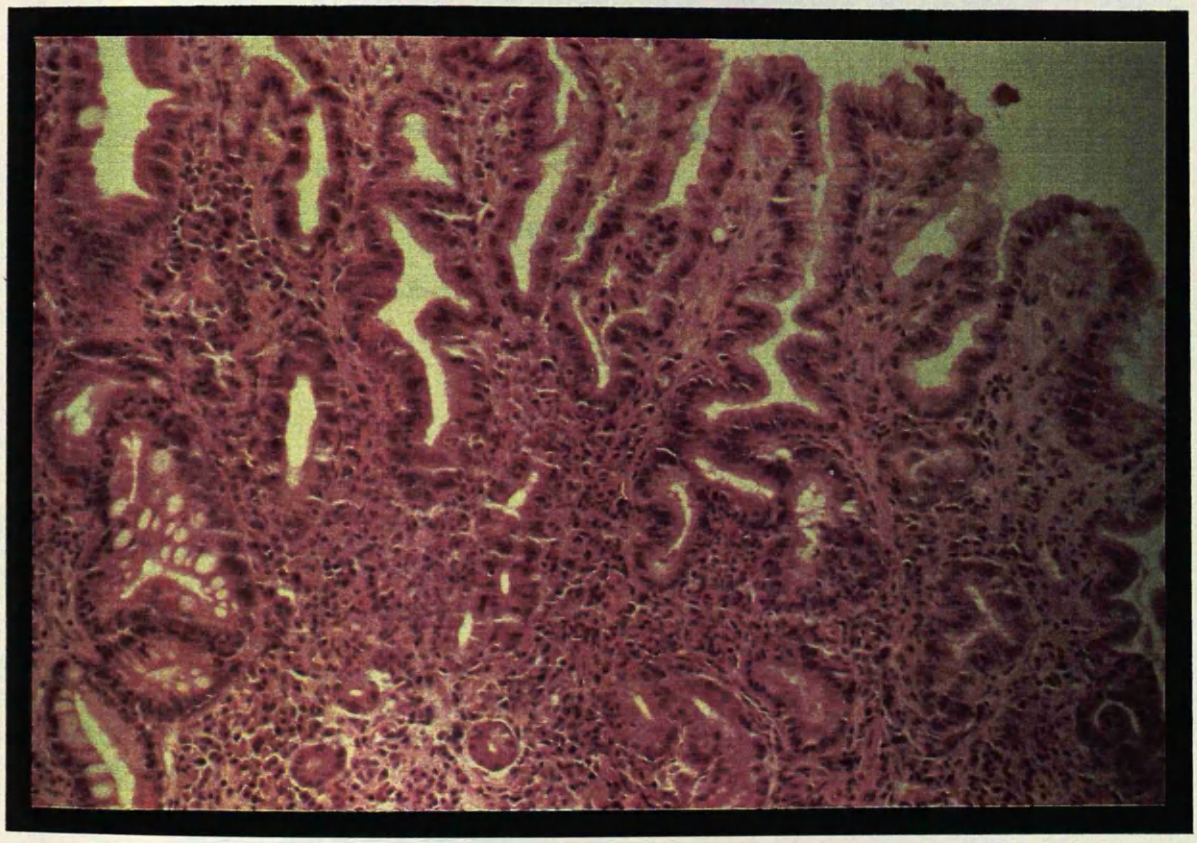


**Figure 12:** H & E section of severe gastritis (Grade 5) with intestinal metaplasia in the lower left of the section.(x25)

Duodenal Mucosa

Inflammatory changes in the duodenal mucosa were graded according to the criteria of Whitehead et al (202). The density of the inflammatory infiltrate and epithelial evidence of neutrophils and metaplasia are noted separately (Figs 13,14).

Duodenitis

0) Normal

1) Superficial epithelium normal, but increased cellularity of the lamina propria.

2) Changes as above, along with abnormality of the surface epithelium such as flattening of the cell, and nuclear hyperchromasia.

3) Erosion of the surface epithelium.

A score of 2 or 3 was taken to be consistent with significant duodenitis.

**Staining Techniques**

Cresyl Fast Violet

Reagents

1) 0.2% Cresyl violet acetate.

2) Cresyl violet differentiator; 95% alcohol - 90ml; chloroform - 10ml, acetic acid - 3 drops.

Technique

1) Remove paraffin in Xylene for 10 minutes.

2) Remove Xylene with absolute alcohol.

3) Rehydrate by immersion in graded alcohol.

4) Stain with 0.2% cresyl violet acetate for 5 minutes.

5) Rinse in water.

6) Rinse in 95% alcohol.

7) Differentiate in cresyl violet differentiator.

8) Rinse in absolute alcohol.

**Figure 13:** H & E section of normal duodenum. (x25)

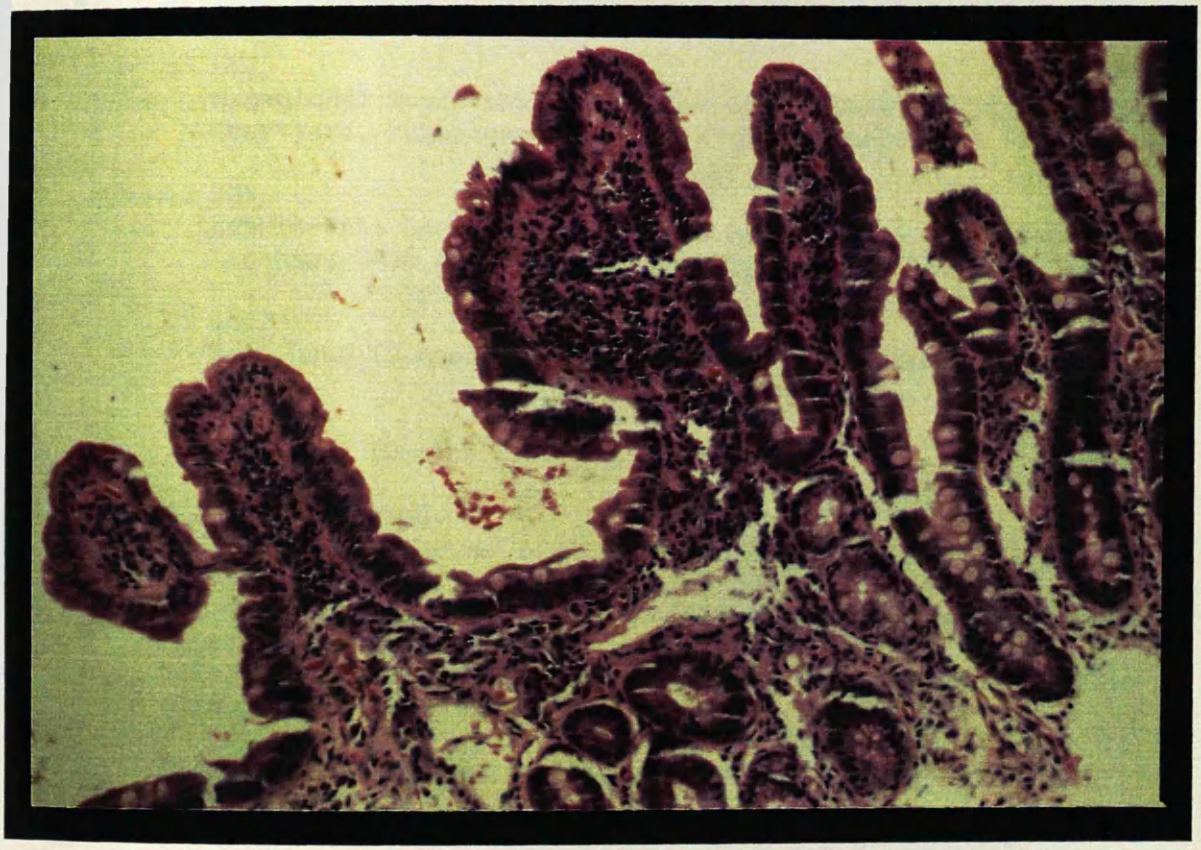


**Figure 14:** H & E section of duodenitis (Grade 2).(x25)

9) Clear section and mount.
This method will stain nuclei violet, the cytoplasm will be colourless and Helicobacter pylori will show as a deep blue violet.

**Immunohistochemistry**

Reagents

1) Blocker - 2% Bovine serum albumin.

2) Enzyme substrate - CMV, HSV- Diaminobenzidine 0.05%.

LEU2,LEU3-Levamisoloe 2.5mg, Fast red violet 5mg, 20ml veronal acetate/HCl buffer pH 9.2.

4) Primary Antibody

a) CMV
Monoclonal mouse anti CMV, clone CCH2. (DAKO Ltd,16 Manor Courtyard, Hughenden Ave, High Wycombe, Bucks HP13 5RE).

b) HSV
Rabbit anti Herpes Simplex Virus I & II H243A. (Immunotag, Lipshaw, 7446 Central Ave, Detroit, Michigan 48210).

c) Leu2
Anti Leu2 (Becton Dickenson Ltd, Cowely, Oxfordshire).

d) Leu3
Anti Leu3 (Becton Dickenson Ltd).

5) Secondary antibody
CMV Sheep anti mouse biotin peroxidase conjugate (Amersham International plc, Amersham UK).
HSV Donkey anti rabbit biotin peroxidase conjugate (Amersham International).
LEU2, LEU3 Rabbit anti mouse alkaline phosphatase conjugate (Becton Dickenson Ltd)

6) Streptavidin, biotin, peroxidase complex(Amersham International).

**Avidin Biotin Technique**

1) Remove paraffin in xylene for 10 minutes.

2) Remove xylene with absolute alcohol.

3) Rehydrate by immersion in graded alcohol.

4) Remove endogenous peroxidase by immersion in Methanol and Hydrogen Peroxide for 30 minutes (60 parts : 1 part).

5) Wash in water.

6) Treat sections with blocker for 10 minutes.

7) Remove blocker and treat sections with diluted antibody, at 4°C for 16 hours.

8) Wash sections with phosphate buffered saline for 30 minutes.

9) Apply secondary antibody at room temperature for 30 minutes.

10) Wash with phosphate buffered saline for 30 minutes.

11) Treat with ABP for 30 minutes at room temperature.

12) Wash with phosphate buffered saline for 30 minutes.

13) Treat with diaminobenzidine for 5 minutes.

14) Wash in water, stain nuclei with eosin, clear and mount.

**Alkaline Phosphatase Technique**

1) Fix in acetone for 10 minutes.

2) Treat with blocker for 10 minutes.

3) Incubate with primary antibody for one hour at room temerature.

4) Wash with tris buffered saline.

5) Apply second layer antibody for one hour.

6) Wash with tris buffered saline.

7) Add enzyme substrate for 10 minutes.

8) Wash with water and fix in buffered formalin for 10 minutes.

9) Counterstain with haematoxylin.

**Statistical Analysis**

Statistical analysis was by the Chi squared test with a Yates correction or the Fishers exact test for 2 by 2 contingency tables and by the two sample t test and Mann-Whitney U test for parametric and non parametric data respectively.

# PART II

# RESULTS

# CHAPTER 4

# CLINICAL RESULTS

**Patient Recruitment**

The study was performed between April 1988 and May 1989. All patients who had undergone renal transplantation between February 1988 and March 1989 were eligible for inclusion, producing a potential study group of 69 patients. Prior to invitation the patients' clinical history was reviewed and decisions taken to exclude patients on clinical grounds. In total 18 patients were excluded for the reasons outlined in Table 1.

Fifty one patients were therefore invited to attend. Seventeen patients declined to participate in the study, leaving 34 patients who underwent endoscopy. One endoscopic examination was a technical failure. Thirty three patients completed the study protocol and comprise the study group in this thesis.

**Non-Attenders**

Information was obtained on those patients who declined the invitation to participate. This included information on H2 receptor antagonist (H2RA) use, dyspeptic symptoms, renal function, serum cyclosporine and prednisolone dosage. This information is summarised in Table 2.

## TABLE 1

## DETAILS OF PATIENTS EXCLUDED

| | |
|---|---|
| Moved or lived outwith area | 6 |
| Allograft failure | 4 |
| Patient death | 3 |
| Major rejection episodes | 2 |
| Other medical complications | 2 |
| Under age | 1 |
| **Total** | **18** |

**Study Group**

The study group comprises 33 patients. The data on dyspeptic symptoms, H2RA use, renal function, serum cyclosporine and prednisolone dosage are shown in Table 2 for comparison with the non-attenders. In addition the study group was divided into symptomatic and asymptomatic and the data for both of these groups is illustrated in Table 3. Symptoms refer to post transplantation.

**Comparison of Groups**

There were no significant differences between the study group and the non-attenders in terms of symptoms, H2RA use, renal function cyclosporine levels and prednisolone dose. Comparison of the two subdivisions of the study group revealed a significantly higher prevalence of H2RA use in the symptomatic group. There was a tendency for the symptomatic group to be older to have higher serum levels of urea, creatinine, cyclosporine and higher prednisolone dose, but these differences did not attain statistical significance.

**Analysis of Symptoms and H2RA Use**

Twenty of the patients attending for endoscopy (60%) had symptoms referrable to the upper GI tract compared to 8 (47%) of those who did not attend.

In the study group there was a non significant increase in symptoms following transplantation, although this change was not seen in those who did not participate in the study. Details of the symptoms in both groups are illustrated in Table 4.

Eight of the non-attenders (47%) were on ranitidine at the time

of the study, compared with 15 patients (45%) in the study group. This comprised 12 in the symptomatic group (60%) and 3 in the asymptomatic group (23%) ($p=0.02$). In the study group only 5 of the 15 patients (33%) experienced an improvement in their symptoms following administration of H2 receptor antagonists.

**TABLE 2**

**COMPARISON OF NON-ATTENDERS AND STUDY GROUP**

| | Non-attenders (N = 17) | Study Group (N = 33) |
|---|---|---|
| Dyspepsia | 8 (47%) | 20 (60%) |
| H2RA | 7 (41%) | 15 (45%) |
| Mean urea (mmol/l) | 11.27 | 10.7 |
| Mean creatinine (umol/l) | 189 | 166 |
| Mean serum Cyclosporine (nmol/l) | 161 | 129 |
| Mean prednisolone dose (mg) | 17 | 18.4 |

## TABLE 3

## COMPARISON OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS

| | SYMPTOMATIC (N=20) | ASYMPTOMATIC (N=13) |
|---|---|---|
| Mean age | 41.9 | 38.2 |
| H2RA | 12 | 3* |
| Mean urea (mmol/l) | 11.4 | 9.8 |
| Mean creatinine (umol/l) | 179 | 197 |
| Mean serum Cyclosporine (nmol/l) | 134 | 121 |
| Mean prednisolone dose(mg) | 20.1 | 15.8 |

*** p=0.02 (Fishers exact test)**

TABLE 4

DETAILS OF PRE-TRANSPLANT AND POST-TRANSPLANT

SYMPTOMS IN BOTH GROUPS

| Symptoms | Study Group (N=33) | | Non-attenders (N=17) | |
|---|---|---|---|---|
| | Pre-Tx | Post-Tx | Pre-Tx | Post-Tx |
| Heartburn | 8 | 15 | 4 | 4 |
| Dyspepsia | 7 | 12 | 3 | 5 |
| Nausea | 4 | 1 | 1 | 1 |
| Anorexia | 2 | 1 | 1 | 1 |
| Total Patients | 10(30%) | 20(60%) | 7(41%) | 8(47%) |

## Endoscopic Findings

The endoscopic findings are summarised in Table 5. The inflamamtory mucosal changes refer to endoscopic appearances only. The histological assessment of these changes will be discussed in the following section.

There was no significant difference in the number of abnormalities detected in the symptomatic and asymptomatic groups. There was also no significant difference in the prevalence of each endoscopic abnormality between the two groups.

## Histological Assessment of the Gastroduodenal Mucosa

### Gastric Mucosa

The gastric mucosa was assessed for the presence of intestinal metaplasia, atrophy and gastritis. Each of these was scored from 0-5. A score of 0-2 was taken to be within normal limits, and a score of 3-5 was viewed as an abnormal result. The findings are shown in Table 6. There was no significant difference in the prevalence of gastritis, atrophy or metaplasia between the two groups.

### Duodenal Mucosa

A similar scoring system was employed for assessment of the duodenal mucosa and is shown in Table 7. For the assessment of duodenitis a score of 0 or 1 was taken to be normal and a score of 2 or 3 was regarded as consistent with significant duodenitis. Once more there was no significant difference between the two groups.

## Summary

In this study group as a whole 67% of patients had an identifiable abnormality on either endoscopic or histological assessment of the upper GI tract. There were 15 patients in the symptomatic group (75%) with an abnormal examination, compared to 7 in the asymptomatic group (53%). This difference was not significant.

Four patients, all symptomatic, were shown to have a gastric ulcer, although there were no duodenal ulcers identified in the study group. Histological gastritis was identified in 10 patients and histological duodenitis in 16. There was no demonstrable relationship between upper GI pathology and renal function or immunosuppression.

TABLE 5

ENDOSCOPIC ABNORMALITIES IN THE STUDY GROUP

| Finding | Symptomatic (N = 20) | Asymptomatic (N = 13) | Total (N=33) |
|---|---|---|---|
| Oesophagitis | 2 | 1 | 3 |
| Gastritis | 7 | 2 | 9 |
| Gastric ulcer | 4 | 0 | 4 |
| Duodenitis | 5 | 3 | 8 |
| Duodenal ulcer | 0 | 0 | 0 |
| Duodenal polyps | 5 | 4 | 9 |
| **Total Number of patients** | 14 (70%) | 7 (54%) | 21 (63%) |

TABLE 6

HISTOLOGICAL ABNORMALITIES IN THE GASTRIC MUCOSA

| | Symptomatic (N = 20) | Asymptomatic (N = 13) | Total (N=33) |
|---|---|---|---|
| Intestinal metaplasia | 1 | 0 | 1 |
| Atrophy | 5 | 3 | 8 |
| Gastritis | 8 | 2 | 10 |
| **Total Patients** | 9 (45%) | 4 (31%) | 13 (39%) |

TABLE 7

HISTOLOGICAL ABNORMALITIIES IN THE DUODENAL MUCOSA

| | Symptomatic (N = 20) | Asymptomatic (N = 13) | Total (N=33) |
|---|---|---|---|
| Gastric metaplasia | 7 | 6 | 13 |
| Duodenitis | 8 | 8 | 16 |
| **Total Patients** | 10(50%) | 9(69%) | 19(57%) |

**Discussion**

This is obviously not a comprehensive review of the prevalence of upper gastrointestinal disease in renal transplant recipients, a subject which has been widely studied in the past. The major limitation in achieving this is that when fully informed consent is required for this type of invasive procedure there will inevitably be a proportion of patients who do not wish to participate. In addition it was decided, for practical reasons, to exclude patients who lived at a distance from the hospital, and to exclude patients who were unwell either due to rejection or to other complications. It was also decided that it would not be ethical to stop H2RA therapy prior to endoscopy and this may obviously alter the spectrum of disease.

In spite of these limitations, however, those who took part in the study and those who declined were comparable for all of the parameters studied and particularly were comparable for the prevalence of dyspeptic symptoms and H2 receptor antagonist use. It therefore seems unlikely that we have preselected a group of patients who have an increased prevalence of upper gastrointestinal disease when compared to transplant recipients as a whole.

**Peptic Ulcer**

The prevalence of peptic ulcer in the study group was 12%, a figure in keeping with many other reports (1,2,12). All of the lesions were chronic peptic ulcers and not mucosal erosions. The lesions were also pre-pyloric with no duodenal ulcers, a finding which is at variance with other reports. It is of course possible that H2RA have modified the prevalence of duodenal ulceration.

All of the patients with peptic ulceration had symptoms of dyspepsia, although in two patients the symptoms were mild and these two were not on H2RA therapy. During the study period there were no complications of peptic ulceration in any of the transplant recipients. This observation is in keeping with reports which have demonstrated a decreasing incidence of complications, since the very high rates reported between 10 and 20 years ago (9,10).

**Mucosal Inflammatory Lesions**

By far the majority of abnormalities detected at endoscopy were mucosal inflammatory lesions without ulceration accounting for 81% of the abnormalities detected and affecting 66% of the study group.

**Duodenitis**

Duodenitis was the most common abnormality in the study group, occuring in 16 (48%) patients of whom 8 had dyspeptic symptoms. These figures relate to histological assessment since endoscopic assessment is known to correlate poorly with histological changes (203).

The relationship of duodenitis to dyspeptic symptoms remains controversial. Cheli et al reported that duodenitis was uncommon in asymptomatic patients, being identified in only 6% of healthy individuals (204). These findings, however, were at variance with a previous report by Kreuning et al who identified a higher prevalence of duodenitis in asymptomatic volunteers (205). One significant difference between the two studies was the age of the subjects studied. In Cheli's report the subjects were all under twenty five,

whereas in the report by Kreuning an older population was studied. It may be, therefore, that the distribution of duodenitis in the population is age dependent as has been recognised with gastritis.

Once a disease process has been identified in asymptomatic individuals its role as a cause of symptoms can be questioned. This has certainly been the case with type B gastritis and has also been suggested with respect to duodenitis(206). This, however, does not seem to be the case and there is strong evidence to show symptomatic improvement with H2 receptor antagonists (203,207). In addition Kreuning et al identified duodenitis in 80% of patients with non ulcer dyspepsia (205). Other authors have reported a much lower prevalence of duodenitis in symptomatic individuals. Thomson et al could identify duodenitis in only 2.8% of symptomatic patients (208). This study, however, relied on endoscopic appearances only and the results are therefore not comparable to histological studies of the duodenal mucosa.

The relationship of duodenitis to duodenal ulceration has also been the subject of some debate. It is certainly true that H2 receptor antagonists can produce symptomatic and histological improvement (207), although histological improvement is disputed by some authors (206). There is a strong association between duodenitis and type B gastritis (204,206) which would be in keeping with the recognised association between type B gastritis and duodenal ulceration, and duodenitis has been demonstrated in up to 100% of patients with duodenal ulceration (205). Further evidence to support a relationship with duodenal ulceration comes from follow up of patients with duodenitis. Thomson et al (208) found that 42% of

patients with duodenitis developed duodenal ulceration during a mean follow up of 3.5 years, a finding similar to that reported by Jonsson (203).

In the study group seven patients with duodenitis were on H2RA therapy producing symptomatic improvement in two. It was also apparent that duodenitis was as common in the asymptomatic group, occuring in 61% which is much higher than would be expected from the reports discussed above. One possible explanation is that H2 receptor antagonists produced symptomatic improvement but did not have an effect on the histological abnormality. This, however, does not seem to be a likely explanation since only 25% of the asymptomatic patients with duodenitis were on H2RA therapy at the time of the study.

It is difficult to be sure of the significance of duodenitis in the study group. It is certainly a common abnormality but was seen to be as common in the asymptomatic group as it was in the symptomatic group. Current knowledge of duodenitis in the general population would suggest that it is usually associated with symptomatic dyspepsia and may therefore be a cause of symptoms in some of the study group.

**Gastritis**

Antral gastritis was the second commonest abnormality, occurring in 10 (30%) patients, 8 of whom had symptomatic dyspepsia. The role of gastritis in symptomatic dyspepsia has been the centre of some debate for many years (57, 206), but studies of Helicobacter pylori in the past 8 years have demonstrated resolution of gastritis

and improvement in symptoms following eradication of the organism (73,209).

Five of the patients were on H2RA therapy and had not experienced any improvement in their dyspeptic symptoms. This is not particularly surprising since it is widely recognised that H2RA have no demonstrable effect on the severity of gastritis and are no more effective than placebo in relieving the symptoms of dyspepsia (57,78).

**Oesophagitis**

This was the least common endoscopic abnormality being identified in only 3 patients, 2 with symptoms. The low prevalence of oesophagitis is rather surprising since it has been reported in up to 36% of transplant recipients (19). Once again however H2RA may have modified the clinical picture.

**Duodenal Polyps**

These were an unexpected and common finding in the study group, occurring in 9 (27%) patients, 5 in the symptomatic group and 4 in the asymptomatic group. The lesions were broad based and multiple usually less than 5mm in diameter, affecting the duodenal cap. In all but 2 patients they were associated with duodenitis. The inflammatory changes, however, were always mild and the polyps did not have the appearance of pseudopolyps which can be seen in association with severe duodenitis. Histological examination of these polyps revealed duodenal mucosa only and it seems likely that these lesions are submucosal.

There have been no previous descriptions of these lesions in the literature. While many different polypoidal lesions have been described in the duodenum, such as adenomas, Brunners gland adenomas and hamartomatous polyps, these lesions are uncommon and one would not expect a prevalence of 27% in an unselected group of patients (210). One interesting possibility is that these represent areas of submucosal lymphoid hyperplasia which has been described producing polypoidal lesions associated with cytomegalovirus infections in the small bowel of patients with the AIDS (149). The relationship of these polyps to cytomegalovirus will be discussed in chapter 6.

**Symptomatic Dyspepsia**

Endoscopy has revealed that a high proportion of patients (72%) had one or more abnormality in the upper GI tract. It seems unlikely that the polyps described above could be responsible for symptoms, but all of the patients with duodenal polyps had at least one other endoscopic or histological abnormality and, therefore, if these polyps are excluded the number of patients with an abnormality of the upper GI tract remains unchanged. Of the patients with symptomatic dyspepsia 70% had an abnormality compared with 76% of the asymptomatic group (polyps excluded). This difference is not significant. There were, however, more patients in the symptomatic group with multiple lesions. The symptomatic group had a mean of one lesion per patient compared with 0.46 in the asymptomatic group ($p<0.05$).

An important point to note is that 81% of patients with an abnormality suffered from a mucosal lesion, without ulceration. In

particular the symptomatic group comprised 14 patients with an endoscopic lesion of whom only 4 (29%) had a peptic ulcer.

The importance of non-ulcer dyspepsia has been poorly recognised previously in transplant recipients. Chisholm did note a high prevalence of dyspepsia in patients with a normal barium meal examination, but did not have endoscopic findings to explain this (1). It is, of course, possible that the early studies using contrast radiology underestimated the prevalence of mucosal inflammation in view of the recognised inability of this technique to diagnose these lesions. However, even after the introduction of endoscopic techniques, mucosal inflammatory lesions were not widely reported and the prevalence has varied.

Schiessel et al reported erosions and ulcers together, but did not specify the prevalence of each type of lesion (7). Alexander identified oesophagitis, gastritis and duodenitis in 36%, 22% and 36% respectively in liver transplant recipients (19). Franzin reported a similar prevalence of gastritis and duodenitis (8), but neither of the studies specified the total number of patients affected by mucosal lesions.

The highest prevalence of gastroduodenal lesions is that reported by Alijani of 72% (211). In these patients, however, the endoscopy was performed within 96 hours of transplantation and within 48 hours of nasogastric intubation, and the authors do not give sufficient details of the lesions to be sure that these were not traumatic.

Two further publications report a lower prevalence. Cohen (11) found histological gastritis in only 3 of 573 patients (0.005%) and

Timoney (12) reported duodenitis in 8% of transplant recipients.

The reasons for the wide discrepancy in the studies reported above may be related to the diagnostic methods used and to the timing of endoscopy. As discussed previously the correlation of endoscopic and histological abnormalities is poor, and it may not be valid to assume that the prevalence of upper GI lesions is independent of time elapsed since transplantation. Indeed available evidence would suggest that the factors which may contribute to upper GI tract disease will improve with time. Steroid administration and immunosuppressive therapy will be reduced, gastric acid secretion will return towards normal levels (17) and peak incidence of viral infection will occur within the first 2 months following transplantation (122).

If the studies reporting extremes of prevalence are examined in detail we find that histological evidence was not used (12,211), whereas in the studies reporting a prevalence of 35-55% histological assessment of the upper GI tract was also available (8,19),and are in keeping with the data from the study group.

**Relationship to Renal Function and Immunosuppression**

There was no significant association between any of the indentified pathologies and serum urea, creatinine, cyclosporine or prednisolone dose. The mean urea and creatinine in the study group were 10.7mmol/l and 166umol/l respectively and, although outside of the normal reference range, they are not markedly abnormal. Previous work would not lead one to expect a profound influence on the upper GI tract since none of the reported studies have demonstrated any

convincing association between upper GI tract disease and renal function (7,12,24). The mean prednisolone dose was 18.4mg and would not necessarily be associated with a high risk of upper GI tract disease(37,39). The influence of cyclosporine on the upper GI tract is more difficult to assess. Many of the early studies into peptic ulceration in transplant recipients were performed prior to the introduction of cyclosporine. Knechtle (9,10) demonstrated a reduction in the complications of peptic ulceration following the introduction of cyclosporine but the other variables discussed in chapter 1 also changed over the same period of time.

**Conclusions**

The results of the endoscopic and histological examination of the gastroduodenal mucosa has highlighted a high prevalence of mucosal inflammatory lesions without ulceration. None of these lesions however were significantly associated with symptomatic dyspepsia. There was a strong tendency for gastritis to be commoner in symptomatic patients and it may be that a significant difference would have been observed if larger patient numbers could have been recruited. Duodenitis, however, was commoner in the asymptomatic group which is surprising in view of the association with symptomatic dyspepsia in the general population (204,205). The prevalence of ulceration in the study group was 12% which is in keeping with published reports although the preponderance of gastric ulcers is slightly unusual. It is possible, however, that H2RA therapy may have influenced this. There was no association between GI pathology and renal function or immunosuppression and the aetiology of these

lesions has not been explained. This will be discussed in more detail in the following two chapters with respect to Helicobacter, Cytomegalovirus and Herpes simplex.

# CHAPTER 5

# HELICOBACTER PYLORI

**Prevalence of Helicobacter Pylori**

Helicobacter pylori was detected by either urease slide test or by histology in 16 patients. The sites of colonisation and the distribution in the two subgroups is shown below in Table 8.

All of the patients colonised by H pylori had involvement of the gastric antrum. In two patients the organism was also identified histologically in the duodenal biopsy specimens. The prevalence of H pylori was significantly higher in the symptomatic group ($p=0.02$), although there was no association with any particular symptom (Table 9).

**Comparison of Detection Methods**

Of the 16 patients identified as H pylori positive this was identified histologically in 15, and by the urease slide test in eleven. In one patient the slide test alone was positive, leaving 5 patients diagnosed solely by histology (Table 8).

**TABLE 8**

**PREVALENCE AND DISTRIBUTION OF H PYLORI**

| Method | Number Positive: Symptomatic (N=20) | Asymptomatic (N=13) | By Each Method (N=33) |
|---|---|---|---|
| Urease slide (Gastric only) | 10 | 1 | 11 |
| Histology (Gastric) | 12 | 3 | 15 |
| Histology (Duodenal) | 2 | 0 | 2 |
| Total patients colonised | 13 (65%) | 3 (23%)* | 16 (48%) |

*p=0.02 (Fishers exact test)

**TABLE 9**

**ASSOCIATION OF H PYLORI WITH UPPER GI SYMPTOMS**

| Symptom | H pylori + (N = 13) | H pylori - (N = 7) | Total (N=20) |
|---|---|---|---|
| Pain | 8 (61%) | 5 (71%) | 13 |
| Heartburn | 0 | 1 (14%) | 1 |
| Anorexia | 0 | 1 (14%) | 1 |
| Nausea | 9 (69%) | 5 (71%) | 14 |

**Relationship of H Pylori to Endoscopic and Histological Abnormalities**

The association of H pylori with endoscopic abnormalities and with histological gastritis and duodenitis was assessed. These results are summarised in Table 10.

The results demonstrate a significant association between H pylori colonisation gastritis, and gastric ulceration, although there was no significant association with oesophagitis, duodenitis, or duodenal polyps.

**Relationship of H Pylori to Atrophy and Metaplasia**

Table 11 illustrates the relationship of H pylori to metaplasia and atrophy in gastric and duodenal specimens.

There was no significant difference in the distribution of gastric H pylori in patients with intestinal metaplasia and gastric atrophy of the antral mucosa or gastric metaplasia of the duodenal mucosa. The effects of duodenal colonisation could not be assessed since only 2 patients were affected, one with gastric metaplasia and one without.

TABLE 10
RELATIONSHIP OF H PYLORI TO ENDOSCOPIC AND HISTOLOGICAL ABNORMALITIES IN THE UPPER GI TRACT

| Abnormalities | H pylori + (N=16) | H pylori - (N=17) | Total (N=33) |
|---|---|---|---|
| Oesophagitis | 1 | 2 | 3 |
| Gastritis | 10 | 0 | 10 * |
| Gastric ulcer | 4 | 0 | 4 ** |
| Duodenitis | 7 | 9 | 16 |
| Duodenal polyps | 4 | 5 | 9 |

* P=0.0001 (Fishers exact test)

** P=0.04 (Fishers exact test)

TABLE 11

RELATIONSHIP OF H PYLORI TO ATROPHY AND METAPLASIA

| | H pylori + (N=16) | H pylori - (N=17) | Total (N=33) |
|---|---|---|---|
| **Gastric Mucosa** | | | |
| Gastric atrophy | 5 | 3 | 8 |
| Metaplasia | 5 | 1 | 6 |
| **Duodenal Mucosa** | | | |
| Metaplasia | 5 | 6 | 11 |

**Relationship to Renal Function, Immunosuppression and Age**

The prevalence of H pylori colonisation was also assessed with respect to renal function, immunosuppression and age. These results are summarised in Table 12.

There was no significant difference in mean age or mean Cyclosporine levels between the 2 groups. There was a tendency towards higher serum urea and creatinine and a higher Prednisolone dose in those who were H pylori positive, but this was not significant. There was no significant difference in the mean age of the two groups. In addition there was no significant difference in the prevalence of H pylori in different age groups (44% in those greater than 40 and 53% of those 40 and under).

**Time Elapsed Since Transplantation**

The mean time between transplantation and endoscopy was 13.5 weeks (range 8-19). For H pylori positive patients this was 13.4 weeks (range 10-18) and for H pylori negative it was 13.6 weeks (range 8-19): a non-significant difference.

**Summary**

Sixteen of the patients in the study group (48%) had Helicobacter pylori in the gastric antrum. In addition 2 of these patients had colonisation of the duodenum. Helicobacter pylori was significantly more common in patients with symptomatic dyspepsia and all of the patients in the study group with gastritis or gastric

ulcer were H pylori positive ($p=0.0001$, $p=0.04$). There was no significant association between H pylori and other endoscopic or histological abnormalities, with serum urea, creatinine, prednisolone dose, Cyclosporine levels, age or time elapsed since transplantation.

TABLE 12

RELATIONSHIP OF H PYLORI TO RENAL FUNCTION, IMMUNOSUPPRESSION AND AGE

| | H pylori + (N=16) | H pylori - (N=17) |
|---|---|---|
| Mean urea (mmol/l) | 11.9 | 9.6 |
| Mean creatinine (umol/l) | 182 | 152 |
| Mean serum Cyclosporine (nmol/l) | 126 | 133 |
| Mean Predinoslone dose (mg/day) | 20.4 | 16.4 |
| Mean Age(years) | 42.5 | 38.8 |

## Discussion

### Prevalence

The prevalence of H pylori in the study group was 48%. The difficulty in interpreting this lies in the recognised high prevalence of the organism in the general population. Ideally a control group should have been used, but obviously patients attending for upper GI endoscopy are a pre-selected population who are likely to have a high prevalence of H pylori. To obtain a truly representative group therefore, healthy volunteers would have to have been employed, age and sex matched for the study group. There are, however, many difficulties in conducting invasive investigations in healthy individuals.

For this reason the precise prevalence of H pylori is difficult to determine. Most studies have relied on indirect methods such as urea breath testing or serology. Studies using these methods have demonstrated a prevalence of 20-30%, but have also demonstrated striking variations with age, socioeconomic status and ethnic origins (111,212).

Serological studies have demonstrated a high prevalence of H pylori antibodies in up to 32% of asymptomatic individuals and have confirmed an increasing prevalence with age, rising from 10% in those under 25 to 60% in those over 55 (104,112).

It is likely however, that serological studies will overestimate the prevalence of infection. Rathbone et al demonstrated detectable H pylori antibodies in all patients with non

ulcer dyspepsia, and although the mean IgG and IgA titres were higher in patients who were H pylori positive, there was considerable overlap with those who were H pylori negative (106). Von Wulfen et al found positive antibody titres in 41% of H pylori negative patients and in 46% of H pylori positive patients (104), and Vaira demonstrated a fall in antibody titres following eradication of the organism, but the titres did not return to normal levels (105). It seems, therefore, that positive titres, while indicative of previous exposure to the organism, correlate poorly with active infection.

Urea breath testing on the other hand has been demonstrated to have a high specificity for H pylori and this technique is likely to give a more accurate estimate of the prevalence in the general population (213). Graham et al studied asymptomatic individuals by urea breath testing and found an increase in prevalence from 5% in patients under 45 to 75% in those over 65 (212). The same authors also demonstrated a 20% prevalence in US citizens compared to 46% in Indians and 60% in Chinese (111).

In the study group the mean age of H pylori positive patients was 42.5 compared to 38.8 in those who were H pylori negative. This difference was not significant and, in addition, there was no significant difference in the prevalence of the organism in patients under 40 when compared to those 40 and over.

When these factors are taken into account therefore, there appears to be a higher prevalence of H pylori in the study group than would be expected in an unselected group of normal individuals.

Detection of H Pylori

In all but one patient who was found to be H pylori positive this was detected on histological examination, leaving only one patient diagnosed by urease slide test alone. In the 15 patients with histological evidence of H pylori CLO test was positive in 11 (73%) which is consistent with previous reports for single biopsy CLO test (60,189).

Distribution and Relationship to GI Pathology

All 16 patients positive for H pylori had evidence of gastric colonisation and 2 of them (12.5%) had H pylori identified in the duodenum. This distribution is in keeping with previous reports in the general population which have demonstrated a much higher prevalence of H pylori in the antrum, with duodenal involvement in between 2% and 18% of cases (214,215). In addition duodenal involvement is almost invariably associated with antral colonisation (62,215).

In our study group there was a strong association between H pylori and gastritis. All ten patients with histological gastritis had evidence of H pylori in the gastric antrum. Unusually perhaps, six patients with H pylori did not have gastritis using our scoring system. Part of the explanation for this is the arbitrary cut off which was chosen and all H pylori patients with one exception, did have gastritis graded at one or above. Interestingly the H pylori positive patient who did not have histological evidence of gastritis

was the patient identified by urease slide test alone. It may be, therefore, that this represents a false positive CLO test, which would be unusual (60), or it reflects the patchy nature of H pylori and gastritis which is perhaps the more likely explanation (189).

There was also a positive correlation with gastric ulceration, all 4 of our patients being H pylori positive. This is a slightly higher percentage of patients than might be expected (94,95) but probably reflects the small number identified in the study group.

In the study group there was no demonstrable association between H pylori and oesophagitis, duodenitis or duodenal polyps.

The relationship of H pylori to oesophagitis seems likely to be a casual association. Walker et al identified H pylori in only 25% of patients with oesophagitis, but this was invariably associated with gastric Helicobacter (215). In addition they identified oesophageal H pylori in only 29% of patients with Barrats oesophagus where a high prevalence of H pylori would perhaps be expected.

The importance of Helicobacter as a cause of duodenitis is difficult to ascertain. As discussed above H pylori is found in up to 18% of duodenal biopsies but is almost always accompanied by antral involvement, leading some authors to suggest that it is merely a commensal (215). Other authors, however, have reported a much stronger association between H pylori and duodenitis. Johnstone et al reported duodenal H pylori in 100% of patients with active duodenitis and no H pylori identified in patients without duodenitis (62). Wyatt et al identified gastric Helicobacter in 88% of patients

with duodenitis and duodenal H pylori in 53% (91). In an earlier study Steer identified H pylori adjacent to duodenal ulcers in 73% of patients (216).

In our group 43% of patients with duodenitis had H pylori in the gastric antrum but in the duodenum in only 12%. In addition 56% of patients with gastric Helicobacter had no evidence of duodenitis. If we relax our diagnostic criteria and include a score of one or above we can show duodenitis in 87% of H pylori positive individuals, but also duodenitis in 94% of H pylori negative individuals.

Although our results are at variance with those of Wyatt, Johnstone and Steer they are compatible with those reported by Walker (215). They are also in keeping with a recent report by Shousha et al who found H pylori in the duodenum of 9% of patients, despite identifying gastric metaplasia of the duodenal mucosa in 62% (90). Interestingly this occurred in a group of patients with renal failure on dialysis. The authors concluded that the duodenal environment in haemodialysis patients is hostile to H pylori, although the reasons why this should be are unclear.

Relationship to Symptomatic Dyspepsia

There was a significantly higher prevalence of H pylori in patients with symptomatic dyspepsia compared to those who were asymptomatic; thirteen out of twenty in the symptomatic group, compared with three out of thirteen asymptomatic patients. If the symptomatic group are subdivided, however, there is no significant

association with any particular upper GI symptom.

The relationship of H pylori and gastritis to symptomatic dyspepsia has already been discussed in Chapters 2 and 4 and the results are consistent with previous reports.

Relationship to Renal Function and immunosuppression

The relationship of H pylori to renal function is difficult to ascertain. In the study group the mean serum urea and creatinine were no higher in H pylori positive patients. Only one previous study has reported H pylori in renal failure patients on haemodialysis (90). This revealed a very low prevalence of H pylori (2.5%), although the report dealt with duodenal involvement only and did not comment on gastric colonisation. There are theoretical reasons why an elevated blood urea may encourage H pylori by increasing the substrate available for urease, thereby creating a favourable environment for growth of the organism. In transplant recipients and haemodialysis patients, however, it seems unlikely that blood urea will be sufficiently elevated for this to be an important factor and there are no reports of H pylori in untreated renal failure.

A complicating factor in the study group is that renal function may not be independent of immunosuppression. Compromised renal function is often a manifestation of rejection and will be accompanied by an increased dose of immunosuppressive drugs. In the study group the mean prednisolone dose was not significantly higher

in H pylori positive patients, and there was no difference in serum cyclosporine levels.

The precise role of immunosuppression in helicobacter infection is unclear. There are no reports in the literature of H pylori in transplant recipients. One recent publication by Francis et al has reported the prevalence of H pylori in patients who are HIV positive (217). The authors demonstrated that 14% of HIV positive patients were H pylori positive compared with 48% of control subjects, and the authors concluded that the immunosuppression associated with AIDS was not of importance in the normal immune response to H pylori. There are, however, several problems with this interpretation. Firstly the control group were drawn from a population of dyspeptic patients who are likely to have a high prevalence of H pylori and, secondly, the study group comprised 51 patients who were HIV positive only 16 of whom had AIDS. The immune status of these patients, therefore, was unknown and it is difficult to be certain that T cell suppression is not a factor in H pylori infection. The role of the immune response to H pylori and the effects of immunosuppression will be discussedin Chapter 8.

**Conclusions**

The study group has been demonstrated to have a high prevalence of H pylori (48%). It is difficult to be certain that this is higher than in the general population, primarily because of the difficulty in establishing the precise prevalence in a healthy population. The

available evidence would suggest, however, that the prevalence in an age matched group of healthy individuals is likely to be less than 20%. One important factor which supports a pathogenic role is the significantly higher prevalence of H pylori in the symptomatic group. It is unfortunate that a larger patient group was not available to allow a definite answer to be obtained. There are inevitable difficulties in performing large endoscopic population studies and perhaps it would now be appropriate to study transplant recipients by non invasive methods such as urea breath testing.

# CHAPTER 6

# CYTOMEGALOVIRUS AND HERPES SIMPLEX

**Prevalence of Cytomegalovirus in the Study Group and Control Tissue**

The prevalence of cytomegalovirus was determined in biopsy material from the gastroduodenal mucosa of the study group and in normal gastric and duodenal biopsies obtained from fixed tissue stored in the Department of Pathology. In the study group there were 33 biopsy specimens from each site and in the control group there were 36 gastric and 25 duodenal biopsies. All of the specimens were examined by immunohistochemistry.

The number of positive results detected by immunohistochemistry is summarised in Table 13. In the control group the gastric and duodenal biopsies were obtained from different subjects and the data, therefore, is expressed as the number of positive biopsies. There was a significantly higher prevalence of cytomegalovirus in the study group as a whole and a significantly higher prevalence in the duodenal mucosa. There was, however, no difference in the prevalence of cytomegalovirus in the gastric mucosa of the study group when compared to the control subjects.

In the study group 19 biopsy specimens were positive. In three patients both sites were involved and in the remainder only one site was found to contain virus. Sixteen patients (48%), therefore, had evidence of cytomegalovirus in the gastroduodenal mucosa (Figs 15-17).

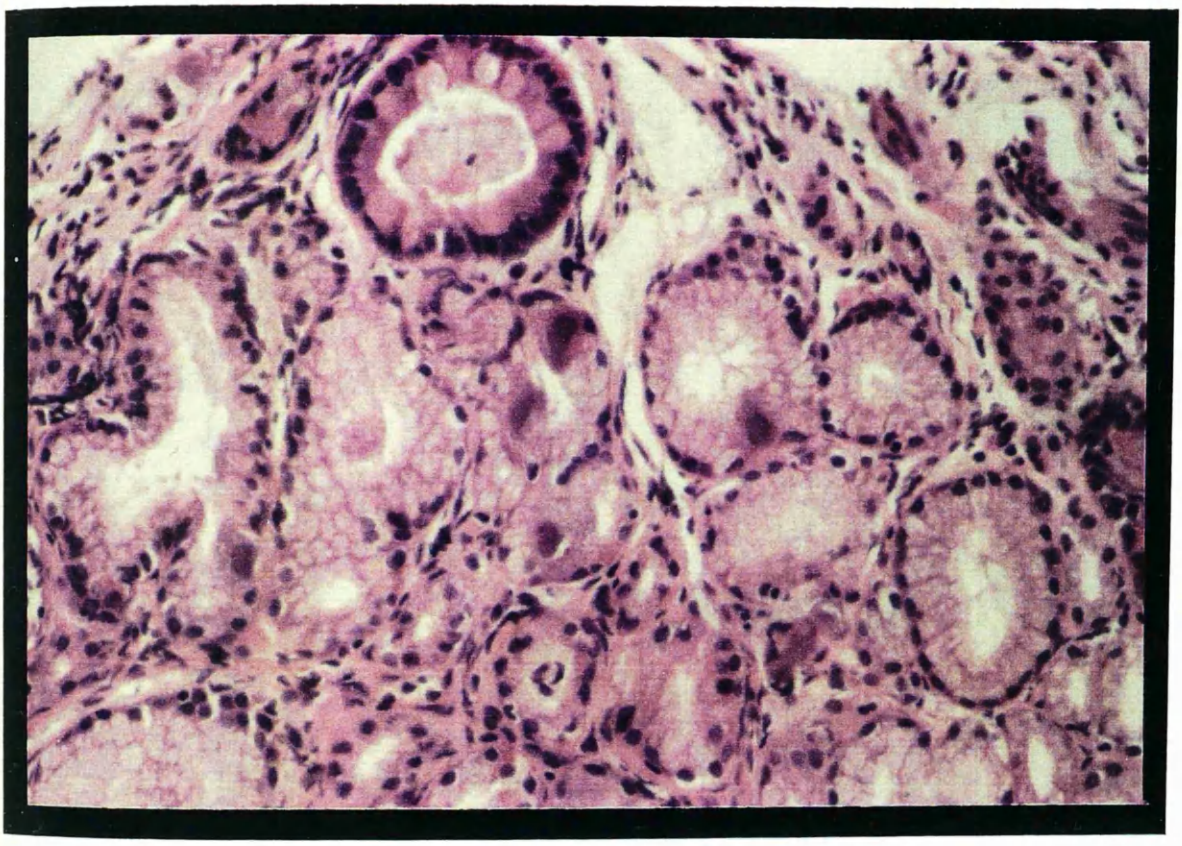


**Figure 15:** H & E section of duodenal mucosa showing cytomegalic cells the glandular epithelium.(x40)

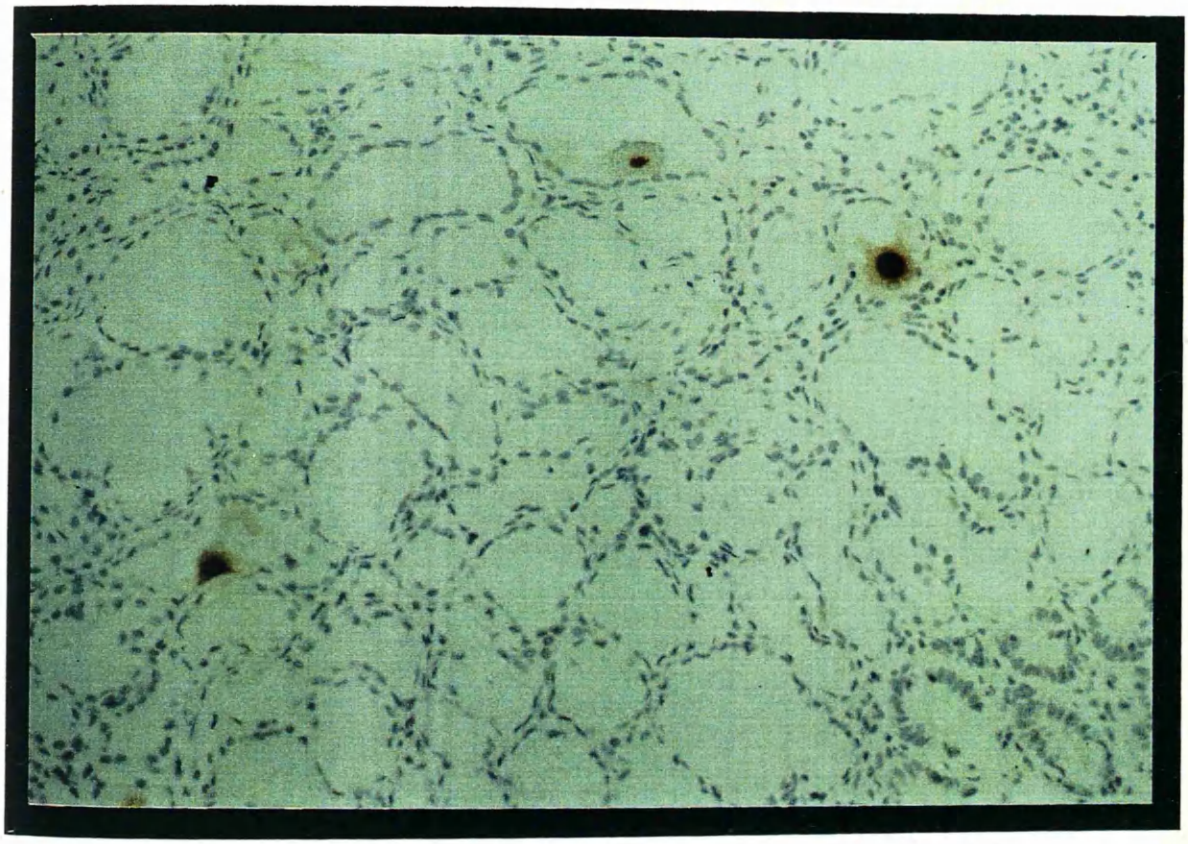


**Figure 16:** Immunohistochemical section of duodenal mucosa stained with anti CMV. The cytomegalic cells in the epithelium stain brown with peroxidase.(x25)

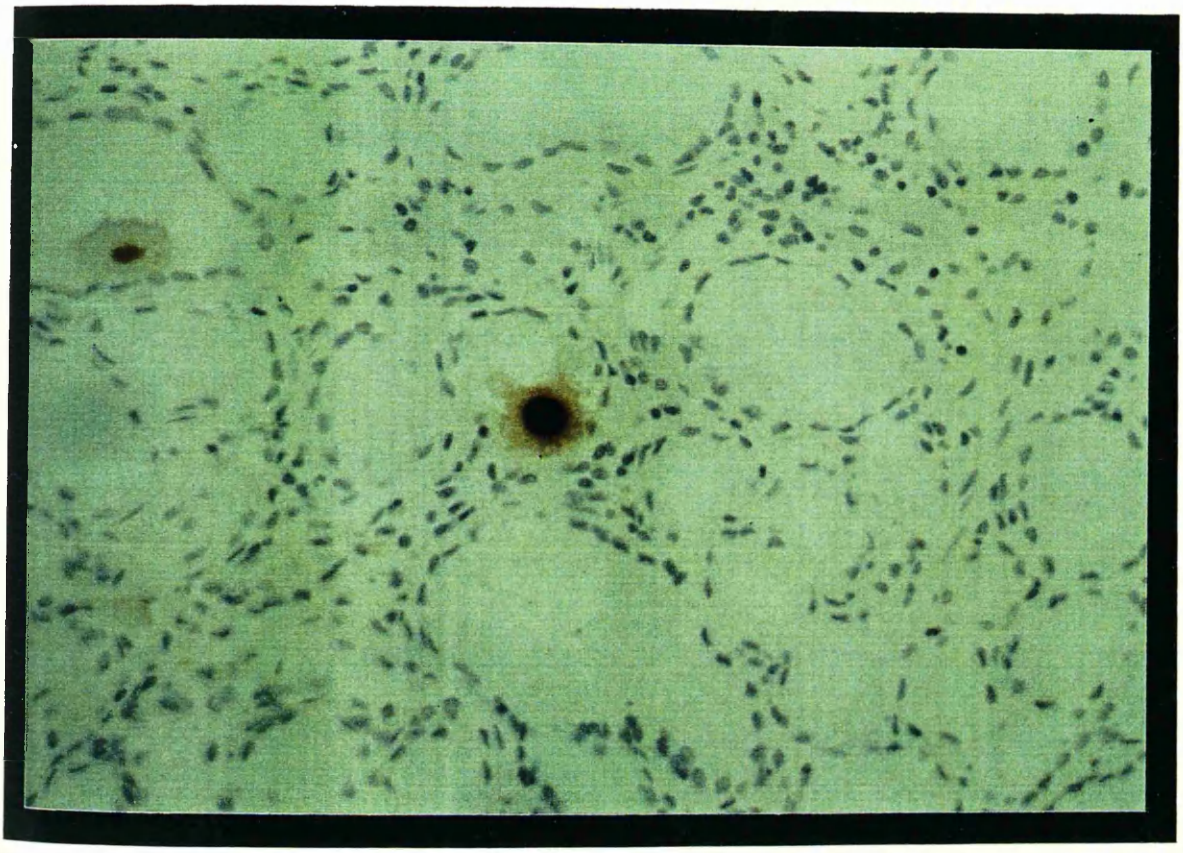


**Figure 17:** Higher magnification of Figure 16 illustrating the striking nuclear enlargement when compared to the normal epithelial nuclei.(x40)

TABLE 13

**PREVALENCE OF CYTOMEGALOVIRUS IN THE GASTRODUODENAL MUCOSA OF TRANSPLANT RECIPIENTS AND CONTROL SUBJECTS**

| | Control Group N = 61 | Study Group N = 66 |
|---|---|---|
| Gastric | 5/36 | 6/33 |
| Duodenal | 2/25 | 13/33* |
| **Total** | 7 | 19** |

* p = 0.006 (Fishers exact test)
** p = 0.027 (Chi squared test)

**Distribution within the Gastroduodenal Mucosa**

Cytomegalovirus was identified in the gastric mucosa of six patients and in the duodenal mucosa of thirteen patients. In the gastric mucosa all positive staining was in the lamina propria and there was no evidence of epithelial involvement. In the duodenal biopsies positive staining was seen in the lamina propria in nine and in the epithelium in seven. In three patients, therefore, both sites were involved (Table 14)

**Relationship of Cytomegalovirus to Pathology and Dyspepsia**

The prevalence of cytomegalovirus in each pathological abnormality is shown in Table 15. Oesophageal biopsy material was not available and therefore oesophagitis has been omitted. The data for gastric and duodenal pathologies relate to cytomegalovirus in the gastric and duodenal mucosa respectively. Cytomegalovirus was significantly associated with duodenitis, but not with gastritis, gastric ulceration or duodenal polyps.

In the control group the gastric and duodenal mucosa was normal and therefore a further control group was employed matched to the study group for gastritis and duodenitis, consisting of ten and sixteen patients respectively. No CMV was identified in any of the biopsy material from this second control group ($p<0.001$, Fisher's exact test).

There was no association between cytomegalovirus and symptomatic dyspepsia, virus being identified in 45% of symptomatic patients and in 53% of asymptomatic patients (Table 16).

TABLE 14

DISTRIBUTION OF CYTOMEGALOVIRUS WITHIN THE GASTRODUODENAL MUCOSA

| | Lamina Propria | Epithelium | Total Patients |
|---|---|---|---|
| Duodenal | 9 | 7 | 13 |
| Gastric | 6 | 0 | 6 |
| Total | 13 | 7 | |

TABLE 15

RELATIONSHIP OF CYTOMEGALOVIRUS TO GASTRODUODENAL PATHOLOGY

| | CMV+ N = 16 (6 gastric, 13 duodenal) | CMV- N = 17 | Total |
|---|---|---|---|
| Gastritis | 2 | 8 | 10 |
| Gastric ulcer | 0 | 4 | 4 |
| Duodenitis | 11 | 5 | 16* |
| Duodenal polyps | 2 | 7 | 9 |

*p = 0.001 (Fishers exact test)

**Relationship of Gastroduodenal Cytomegalovirus to Systemic Cytomegalovirus Infection**

Data on pre and post transplant CMV serology and donor serology was available for twenty five patients and is summarised in table 17. Primary infection was defined as infection ocurring in a seronegative recipient and reccurrent when in a seropositive recipient. Infection was detected by complement fixation test and by IgM titres. No significant association could be demonstrated between CMV serology and identification of the virus in the gastrointestinal tract.

**Relationship of Cytomegalovirus to Renal Function and Immunosuppression**

The mean serum urea,creatinine and cyclosporine,and the mean prednisolone dose are shown in table 18 for CMV positive and for CMV negative patients. There was no relationship between urea or cyclosporine and gastroduodenal cytomegalovirus. There was a tendency for the CMV positive patients to have a higher mean prednisolone dose, but this did not reach statistical significance ($p=0.3$). There was also a tendency towards a higher mean creatinine in the CMV negative patients but once more this did not achieve statistical significance($p=0.2$).

**Relationship of Cytomegalovirus to Age and Time Elapsed Since Transplantation**

The mean age and the time elapsed since transplantation for both the positive and negative groups are summarised in Table 17. There was no statistical association between either of these

parameters and CMV infection.

**Herpes Simplex Virus in the Upper Gastrointestinal Tract**

Herpes simplex virus was looked for in the gastric and duodenal mucosa by immunohistochemistry in the study group and in control tissue. This comprised thirty three biopsies from the stomach and from the duodenum in the study group and thirty six gastric and twenty five duodenal biopsies in the control group; one hundred and twenty seven seperate specimens. No positive staining could be identified in any of the biopsy specimens

## TABLE 16

## RELATIONSHIP OF CYTOMEGALOVIRUS TO SYMPTOMATIC DYSPEPSIA

| | | Symptomatic N = 20 | Asymptomatic N = 13 |
|---|---|---|---|
| Gastric | CMV | 4 | 2 |
| Duodenal | CMV | 7 | 6 |
| Total patients positive | | 9 | 7 |

TABLE 17

RELATIONSHIP OF GASTRODUODENAL CYTOMEGALOVIRUS

TO SYSTEMIC CMV INFECTION

| | Gastroduodenal CMV | | |
|---|---|---|---|
| | CMV + (N=10) | CMV - (N=15) | Total |
| Seronegative | 2 | 1 | 3 |
| Seropositive no Recurrence | 2 | 4 | 6 |
| Primary Infection | 4 | 3 | 7 |
| Secondary Infection | 2 | 7 | 9 |

## TABLE 18

## THE RELATIONSHIP OF CYTOMEGALOVIRUS TO RENAL FUNCTION AND IMMUNOSUPPRESSION

| | CMV+ N=16 | CMV - N=17 |
|---|---|---|
| Mean urea (mmol/l) | 10.1 | 11.3 |
| Mean creatinine (umol/l) | 145 | 187 |
| Mean cyclosporine (nmol/l) | 131 | 128 |
| Mean prednisolone dose (mg) | 20.8 | 16.2 |
| Mean age (years) | 39.7 | 42.5 |

## Discussion

### Prevalence

The prevalence of cytomegalovirus in the study group was 48% and was significantly higher than in the control group. This prevalence is comparable to 45% reported by Franzin (8) and 33% reported by Alexander (19). Neither of these studies, however, had a normal control group and it was impossible to be certain that this apparent high prevalence was not seen in the general population. The results of this study have shown that the virus can be identified in the gastroduodenal mucosa of normal individuals but at a lower prevalence than is seen in transplant recipients.

One potential criticism could be that the control biopsy specimens were of normal gastroduodenal mucosa, and it may be argued that if specimens with gastritis and duodenitis had been used as controls the prevalence may have been higher. For this reason a second control group was used, matched to the study group for changes of gastritis and duodenitis. In the second control group no CMV was identified in ten biopsies with gastritis or in sixteen biopsies with duodenitis ($p<0.001$).

### Association with Pathology and Dyspepsia

Duodenitis was the only pathological abnormality associated with cytomegalovirus. Eleven of 16 patients with histological duodenitis had CMV in the duodenal mucosa and only two patients with duodenal CMV had a histologically normal mucosa. This is in keeping with the report by Alexander et al (19) who found a significant association with duodenitis but not with gastritis or oesophagitis.

Franzin, however, could demonstrate no significant association between CMV and inflammatory lesions of the gastric or duodenal mucosa (8). The other reports which had studied CMV in the gastroduodenal mucosa did not include any transplant recipients with a normal mucosa and could not comment on the association of CMV with particular pathological lesions (11,15,142).

In spite of a significantly increased prevalence of CMV in the study group and a significant association with duodenitis there was no demonstrable association between CMV infection and symptomatic dyspepsia. Once more this finding is entirely in keeping with Alexander's report and also with Franzin's findings relating to symptomatology.

In contrast to the anecdotal and uncontrolled reports these results do not support the veiw that CMV is a cause of peptic ulceration or gastritis. In our study group there was no association with peptic ulcer as suggested by Franzin and Cohen (8,11). These papers, however, were retrospective and did not have control specimens from non-transplant patients and the association may have been coincidental. Cohen reported cytomegalovirus in 62% of peptic ulcers or erosions in renal transplant recipients and concluded that CMV played an important role in the pathogenesis of these lesions (11). The prevalence of 62% and small numbers involved could well have given rise to a casual association with peptic ulceration and is not widely at variance with the prevalence in our study group as a whole. Similarly the prevalence reported by Millard at post-mortem of 12% (142) is much lower than ours and does not support a pathogenic role. Diethelm et al reported CMV as a cause of

haemorrhagic gastritis, (15) and while this may occur it would not appear to be a common complication in the upper GI tract.

A further factor which may have a bearing on this is the small number of peptic ulcers in our study group. As discuused previously the use of H2 receptor antagonists may have modified the spectrum of disease. Most reports would suggest a peptic ulcer prevalence of around 10% in transplant recipients and therefore a large study population would be required to highlight any significant relationship with CMV infection.

There was also no relationship between CMV and duodenal polyps. As discussed in Chapter 4 the mucosa of these polyps was either normal or showed changes of duodenitis. This therefore suggested that the lesions were submucosal and gave rise to the interesting possibility that these were cases of submucosal lymphoid hyperplasia which has been reported to occur in association with CMV (149). From our results this does not apear to be the case and the aetiology of these polypoidal lesions remains unclear.

Distribution within the Gastroduodenal Mucosa

Previous reports of cytomegalovirus in the upper and lower GI tract have tended to show contradictory results with respect to the distribution of virus within the epithelium and lamina propria.

In our study group all gastric lesions were in the lamina propria with no epithelial involvement. In contrast the duodenal epithelium was seen to be involved in seven out of 13 patients, although in 9 of the 13 the lamina propria was involved leaving only four patients with purely epithelial involvement.

These results are consistent with Cohen who reported involvement of the lamina propria in all of eight patients and concurrent epithelial involvement in seven (11). Included in this group were four gastric specimens three of which showed evidence of virus within the epithelial cells: a feature which we did not see. Hinnant also reported a preponderance of virus within the lamina propria in the lower GI tract, finding that only 10% of the involved cells were epithelial (147).

Conversely Franzin et al identified a preponderance of epithelial cells affecting the surface and glandular cells of both gastric and duodenal specimens, with relative sparing of the lamina propria (8).

A possible explanation for these discrepancies is that Franzin identified viral infection by cytomegalia alone, and in our series the only cytomegalic cells identified were epithelial, all cells in the lamina propria being identified by immunohistochemistry. If our study had, therefore, been performed without special histological techniques we too would have identified only epithelial involvement and only duodenal involvement along with a much lower prevalence of viral infection.

Comparison of Detection Methods

CMV was identified by immunohistochemistry in sixteen patients. An important feature was the demonstration of CMV in 10 of the 16 who did not show evidence of CMV on routine histological examination. This difference was particularly marked in the lamina propria. This is in keeping with reports by Jiwa and Niedobatek (196,195) who

identified histological changes in only 43% and 60% respectively of patients who were positive by immunohistochemistry. Both of these publications reported on a wide variety of tissues although neither reported on the gastrointestinal tract and CMV has not been studied previously in the GI tract by immunohistochemistry.

The increased detection rate of CMV by the use of these methods would suggest that previous reports, relying on histology alone, have underestimated the prevalence of infection in the upper GI tract (8,11). One report on the results of culture of endoscopic biopsies would suggest that this too is a sensitive method although is perhaps technically more difficult, requiring rapid transport of specimens and access to virology facilities (19). The advantage of the method used in this study is the ability to perform the techniques on endoscopic biopsy material processed for standard histological examination.

Gastroduodenal Cytomegalovirus and Systemic Infection

There was no demonstrable relationship between CMV serology and identification of the virus in the GI tract. The most difficult aspect of this to explain is the presence of virus in two seronegative patients. Seronegativity wold imply that the patient has never been exposed to the virus and therefore the immunohistochemistry gave rise to spurious results. With this in mind the sections relating to these two patients were reviewed and cytomegalic cells were identified staining positively for CMV.

Another possible explanation is that the patients had not seroconverted at the time of endoscopy and that follow up with

further blood sampling for antibody titres would have revealed increasing antibody titres over a period of time. It is also possible that the patients had been exposed to the virus but that their antibody levels had fallen to undetectable levels. This last option is likely to be a rare occurrence although it has been reported (218).

Only one of the previous reports on gastrointestinal CMV studied patient serology and all of the patients were seropositive. No conclusions could be drawn, therefore, from this study regarding the influence of the patients' serological status (19).

Relationship to Immunosuppression and Renal Function

There was no significant relationship between gastrointestinal CMV and the immunosuppressive regime or renal function. While there is a well recognised relationship between CMV, the increased immunosuppression and deteriorating renal function associated with graft rejection (161) none of the patients in the study group suffered from acute rejection episodes at the time of endoscopy and consequently any differences in immunosuppression and renal function were modest and would be unlikely to show any significant difference.

**Conclusions**

This study has shown a high prevalence of CMV in the gastroduodenal mucosa of renal transplant recipients as suggested by previous reports (8,19). In addition it has demonstrated that the prevalence of CMV is higher than in the normal population and has defined the prevalence in the general population to be around 11%.

It has confirmed the poor specificity of routine histology in detecting cytomegalovirus, which was recognised in some organ systems, but was not previously recognised in the gastrointestinal tract (195, 196).

The presence of CMV was associated with duodenitis but no other pathological abnormality, and although CMV is present in a high proportion of transplant recipients it is not a major cause of upper GI pathology as suggested by some authors (8,11,15,142), and the presence of CMV inclusions in peptic ulcers may merely be a casual association. CMV was not, however, associated with symptomatic dyspepsia and its importance in dyspepsia is more difficult to evaluate. The presence of CMV in asymptomatic individuals does not, however, necessarily rule out cytomegalovirus as a cause of dyspepsia in some individuals.

**Herpes Simplex Virus**

One hundred and twenty seven gastric and duodenal biopsies were examined by immunohistochemistry for Herpes simplex virus with no positive staining. This is perhaps a slightly surprising result in view of the prevalence of HSV infection in renal transplant recipients and previous reports of HSV infection in the gastrointestinal tract.

The prevalence of Herpes simplex infection in transplant recipients has been reported to be between 14 and 70%, usually occurring within the first six months following transplantation, (165,219), and although gastrointestinal HSV is an uncommon event (178,182) some series have reported a high prevalence in

immunosuppresed patients (176).

There are several possible explanations why our results are different from those which might have been expected. These include the site of involvement, the population under study and the detection methods used.

The largest study of gastrointestinal Herpes simplex was that reported by Buss and Scharyj in 1979 (176). This was a post mortem study of patients who had died of disseminated HSV infection. Fifty six patients were studied, fifty of whom were found to have herpetic oesophagitis. An important factor in this report which is of relevance to the work contained in this thesis is that only one of the fifty patients had evidence of herpetic infection in the gastric mucosa. In our study biopsy material was not taken from the oesophagus.

The report by Buss and Scharyj also differs from ours in the patient population studied. Firstly the patients had died of disseminated Herpes simplex infection, although only seven of the patients were recognised as having clinical HSV ante mortem. The second difference is that the study population consisted of patients undergoing chemotherapy for malignant disease and were more profoundly immunosuppressed than would be normal in renal transplant recipients.

The third factor to be taken into account are the methods employed to detect Herpes simplex. The majority of reports, including that by Buss and Scharyj, relied on histopathological detection of the viral cytopathic effect (176,182,220,221). The typical histological features consist of intranuclear inclusions and

multinucleate giant cells, usually found in the epithelium adjacent to ulcer margins (220). These feature, however, are not specific and can be produced by Varicella zoster and Cytomegalovirus and apparent nuclear inclusions have also been demonstrated in the absence of viral disease (222). None of these histological features were identified in our biopsy material.

It is possible that immunohistochemistry failed to identify virus present in the biopsy material. This, however, is an unlikely possibility. Immunohistochemistry obviously relies on the presence of viral protein as the antigen and it could be argued that viral DNA may be present and could be detected by in situ hybridisation. This is unlikely, however, since viral DNA in the absence of viral protein would imply that the gastroduodenal mucosa is a site of viral latency and this has certainly not been recognised to date. Further study of the biopsy material, however, will be performed using viral DNA probes to ensure that this is not the case.

Since one would expect immunohistochemistry to be a more sensitive detection method than histology alone, and should be as sensitive as insitu hybridisation, the conclusion must be that Herpes simplex is not present in the gastric or duodenal mucosa of renal transplant recipients.

# CHAPTER 7

# MUCOSAL T LYMPHOCYTE SUBSETS

## INTRODUCTION

Mucosal T lymphocytes were studied in gastric and duodenal biopsies which were frozen in liquid nitrogen and subsequently stored. Tissue was available from the stomach and duodenum of fifteen transplant recipients. In addition a prospective group of normal control tissue was obtained and frozen. In this control group tissue was obtained for urease slide test analysis and the patients were matched to the transplant recipients for age and Helicobacter status.

The antibodies used were for Leu 2 (Suppressor/cytotoxic) and Leu 3 (Helper/inducer) and the results are expressed as the mean T lymphocyte count per $mm^2$. The ratio of Leu3 to Leu 2 was also calculated and is expressed as the mean of the ratios.

### Transplant Recipients and Controls

The data for T lymphocyte subsets in the gastroduodenal mucosa of both the study group and the control group are summarised in table 19. There was no significant difference between either subset or the Leu3:Leu2 ratio in the two groups.

### Relationship to Immunosuppression and Renal Function

Each of the T cell markers and the Leu3:Leu2 ratio was assessed with respect to serum urea, cyclosporine, creatinine and prednisolone dose for both the gastric and duodenal mucosa in the transplant recipients.

TABLE 19

T LYMPHOCYTE SUBSETS IN TRANSPLANT RECIPIENTS AND CONTROLS

| | TRANSPLANT (N=15) | CONTROLS (N=15) |
|---|---|---|
| Leu2 (gastric) | 133 | 154 |
| Leu2 (duodenal) | 88 | 94 |
| Leu3 (gastric) | 189 | 243 |
| Leu3 (duodenal) | 194 | 223 |
| L3:L2 ratio (gastric) | 1.71 | 2.08 |
| L3:L2 ratio (Duodenal) | 2.58 | 2.69 |

There was a positive correlation between Leu3 and serum creatinine in the duodenal mucosa ($r=0.52, p=0.04$) and a positive correlation between the Leu3:Leu2 ratio and both urea and creatinine in the duodenum ($r=0.54$, $p=0.03$ for both). These relationships are summarised in figures 18,19 and 20. There was no significant relationship between T lymphocyte subsets and the other parameters. The correlation coefficients and significance levels are illustrated in table 20.

**Relationship to Gastritis and Duodenitis**

The influence of gatritis and duodenitis on T cell subsets is summarised in table 21. There was a tendency towards an increase in absolute numbers of Leu2 positive and Leu3 positive cells in gastritis but these did not achieve statistical significance ($p=0.09$ and $p=0.08$ respectively). There was no significant change in the Leu3:Leu2 ratio or any association with duodenitis.

**The Influence of Helicobacter Pylori**

The effects of H pylori infection was assessed in the gastric mucosa only since the organism was not identified in the duodenum. Assessment was performed on the study group and the control group in combination and separately.

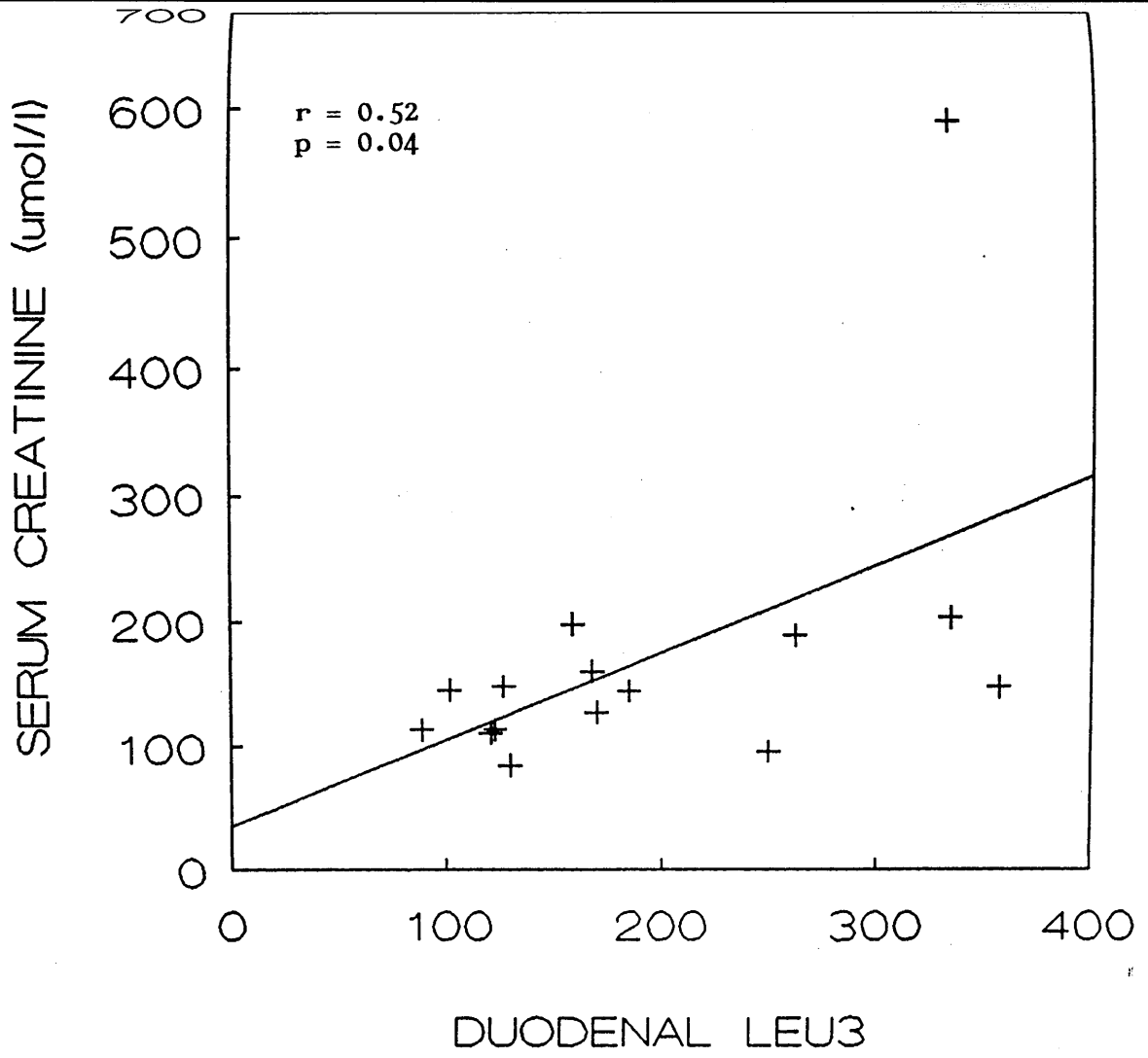



FIGURE 18
DUODENAL LEU3 CREATININE

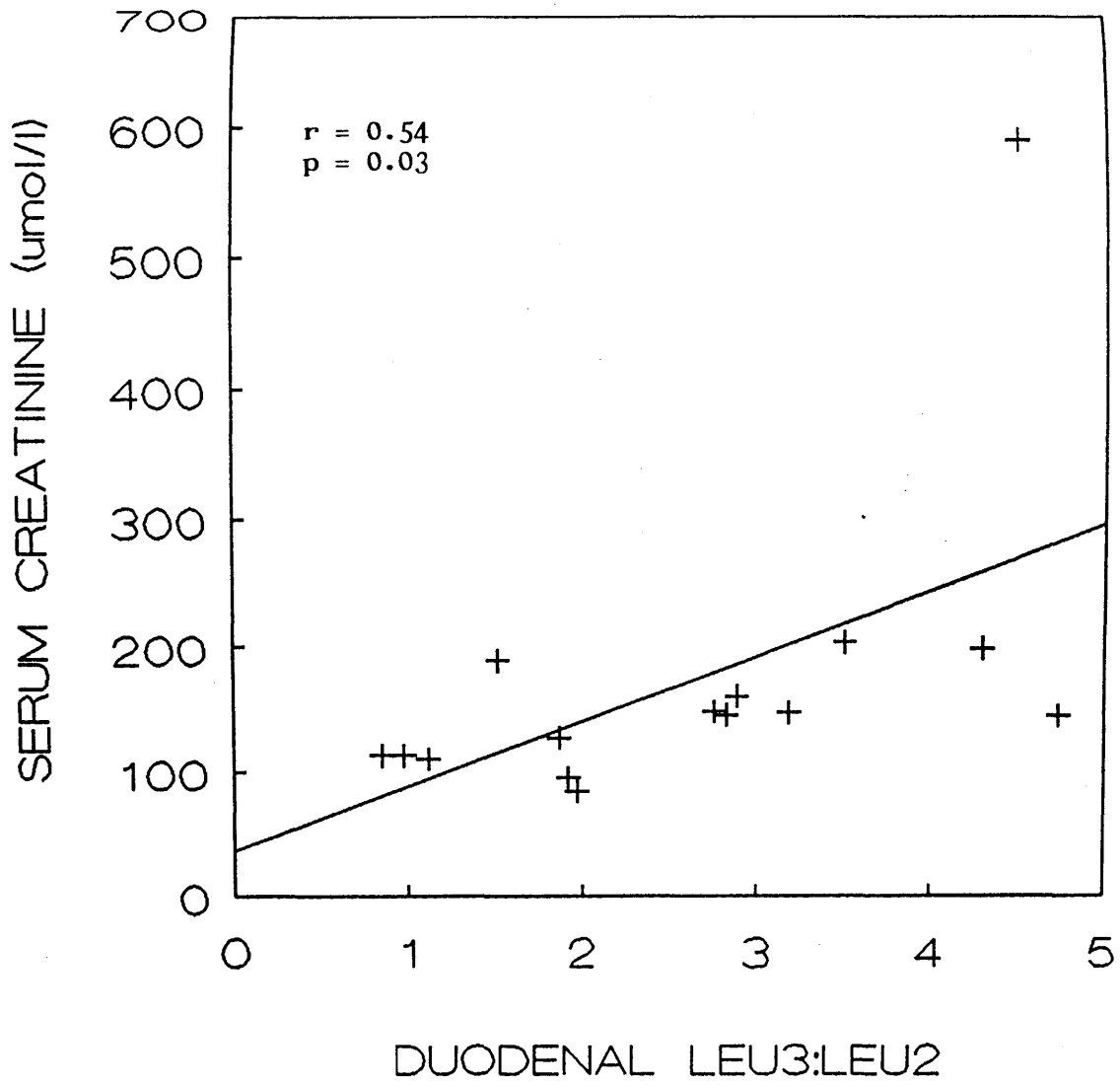



FIGURE 19

DUODENAL LEU3:LEU2 CREATININE

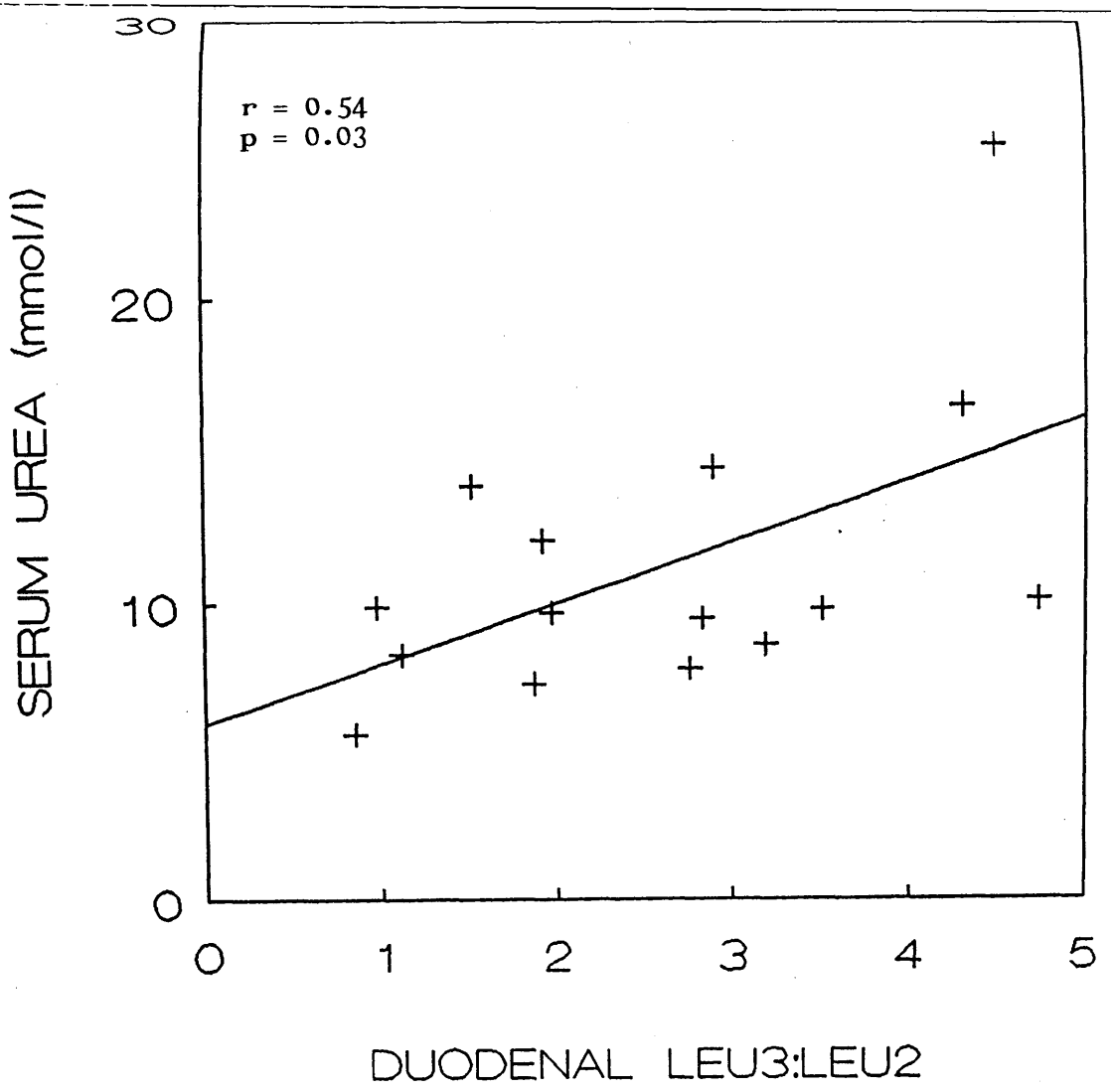



FIGURE 20
DUODENAL LEU3:LEU2 UREA

TABLE 20

T LYMPHOCYTE SUBSETS, RENAL FUNCTION

AND IMMUNOSUPPRESSION

CORRELATION COEFFICIENT

| | Cyclosporine | Prednisolone | Urea | Creatinine |
|---|---|---|---|---|
| Leu2(gastric) | -0.282 | 0.043 | -0.252 | -0.254 |
| Leu2(duodenal) | 0.14 | 0.16 | -0.119 | -0.098 |
| Leu3(gastric) | -0.294 | 0.268 | -0.224 | -0.146 |
| Leu3(duodenal) | -0.166 | -0.104 | 0.42 | 0.52* |
| L3:L2(gastric) | -0.31 | 0.45 | 0.45 | -0.307 |
| L3:L2(duodenal) | -0.197 | -0.45 | 0.54** | 0.54** |

* p = 0.04

** p = 0.03

**TABLE 21**

**T LYMPHOCYTE SUBSETS**

**GASTRITIS AND DUODENITIS**

| | Gatritis (N=5) | No Gastritis (N=10) | Duodenitis (N=8) | No Duodenitis (N=7) |
|---|---|---|---|---|
| Leu2 | 211 | 94 | 92 | 83 |
| Leu3 | 267 | 149 | 207 | 178 |
| L3:L2 | 1.97 | 1.59 | 2.37 | 2.82 |

There was no significant difference in the absolute numbers of Leu3 positive cells or the Leu3:Leu2 ratio in the H pylori positive patients when compared to the H pylori negative patients. There was a tendency for the numbers of Leu2 positive cells to be higher in the presence of H pylori, but this did not achieve statistical significance ([p=0.18] [table 22]). When the data for transplant recipients and controls was examined seperately no differences could be identified (table 23).

**The Influence of Cytomegalovirus Infection**

Data on gastroduodenal CMV was available for the study group only and are summarised in table 24. There was a significant increase in the absolute numbers of Leu3 positive cells in the CMV positive patients (p=0.016), although this was not reflected in any alteration of the Leu3:Leu2 ratio. The majority of CMV infection in the study group was in the duodenum (6 out of 8) and therefore the data was analysed for duodenal mucosa only. On this occassion there was no significant increase in the Leu3 population (table 25).

The data was also analysed with respect to serological evidence of active CMV infection and, once more, no significant relationship could be demonstrated (Tables 26,27)

## TABLE 22

## T LYMPHOCYTE SUBSETS AND HELICOBACTER PYLORI (A)

| | H pylori + (N=18) | H pylori - (N=12) |
|---|---|---|
| Leu2 | 163 | 114 |
| Leu3 | 233 | 189* |
| L3:2 ratio | 1.6 | 2.2 |

p=0.18

TABLE 23

T LYMPHOCYTE SUBSETS AND HELICOBACTER PYLORI (B)

| | TRANSPLANT | | CONTROLS | |
|---|---|---|---|---|
| | H pylori + (N=9) | H pylori - (N=6) | H pylori + (N=9) | H pylori - (N=6) |
| Leu 2 | 150 | 107 | 177 | 121 |
| Leu 3 | 204 | 165 | 263 | 212 |
| L3:L2 | 1.80 | 1.68 | 1.47 | 2.98 |

## TABLE 24

### T LYMPHOCYTE SUBSETS AND CYTOMEGALOVIRUS

| | CMV + (N=8) | CMV - (N=22) |
|---|---|---|
| Leu2 | 123 | 106 |
| Leu3 | 267 | 164* |
| L3:L2 | 2.57 | 1.99 |

**p=0.018 (Students t test)**

## TABLE 25

## T LYMPHOCYTE SUBSETS AND DUODENAL CYTOMEGALOVIRUS

| | CMV + (N=6) | CMV - (N=9) |
|---|---|---|
| Leu2 | 111 | 82 |
| Leu3 | 225 | 170* |
| L3:L2 | 2.62 | 1.99 |

**p=0.26 (Students t test)**

TABLE 26

GASTRIC T LYMPHOCYTE SUBSETS AND CMV SEROLOGY

| | CMV+ (N=7) | CMV- (N=6) |
|---|---|---|
| Leu2 | 120 | 172 |
| Leu3 | 172 | 212 |
| L3:L2 | 1.98 | 1.28 |

TABLE 27

DUODENAL T LYMPHOCYTE SUBSETS AND CMV SEROLOGY

| | CMV+ (N=7) | CMV- (N=6) |
|---|---|---|
| Leu2 | 73 | 89 |
| Leu3 | 201 | 187 |
| L3:L2 | 2.95 | 2.54 |

## Discussion

### T Lymphocyte Subsets in Normal Gastroduodenal Mucosa

#### Gastric

The findings are expressed as T cells per $mm^2$. Information on the normal values are difficult to obtain from the current literature. Information, however, can be obtained from control subjects utilised in studies of abnormal gastric mucosa. Kaye et al studied mucosal T cell subsets in patients with pernicious anaemia by immunohistochemistry (223). Included in this publication were 12 subjects with a normal gastric mucosa. The mean number of Leu3 (T Helper) cells in this group were 147 $\pm$ 97 and 292 $\pm$ 71 for Leu2 (T Suppressor) subset. The authors also noted slight variations in the distribution of T lymphocytes at different levels within the mucosa. In the study group in this thesis, however, the numbers were assessed for the entire biopsy specimen and were comparable to the figures reported above.

#### Duodenal

As with normal gastric mucosa the absolute numbers of T lymphocytes in normal duodenal mucosa must be obtained from control groups in studies of duodenal pathology. Jenkins et al studied duodenal biopsy specimens from patients with coeliac disease and included 20 patients with a normal duodenal mucosa (224). The results are expressed per $mm^2$ but the subsets were counted separately for surface epithelium, crypt epithelium and lamina propria and were not calculated for the full thickness of the biopsy specimens. Arithmetically however, 89% of Leu3 and Leu2 lymphocytes were in the lamina propria with mean values of 255 and 103 respectively, giving

a mean ratio of 2.07. These figures are comparable with our own for the full thickness of the duodenal mucosa.

Mucosal T Lymphocyte Subsets in Transplant Recipients

Only one study has reported on T lymphocytes in the gastroduodenal mucosa of transplant recipients (225). This study differs from ours in two important respects. Firstly the patients were bone marrow recipients and may not be strictly comparable to our study group, and secondly, the results were expressed as cells per twenty crypts for the epithelium and as cells per grid area ($0.0625mm^2$) for the lamina propria. It is possible, however to overcome this second problem by employing a conversion factor of 256 to express the T lymphocytes in the lamina propria per $mm^2$. The authors found a total Leu3 count of $796/mm^2$ and a total Leu2 count of $727/mm^2$ giving a Leu3:Leu2 ratio of 1.09. The authors also demonstrated an increase in the absolute numbers of both cell types in graft versus host disease, giving $2432/mm^2$ and $1735/mm^2$ respectively and a ratio of 0.71.

The absolute numbers in transplant recipients are higher than in the gastroduodenal mucosa of normal individuals and are increased significantly in graft versus host disease. It is difficult, however, to be certain of the importance of this in renal transplant recipients, since they have a much lower incidence of graft versus host disease and the immunosupressive regime is very different. The figures are obviously widely at variance with our own data for transplant recipients.

The Relationship to Renal Function and Immunosuppression

There was a significant positive correlation between the Leu3 subset and serum creatinine in the duodenal mucosa and a significant positive correlation between the Leu3:Leu2 ratio in the duodenal mucosa and both urea and creatinine. This may be due to the relationship between renal function and cylosporine levels. In the study group there was a weak negative correlation between cyclosporine levels and renal function. This would mean that high urea and creatinine levels tend to be associated with a decreased serum cyclosporine level. There was, however, no significant relationship between the T lymphocyte subsets and either cyclosporine levels or prednisolone dose.

The Influence of H Pylori on Mucosal T Cell Subsets

Information on the local T cell response to H pylori is difficult to come by in the literature. At present only one report, published as an abstract, is available for comparison with our work (109). This report by Rathbone et al studied Leu3 and Leu2 subsets in the gastric mucosa of normal individuals and those with H pylori associated gastritis.

The authors reported an increase in the absolute numbers of Leu3 (T Helper) cells in the lamina propria of patients with H pylori gastritis and a decrease in Leu2 (T Suppressor) cells. They also identified an increased percentage of Leu3 lymphocytes expressing the CD7 marker which is indicative of T cell blastogenesis, suggesting activation of a cell mediated response, presumably to H pylori.

In our data there was an increase in the absolute numbers of

Leu 3 positive cells in patients with H pylori (233 v 189). This did not, however, achieve statistical significance(p=0.18)

The Influence of Cytomegalovirus on Mucosal T Cell Subsets

There is no guidance in the literature on the expected pattern of T lymphocyte subsets in the gastroduodenal mucosa in cytomegalovirus infection. There are, however, many reports of circulating T lymphocyte subpopulations accompanying CMV infection in the general population and in transplant recipients.

Maher et al demonstrated inversion of the Leu3: Leu2 ratio in transplant recipients with CMV infection (226). This inversion was frequently seen before a rise in antibody titres and in some cases remained inverted for several years. This finding was similar to that of Dafoe, who further demonstrated that the inversion was due to an absolute increase in the Leu2 subset (227). The finding of increased Leu2 cells was confirmed by Schooley et al (228). They also found that inversion of the Leu3:Leu2 ratio was prolonged in cadaveric transplants, but was transient in living related donor transplants and was associated only with active CMV infection. This difference was presumed to be due to the less aggressive immunosuppression in the latter group.

Not all authors, however, have demonstrated this feature. Von Es et al, in a large series, found no change in the Leu3:Leu2 ratio with CMV infection (229), findings confirmed by Rinaldo (163).

In one study of gastrointestinal CMV infection Brouillete et al demonstrated an inversion of the Leu3:Leu2 ratio (230). This difference was observed in peripheral blood lymphocytes, and the

authors did not study mucosal T lymphocytes.

In the study group of this thesis there was an increase in the Leu3 subset in CMV infection, but no change in the Leu3:Leu2 ratio. The majority of CMV in the study group was in the duodenal mucosa and when the gastric mucosal lymphocyte counts were excluded from the analysis the difference was no longer significant. It may be, therefore, that the former result was spurious or that removal of the gastric CMV reduced the numbers so that statistical significance was not achieved. We certainly did not observe the increase in the Leu2 subset or inversion of the Leu3:Leu2 ratio, which has been reported in peripheral blood, reflected in the gastric or duodenal mucosa, nor was there any relationship between mucosal T cell subsets and serological evidence of active CMV infection.

**Conclusions**

The results of T lymphocyte subset analysis are disappointing and inconclusive. There were minor trends identified but no important significant results. It is particularly disappointing that no convincing relationship could be demonstrated between mucosal T lymphocytes and either CMV or H pylori infection. While the systemic T cell response to CMV infection has been described extensively the importance of the T cell response to Helicobacter infection is unknown and the results of this study have not clarified the situation.

There are perhaps two important reasons for these inconclusive results. The first relates to the small numbers of patients involved. There were 15 patients in each group giving a total of sixty

individual biopsies each of which was examined for Leu2 and Leu3. The difficulties may have arisen when the groups were subdivided into transplant and controls and further divided into duodenal and gastric mucosa, thereby reducing the numbers to fifteen in each group. A second and possibly more important reason is the subjectivity involved in counting the T cells. There is no automated way in which this can be achieved and counting has to be done manually. All of our slides were counted by two independent observers and the mean error was 36%, highlighting the poor reproduceability of the technique. With this margin of error relatively small differences may be lost.

# PART III

# CONCLUSIONS

# CHAPTER 8

# CONCLUSIONS

## THE PREVALENCE OF UPPER GI PATHOLOGY AND DYSPEPSIA

Although the prevalence of peptic ulceration and the incidence of complications in transplant recipients has been recognised for many years, the importance of non-ulcer dyspepsia has been poorly recognised and the high prevalence of mucosal inflammatory lesions has only been appreciated recently.

This study has identified symptomatic dyspepsia in 60% of renal transplant recipients which was resistant to H2 receptor antagonist therapy in 67%. The prevalence of peptic ulceration was 12% in the study group as a whole and 20% in the symptomatic group, which is consistent with previous reports (1,9,10,12). This does, however, mean that 80% of patients with dyspeptic symptoms did not suffer from peptic ulceration at the time of endoscopy.

A striking feature was the high prevalence of mucosal inflammatory lesions but, even taking these into account, 30% of the dyspeptic patients had no identifiable abnormality in the upper gastrointestinal tract. A further problem is the high prevalence of mucosal inflammatory lesions in the asymptomatic group.

There was a trend for gastritis to be commoner in symptomatic patients but this did not achieve statistical significance. It may be, however, that a significant association would have been demonstrated if a larger study population had been available. The relationship of gastritis to symptomatic dyspepsia has been discussed in Chapters 2 and 4 and the subject remains controversial. Evidence based on the eradication of H pylori would suggest, however, that resolution of gastritis is accompanied by improvement in dyspeptic symptoms (73, 209, 231).

The most common abnormality detected was duodenitis and, as with gastritis, its role in dyspepsia has been the subject of some debate. Cheli et al in 1982 suggested that duodenitis was very uncommon in asymptomatic individuals (204), although this was at variance with other reports (205,232). The results from this study would suggest that duodenitis does occur in asymptomatic transplant recipients.

In view of these uncertainties it would be possible to argue that gastritis and duodenitis are not important in the aetiology of dyspepsia in transplant recipients. This point of view, however, is not necessarily valid and it is entirely reasonable to suggest that gastritis and duodenitis can give rise to symptoms in some patients, but not in others. It is widely recognised that asymptomatic peptic ulcer occurs commonly in the general population and yet few people would argue that peptic ulceration is not responsible for symptoms in many patients.

It seems, reasonable, therefore, to suggest that gastritis and duodenitis are responsible for dyspeptic symptoms in a significant proportion of transplant recipients and that they are also identified in patients who are asymptomatic. This is especially true since the mechanism of dyspeptic pain is poorly understood and it is recognised that many patients with peptic ulceration are asymptomatic (233).

Identification of the pathological processes highlighted above does not however explain their aetiology. The relationship of these abnormalities to H pylori and Cytomegalovirus will be discussed in the following sections.

## THE ROLE OF HELICOBACTER PYLORI

The overall prevalence of H pylori in the study group was 48% and the organism was identified in 65% of those with symptomatic dyspepsia. The problems in obtaining a truly representative control group give rise to difficulty in interpreting the importance of the absolute prevalence, since the organism is widespread in the general population. Current data, however, would suggest that the prevalence is higher than would be expected in an age matched group of normal individuals (111, 212, 213). It would be an attractive theory to suggest that H pylori is found more commonly in transplant recipients because of immunosuppression and poor renal function. Such a relationship was not demonstrated in the study group, although there was probably insufficient variation in renal function and the immunosuppressive regimes to highlight any significant association.

In practice, however, the absolute prevalence of H pylori is of lesser importance than its relationship to gastritis and dyspeptic symptoms. In the study group there was a significant association with gastritis, gastric ulceration and with symptomatic dyspepsia.

The importance of H pylori, therefore, lies in its role in gastritis, non ulcer dyspepsia and gastric ulceration which is consistent with its role in the general population. This study has confirmed the high prevalence of dyspepsia in transplant recipients and has demonstrated a significant association between dyspeptic symptoms and H pylori colonisation.

It would be premature to advocate widespread use of colloidal bismuth and antibiotics in the routine management of transplant dyspepsia at this time. Further work must be carried out using if

necessary, non invasive methods of assessing H pylori colonisation. This should be followed up by controlled trials of therapy with monitoring of the symptomatic response to eradication of Helicobacter. In clinical practice the management of transplant dyspepsia still requires pre-treatment investigation, ideally by endoscopy, along with biopsy of the gastric antrum for the assessment of H pylori coloniseation.

**THE ROLE OF CYTOMEGALOVIRUS**

Cytomegalovirus was identified in 48% of the study group compared with 11% of the normal controls. The distribution of CMV in the upper GI tract was not uniform and the prevalence of virus in the gastric mucosa was not significantly different in the study group and in the control group. The major difference, however, was in the distribution in the duodenal mucosa. Virus was identified in the duodenal mucosa in 39% of transplant recipients but in only 8% of the control group (p=0.006).

The virus was identified by cytomegalic changes in seven patients but by immunohistochemistry in nineteen. This feature has been reported in other tissues but has not been previously reported in the GI tract (195,196). The implication of this finding, therefore, is that previous reports relying on histology alone are likely to have underestimated the prevalence of CMV infection (8,11,142).

The prevalence of cytomegalovirus in the upper GI tract of transplant recipients has been reported previously to be between 12% and 62% (8,11,19,142). The results of this study obviously lie

within this range notwithstanding the different detection methods. Prior to the work of this thesis, however, the prevalence in the normal population was unknown and it was not possible to draw any definite conclusions regarding the possible pathogenic effect of the virus in transplant recipients.

Identification of CMV in the upper gastrointestinal tract is not, however, proof of a pathogenic role. The only pathological process which was associated with CMV infection was duodenitis. There were sixteen patients in the study group with histological duodenitis, eleven of whom had CMV identified in the duodenal mucosa. More significant, perhaps, was the fact that only two patients with duodenal CMV had a normal mucosa.

Duodenitis has only been reported in small numbers in transplant recipients although it is a common problem in the general population and is generally thought to be part of the spectrum of duodenal ulceration (104,232). The possibility that duodenitis in the general population is due to cytomegalovirus was therefore considered and control tissue was obtained from non transplant recipients with duodenitis. No virus was identified in these patients ($p<0.001$).

If CMV is a cause of duodenitis it is only of importance if it is related to symptoms. In the study group in this thesis there was no association of CMV and duodenitis with symptomatic dyspepsia. In the light of studies in the general population this is perhaps rather surprising since duodenitis is an uncommon finding in asymptomatic individuals (204,205). It is of course possible to suggest that duodenitis may give rise to symptoms in some patients and may be

asymptomatic in others. This is certainly the case in Helicobacter associated gastritis which has been studied extensively in recent years.

Having established a statistical link between duodenitis and CMV infection there is still the question of its causal role. Unfortunately it is not possible to give a definite answer to this question based on the results of this work. Cytomegalovirus was not identified in duodenitis from normal control subjects but it is possible that its identification in the transplant recipients is merely a manifestation of the high prevalence of the virus in this group. To prove a causal role it would be necessary to establish that eradication of the virus is accompanied by histological improvement in the duodenal mucosa and this has not been done. It could indeed be argued that this type of study is not likely to be performed since CMV infection is difficult to treat and the toxicity of the chemotherapy is not justified where the symptoms are generally mild.

There is certainly no evidence to support the view that CMV is implicated in the pathogenisis of peptic ulceration in transplant recipients as suggested by some authors (8,11,142). The prevalence of peptic ulceration in the study group was 12% which is in keeping with the prevalence in other series and it seems likely that a much larger study group would be required along with appropriate controls to investigate a link with peptic ulceration.

## THE ROLE OF HERPES SIMPLEX VIRUS

The findings for Herpes simplex virus were negative and the virus was not identified in the gastroduodenal mucosa. This does not necessarily mean that Herpes simplex is not present in the upper GI tract. Previous reports in non transplant immunosuppressed patients have identified the virus in the oesophagus but have identified gastric involvement in only 2% of patients with herpetic oesophagitis (176). There is, however, no evidence of Herpes simplex virus in either the gastric or duodenal mucosa of the study group or the control subjects.

## MUCOSAL T LYMPHOCYTE SUBSETS

Prior to the beginning of this study it was hoped that viral or bacterial infection in transplant recipients would be accompanied by changes in the normal pattern of mucosal lymphocytes. It was an attractive theory to suggest that the upper gastrointestinal complications were due to the immunosuppressed condition of these patients and that a study of the mucosal lymphocyte subsets would shed some light on the immune response to these infective agents. This was particularly important with respect to H pylori where the importance of the cell mediated response is unclear.

Unfortunately the data obtained from this work has done little to clarify these points. There was no significant difference in the T cell subsets between the study group and the control group, although the study group are undoubtedly immunosuppressed. It may be that a simple numerical process is not sufficiently sensitive to detect differences in immune function. It is also probable that the

subjectivity of cell counting is not sufficiently accurate to detect relatively small differences.

The data did demonstrate an increase in the Leu3 subset in H pylori infection, previously reported by Rathbone et al (109), although this did not achieve statistical significance.

**FINAL CONCLUSIONS**

This study has confirmed a relatively high prevalence of peptic ulceration in transplant recipients which is in keeping with previous reports. It has also highlighted a high prevalence of non ulcer dyspepsia, gastritis and duodenitis which have been poorly recognised in the past.

Antral gastritis was significantly associated with H pylori colonisation and the organism was identified in 48% of the study group. H pylori was also significantly associated with gastric ulceration and dyspeptic symptoms. This prevalence is likely to be higher than would be expected in an age matched group of unselected non transplant recipients. There is, however, no clear evidence from the study that abnormalities of mucosal T lymphocytes contribute to an increased risk of H pylori infection.

Duodenitis was significantly associated with Cytomegalovirus in the duodenal mucosa although neither of these factors appeared to be related to symptomatic dyspepsia. Once more there was no clear evidence of mucosal lymphocyte abnormalities associated with CMV infection.

In practical terms dyspepsia in transplant recipients is a multifactorial process which is likely to involve an interaction of

acid secretion, corticosteroids and Helicobacter pylori, and may also involve CMV infection in the duodenum. Dyspepsia in this group of patients should be fully investigated and the high prevalence of mucosal inflammatory lesions would suggest that this would be best achieved by endoscopy and biopsy for the assessment of H pylori.

It is difficult to be certain of the best way to investigate further the role of CMV in duodenitis and there is insufficient information on the response of duodenitis to H2 receptor antagonists in this group to be sure that it is the same pathological process seen in the general population. Further work is required to study the effects of H pylori eradication on symptoms and on histological gastritis and this would appear to be the most promising avenue for further research.

# PART IV

# APPENDICES

## APPENDIX 1(a)
## STUDY GROUP

| Patient Number | Age | Symptoms | H2 Antagonists | Prednisolone Dose | Serum biochemistry Urea | Creatinine | Cyclosporine |
|---|---|---|---|---|---|---|---|
| 821041 | 38 | Yes | No | 20 | 9.9 | 113 | 67 |
| 821748 | 53 | Yes | Yes | 15 | 8.6 | 147 | 120 |
| 779860 | 40 | Yes | Yes | 20 | 9.2 | 103 | 116 |
| 853773 | 39 | Yes | No | 20 | 8.4 | 110 | 145 |
| 865820 | 31 | Yes | No | 15 | 13.9 | 189 | 177 |
| 733701 | 55 | Yes | No | 20 | 13.8 | 332 | 78 |
| 745539 | 45 | Yes | Yes | 15 | 7.8 | 122 | 126 |
| 779162 | 47 | Yes | Yes | 15 | 9.5 | 145 | 206 |
| 855418 | 30 | Yes | No | 20 | 10.4 | 168 | 122 |
| 608902 | 49 | Yes | Yes | 20 | 9.2 | 120 | 154 |
| 858614 | 49 | Yes | Yes | 20 | 8.7 | 152 | 156 |
| 852379 | 33 | Yes | No | 15 | 7.8 | 134 | 117 |
| 829323 | 42 | Yes | Yes | 20 | 24.9 | 590 | 122 |
| 853214 | 44 | Yes | No | 15 | 8.6 | 147 | 120 |
| 676774 | 48 | Yes | Yes | 20 | 9.7 | 84 | 161 |
| 342534 | 43 | Yes | Yes | 12.5 | 16.5 | 198 | 225 |
| 818178 | 57 | Yes | Yes | 15 | 14.5 | 160 | 138 |

## APPENDIX 1(a)
## STUDY GROUP

| Patient Number | Age | Symptoms | H2 Antagonists | Prednisolone Dose | Serum Biochemistry | | |
|---|---|---|---|---|---|---|---|
| | | | | | Urea | Creatinine | Cyclosporine |
| 754884 | 41 | Yes | No | 15 | 7.8 | 148 | 160 |
| 675422 | 32 | Yes | Yes | 15 | 21.8 | 302 | 93 |
| 865086 | 38 | Yes | Yes | 15 | 7.3 | 127 | 201 |
| 499439 | 36 | No | No | 20 | 8.7 | 144 | 96 |
| 611270 | 19 | No | No | 15 | 9.6 | 94 | 131 |
| 853215 | 59 | No | No | 15 | 10.2 | 134 | 175 |
| 638747 | 39 | No | No | 15 | 8.0 | 131 | 114 |
| 866240 | 33 | No | Yes | 15 | 12.1 | 95 | 152 |
| 744548 | 37 | No | No | 20 | 19.1 | 251 | 83 |
| 178868 | 37 | No | Yes | 15 | 4.2 | 141 | 98 |
| 863280 | 42 | No | No | 15 | 9.8 | 203 | 119 |
| 839531 | 31 | No | No | 20 | 8.3 | 110 | 107 |
| 858261 | 65 | No | No | 15 | 14.0 | 219 | 103 |
| 82116 | 28 | No | No | 15 | 5.6 | 113 | 190 |
| 852274 | 33 | No | No | 15 | 7.5 | 135 | 77 |
| 803808 | 27 | No | Yes | 10 | 10.1 | 144 | 132 |

## APPENDIX 1(b)
## STUDY GROUP

| Patient Number | Endoscopic Findings | Gastric Histology | | | Duodenal Histology | |
|---|---|---|---|---|---|---|
| | | Gastritis | Atrophy | Metaplasia | Duodenitis | Metaplasia |
| 821041 | Polyps | 3 | 1 | 0 | 1 | - |
| 821748 | Duodenitis | 1 | 0 | 0 | 2 | + |
| 779860 | Normal | 3 | 3 | 1 | 1 | - |
| 853773 | Gastritis | 1 | 1 | 0 | 1 | + |
| 865820 | Polyps | 0 | 1 | 0 | 0 | + |
| 733701 | Polyps | 1 | 3 | 0 | 2 | + |
| 745539 | Normal | 1 | 0 | 0 | 1 | - |
| 779162 | Polyps | 0 | 0 | 0 | 1 | - |
| 855418 | Normal | 1 | 0 | 0 | 2 | + |
| 608902 | Normal | 1 | 1 | 0 | 1 | - |
| 858614 | Polyps | 1 | 1 | 0 | 2 | + |
| 852379 | Normal | 0 | 0 | 0 | 1 | - |
| 829329 | GU | 3 | 3 | 0 | 1 | - |
| 853214 | GU | 3 | 1 | 0 | 1 | - |
| 676774 | GU | 1 | 1 | 0 | 2 | - |
| 342534 | Normal | 0 | 0 | 0 | 1 | - |
| 818178 | Gastritis | 3 | 1 | 0 | 2 | - |

## APPENDIX 1(b)
## STUDY GROUP

| Patient Number | Endoscopic Findings | Gastric Histology | | | Duodenal Histology | |
|---|---|---|---|---|---|---|
| | | Gastritis | Atrophy | Metaplasia | Duodenitis | Metaplasia |
| 754884 | GU | 3 | 3 | 1 | 3 | + |
| 675422 | Normal | 3 | 1 | 1 | 0 | - |
| 865086 | Normal | 5 | 3 | 3 | 2 | - |
| 499439 | Normal | 3 | 3 | 1 | 1 | - |
| 611270 | Normal | 1 | 1 | 0 | 2 | - |
| 853215 | Normal | 1 | 1 | 0 | 2 | + |
| 638747 | Polyps | 0 | 0 | 0 | 1 | + |
| 866240 | Normal | 1 | 1 | 0 | 2 | - |
| 744548 | Normal | 1 | 0 | 0 | 1 | - |
| 785868 | Polyps | 1 | 1 | 1 | 2 | + |
| 863280 | Duodenitis | 1 | 3 | 0 | 2 | + |
| 839531 | Normal | 1 | 0 | 0 | 1 | - |
| 858261 | Polyps | 1 | 1 | 0 | 2 | + |
| 821116 | Normal | 1 | 3 | 0 | 2 | + |
| 852274 | Polyps | 3 | 1 | 0 | 3 | + |
| 803808 | Oesophagitis | 1 | 0 | 0 | 1 | - |

## APPENDIX 1(c)
## STUDY GROUP

| Patient Number | Urease Slide Test | Helicobacter Histology Gastric | Helicobacter Histology Duodenal | HSV Histology Gastric | HSV Histology Duodenal |
|---|---|---|---|---|---|
| 821041 | + | + | - | - | - |
| 821748 | + | + | + | - | - |
| 779860 | + | + | - | - | - |
| 853773 | + | + | - | - | - |
| 865820 | + | - | - | - | - |
| 733701 | - | - | - | - | - |
| 745539 | - | - | - | - | - |
| 779162 | - | - | - | - | - |
| 855418 | - | - | - | - | - |
| 608902 | - | - | - | - | - |
| 858614 | - | - | - | - | - |
| 852379 | - | - | - | - | - |
| 829323 | + | + | - | - | - |
| 853214 | + | + | - | - | - |
| 676774 | - | + | - | - | - |
| 342534 | + | + | - | - | - |

## APPENDIX 1(c)
## STUDY GROUP

| Patient Number | Urease Slide Test | Helicobacter Histology | | Herpes Simplex Histology | |
|---|---|---|---|---|---|
| | | Gastric | Duodenal | Gastric | Duodenal |
| 754884 | + | + | - | - | - |
| 675422 | - | + | + | - | - |
| 865086 | + | + | - | - | - |
| 499439 | - | + | - | - | - |
| 011270 | - | - | - | - | - |
| 853215 | - | - | - | - | - |
| 638747 | - | - | - | - | - |
| 866240 | - | - | - | - | - |
| 744548 | - | - | - | - | - |
| 785868 | - | - | - | - | - |
| 863280 | - | - | - | - | - |
| 839531 | - | - | - | - | - |
| 858261 | - | + | - | - | - |
| 821116 | - | - | - | - | - |
| 852274 | + | + | - | - | - |
| 803808 | - | - | - | - | |

## APPENDIX 1(d)
## STUDY GROUP CMV STATUS

| Patient Number | Serology | | Donor status | Gastric CMV | Duodenal CMV |
|---|---|---|---|---|---|
| | Pre Transplant | Post Transplant | | | |
| 821041 | - | - | - | + | - |
| 821748 | NK | ++ | - | - | + |
| 779860 | + | ++ | - | - | - |
| 853773 | + | NK | - | - | - |
| 865820 | - | - | NK | - | - |
| 733701 | + | ++ | + | - | - |
| 745539 | + | ++ | NK | - | - |
| 779162 | - | ++ | - | - | - |
| 855418 | NK | NK | NK | + | + |
| 608902 | NK | ++ | + | - | + |
| 858614 | - | ++ | + | + | + |
| 852379 | + | + | + | - | - |
| 829323 | NK | ++ | NK | - | - |
| 853214 | NK | NK | + | - | - |
| 676774 | + | + | + | - | + |
| 342534 | + | ++ | + | - | + |
| 818178 | + | ++ | - | - | - |

Post transplant serology + = seropositive, ++ = serological evidence of active infection

## APPENDIX 1(d)
## STUDY GROUP CMV STATUS

| Patient Number | Serology | | Donor Status | Gastric CMV | Duodenal CMV |
|---|---|---|---|---|---|
| | Pre Transplant | Post Transplant | | | |
| 754884 | + | + | NK | - | - |
| 675422 | - | NK | NK | - | + |
| 865086 | + | ++ | NK | - | - |
| 499439 | - | - | + | + | - |
| 611270 | NK | NK | NK | - | + |
| 853215 | NK | ++ | + | - | + |
| 638757 | + | ++ | NK | - | - |
| 866240 | - | - | - | - | + |
| 744548 | + | + | NK | - | - |
| 785868 | + | ++ | - | - | + |
| 863280 | + | + | - | - | + |
| 839531 | _ | + | + | - | - |
| 858261 | _ | ++ | + | - | - |
| 821116 | - | NK | - | + | + |
| 852274 | + | ++ | + | - | - |
| 803808 | + | + | NK | - | - |

Post transplant serology + = seropositive, ++ = serological evidence of active infection

## APPENDIX 2
## NON-ATTENDERS

| Patient Number | Age | Symptoms | H2 Antagonists | Prednisolone Dose | Serum Biochemistry Urea | Creatinine | Cyclosporine |
|---|---|---|---|---|---|---|---|
| 767314 | | No | Yes | 20 | 30.2 | 921 | 244 |
| 757187 | | No | Yes | 15 | 6.1 | 106 | 73 |
| 414853 | | Yes | Yes | 15 | 7.1 | 126 | 132 |
| 800172 | | Yes | No | 20 | 4.9 | 113 | 215 |
| 833776 | | No | No | 20 | 9.5 | 73 | 232 |
| 764098 | | No | No | 20 | 12.8 | 193 | 197 |
| 392717 | | Yes | No | 15 | 13.4 | 148 | 117 |
| 853516 | | No | No | 15 | 7.9 | 69 | 113 |
| 854787 | | No | No | 15 | 7.3 | 140 | 94 |
| 780310 | | Yes | Yes | 15 | 8.4 | 113 | 122 |
| 854943 | | Yes | No | 20 | 9.7 | 174 | 26 |
| 814483 | | Yes | No | 20 | 16.7 | 216 | 286 |
| 680478 | | No | Yes | 20 | 9.5 | 98 | 223 |
| 739933 | | No | Yes | 20 | 8.8 | 160 | 82 |
| 813957 | | Yes | Yes | 15 | 11.6 | 171 | 113 |
| 764262 | | Yes | Yes | 12.5 | 7.5 | 130 | 309 |
| 800317 | | No | No | 12.5 | 20.3 | 200 | 165 |

## APPENDIX 3
## T LYMPHOCYTE SUBSETS (a) STUDY GROUP

| Patient Number | Biopsy Site | CMV | H pylori | Leu2 | Leu3 | Leu3/Leu2 |
|---|---|---|---|---|---|---|
| 821041 | Gastric | + | - | 308 | 462 | 1.5 |
| 821041 | Duodenal | - | + | 90 | 89 | 0.98 |
| 818178 | Gastric | - | + | 343 | 413 | 1.2 |
| 818178 | Duodenal | - | - | 58 | 168 | 2.89 |
| 863280 | Gastric | - | - | 227 | 247 | 1.09 |
| 863280 | Duodenal | + | - | 95 | 334 | 3.51 |
| 676774 | Gastric | - | + | 88 | 160 | 1.81 |
| 676774 | Duodenal | + | - | 66 | 130 | 1.97 |
| 779162 | Gastric | - | - | 88 | 152 | 1.73 |
| 779162 | Duodenal | - | - | 36 | 102 | 2.83 |
| 865086 | Gastric | - | + | 151 | 128 | 0.85 |
| 865086 | Duodenal | - | - | 91 | 170 | 1.87 |
| 865820 | Gastric | - | + | 65 | 92 | 1.42 |
| 865820 | Duodenal | - | - | 199 | 262 | 1.32 |
| 866240 | Gastric | - | - | 31 | 28 | 0.90 |

## APPENDIX 3
## T LYMPHOCYTE SUBSETS (a) STUDY GROUP

| Patient Number | Biopsy Site | GI CMV | H pylori | Leu2 | Leu3 | Leu3/Leu2 |
|---|---|---|---|---|---|---|
| 866240 | Duodenal | + | - | 130 | 250 | 1.92 |
| 829323 | Gastric | - | + | 24 | 130 | 5.42 |
| 829323 | Duodenal | - | - | 74 | 332 | 4.49 |
| 821116 | Gastric | + | - | 97 | 323 | 3.33 |
| 821116 | Duodenal | + | - | 144 | 123 | 0.85 |
| 342534 | Gastric | - | + | 53 | 54 | 1.02 |
| 342534 | Duodenal | + | - | 37 | 159 | 4.30 |
| 821748 | Gastric | - | + | 92 | 198 | 2.15 |
| 821748 | Duodenal | + | + | 112 | 357 | 3.19 |
| 839531 | Gastric | - | - | 89 | 134 | 1.51 |
| 839951 | Duodenal | - | - | 108 | 121 | 1.12 |
| 754884 | Gastric | - | + | 230 | 205 | 0.89 |
| 754884 | Duodenal | - | - | 46 | 127 | 2.76 |
| 03808 | Gastric | - | - | 114 | 111 | 0.97 |
| 803808 | Duodenal | - | - | 39 | 185 | 4.74 |

## APPENDIX 3
## MUCOSAL T LYMPHOCYTES (b) CONTROL GROUP

| Patient Number | Biopsy Site | H pylori | Leu2 | Leu3 | Leu3/Leu2 |
|---|---|---|---|---|---|
| 808908 | Gastric | - | 49 | 383 | 7.81 |
| 808908 | Duodenal | - | 79 | 95 | 1.20 |
| 572416 | Gastric | - | 138 | 135 | 0.97 |
| 572416 | Duodenal | - | 135 | 352 | 2.61 |
| 555532 | Gastric | + | 210 | 435 | 2.07 |
| 555532 | Duodenal | - | 104 | 294 | 2.82 |
| 737662 | Gastric | + | 115 | 128 | 1.11 |
| 737662 | Duodenal | - | 47 | 163 | 3.47 |
| 876833 | Gastric | | 252 | 109 | 0.43 |
| 876838 | Duodenal | - | 59 | 92 | 1.56 |
| 877412 | Gastric | + | 449 | 608 | 1.35 |
| 877412 | Duodenal | - | 47 | 160 | 3.40 |
| 538946 | Gastric | + | 124 | 153 | 1.23 |
| 538946 | Duodenal | - | 43 | 216 | 5.02 |
| 721797 | Gastric | + | 88 | 81 | 0.92 |

## APPENDIX 3
## MUCOSAL T LYMPHOCYTES (b) CONTROL GROUP

| Patient Number | Biopsy Site | H pylori | Leu2 | Leu3 | Leu3/Leu2 |
|---|---|---|---|---|---|
| 721797 | Duodenal | - | 99 | 322 | 3.25 |
| 349576 | Gastric | - | 71 | 185 | 2.60 |
| 349576 | Duodenal | - | 83 | 299 | 3.60 |
| 775406 | Gastric | - | 169 | 211 | 1.25 |
| 775406 | Duodenal | - | 77 | 144 | 1.87 |
| 066278 | Gastric | + | 106 | 198 | 1.87 |
| 066278 | Duodenal | - | 131 | 300 | 2.29 |
| 369316 | Gastric | + | 119 | 189 | 1.59 |
| 369316 | Duodenal | - | 126 | 190 | 1.51 |
| 204584 | Gastric | + | 234 | 339 | 1.49 |
| 204584 | Duodenal | - | 151 | 402 | 2.66 |
| 310505 | Gastric | + | 148 | 243 | 1.64 |
| 310505 | Duodenal | - | 193 | 144 | 0.75 |
| 877430 | Gastric | - | 52 | 251 | 4.83 |
| 877430 | Duodenal | - | 40 | 176 | 4.40 |

# REFERENCES

1) Chisholm GD, Mee AD, Williams G, Castro JE, Baron JH. Peptic ulceration, gastric secretion and renal transplantation. Br Med J 1977; **1**: 1630-32.

2) Moore TC, Hume DM. The period and nature of hazard in renal transplantation. Annals Surg 1969; **170**: 1-11.

3) Libertino JA, Zinman L, Dowd JB. Gastrointestinal complications related to human renal homotransplantation. Surgical Clinics of North America 1971; **51**: 733-37.

4) Hadjiyannakis EJ, Evans DB, Smellie WAB, Calne RY. Gastrointestinal complications after renal transplantation. Lancet 1971; **ii**: 781-84.

5) Canavan JSF, Briggs JD, Bell PRF. Gastric acid secretion following renal transplantation. Br J Surg 1975; **62**: 737-40.

6) Gordon EM, Johnson AG, Williams G. Gastric assessment of prospective renal transplant recipients. Lancet 1972; **i**: 226-29.

7) Schiessel R, Starlinger M, Wolf A et al. Failure of cimetidine to prevent gastroduodenal ulceration and bleeding after renal transplantation. Surgery 1981; **90**: 456-58.

8) Franzin G, Muolo A, Griminelli T. Cytomegalovirus inclusions in the gastroduodenal mucosa of patients after renal transplantation. Gut 1981; **22**: 698-701.

9) Knechtle SJ, Kempf K, Bollinger RR. Peptic ulcer disease following renal transplantation. Transplant Proc 1987; **19**: 2233-36.

10) Knechtle SJ, Bollinger RR. Perforation of the gastrointestinal tract following renal transplantation. Transplant Proc 1989; **21**: 2143-44.

11) Cohen EB, Komorowski RA, Kauffman HM, Adams M. Unexpectedly high incidence of cytomegalovirus infection in apparent peptic ulcers in renal transplant recipients. Surgery 1985; **97**: 606-612.

12) Timoney AG, Man WK, Spencer J, Taylor H, Williams G. Histamine and duodenal ulceration in renal transplant recipients. Gut 1989; **30**: 65-71.

13) Peterson PK, Balfour HH, Marker SC, Fryd DS, Howard RJ, Simmons RL. Cytomegalovirus disease in renal allograft recipients: A prospective study of the clinical features risk factors and impact on renal transplantation. Medicine 1980; **59**: 283-300.

14) Shepherd AMM, Stewart WK, Wormsley KG. Peptic ulceration in chronic renal failure. Lancet 1973; **i**: 1357-59.

15) Ventkatesweran PS, Jeffers A, Hocken AG. Gastric acid secretion in chronic renal failure. Brit Med J 1972; **4**: 22-23.

16) Alijani MR, Benjamin SB,Collen MJ, Foegh ML. Misoprostil : A prostaglandin E1 analogue versus antacid in the prevention of stress ulcers in kidney transplant patients. Transplant Proc 1989; **21**:2145-46.

17) Doherty CC, McGeown MG. Peptic ulceration, gastric acid secretion and kidney transplantation. Dialysis & Transplantation 1979; **8**: 970-77.

18) Diethelm AG, Gore I, Chien LT, Sterling WA, Morgan JM. Gastrointestinal haemorrhage secondary to cytomegalovirus after renal transplantation. Am J Surg 1976; **131**: 371-74.

19) Alexander JA, Cueller RE, Fadden RJ, Genovese JJ, Gavaler JS, Van Thiel DA. Cytomegalovirus infection of the upper GI tract before and after liver transplantation. Transplantation 1988; **46**: 378-382.

20) Clarke SD, Neill DW, Wellbourn RB. The effects of corticotrophin and corticoids on secretion from denervated gastric pouches in dogs. Gut 1960; **1**: 36-43.

21) Cooke AR, Grossman MI. Effect of hydrocortisone on secretion of acid and pepsin by Heidenhain pouches. Proc Soc Exp Biol Med 1960; **123**: 704-10.

22) Doherty CC. Gastric secretion in chronic uraemia and after renal transplantation. Ir J Med Sci 1980; **140**: 5-9.

23) McConnel JB, Thjodleifsson B, Stewart WK, Wormsley KG. Gastric function in chronic renal failure, effects of maintenance haemodialysis. Lancet 1975; **ii**: 1121-23.

24) Walter S, Thorup Andersen J, Christensen V et al. Effect of cimetidine on upper gastrointestinal bleeding after renal transplantation: a prospective study. Br Med J 1984; **289**: 1175-76.

25) Jones RH, Rudge CJ, Bewick M, Parsons V, Weston MJ. Cimetidine: prophylaxis against upper gastrointestinal haemorrhage after renal transplantation. Br Med J 1978; **1**: 398-400.

26) Garvin PJ, Carney K, Castenada M, Codd JE. Peptic ulcer disease following transplantation: The role of cimetidine. Am J Surg 1982; **144**: 545-48.

27) Doherty CC. Effect of cimetidine on upper gastrointestinal bleeding after renal transplantation. Br Med J 1984; **289**: 1539-40.

28) Christiansen DM, Johansen A. Single gastric biopsy in subjects with low acid secretion after maximal histamine stimulation. Scand J Gastroent 1966; **1**: 86-93.

29) Korman MG, Laver MC, Hansky J. Hypergastrinaemia in chronic renal failure. Br Med J 1972; **1**: 209-10.

30) Paimela H, Harkonen H, Karonen SL, Tallgren LG, Ahonen J. The effect of renal transplantation on gastric acid secretion and on serum levels of gastrin and group pepsinogens. Ann Clin Res 1983; **17**: 105-09.

31) Crean GP, Marshall MW, Rumsey RDE. Parietal cell hyperplasia induced by the administration of pentagastrin (ICI 50, 123) to rats. Gastroenterol 1969; **57**: 147-55.

32) Barreras RF, Donaldson RM. Role of calcium in gastric hypersecretion. N Eng J Med 1967; **276**: 1122-24.

33) Peden NR, Callachan H, Shepherd DM, Wormsley KG. Gastric mucosal histamine and histamine methyltransferase in patients with duodenal ulcer. Gut 1982; **23**: 58-62.

34) Man WK, Saunders JH, Ingoldby C, Spencer J. Effect of cimetidine on the amounts of histamine in the gastric mucosa of patients with duodenal or gastric ulcers. Gut 1981; **22**: 923-26.

35) Meltzer LE, Bockmann AA, Kanenson W, Cohen A. The incidence of peptic ulcer among patients on long-term prednisone therapy. Gastroenterol 1958; **35**: 351-56.

36) Bollet AJ, Black R, Bunim JJ. Major undesirable side effects resulting from prednisolone and prednisone. J Am Med Assoc 1955; **158**: 459-63.

37) Conn HO, Blitzer BL. Non-association of adrenocorticosteroids and peptic ulcer. N Engl J Med 1976; **294**: 473-79.

38) Messer J, Reitman D, Sacks HS, Smith H Jr, Chalmers TC. Association of adrenocorticosteroid therapy and peptic ulcer disease. N Engl J Med 1983; **309**: 21-24.

39) Conn HO, Poynard T. Adrenocorticosteroid administration and peptic ulcer: a critical analysis. J Chronic Dis 1985; **38**: 457-68.

40) Doherty CC, McGeown MG. Peptic ulceration, gastric acid secretion and kidney transplantation. Dialysis and Transplantation 1979; **8**: 970-77.

41) Max M, Menguy R. Influence of adrenocorticotrophin, cortisone, asprin and phenylbutazone on the rate of exfoliation and the rate of renewal of gastric mucosal cells. Gastroenterol 1970; **58**: 329-336.

42) Janowicz HD, Weinstein VA, Shaer RG, Cereghini JF, Hollander F. The effects of cortisone and corticotrophin on the healing of gastric ulcer: an experimental study. Gastroenterol 1958; **34**: 11-20.

43) Croft DN. Cell turnover and loss and the gastric mucosal barrier. Am J Dig Dis 1977; **22**: 383-86.

44) Doenges JL. Spirochaetes in the gastric glands of macacus rhesus and of man without related disease. Arch Pathol 1939; **27**: 469-77.

45) Warren JR, Marshall BJ. Unidentified bacilli on gastric epithelium on active chronic gastritis. Lancet 1983; **i**: 1273-75.

46) Marshall BJ, Royce H, Annear DI et al. Original isolation of campylobacter pyloridis from human gastric mucosa. Microbios Letters 1984; **25**: 83-88.

47) Marshall BJ, Goodwin CS. Revised nomenclature of campylobacter pyloridis. Int J Syst Bacteriol 1987; **37**: 68.

48) Goodwin CS, Armstrong JA, Chilvers T et al. Transfer of Campylobacter pylori and Campylobacter mustelae to Helicobacter gen nov as Helicobacter pylori comb nov and Helicobacter mustelae comb nov respectively. Int J Syst Bacteriol 1989; **39**: 397-405.

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

50) Kasper GF. Natural sources and microbiological characteristics of campylobacter pylori. Scand J Gastroenterol 1988; **23**: Suppl 142: 14-15.

51) Owen RJ, Martin SR, Borman P. Rapid urea hydrolysis by gastric campylobacter. Lancet 1985; **i**: 111.

52) Hazell SL, Lee A. Campylobacter pyloridis, urease, hydrogen ion back diffusion and gastric ulcer. Lancet 1986; **ii**: 15-17.

53) Strickland RG, Mackay IR. A reappraisal of the nature and significance of chronic atrophic gastritis. Am J Dig Dis 1973; **18**: 426-440.

54) Marshall BJ. Campylobacter pyloridis and gastritis. J Infect Dis 1986; **153**: 650-57.

55) Cheli R, Gioacosa A, Molinari F. Chronic gastritis and duodenogastric reflux. Scand J Gastroenterol 1981;16,Suppl **67**: 125-27.

56) Jones R. Dyspeptic symptoms in the community. Gut 1989; **30**: 893-98.

57) Talley MJ. The pathogenesis of non-ulcer dyspepsia. Med J Aust 1985; **143**: 173-174.

58) Fung WP, Papadimitriou JM, Matz LR. Endoscopic, histological and ultrastructural correlations in chronic gastritis. Am J Gastroenterol 1979; **71**: 269-79.

59) Vaira D, Holton J, Osborne J, Dowset J, McNeil I, Hatfield A. Use of endoscopy in patients with dyspepsia. Br Med J 1989; **299**: 237.

60) Marshall BJ, Warren JB, Francis GJ, Langton SR, Goodwin CS, Blincow ED. Rapid urease test in the management of campylobacter pyloridis associated gastritis. Am J Gastroenterol 1987; **82**: 200-10.

61) Wyatt JI, Rathbone BJ, Heatley RL. Local immune response to gastric campylobacter in non-ulcer dyspepsia. J Clin Pathol 1986; **39**: 863-70.

62) Johnston BJ, Reed PI, Ali MH. Prevalence of campylobacter pylori in duodenal and gastric mucosa - relationship to inflammation. Scand J Gastroenterol 1988; **23**, Suppl 142: 69-75.

63) O'Connor HJO, Axon ATR, Dixon MF. Campylobacter like organisms unusual in type A (pernicious anaemia) gastritis. Lancet 1984; **ii**: 1091.

64) Offerhaus GJA, Riev PNMA, Jansen JBMJ, Joosten HJM, Lamers CBHW. Prospective comparative study of the influence of post-operative bile reflux on gastric mucosal histology and campylobacter pylori infection. Gut 1989; **30**: 1552-57.

65) Axon ATR. Campylobacter pyloridis: what role in gastritis and peptic ulcer? Br Med J 1986; **293**: 772.

66) Ramsey EJ, Carey KV, Peterson WL et al. Epidemic gastritis with hypochlorhydria. Gastroenterol 1979; **76**: 1449-57.

67) Gledhill T, Leicester RJ, Addis B et al. Epidemic hypochlorhydria. Br Med J 1985; **290**: 1383-86.

68) Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfill Koch's postulates for pyloric campylobacter. Med J Aust 1985; **142**: 436-39.

69) Morris AM, Micholson G. Ingestion of campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastroenterol 1987; **82**: 192-199.

70) Marshall BJ, Goodwin CS, Warren JR et al. Long-term healing of gastritis and low duodenal ulcer relapse after eradication of campylobacter pyloridis: A prospective double blind study. Gastroenterol 1987; **92**: 1518.

71) McNulty CAM, Gearty JC, Crump B. Campylobacter pyloridis and associated gastritis: investigator blind placebo controlled trial of bismuth salicylate and erythromycin ethylsuccinate. Br Med J 1986; **293**: 645-49.

72) Coghlan JG, Humphries H, Dooley C et al. Campylobacter pylori and recurrence of duodenal ulcers : a 12 month follow-up study. Lancet 1987; **ii**: 1109-11.

73) Rokkas T, Pursey C, Uzoechina E et al. Non-ulcer dyspepsia and short-term De-Nol therapy with particular reference to the role of campylobacter pylori. Gut 1988; **29**: 1386-91.

74) Lambert JR, Dunn K, Borromeo M, Korman MG, Hansky J. Campylobacter pylori - A role in non-ulcer dyspepsia? Scand J Gastroenterol 1989; **24**, Suppl 160: 7-13.

75) Johnston BJ, Reed PI, Ali MH. Prevalence of campylobacter pylori in duodenal and gastric mucosa - relationship to inflammation. Scand J Gastroenterol 1988; **23** Suppl 142: 69-75.

76) Marshall BJ, Waren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984 **i**: 1311-14.

77) Von Wulffen H, Heesemen J, Batzow GH, Loning T, Laufs R. Detection of campylobcter pylori in patients with antrum gastritis and peptic ulcers by culture, complement fixation tests and immunoblot. J Clin Microbiol 1986; **24**: 716-20.

78) McKenna D, Humphreys H, Dooley C et al. C pylori and histological gastritis in duodenal ulcer. A controlled prospective randomised trial. Gastroenterol 1987; **92**: 1528.

79) Lamouliatte H, Megraud F, De Mascarel A, Roux D, Quinton A. C pylori and epigastric pain, endoscopic, histological and bacteriological correlations. Gatroenterol Clin Biol 1987; **11**: 212-16.

80) Girdwood RWA, Fricker CR, Forrest JAH, Burnett RA. Campylobacter pyloridis in biopsy samples of normal and diseased stomachs: Pathological, serological and bacteriological findings. In: Pearson AD, Skirrow MB, Lior H. Eds. Campylobacter III, Proceedings of the Third International Workshop on Campylobacter Infections. Public Health Laboratory Service, London 1985; 180.

81) Rollasson TP, Stone J, Rhodes JM. Spiral organisms in endoscopic biopsies of the human stomach. J Clin Pathol 1984; **37**: 23-26.

82) Wormsley KG. Ulcer disease: epidemiology. In: Taylor TV, Gillespie IE. Current opinion in Gastroenterology 1987; **3**: 945-51. Gower Academic Journals, London

83) Schrager J, Spink R, Mitra S. The antrum in patients with duodenal and gastric ulcers. Gut 1967; **8**: 497-508.

84) Goodwin CS, Blake P, Blincow E. The minimum inhibitory and bactericidal concentrations of antibiotics and anti-ulcer agents against C pylori. J Antimicrob Chemother 1986; **17**: 309-14.

85) Moshal MG, Gregory MA, Pillay C, Spitaels JM. Does the duodenal cell ever return to normal? A comparison between treatment with Cimetidine and Denol. Scand J Gastroenterol 1979; **14**, Suppl 54: 48-51.

86) Martin DF, Hollanders D, May SJ, Ravenscroft MM, Tweedle DEF, Miller JP. Difference in relapse rates of duodenal ulcer after healing with cimetidine or tripotassium dicitrato bismuthate. Lancet 1981; **1**: 7-10.

87) Hall DWR. Review of the modes of action of colloidal bismuth subcitrate. Scand J Gastroenterol 1988;23, Suppl **157**: 3-6.

88) Kerrigan DD, Taylor ME, Read NW, Houghton LA, Johnson AG. Duodenal bulb acidity and the natural history of duodenal ulceration. Lancet 1989; **ii**: 61-63.

89) Patrick WJA, Denham D, Forrest APM. Mucous change in the human duodenum: A light and electron microscopic study and correlation with disease and gastric acid secretion. Gut 1974; **15**: 767-76.

90) Shousha S, Keen C, Parkins RA. Gastric metaplasia and campylobacter pylori infection of duodenum in patients with chronic renal failure. J Clin Pathol 1984; **42**: 348-51.

91) Wyatt JI, Rathbone BJ, Dixon MF, Heatley RV. Campylobacter pyloridis and acid induced gastric metaplasia in the pathogenesis of duodenitis. J Clin Pathol 1987; **40**: 841-848.

92) Carrick J, Lee A, Hazell S, Ralston M, Daskalopoulos G. Campylobacter pylori, duodenal ulcer, and gastric metaplasia: possible role of functional heterotopic tissue in ulcerogenesis. Gut 1989: **30**: 790-97.

93) Levi S, Beardshall K, Haddad G, Playford R, Ghosh P, Calam J. Campylobacter pylori and duodenal ulcers; the gastrin link. Lancet 1989; **i**: 1167-68.

94) Burnett RA, Forrest JAH, Girdwood RWA, Fricker CR. Campylobacter like organisms in the stomach of patients and healthy individuals. Lancet 1984; **i**: 1349.

95) Hui WM, Lam SK, Chau PY. Pathogenetic role of campylobacter pyloridis in gastric ulcer. J Gastroenterol Hepatol 1987; **2**: 309-16.

96) Yeomans ND. Bacteria in ulcer pathogenesis in Piper DW Ed. Ballieres Clinical Gastroenterology, London 1988, **2**(3): 573-91.

97) Humphreys H, Bourke S, Dooley C et al. Effect of treatment on campylobacter pylori in peptic disease: a randomised prospective trial. Gut 1988; **29**: 279-83.

98) Tytgat GNJ. Colloidal bismuth subcitrate in peptic ulcer - A review. Digestion 1987; **37** Suppl 2: 31-41.

99) Tytgat GNJ, Hameeteman W, van Olffen GH. Sucralfate, bismuth compounds substituted benzimidazole, trimipramine and pirenzipine in the short and long-term treatment of duodenal ulcer. Clin Gastroenterol 1984; **13**: 543-68.

100) Tytgat GNJ, Rauws E, Langenberg W. The role of colloidal bismuth subcitrate in gastric ulcer and gastritis. Scand J Gastroenterol 1986; **121** Suppl 122: 22-29.

101) Johnston BJ, Reed PI, Hali M. Campylobacter like organisms in duodenal and antral endoscopic biopsies: relationship to inflammation. Gut 1986; **27**: 1132-37.

102) Meyrick-Thomas J, Pointer D, Gooding C et al. Gastric spiral bacteria. Lancet 1984; **i**: 100.

103) Buck GE, Gourlay WK, Lee WK, Subramanjam K. Relationship of campylobacter pyloridis to gastritis and peptic ulcer. J Inf Dis 1986; **153**: 664-69.

104) Von Wulffen H, Grote HJ, Gatermann S, Loning T, Berger B, Buhl C. Immunoblot analysis of immune response to campylobacter pylori and its clinical associations. J Clin Pathol 1988; **41**: 653-59.

105) Vaira D, Holton J, Cairns SR et al. Antibody titres to campylobacter pylori after treatment for gastritis. Br Med J 1988; **297**: 397.

106) Rathbone BJ, Wyatt JI, Worsley BW et al. Systemic and local antibody response to gastric campylobacter pyloridis in non-ulcer dyspepsia. Gut 1986; **27**: 642-647.

107) Barer MR, Elliot TSJ, Berkeley D, Thomas JE, Eastham EJ. Cytopathic effects of campylobcter pylori urease. J Clin Pathol 1988; **41**: 597.

108) Yrios JW, Balish E. Immune response of athymic and euthymic germfree mice to campylobacter spp. Infect Immuun 1986; **54**: 339-46.

109) Rathbone BJ, Wyatt J, Trejdosiewicz LK, Heatley RV, Losowsky MS. Mucosal T cell subsets in normal gastric antrum and C pylori associated gastritis. Gut 1988; **29**: A1438.

110) Rauws EAJ, Langenberg W, Houthoff HJ, Zanen HC, Tytgat GN. Campylobacter pyloridis associated chronic active antral gastritis. A prospective study of its prevalence and the effects of antibacterial and antiulcer treatment. Gastroenterol 1988; **94**: 33-40.

111) Graham DY, Klein PD, Opekun AR et al. Epidemiology of campylobacter pylori infection: Ethnic considerations. Scand J Gastroenterol 1988; **23** Suppl 142: 9-13.

112) Kosunen TU, Hook J, Rautelin HI, Myllyla G. Age dependent increase in campylobacter pylori antibodies in blood donors. Scand J Gastroenterol 1989; **24**: 110-14.

113) Walker SJ, Murray E. A review of campylobacter pylori in upper gastrointestinal disease. Br J Hosp Med 1988; **40**: 27-34.

114) Marshall BJ, Whisson M, Francis G, McGechie D. Clinical and serological studies on Australian bloo donors using a passive haemagglutination test for campylobacter pyloridis. In: Pearson AD, Skirrow MB, Lion H Eds. Campylobacter III Proceedings of the Third International Workshop on Campylobacter Infections: London: PHLS 1985: 188-89.

115) Mitchell HM, Lee A, Carrick J. Increased incidcence of C pylori infection in gastroenterologists: Further evidence to support person to person transmission of C pylori. Scand J Gastroenterol 1989; **24**: 396-400.

116) Mitchell HM, Bohane TD, Berkowicz J, Hazell SL, Lee A. Antibody to campylobacter pylori in families of index children with gastrointestinal illness due to C pylori. Lancet 1987; **ii**: 681-82.

117) Berkowicz J, Lee A. Person to person transmission of C pylori. Lancet 1987; ii: 680-81.

118) Vaira D,D'Anastasio C, Holton J et al. Campylobacter pylori in abbatoir workers. Gut 1988; **29**: A1437.

119) Meiselman MS, Miller-Catchpole R, Christ M, Randal E. Campylobacter pylori gastritis in the acquired immune deficiency syndrome. Gastroenterol 1988; **95**: 209-12.

120) Krech U. Complement fixing antibodies against cytomegalovirus in different parts of the world. Bull WHO 1973; **49**: 103-06.

121) Olson LC, Ketusinha R, Mansuwan P, Snitbhan R. Respiratory tract excretion of cytomegalovirus in Thai children. J Paediatr 1970; **77**: 499-504.

122) Ho M. Cytomegalovirus, biology and injection. Plenum Medical Book Co., New York & London 1982;

123) Klemola E, Kaaraiainen L. Cytomegalovirus as a possible cause of a disease resembling infections mononucleosis. Br Med J 1965; **2**: 1099-1102.

124) Klemola E, von Essen R, Henle G, Henle W. Infectious mononucleosis like disease with negative heterophile agglutination test. Clinical features in relation to Epstein-Barr virus and cytomegalovirus antibodies. J Infect Dis 1970; **121**: 608-14.

125) Pagano JS. Diseases and mechanisms of persistent DNA virus infection: Latency and cellular transformation. J Infect Dis 1975; **132**: 209-223.

126) Schrier R, Nelson JA, Oldstone MBA. Detection of human CMV in peripheral blood lymphocytes in a natural infection. Science 1985; **230**: 1048-50.

127) Crawford DH, Sissons JGP. Chronic herpes virus infection. In Webster ADB Ed, Immunodeficiency and Disease. Klower Academic Publishers, Dordrecht 1988.

128) Rifkind D, Starzl TE, Marchioro TL, Waddell WR, Rowlands DT Jr, Hill RB Jr. Transplantation pneumonia. J Am Med Assoc 1964; **189**: 808-812.

129) Hedley-White ET, Craighead JE. Generalised cytomegalovirus inclusion disease after renal homotransplantation. N Engl J Med 1965; **272**: 473-475.

130) Naraqi S, Jackson GG, Jonasson O, Yamashiroya HM. Prospective study of prevalence incidence and source of herpesvirus infections in patients with renal allografts. J Infect Dis 1977; **136**: 531-39.

131) Ho M, Wajszczak CP, Hardy A et al. Infections in kidney, heart and liver transplant recipients on Cyclosporine. Transplant Proc 1983; **15**: 2768-2772.

132) Simmons RL, Weil R, Tallent MB et al. Do mild infections trigger the rejection of renal allografts? Transplant Proc 1970; **2**: 419.

133) Von Willebrand E, Pettersson E, Ahonen J, Hayry P. CMV infection, class II antigen expression and human kidney allograft rejection. Transplantation 1986; **42**: 364-67.

134) Ho M, Suwansirikul S, Dowling JN, Youngblood LA, Armstrong JA. The transplanted kidney as a source of cytomegalovirus infection. N Engl J Med 1975; **293**: 1109-12.

135) Betts RF, Freeman RB, Douglas RG, Talley TE, Rundell B. Transmission of cytomegalovirus infection with renal allograft. Kidney Int 1975; **8**: 387-394.

136) Chou S. Acquisition of donor strains of cytomegalovirus by renal transplant recipients. N Eng J Med 1986; **314**: 1418-23.

137) Grundy JE, Super M, Sweny P et al. Symptomatic cytomegalovirus infection in seropositive kidney recipients: Reinfection with donor virus rather than reactivation of recipient virus. Lancet 1988; **i**: 132-35.

138) Chou S, Norma DJ. The influence of donor factors other than serological status on the transmission of cytomegalovirus to transplant recipients. Transplantation 1988; **46**: 89-93.

139) Campbell DA, Piercey JRA, Shnitka TK, Goldsand G, Devine RDO, Weinstein WM. Cytomegalovirus associated gastric ulcer. Gastroenterol 1977; **72**: 533-35.

140) Goodman MD, Porter DD. Cytomegalovirus vasculitis with fatal colonic haemorrhage. Arch Pathol 1973; **96**: 281-84.

141) Wolfe BM, Cherry JD. Haemorrhage from cecal ulcers of cytomegalovirus infection: Report of a case. Ann Surg 1973; **177**: 490-94.

142) Millard PR, Herbertson BM, Nagington J, Evans DB. The morphological consequences and the significance of cytomegalovirus infection in renal transplant recipients. Q J Med 1973; **42**: 585.

143) Diethelm AG, Gore I, Chien LT, Sterling WA, Morgan JM. Gastrointestinal haemorrhage secondary to cytomegalovirus after renal transplantation. Am J Surg 1976; **131**: 371-74.

144) Alexander JA, Cuellar RE, Fadden RJ, Genovese JJ, Gavaler JS, Van Thiel DH. Cytomegalovirus infection of the upper gastrointestinal tract before and after liver transplantation. Transplantation 1988; **46**: 378-82.

145) Frank D, Raicht RF. Intestinal perforation associated with cytomegalovirus infection in patients with acquired immune deficiency syndrome. Am J Gastroenterol 1984; **79**: 201-05.

146) Kavin H, Jonas RB, Chowdhury L, Kabins S. Acalculous cholecystitis and cytomegalovirus infection in the acquired immune deficiency syndrome. Ann Intern Med 1986; **104**: 53-54.

147) Hinnant KL, Rotterdam HZ, Bell ET, Tapper ML. Cytomegalovirus infection in the alimentary tract: A clinicopathological correlation. Am J Gastroenterol 1986; **81**: 944-50.

148) Dietrich DT. Cytomegalovirus: A new gastrointestinal pathogen in immunocompromised patients. Am J Gastroenterol 1987; **82**: 764-65.

149) Mobley K, Rotterdam HZ, Lerner CW, Tapper ML. Autopsy findings in the acquired immune deficiency syndrome. In Sommers SC, Rosen PP, Fechner RE.Eds, Pathol Annual 1985; **20:1** 45-65. Appleton Century Crofts, Connecticut.

150) Nahmias A, Dowdle W. Antigenic and biologic differences in herpesvirus hominis. Prog Med Virol 1968; **10**: 110-159.

151) Buchman TG, Simpson T, Nosal C, Roizman B, Nahmias AJ. Structure of herpes simplex virus DNA and application to molecular epidemiology. Ann NY Acad Sci 1981; **354**: 279-90.

152) Pereira L, Cassai E, Honess R, Roizman B, Terni M, Nahmias A. Variability in the structural polypeptides of herpes simplex virus strains: potential application in molecular epidemiology. Infect Immunol 1976; **13**: 211-220.

153) Rawls W. Herpes simplex virus. In: Field BM, Knipe DM, Channock RM, Melmick JL, Roizman B, Shope RE. Virology 1985; Raven Pres, New York, 527-61.

154) Whitley RJ, Nahmias AJ, Visintine AM, Fleming CL, Alford CA. The natural history of herpes simplex virus infection of mother and newborn. Pediatrics 1980; **60**: 489-94.

155) Nahmias AJ, Josey EW. Herpes simplex virus 1 and 2. In Evans A Ed : Viral infections of humans: epidemiology and control. Plenum Medical, New York; 1982: 351.

156) Wentworth BB, Alexander ER. Seroepidemiology of infections due to members of the herpesvirus group. Am J Epidemiol 1971; **94**: 496-507.

157) Corey L, Spear PG. Infections with Herpes simplex viruses (1). N Engl J Med 1986; **314**: 686-691.

158) Becker TM, Blount JH, Guinan ME. Genital herpes infection in private practice in the United States 1966-1981. J Am Med Assoc 1985; **253**: 1601-03.

159) Embil JA, Stephens RG, Manuel FR. Prevalence of recurrent herpes labialis and aphthous ulcers among young adults on six continents. Can Med Asoc J 1975; **113**: 627-30.

160) Grout P, Barber VE. Cold sores - An epidemiological Survey. J R Coll Gen Pract 1976; **26**: 428-34.

161) Editorial. Cytomegalovirus infection in allograft recipients. Lancet 1989; **1**: 302-303

162) Lopez C, Simmons RL, Mauer M et al. Role of virus infections in immunosuppressed renal transplant patients. Transplant Proc 1973; **5**: 803-808.

163) Rinaldo CR, DeBasio RL, Hamoudi WH, Rabin B, Liebert M, Hakala TR. Effect of herpesvirus infection on T lymphocyte subpopulations and blastogenic responses in renal transplant recipients receiving cyclosporine. Clin Immunol Immunopathol 1986; **38**: 357-66.

164) Haagsma EB, Klompmaker IJ, Grond J et al. Herpes virus infections after orthoptic liver transplantation. Transplant Proc 1987; **19**: 4054-56.

165) Dunn DL, Matas AJ, Fryd DS, Simmons RL, Najarian JS. Association of concurrent herpes simplex and cytomegalovirus with detrimental effects after renal transplantation. Arch Surg 1984; **119**: 812-817.

166) Douglas RG, Anderon MS, Wey JG et al. Herpes simplex virus pneumonia. J Am Med Assoc 1969; **210**: 902-904.

167) Arora KK, Karalakulasingam R, Raff MJ, Martin DG. Cutaneous herpesvirus hominis (type 2) infection after renal transplantation. J Am Med Assoc 1974; **230**: 1174-75.

168) Elliot WC, Houghton DC, Bryant RE, Wickland R, Barry JM, Bennet WM. Herpes simplex type 1 hepatitis in renal transplantation. Arch Intern Med 1980; **140**: 1656-60.

169) Linneman CC, First MR, Alvira MM, Alexander JW, Schiff GM. Herpesvirus hominis type 2 meningoencephalitis following renal transplantation. Am J Med 1976; **61**: 703-708.

170) Pass RF, Whitley RJ, Whelchel JD, Diethelm AG, Reynolds DW, Alford CA. Identification of patients with increased risk of infection with herpes simplex virus after renal transplantation. J Infect Dis 1979; **140**: 487-92.

171) Goodman JL. Possible transmission of herpes simplex virus by organ transplantation. Transplantation 1989; **47**: 609-13.

172) Dummer JS, Armstrong J, Somers J, Kusne S, Carpenter BJ, Rosenthal JT, Ho M. Transmission of infection with Herpes simplex virus by renal transplantation. J Infect Dis 1987; **155**: 202-06.

173) Bastian JF, Kaufman IA. Herpes simplex oesophagitis in a healthy 10 year old boy. J Pediatr 1982; **100**: 426-27.

174) Owensby LC, Stammer JL. Esophagitis associated with herpes simplex infection in an immunocompetent host. Gastroenterol 1978; **74**: 1305-06.

175) Corey L, Spear PG. Infections with herpes simplex virus. N Engl J Med 1986;**314**:749-57.

176) Buss DH, Scharyj JM. Herpesvirus infection of the esophagus and other visceral organs in adults. Incidence and clinical significance. Am J Med 1979; **66**: 457-62.

177) Knyvett AF. Peptic ulcer: Herpes simplex an aetiologicalagent. Med J Aust 1962; **22**: 229-30.

178) Walker DP, Longson M, Lawler W, Mallick NP, Davies JS, Johnson RWG. Disseminated herpes simplex virus infection with hepatitis in adult renal transplant recipient. J Clin Pathol 1981; **34**: 1044-46.

179) Rune SJ, Linde J, Bonnevie O et al. Acyclovir in the prevention of duodenal ulcer recurrence. Gut 1990; **31**: 151-52

180) Goodell SC, Quinn TC, Mkrtichien E, Schuffter MD, Holmes KK, Corey L. Herpes simplex virus proctitis in homoexual men. Clinical sigmoidoscopic and histopathological features. N Engl J Med 1983; **308**: 868-71.

181) Jacobs E. Anal infections caused by herpes simplex virus. Dis Colon Rectum 1976; **19**: 151-57.

182) Adler M, Goldman M, Liesnard C et al. Diffuse herpes simplex virus colitis in a kidney transplant recipient, successfully treated with acyclovir. Transplantation 1987; **43**: 919-21.

183) Calne RY, Alexandre GPJ, Murray JE. A study of the effects of drugs in prolonging survival of homologous renal transplants in dogs. Ann N Y Acad Sci 1962; **99**: 743-61.

184) Starzl TE, Marchioiro TL, Waddell WR. The reversal of rejection in human renal homografts with subsequent developement of homograft tolerance. Surg Gyn Obst 1963; **117**: 385-95.

185) Eickhoff TC, Olin DB, Anderson RJ, Schafer LA. Current problems and approaches to diagnosis of infection in renal transplant recipients. Transplant Proc 1972; **4**: 693-97.

186) D'Apice AJF. In: Morris PJ Ed. Kidney transplantation. Principles and practice, 2nd Ed . Grune and Stratton, London 1984 pp 239-58.

187) Morris PJ. In: Morris PJ Ed. Kidney transplantation. Principles and practice, 2nd Ed. Grune and Stratton, London 1984 pp 261-76.

188) Goodwin CS, Blincow ED, Warren JR, Waters TE, Sanderson CR, Easton L. Evaluation of cultural techniques for isolating Campylobacter pyloridis from endoscopic biopsies of gastric mucosa. J Clin Pathol 1985; **38**: 1123-31.

189) Morris A, Ali MR, Brown P, Lane M, Patton K. Campylobacter pylori infection in biopsy specimens of gastric antrum: laboratory diagnosis and estimation of sampling error. J Clin Pathol 1989; **42**: 727-32.

190) Burnett RA, Brown IL, Findlay J. Cresyl fast violet staining method for campylobacter like organisms. J Clin Pathol 1987; **40**: 353.

191) Van Noorden S, Polak J. Immunocytochemistry today. In:Polak J, Van Noorden S Eds. Immunocytochemistry: Practical applications in pathology and biology. John Wright & Sons, Bristol 1983.

192) Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. Proc Soc Exp Biol Med 1941; **47**: 200-202.

193) Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 1981; **29**: 577-80.

194) Gatter KC, Falini B, Mason DY. The use of monoclonal antibodies in histopathological diagnosis. In: Anthony PP, MacSween RNM. Recent advances in histopathology 12. Churchill Livingstone, Edinburgh 1984.

195) Niedobitek G, Finn T, Herbst H, et al. Detection of cytomegalovirus by in situ hybridisation and immunohistochemistry using a new monoclonal antibody CCH 2: A comparison of methods. J Clin Pathol 1988; **41**: 1005-09.

196) Jiwa NM, Raap AK, van de Rijke FM, et al. Detection of cytomegalovirus antigens and DNA in tissues fixed in formaldehyde. J Clin Pathol 1989; **42**: 749-54.

197) Huang S, Minassian H, More JD. Application of immunofluorescent staining in paraffin sections improved by trypsin digestion. Lab Invest 1976; **35**: 383-91.

198) Naoumov NV, Alexander GJM, Eddleston ALWF, Williams R. In situ hibridisation in formalin fixed, paraffin wax embeded liver specimens: method for detecting human and viral DNA using biotinylated probes. J Clin Pathol 1988; **41**: 793-98.

199) Burns J, Redfern DRM, Esini MM, McGee J O'D. Human and viral gene detection in routine paraffin embeded tissue by in situ hybridisation with biotinylated probes: viral localisation in herpes simplex. J Clin Pathol 1986; **39**: 1066-73.

200) Olsiewski PJ, Thalenfield BE, Engelhardt DL. Applications of non-radioactive DNA probe technology. Amer Clin Prod Review 1984; **3**: 30-33.

201) Watt PCH, Sloan JM, Kennedy TL. Changes in gastric mucosa after vagotomy and gastrojejunostomy for duodenal ulcer. Br Med J 1983; **287**: 1407-1410.

202) Whitehead R. Morphological aspects of duodenitis. Scand J Gastroenterol 1982; **17** Suppl 79:80-83.

203) Jonsson KA, Bodemar G, Norrby K, Walan A, Tysk C. Ten other investigators. Are endoscopic and/or histological findings in gastroduodenal mucosa a predictor of clinical outcome in peptic ulcer disease? Scand J Gastroenterol 1988; **23**: 199-208.

204) Cheli R. Symptoms in chronic non specific duodenitis.Scand J Gastroenterol 1982; **17** Suppl 79:84-87.

205) Kreuning J,van de Wal AM, Kuiper G, Lindeman J.Chronic nonspecific duodenitis.A multiple biopsy study of the duodenal bulb in health and disease. Scand J Gastroenterol 1989; **24** suppl 167:16-20

206) Jonsson KA, Gothard R, Bodemar G, Brodin V. The clinical relevance of endoscopic and histologic inflammation of gastroduodenal mucosa in dyspepsia of unknown origin. Scand J Gastroenterol 1989; **24**: 385-95.

207) Niv Y. Duodenitis: a pre-ulcer state. Arch Intern Med 1986; **146**: 2089.

208) Thomson WO, Joffe SN, Robertson AG, Lee FD, Imrie CW, Blumgart LH. Is duodenitis a dyspeptic myth? Lancet 1977; **i**: 1197-98.

209) Borody T, Daskalopoulos G, Brandl S, Carrick J, Hazell S. Dyspeptic symptoms improve following eradication of gastric campylobacter pyloridis. Gastroenterol 1987; **92**: 1324.

210) Rosai J. Ackermans Surgical Pathology 7th Edition. CV Mosby, St Louis, 1989. 536-38

211) Alijani MR, Benjamin SB, Collen MJ, Foegh ML. Misoprostil: A prostaglandin E1 analogue versus antacid in the prevention of stress ulcers in kidney transplant patients. Transplant Proc 1989; **21**: 2145-46.

212) Graham DY, Klein PD, Opekun AR. Epidemiology of campylobacter pyloridis infection. Gastroenterol 1987; **92**: 1411.

213) Rauws EAJ, Royen EAV, Langenberg W, Woensel JV, Vrij AA, Tytgat GN. $^{14}C$ urea breath test in C pylori gastritis. Gut 1989; **30**: 798-803.

214) Gad A, Hradsky M, Furugard K, Malmodin B, Nyberg O. Campylobacter pylori and non-ulcer dyspepsia. A prospective study in a Swedish population. Scand J Gastroenterol 1989; **24** Suppl 167: 44-48.

215) Walker SJ, Birch PJ, Stewart M, Stoddard CJ, Hart CA, Day DW. Patterns of coloniseation of campylobacter pylori in the oesophagus, stomach and duodenum. Gut 1989; **30**: 1334-38.

216) Steer HW. Surface morphology of the gastroduodenal mucosa in duodenal ulceration. Gut 1984; **25**: 1203-10.

217) Francis ND, Logan RPH, Walker WM et al. Campylobacter pylori in the upper gastrointestinal tract of patients with HIV 1 infection. J Clin Pathol 1990; **43**: 60-62.

218) Stagno S, Reynolds DW, Tsiantos A, et al.Comparative virologic and serologic studies of symptomatic and subclinical congenitally and natally acquiered cytomegalovirus infection. J Infect Dis 1975; **132**: 568-577.

219) Toussaint C, Thiery L, Vereerstraeten P et al. Clinical aspects of infections due to herpesvirus in renal transplant recipients.Transplant Clin Immunol 1978; **9**: 5.

220) Strano AJ. Light microscopy of selected viral diseases (morphology of viral inclusion bodies) Pathol Annual 1976; **11**: 53-75.

221) Nash G, Ross JS. Herpetic oesophagitis; A common cause of oesophageal ulceration. Hum Pathol 1974; **5**: 339-45.

222) Sobel HJ, Schwarz R, Marquet E. Nonviral nuclear inclusions. Arch Pathol 1969; **87**: 179-192.

223) Kaye MD, Whorwell PJ, Wright R. Gastric mucosal lymphocyte subpopulations in pernicious anaemia and in normal stomach. Clin Immunol Immunopathol 1983; **28**: 431-40.

224) Jenkins D, Goodall A, Scott BB. T lymphocyte subpopulations in normal and coeliac small intestinal mucosa defined by monoclonal antibodies. Gut 1986; **27**: 1330-37.

225) Nakleh R, Snover DC, Weisdorf S, Platt JL. Immunopathology of graft versus host disease in the upper gastrointestinal tract. Transplantation 1989: **48**: 61-65.

226) Maher P, O'Toole CM, Wreghit TG, Speigelhalter DJ, English TAH. Cytomegalovirus infection in cardiac transplant recipients associated with chronic T cell subset ratio inversion with expansion of a Leu 7+ Ts-c+ subset.Clin Exp Immunol 1985; **62**: 515-24.

227) Dafoe DC, Stoolman LM, Campbell DA, Lorber MI, Waskerwitz J, Turcotte JG. T cell subset patterns in cyclosporine treated renal transplant recipients with primary cytomegalovirus disease. Transplantation 1987; **43**: 452-54.

228) Schooley RT, Hirsch MS, Colvin RB. Association of herpes virus infections with T lymphocyte subset slterations, glomerulopathy and opportunistic infections after renal transplantation. N Engl J Med 1983; **308**: 307-313.

229) Van Es A, van Gemert GW, Baldwin WM, Oljans PJ, Tanke HJ, Ploem BJ, Van Es BLA. Viral infections and T lymphocyte subpopulations in renal transplant recipients. N Eng J Med 1983; **309**: 110-111.

230) Brouillette DE, Alexander J, Yoo YK, Chien MC,Tartar RE, Gavaler J, Van Thiel DH. T cell populations in liver and renal transplant recipients with infectious esophagitis. Dig Dis Sci 1989; **34**: 92-96.

231) McNulty CAM. Pathogenicity of Campylobacter pylori- A causitive factor in gastritis. Scand J Gastroenterol 1989; **24** (Suppl 160); 3-6.

232) Joffe SN. Relevance of duodenitis to non ulcer dyspepsia and peptic ulceration. Scand J Gastroenterol 1982; **17** (Suppl 79): 88-97.

233) Texter EC. Ulcer pain mechanisms. Scand J Gastroenterol 1987; **22** (Suppl 134): 1-20.