LASER DOPPLER ASSESSMENT OF GASTRIC MUCOSAL BLOOD FLOW IN NORMALS AND ITS RELATIONSHIP TO THE SYSTEMIC ACTIVITY OF GROWTH PEPTIDES IN HEALING AND NON HEALING GASTRIC ULCERS

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DECLARATION

This thesis is submitted for the degree of MMed.Sci. The thesis represents

my own work, conducted under the auspices of the Department of General

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supervised by Professor SR Thomson.

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DEDICATION

This thesis is dedicated to my darling wife Elizabeth who endured three years of the laser Doppler, helped with the literature review and never lost faith in me, my parents and brothers Mark and Blaise, who stood by me in all my efforts and lastly to Mr Thomas Atkins who constantly reminded to do my duty in all things.

"Then let us pray that come it may,
As come it will for 'a that;
That sense and worth, o'er a' the earth,
May bear the gree, and a' that.
For a' that and a' that,
It's coming yet, for a' that,
That man to man the warld o'er
Shall brothers be for a' that"

Robbie Burns (1759-1796)

Fir Sandie

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ETHICAL APPROVAL

This work was approved by the Postgraduate and Ethics Committee of the Medical Faculty of the University of Natal.

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LIST OF ABBREVIATIONS

H.pylori	Helicobacter pylori
MAO	Maximum Acid Output
MHR	Maximum Histamin Response
NSAID's	Non Steroidal Anti-Inflammatory Drugs
EGF	Epidermal Growth Factors
EGFR	Epidermal Growth Factor Receptor
b FGF	Basic Fibroblast Growth Factor
TGF	Transforming Growth Factor
DNA	Deoxyribonucleic Acid
m-RNA	Messenger Ribonucleic Acid
ATP	Adenosine Triphosphate
HCL	Hydrochloric Acid
PPI	Proton Pump Inhibitor
ELISA	Enzyme Linked Immunoassay
V	Volts
ASA	Amino Salicyclic Acid
NaCl	Sodium Chloride
PAF	Platelet Activating Factor
L-NNA	NG-nitro-L-arginine
L-NMMA	NG-monomethyl-L-arginine
PCNA	Proliferating cell nuclear antigen
RT	Reverse Transcriptase
PCR	Polymerase Chain Reaction
PGI2	Prostaglandin I2

SUMMARY

The pattern of mucosal blood flow in normal human stomachs, and benign gastric ulcers was assesed with laser Doppler flowmetry and the relationship between a single determination of ulcer blood flow and the systemic level of growth factors was investigated.

A significant ascending gradient in mucosal blood flow from the antrum to fundus was demonstrated. Different levels of cellular activity in the regions of the stomach may explain this gradient. In the gastric ulcers that healed on standard medical therapy mucosal blood flow was significantly increased in comparison to normal stomachs. In the ulcers that were refractory to standard medical therapy mucosal blood flow was significantly lower than in normal stomachs and healing ulcers. Higher systemic levels of the growth factor bFGF were demonstrated in healing ulcers compared to non-healing ulcers.

Gastric mucosal blood flow can increase in response to the increased metabolic demands of healing, however impairment of this response may be an important factor preventing healing of benign gastric ulcers. It would appear that non-healing of gastric ulcers can be predicted at initial diagnosis by reduced peri-ulcer gastric mucosal blood flow and low blood levels of bFGF.

CHAPTER 1

INTRODUCTION

1.1 THE PATHOGENESIS OF BENIGN GASTRIC ULCERATION

1.1.1 Early descriptions of gastric ulceration

Gastric ulceration was known to the Ancients. Diocles (350-325 BC), Galen (131-201 AD) and Palus Aegineta (625-690 AD) each described gastric ulceration and the complications of haematemesis and melaena.[1] Marcelius Donatus of Mantua described gastric ulcers at necropsy, around 1586.[2] Matthew Baillie[3] of London published "The morbid anatomy of some of the most important parts of the human body" in 1793. This is regarded as the first accurate description of the pathological appearance and the symptomatology of gastric ulceration. The great French clinician Jean Cruveilhier[4] made the clear distinction between benign and malignant gastric ulceration. His contribution was highly regarded in his native land. With true Gallic "*elan*" benign gastric ulceration was referred to in France as "the ulcer of Cruveilhier" for many decades following his death.

However, it was the Germans who dominated surgery in the 19th Century, and the surgical treatment of benign gastric ulceration was no exception. Rydygier, a Prussian, performed the first successful partial gastrectomy for a benign gastric ulcer in 1881.[5] His 30-year-old patient was discharged from hospital on the 13th post-operative day. The editor's footnote at the end of Rydiger's report of this first gastrectomy for a benign gastric ulcer read "Let us hope also the last". Rydiger also proposed operating for life threatening haemorrhage from a gastric ulcer.

Rydiger never achieved the fame that was his due, probably because the honour of doing the first successful gastrectomy had gone to Christian Albert Theodor Billroth, for malignant disease, earlier in 1881. Although gastric ulceration had been known and described for a long time its aetiology and pathogenesis remained unclear.

1.1.2 Historical concepts of gastric ulcer pathogenesis

The first scientific theories about the pathogenesis of benign gastric ulceration arose in the mid-twentieth century. Daintree Johnson (1910-1980) made a seminal contribution to the understanding of gastric ulceration and its pathogenesis. He stated in 1957, "Every ulcer and no less a peptic one, may be considered as the outcome of a conflict between attack and defence, and sometimes the violence of the former and sometimes the poverty of the latter, will be the paramount factor in pathogenesis".[6]

The realisation that gastric ulceration occurs in patients who are not acid hyper-secretors led Johnson in 1957 to classify gastric ulcers into three groups.[6] This classification was based on the presumed aetiology of the ulcers and was believed to have implications for the surgical management of these ulcers.

A type I ulcer was confined to the lesser curvature without any pathology in the duodenum, pylorus or pre-pyloric region. Type I ulcers were usually associated with a normal or hypo-secretory acid state. This implied that these ulcers should be treated by resectional surgery without vagotomy. Type II gastric ulcers were gastric ulcers associated with duodenal ulcers. Type III ulcers were pre-pyloric in

location. Type II and type III ulcers were associated with acid hyper-secretion, and were felt to behave like duodenal ulcers. This implied that they should be managed surgically by acid reducing operations such as vagotomy rather than by resection. However, it has been shown that the incidence of recurrent ulceration is 20% higher in pre-pyloric ulcers treated by a truncal or highly selective vagotomy than in duodenal ulcers treated with the same operations.[7,8] Nevertheless, Johnson's sound observational data laid the basis for subsequent investigators.

In 1959 Marks,[9] proposed an opposing view when he concluded that gastric ulceration and duodenal ulceration had a similar aetiology. Marks stated, "It is probable that in their pathogenesis pre-pyloric and other gastric ulcers do not differ from gastric ulcers associated with duodenal ulcers: the same conflict of acid-pepsin aggression versus mucosal defence applies to all." His conclusion was based on his observations of Maximum Acid Output (MAO) and Maximum Histamine Response (MHR) in 33 gastric ulcer patients.

In pre-pyloric ulcers MAO and MHR were similar to or slightly higher than normal controls. In combined gastric and duodenal ulcers the MAO was slightly higher than normal and slightly lower than in uncomplicated duodenal ulcers. In isolated gastric ulcers MAO and MHR depended on the duration of symptoms. In long standing ulcers acid output was low whilst in acute ulcers acid output was high.

Marks concluded that the reduction in acid secretion was a consequence of gastric ulceration. He felt that peptic (gastric or duodenal) ulceration occurred in

normal mucosa exposed to high acid-pepsin secretion or in mucosa abnormally susceptible to injury even in the face of diminished acid pepsin secretion.

Du Plessis[10] in 1965 emphasised the role of refluxing duodenal contents in gastric ulcer pathogenesis. He identified severe chronic gastritis extending from the pylorus for a variable distance proximally in 61 of 75 stomachs, which had been resected for benign gastric ulceration. The gastric ulcers were all situated in the area of atrophic gastritis. Du Plessis aspirated resting stomach juice from patients with gastric ulcers, dyspepsia, duodenal ulcers and normal stomachs. The aspirates in the patients with gastric ulcers had high concentrations of bile-acid conjugates. He concluded that gastric ulceration is preceded by chronic gastritis secondary to refluxing of duodenal contents. He postulated that refluxing alkaline duodenal contents interfered with the protective layer of mucous and allowed acid and pepsin to come into contact with the mucosa. This promoted ulceration.

The unifying principle behind these concepts is that benign gastric ulceration arises from an imbalance between aggressive luminal factors and the integrity of the mucosal barrier. The role of the mucosal barrier is pivotal to mucosal defence. Our current understanding of the pathogenesis of benign gastric ulceration emphasises this concept.

1.1.3 Current concepts in gastric ulcer pathogenesis

In the early 1980's Marshall revolutionised the world's understanding of the pathogenesis of peptic ulcer disease.[11-13] Marshall isolated a spiral urease-

producing organism, in the interface between the epithelial cells of the gastric mucosa and the overlying mucous layer, which was called *Campylobacter pylori*. *Campylobacter pylori*, was subsequently renamed *Helicobacter pylori (H. pylori)*, and became the subject of intense investigation.

A strong causal relationship between *H. pylori* infection and upper gastrointestinal disease has been documented. The overwhelming majority of duodenal ulcers are *H. pylori* positive.[14-18] Furthermore successful eradication of *H. pylori* infection markedly reduces the rate of duodenal ulcer recurrence and offers a permanent cure. However in gastric ulceration the association with *H. pylori* is less striking. Only 70 to 90% of patients with benign gastric ulcers are infected with *H. pylori*.[19] Borody *et al.* [20] reported a series of 115 patients with gastric ulcers. Only 71 (62%) were *H. pylori* positive, and 44 (38%), were *H. pylori* negative.

Other factors, such as cigarette smoking and ingestion of Non-Steroidal Anti-Inflammatory Drugs (NSAID's), are implicated in the pathogenesis of benign gastric ulceration.[21-23] However in the study of Borody *et al.*, 13 (30%) of the *H. pylori* negative group had no identifiable cause of a gastric ulcer.[20] This may merely represent the inability to identify *H. pylori* due to poor techniques, or suppression of *H. pylori* rather than eradication, secondary to the use of inappropriate antibiotics.

The relationship between *H. pylori* and NSAID use remains unclear. There is evidence that *H. pylori* associated and NSAID associated gastric ulcers are

distinct pathological entities. Laine points out that there does not appear to be a difference between NSAID users and non-users as far as the incidence of *H. pylori* infection is concerned.[19] NSAID's cause sub-epithelial haemorrhage and erosions but do not cause the increase in inflammatory infiltrate in the gastric mucosa which is typical of *H. pylori* associated gastritis. *H. pylori* positive NSAID users with gastric ulcers have more inflammation than *H. pylori* negative NSAID users with gastric ulcers. Furthermore patients with NSAID associated gastric ulcers tend to have a lower prevalence of *H. pylori* and less gastritis than non-NSAID users. However, a patient who is *H. pylori* positive and who uses NSAID's, is at higher risk of developing a gastric ulcer due to the synergistic effect of two noxious stimuli on the gastric mucosa. Laine[19] feels that NSAID associated ulcers represent a subset of gastric ulceration, which do not require *H. pylori* to develop.

To paraphrase Johnson,[6] gastric ulcers are increasingly seen as local areas of mucosa in which the effects of noxious luminal stimuli (acid, pepsin) have overcome the ability of the gastric mucosa to maintain mucosal integrity. Ulceration occurs secondary to impaired mucosal resistance. The causes of this impaired mucosal defence are numerous, but include *H. pylori* infection, cigarette smoke and NSAID use. The common pathway of the impaired mucosal defence is unclear. Reduced mucosal blood flow may be an important part of the final common pathway.

1.1.4 Mucosal blood flow and mucosal ulceration

Mucosal defence and ulceration is a dynamic process. Mucosal blood flow plays an important role in maintaining mucosal integrity through the removal of back

diffusing Hydrogen ions and in providing adequate nutrients to the mucosa. Alterations in mucosal blood flow are important in the breakdown of this fine balance between defence and ulceration.[24] This is the situation in acute mucosal injury.[25] The role of mucosal blood flow in chronic ulceration is less clearly defined but appears to be equally important.

Mucosal blood flow also plays a central role in mucosal healing.[24] An adequate supply of oxygen and nutrients is essential for the high energy demanding processes involved in mucosal healing. These include in-growth of epithelial cells and angiogenesis from intact vessels surrounding the ulcer and removal of metabolic waste products.

1.1.5 Growth Factors and mucosal blood flow in gastric ulcer healing.

Gastric ulcers share many features with wounds in other sites. Healing requires epithelial cell migration, epithelial cell proliferation and the growth of granulation tissue.[26] Cell proliferation and migration are triggered and regulated by growth factors and cytokines through their action on specific receptors. Growth factors and cytokines are both extra-cellular signalling peptides. Cytokines are considered to be local mediators in cell to cell communication whereas growth factors were originally defined on the basis of their stimulating cell growth and cell division. Several growth factors have been shown to exhibit gastro-protective, mitogenic and angiogenic activities and to accelerate gastric healing. Healing is initiated and controlled by secretion of growth factors at the ulcer edge. These peptide growth factors modulate cell proliferation by acting as ligands at specific trans-membrane receptors. The role of many growth factors and cytokines is still

unclear and there is considerable overlap in their biological functions.[27-30]

Different ulcer types (skin, gastric and intestinal ulcers) demonstrate similar mechanisms of ulcer healing.[26] Subsequent to ulceration, cells adjacent to the crater rapidly de-differentiate in response to growth factors. A novel cell line grows from the base of existing crypts. This cell line contains Epidermal Growth Factor (EGF), which is a potent stimulator of cell proliferation.[30] Parallel to secretion of growth factors, inflammatory infiltration occurs close to the necrotic tissue and the ulcer crater. Granulation tissue develops below the ulcer base as part of the repair process and may prevent perforation. Epithelial cells are stimulated by growth factors and migrate over the ulcer crater.

During the initial acute phase of ulceration and healing, inflammatory cells are seen prominently around the ulcer circumference. Later fibroblasts and micro-vessels replace these inflammatory cells. Angiogenesis (in-growth of new vessels) supports healing by improving nutrient and oxygen delivery. After re-epithelialisation all the layers of the gastric wall including the mucosa, muscularis, submucosa, muscularis propria and serosa are reconstructed.[26]

Peptide growth factors play an important role in promoting ulcer healing. Their function is intimately related to mucosal blood flow, and administration of angiogenic promoting factors to rats has been shown to promote ulcer healing.[26, 31] Angiogenesis ensures an adequate nutrient supply to support the high energy demanding process of mucosal healing. Growth factors modulate cellular growth and angiogenesis by altering genetic transcription.[32-35] Adequate mucosal blood

flow allows efficient buffering of back diffusing Hydrogen ions. The three growth factors which have been most studied, in association with gastrointestinal pathology, are basic Fibroblast Growth Factor (bFGF), EGF and Transforming Growth Factor (TGF). The following section presents an overview of these three growth factors.

1.1.6 Fibroblast Growth Factor

FGF is a potent angiogenic factor, which is expressed in most cells of the body. Initially only two forms of FGF were known, acidic (FGF1) and basic (FGF2). In 1991 the New York Academy of Science divided the FGF family into seven members.[36] Subsequently the number of FGF sub-groups has increased to nine. Basic and acidic FGF are the best characterised members of the family. b-FGF is an 18 kDa polypeptide with a high affinity for heparin and heparin sulphate proteoglycans. b-FGF is stored within the extra-cellular matrix, and is a direct mitogen for vascular endothelial cells. The FGF receptors are a subgroup of the tyrosine kinase family and display a remarkable degree of cross affinity for the different FGF subtypes.

1.1.7 Transforming Growth Factor

TGF was first isolated from culture medium conditioned by Rous sarcoma virus transformed fibroblasts. The addition of the, at the time, still un-named growth factor transformed normal fibroblasts into malignant fibroblasts. The peptide was therefore named Transforming Growth Factor. There are two superfamilies, TGF-α and TGF-β. The members of the TGF-β superfamily have a variable pro-domain and a highly conserved 110-114 amino acid residue mature segment. This

segment contains seven cysteine residues and forms the cysteine knot. TGF-β1 is one of the isoforms of the TGF-β superfamily. TGF-β1 is secreted in a latent form and is converted to the active form at the cell surface and in the extracellular matrix. TGF shares about 35% amino acid sequence homology with EGF and binds to the same receptors as EGF. TGF inhibits gastric acid secretion and is angiogenic. Expression of m-RNA for TGF has been found in the gastric mucosa of guinea pigs, rats, dogs and humans.[37]

1.1.8 Epidermal Growth Factor

EGF was first isolated from the rat submandibular gland. EGF is formed as a pro-molecule of 1217 amino acid residues and includes six cysteine residues that form three disulphide bonds.[30] The EGF receptor (EGFR) is a tyrosine specific "single pass" transmembrane protein of around 1200 amino acid residues. The EGFR has a large glycosylated extra-cellular domain that binds EGF. This leads to the activation of the intracellular tyrosine domain. This transfers phosphate from ATP on selected tyrosine side chains. After binding, down regulation of receptors occurs with lysosomal degradation of EGFR. EGFR 's are not specific for EGF and may also serve as receptors for TGF-α.

EGF is known to stimulate DNA synthesis in the gastric mucosa of rodents.[27,38] Tepperman and Soper,[39] administered a bolus of the nitric oxide synthase inhibitor, NG nitro-L-arginine methyl ester, to rats which had been pre-treated with intra-luminal HCL. This resulted in a significant increase in the area of mucosal haemorrhagic damage, and a significant reduction in mucosal blood flow. The extent of the mucosal damage was greater in rats, which had been

sialoadenectomised. Intravenous administration of EGF however limited the extent of the mucosal damage and prevented the decrease in mucosal blood flow in the sialoadenectomised rats.

1.2 THERAPY FOR GASTRIC ULCERS

1.2.1 Acid suppression and gastric ulcer therapy

Gastric ulcer therapy has evolved and improved from the 1970's. Initially anti-ulcer therapy focused on promoting healing by reducing acid load, hence removing the noxious stimulus and allowing mucosal healing. Acid reducing drugs progressed from the development of the histamine receptor antagonists, to the Proton Pump Inhibitors (PPI). However, ulcer healing can be induced by drugs that do not alter gastric acidity, and the discovery of H. pylori has opened up new forms of therapy. Acid suppression remains an important part of the therapy of benign gastric ulcers.

In 1981 Wright et al.,[40] documented a healing rate of 53% for gastric ulcers on medical therapy. However surgical therapy was eventually required in 56% of the group. The medical therapy of the time was not very efficacious. Wright et al.[41] subsequently compared the efficacy of the histamine receptor antagonists, ranitidine and cimetidine in ulcer healing in 72 patients with benign gastric ulcers. The one-month healing rate was 47% for the ranitidine group and 52% for the cimetidine group. Treatment was continued for a further four weeks in the non-healing ulcers. The final healing rate was improved to 77% in the ranitidine group and 76% in the cimetidine group. There was a trend for larger ulcers to take longer than four weeks to heal.[41] Athough these results were an improvement, a

significant number of gastric ulcers still remained refractory to treatment with acid suppression by histamine receptor antagonists.

The place of anti-secretory drugs in medical management of benign gastric ulceration is well established.[42]   However, gastric ulcers are not always associated with hyper-acidic states and therefore there is less rationale for the use of these agents in gastric ulceration in comparison to duodenal ulceration. Howden *et al.*[43] in a meta-analysis of 56 clinical trials of anti-secretory drugs in benign gastric ulceration, showed that conventional dosing regimens consistently heal about 80% of gastric ulcers by eight weeks. He showed that extending the period of treatment from eight weeks to twelve weeks resulted in almost 100% healing rates. Furthermore he could show no association between healing rates and acid suppression. This was in marked contrast to the situation in duodenal ulceration where there is a marked correlation between the degree of acid suppression and the rate of ulcer healing.[42]

The development of the PPIs enabled a much greater therapeutic level of acid suppression to be achieved than that achieved by the anti-histamines. However healing rates are still short of the desired 100% cure rate. In 1998 Yeomans[44] reported an 83% cure rate for NSAID induced gastric ulcers on Omeprazole at 20mg daily, in comparison with a rate of 93% for duodenal ulcers on identical treatment.

Gastric ulcers take longer to heal than duodenal ulcers, when acid suppression is the sole modality of treatment. Despite a marked improvement in the healing

rates of gastric ulcers over the last two decades, refractory ulceration remains a problem. Today, elective gastric surgery for benign disease is rare. (Rydiger's editor would be pleased to know). However, Soll[45] stated in 1996, "The therapeutic response of gastric ulcers is somewhat slower than for duodenal ulcers, in part because gastric ulcers are generally larger than duodenal ulcers". He recommended that large gastric ulcers should only be considered refractory if they have not healed after twelve weeks of therapy. Gastric ulcers tend to be more refractory than duodenal ulcers. Refractory gastric ulceration remains a clinical problem. The reasons for non-healing are multiple and include failure to eradicate *H. pylori,* poor compliance, and persistent smoking and NSAID use.

1.2.2 Gastric ulcer healing using non-acid suppression therapy

Sucralfate is effective against acute chemical-induced haemorrhagic mucosal lesions and accelerates the healing of chronic gastric ulcers without decreasing gastric acidity.[46-50] The mechanism of action of sucralfate is incompletely understood. It has been shown to reduce micro-vascular injury and to maintain mucosal blood flow in acute lesions in rats. This allows rapid epithelial restitution. In chronic ulcer healing in rats sucralfate has been shown to promote angiogenesis.[32] This may be due to its ability to bind endogenous growth factors and prevent degradation of these factors by luminal acid. The effectiveness of sucralfate in therapy of gastric ulceration has contributed to a new understanding of gastric ulcer pathogenesis and healing, which emphasises the importance of the mucosal barrier and mucosal blood flow in gastric ulceration.

Chen *et al.*[51] showed that sucralfate exerted its effect by increasing mucosal

blood flow. He used an *ex-vivo* gastric chamber preparation and laser Doppler flowmetry to study the effect of sucralfate on gastric mucosal blood flow in rats. Gastric mucosal blood flow was measured in rats following topical application of absolute ethanol alone or after pre-treatment with sucralfate. The total area of haemorrhagic mucosal lesions was measured to document the degree of mucosal damage. Pre-treatment with sucralfate significantly reduced the extent of mucosal damage. Mucosal blood flow was significantly reduced subsequent to ethanol application. This fall in blood flow was attenuated by pre-treatment with sucralfate in both fasted and fed rats. Graded doses of sucralfate resulted in an increase in gastric mucosal blood flow in a dose dependent manner. Chen concluded that sucralfate increases gastric mucosal blood flow in rats and attenuates the reduction in blood flow induced by ethanol.

Preservation and enhancement of gastric mucosal blood flow seem to be the mechanism by which sucralfate promotes healing of gastric ulceration. It would seem that sucralfate interacts with growth peptides and hence promotes mucosal healing. Folkman *et al.*[52] extended this concept to produce a hypothetical model of ulcer formation and healing which also explained the healing effect of sucralfate. FGF is continuously produced by the gastric and duodenal mucosa. However its production is balanced by continuous degradation of peptide by gastric acid. The traditional hypothesis is that acid directly causes the ulcer. Folkman *et al.* however postulated that mucosal ulceration occurs only if FGF is broken down.[52] He proposed that sucralfate binds to endogenous FGF and prevents its breakdown so facilitating ulcer healing. This is achieved without altering acid concentration.

1.2.3 Growth factors and gastric ulcer therapy

Experimentally, peptide growth factors have been shown to enhance gastric ulcer healing. Folkman *et al.*[52] showed that an acid stable form of bFGF could be administered orally to rats with duodenal ulcers. The peptide induced a nine-fold increase in angiogenesis in the ulcer bed and accelerated ulcer healing more potently than the acid reducing histamine receptor antagonists. Therapeutic intervention in gastric ulceration has mostly been directed against aggressive factors. With the discovery of the potent healing capacity of growth factors, direct treatment of ulcers may be possible regardless of acid and pepsin secretion.

Growth factor therapy is still experimental.[34] However its efficacy provides insight into the healing process in gastric ulceration. Ulcers will heal if the noxious stimuli are removed, by acid suppression and *H. pylori* eradication. However, if mucosal blood flow is enhanced, healing will occur even in the presence of these noxious factors. This illustrates in practical therapeutic terms the inter-relationship between mucosal blood flow, injury and healing.

1.2.4 *Helicobacter pylori* therapy

Since 1983 *H. pylori* has been recognised as a causal factor in gastric ulceration. The American College of Gastroenterology has recommended that all patients with peptic ulceration and proven *H. pylori* infection should be treated with eradication therapy.[14,15,45,53] Decisions about eradication therapy should take account of previous antibiotic exposure and local resistance patterns. A bismuth-metronidazole-tetracycline combination plus proton pump inhibitors or clarithromycin and either metronidazole or amoxicillin plus PPI will achieve a 90%

cure rate probably within one week of therapy.[45] Regardless of *H. pylori* status, conventional therapy based on the principle of reducing the noxious stimuli of mucosal ulceration, is necessary to relieve symptoms and to promote healing.[53]

1.3 TECHNIQUES OF MEASURING GASTRIC MUCOSAL BLOOD FLOW

Much effort has been expended to develop a satisfactory technique of measuring gastric mucosal blood flow in humans. In order for a technique to be widely applicable it must be safe, simple to use, reproducible and reliable. An overview of the most widely available techniques is given in the following section.

1.3.1 Microsphere distribution technique

Microspheres injected into the left ventricle of the heart will be evenly distributed in the blood stream. These microspheres will be distributed to the organs of the body, in direct proportion to the regional perfusion of each organ.[25,54] Radio-labelled or coloured microspheres of the appropriate diameter (15 μm) will lodge in the lamina propria of the gastric mucosa below the gland bases. Here they can be measured and blood flow can be derived from these measurements. Microsphere clearance permits accurate flow measurements of the entire gastric wall as well as the mucosa. It also allows measurement of flow to different regions of the gastric mucosa and is independent of the secretory state of the stomach. There are a number of restraints on the widespread application of the microsphere distribution technique. A limited number of measurements may be made at each sitting. Radioactive material is used and the stomach must be removed prior to blood flow measurement. This restricts its application to animals and makes it costly and time-consuming.

1.3.2 Clearance techniques

In 1966 Jacobson *et al.*[55,56] developed the aminopyrine clearance technique for the determination of gastric mucosal blood flow. The method is based on the permeability of lipid membranes to the undissociated but not to the dissociated form of compounds. At the pH of plasma a weak base such as aminopyrine (pKA=5) is present in the undissociated state and readily traverses the gastric mucosa. At the pH of gastric juice the weak base ionises and aminopyrine is trapped in the stomach lumen. Aminopyrine is cleared from the blood on a single pass through the gastric mucosa. Thus the clearance of aminopyrine into the gastric juice is a direct measure of gastric mucosal blood flow. However, the large doses of aminopyrine required meant that the technique was not suitable for use in humans until Tague and Jacobson in 1976 reported on the use of [14C] aminopyrine to measure gastric mucosal blood flow in dogs.[57] Only a trace amount of this compound was needed to make the measurement. This allowed Guth *et al.* to apply this technique to humans in 1978.[58] They showed that pentagastrin stimulation increased gastric mucosal blood flow. *Archibald et al.*[59] showed that aminopyrine clearance is different in the secreting and non-secreting stomach. They correlated microsphere measured flow with aminopyrine clearance during intravenous infusion of histamine and isoproterenol. In the histamine group the aminopyrine clearance averaged 83% of microsphere determined flow. In the isoproterenol group the correlation between aminopyrine clearance and microsphere clearance was very poor. This implies that aminopyrine clearance underestimates mucosal blood flow in the non-secreting stomach.

There are other limitations with the aminopyrine clearance technique. Aminopyrine clearance represents overall mucosal blood flow and cannot assess regional differences. Furthermore, the technique requires prolonged naso-gastric intubation, aspiration of gastric secretions and collection of systemic blood samples which makes it labour intensive and uncomfortable for the subjects.

1.3.3 Hydrogen gas clearance

This technique, first reported by Murakami et al.,[60] is based on the dissociation of molecular Hydrogen at the surface of a platinum probe into Hydrogen ions and electrons. The rate of removal of Hydrogen from perfused tissue is determined by the rate of blood flow. The Hydrogen gas is inhaled. A platinum electrode is placed on the mucosal surface. This generates a current (due to oxidation of Hydrogen), which is proportional to the concentration of Hydrogen in the tissue. Hydrogen gas clearance has been validated against microsphere technique.

Soybel et al.[61] assessed the effects of changes in luminal pH and the secretory activity of the gastric mucosa on the accuracy of Hydrogen clearance measurements. Hydrogen clearance measurements at different pH levels were compared in vitro and in vivo. Furthermore, blood flow measurements by hydrogen clearance and radioactive microsphere clearance were compared during stimulation of acid secretion by infusion of histamine, and during suppression of acid secretion by infusion of cimetidine. They showed that in vitro hydrogen washout was relatively constant over a spectrum of pH values, ranging from two to eight. For the in vivo experiments they used chambered segments of the canine fundus. In these animals Hydrogen clearance was not significantly

affected by pH of the luminal solutions either in the resting state or at lower blood flows induced during infusion of vasopressin. They demonstrated a close correlation between measurements of mucosal blood flow as determined by Hydrogen clearance and microsphere clearance under resting conditions. This correlation was also demonstrated during intravenous histamine stimulation of acid secretion and after suppression of acid secretion by infusion of cimetidine. They concluded that the hydrogen clearance technique was an accurate method for measuring gastric mucosal blood flow over a range of physiological conditions.

This technique was the first that was easily adapted for human use. There is no limit to the number of measurements that can be made. However, the method is incapable of detecting rapid changes in blood flow as it takes on average thirty minutes to make a single measurement.

1.3.4 Laser Doppler flowmetry

Stern et al.,[62] Bonner and Nossal[63], and Bonner et al.[64] have described the physics of laser Doppler flowmetry. A detailed description of the physics is given in Appendix 2. Numerous investigators have reported on the use of laser Doppler flowmetry in the gastrointestinal tract of both human and animal subjects.

1.3.5 The laser Doppler instrument

The laser Doppler instrument has a laser diode, which generates a wavelength of 780 nm. This is near the isobestic point of oxyhaemoglobin and deoxyhaemoglobin (800 nm). This wavelength is not absorbed by dark tissue and so eliminates the effect of changes in blood oxygenation on the Doppler reading.

A fibre optic cable delivers the laser beam to the tissue and carries the signal back to the photo-detector where the spectrum of frequencies are collected and indirectly measured by a process called heterodyning. The output of the photo-detector is a spectrum of frequencies, which cannot be simply characterised. The signal is processed by a filter using the root mean square band-width of the power spectrum to compute a single value for flow.

1.3.6 The validity of laser Doppler flowmetry

The validity of laser Doppler depends on empirical demonstration that blood flow determined by laser Doppler scales linearly with blood flow detected by an independent technique. Various authors have validated laser Doppler flowmetry against other techniques of measuring mucosal blood flow, and their work is discussed in the following section.

Kvietys et al.[65] measured blood flow in the mucosa of the feline jejunum by laser Doppler flowmetry and Hydrogen gas clearance. Laser Doppler and Hydrogen clearance estimates were compared with measurements of total-wall blood flow and mucosal-submucosal blood flow made by the radio-labeled microsphere technique. They demonstrated a significant linear correlation between laser Doppler flowmetric measurements and total jejunal wall blood flow as measured by the microsphere technique. They also demonstrated a significant correlation between laser Doppler measurements and mucosal-submucosal blood flow as measured by the microsphere technique. The Hydrogen clearance technique tended to over-estimate total blood flow of the wall of the jejunum when compared to the microsphere clearance technique. They postulated that Hydrogen

clearance selectively measured flow in the mucosa. This was felt to be surprising, as hydrogen gas should be subject to counter-current exchange within the villi of the small intestine. The counter-current exchange of hydrogen ions in normally perfused feline small bowel could be as high as 95%. Theoretically this could lead to an underestimation of mucosal blood flow in the small intestine. However, they cautioned that these findings were applicable only to physiological conditions of intestinal blood flow (26 to 74 ml/min/100g of tissue). They felt that this could limit the use of Hydrogen clearance in situations of impaired blood flow. Laser Doppler correlated well with both total jejunal wall blood flow and mucosal-submucosal flow.

Kiel et al.[66] used two laser Doppler flowmeters to simultaneously monitor blood flow in the mucosa and serosa of chambered canine stomachs. In isolated gastric segments vasodilated with isoproterenol, laser Doppler flowmetry demonstrated that mucosal and muscularis blood flows were both linearly related to total electromagnetic blood flow.

Feld et al.[67] compared laser Doppler flowmetry with electromagnetic total blood flow measurements in isolated segments of canine small intestinal mucosa. They demonstrated good correlation between the methods. Administration of vasoactive drugs such as norepinephrine caused a decrease in mucosal perfusion as recorded by the two methods.

Gana et al.[68] compared focal gastric mucosal blood flow values simultaneously obtained by laser Doppler flowmetry and Hydrogen gas clearance. Readings

were made at the same point in a chambered segment model of the gastric fundus in nine anaesthetised dogs. The gastric mucosal blood flow values determined by laser Doppler flowmetry correlated closely with the values determined by Hydrogen clearance.

Ahn et al.[69] evaluated the use of laser Doppler flowmetry to measure gastric blood flow in cats. In five cats flowmeter signals and venous outflow of the stomach were simultaneously recorded. Temporal reproducibility was assessed by comparing the variation of the flowmeter signal during a state of constant blood flow with the variation in flow measured by a drop-counting technique. This was repeated over four recording periods of fifteen minutes each. Spatial reproducibility was assessed in a single cat by moving the probe to a point 1cm distant from the original point and repeating the measurements. The flowmeter recordings were highly reproducible for both temporal and spatial variation with a coefficient of variation varying between 4% and 13%. A significant correlation coefficient was obtained between flowmeter signal and venous outflow of the stomach. However, during complete occlusion of the coeliac plexus, when no venous outflow was detected, the laser Doppler flowmeter still detected a reading, which was approximately 30% of the pre-occlusion reading. This was ascribed to the heterogeneous vascular supply of the stomach.

There are limitations to and controversies surrounding the use of laser Doppler. The depth of tissue sampled by laser Doppler is unclear. In all tissue light will diffuse after passing through 300-600 μm of tissue. This mean path for light diffusion and the spatial separation of the light conducting fibres are the primary

determinants of the tissue volume sampled. Thus for 600 µm fibre separation and typical tissue, the depth of sampled tissue is about 0.6-1.2 mm. The laser Doppler instrument used by Kiel *et al.* was specifically built by Bonner.[66] These authors asserted that their instrument had a penetrative depth of 0.5-1.0 mm. However the penetrative depth of laser Doppler has been the subject of a great deal of investigation, and still remains controversial. Kvietys *et al.*[65] placed a non-perfused layer of tissue between the laser probe and the perfused mucosal surface. The signal was diminished by up to 20%. Occluding the arterial supply of the segment of jejunum decreased mucosal blood flow. The laser Doppler reading made through the interposed non-perfused segment decreased. On reperfusion the laser Doppler reading increased. This implied that their laser Doppler flowmeter (Periflux[®]. Model Pf 1d Perimed, Stockholm) was capable of detecting fluctuations in blood flow through 3 mm of non-perfused tissue. They. concluded that laser Doppler flowmetry measured total wall blood flow rather than mucosal blood flow.

Johansson *et al.*[70] measured blood flow in the mucosa and serosa in 49 patients undergoing small bowel resection, in 36 patients undergoing colonic resection and in 54 patients undergoing gastric resection. Two different laser Doppler flowmeters were used: the PF1d and PF2 (Periflux[®], Perimed, Stockholm, Sweden). Recordings of total venous out-flow were made by isolating a segment of bowel and cannulating the draining vein. Venous out-flow was collected in a graduated glass. Mucosal and serosal flowmeter signals were compared during resting conditions, vascular occlusion, and reactive hyperaemia. The magnitude of the mucosal and serosal flowmeter signals was comparable throughout the

small bowel, large bowel and stomach. Following pentagastrin stimulation a significant increase in laser Doppler flowmetric readings was recorded from the serosal and mucosal surface of the stomach. There was also a significant correlation between total wall blood flow (as measured by total venous outflow) and laser Doppler readings. This was similar in the small and large bowel. Johansson et al.[70] agreed with Kvietys et al.,[65] that laser Doppler flowmetry had a tissue penetration depth of at least 6 mm, and therefore measured whole wall perfusion rather than mucosal perfusion.

Other authors have come to the opposite conclusion. They feel that laser Doppler flowmetry measures mucosal rather than total wall flow. Stern et al.[62] showed that the insertion of a 1 mm thick non-perfused layer between the probe and the mucosa eliminated any signal. They felt this implied that the laser Doppler instrument had a penetrative depth of 1 mm. Kiel et al.[66] determined the penetrative depth of laser Doppler flowmetry by observing reactive hyperaemia following arterial occlusion. Reactive hyperaemia was frequently detected by laser Doppler in the mucosa, but rarely in the muscularis. Placing a layer of non-perfused tissue between the probe and the perfused mucosa abolished the resting laser Doppler mucosal flow signal and attenuated the recording of peak hyperemia by 85%. These findings indicate that laser Doppler flowmetry instruments yield linear, superficial measurements of gastric blood flow in either mucosa or muscularis rather than total wall blood flow.

Shepherd and Riedel[71] also demonstrated that laser Doppler flowmetry measured mucosal rather than total wall blood flow. They recorded laser Doppler flowmetric

measurements and total electromagnetic measurements whilst altering the perfusion pressure in an isolated vascularised segment of canine small bowel. In a second series of experiments they infused either isoproterenol or adenosine intra-arterially to vasodilate the preparations. In a third series they occluded the supply catheter and used the laser Doppler flowmeter to record the reactive hyperaemic response in the mucosa and the muscularis. They showed that after 60 seconds of arterial occlusion, laser Doppler flowmetry demonstrated a hyperaemic response in the mucosa, but not in the muscularis. This was taken as evidence that laser Doppler flowmetry measured blood flow in the mucosa and not the entire bowel wall. When isoproterenol was infused, the expected vasodilatory response was seen first in the mucosa with laser Doppler. The mucosal hyperaemia preceded the total increase in bowel wall blood flow as detected by the electromagnetic flowmeter. This was taken as further evidence that laser Doppler measures mucosal blood flow rather than total bowel wall blood flow. Adenosine, which diverts blood from the mucosa, resulted in a decrease in mucosal blood flow as measured by laser Doppler.

Stern et al.,[62] Kiel et al.[66] and Shepherd and Riedel[71] demonstrated that laser Doppler flowmetry measures mucosal flow rather than total wall flow. Kvietys et al.[65] states that the laser Doppler instrument used by Shepherd and Riedel and Kiel et al. was specifically designed for them by Bonner. Details of the instrument's components are not given in the paper. However it is apparently similar to the instrument used by Stern et al.,[62] for which details are given. Their instrument used a helium-neon laser with a wavelength of 632.8 nm and a beam diameter of 1 mm. The returning light was reflected through a 2 mm aperture

placed at the surface of the tissue, which limited stray, and a second 0.5 mm aperture 1 m away, which selected out a single coherence area. They feel that differences between components such as diameter, separation, and the numerical aperture of the optical fibres could account for the difference in depth sensitivity between different laser Doppler instruments. These differences must be borne in mind when interpreting results obtained using laser Doppler flowmetry.

Laser Doppler has been shown to be accurate under a variety of pathophysiological conditions. Casadevall et al.[72] compared laser Doppler flowmetry, reflectance spectrophotometry and the Hydrogen gas clearance technique in rats under conditions of hypoxia, hyperoxia, acute normovolaemic anaemia and pharmacologically induced vasoconstriction. They, showed a good correlation between laser Doppler and the other techniques under conditions of vasoconstriction, hypoxia and hyperoxia. However under conditions of anaemia laser Doppler flowmetry appeared to be limited by the decreased number of red blood cells in the field under examination.

We felt that laser Doppler flowmetry had been sufficiently validated by the above authors to be used as a scientific tool. Laser Doppler can easily be used during upper endoscopy, making it an attractive tool for experimental and clinical work. The measurements add on average five to ten minutes onto the time taken for a routine upper endoscopy. They are easy to repeat at a subsequent endoscopy and are well tolerated by the patients. Furthermore, laser Doppler allows the measurement of gastic mucosal blood flow at multiple sites in the stomach.

However the controversies and problems with laser Doppler must be borne in mind when interpreting results.

CHAPTER 2

HYPOTHESIS

Mucosal blood flow is dynamic and reflects the functional state of the gastric mucosa. Mucosal ulceration is a state in which the fine balance between acid bathing the mucosa and mucosal defence has broken down. Mucosal healing is an energy dependent process, involving epithelialisation, angiogenesis and cellular differentiation, which depends on adequate blood flow for supply of energy and nutrients and the removal of metabolic waste products. It is postulated that blood flow in the gastric mucosa will vary with pathological conditions of the stomach in response to changing metabolic demands. If such alterations do occur in relationship to benign gastric ulceration they should be detectable with laser Doppler flowmetry. As growth factors are involved in controlling the healing process, fluctuations in the systemic levels of growth factors can be expected in gastric ulceration.

CHAPTER 3

AIM

1. To investigate the blood flow distribution in normal human stomachs by comparing measurements from three different regions (antrum, incisura and fundus).

2. To assess blood flow in chronic benign gastric ulcers from pre-treatment through to healing.

3. To assess the relationship between a single systemic levels of growth factors and the ability of the gastric ulcer to heal.

CHAPTER 4

METHODOLOGY

Mucosal blood flow was measured with laser Doppler flowmetry during routine upper endoscopy. All subjects received conscious sedation in the form of intravenous midazolam. Supplemental oxygen was administered and pulse oximetry and non-invasive blood pressure monitoring were continuously performed on all the subjects. Only haemodynamically stable patients were recruited into the study. The Olympus EVIS video-endoscope was used. A complete upper gastrointestinal endoscopy was performed with the patients in the left lateral position. All patients had fasted overnight.

The laser Doppler flowmeter was a LASERFLO Blood Perfusion Monitor (Vasamedics Inc USA, St Paul Minnesota), using a laser probe of wavelength of 780 nm and a Doppler shift frequency of 30 Hz - 20 000 Hz. The laser Doppler flowmeter was switched on half an hour prior to use, to allow it to "warm up". The tip of the probe was held against the white static surface on the back of the instrument to zero the instrument. This was in compliance with the manufacturer's instructions. Data was captured onto a personal computer and subsequently transferred to a database for statistical analysis. The probe was passed down the biopsy port and held against the gastric mucosa. To ensure consistency in the pressure exerted by the probe, the mucosa was observed to indent but the probe was not allowed to buckle. Air insufflation was kept to a minimum. The probe was allowed to protrude 2 to 4 cm from the tip of the

gastroscope. This allowed for slight movement of the probe with peristalsis and reduced the number of un-couplings from the gastric mucosa. On achieving stabilty readings were made for one minute.

Figure 4.1.   The Laserflo BPM^2 and the personal computer
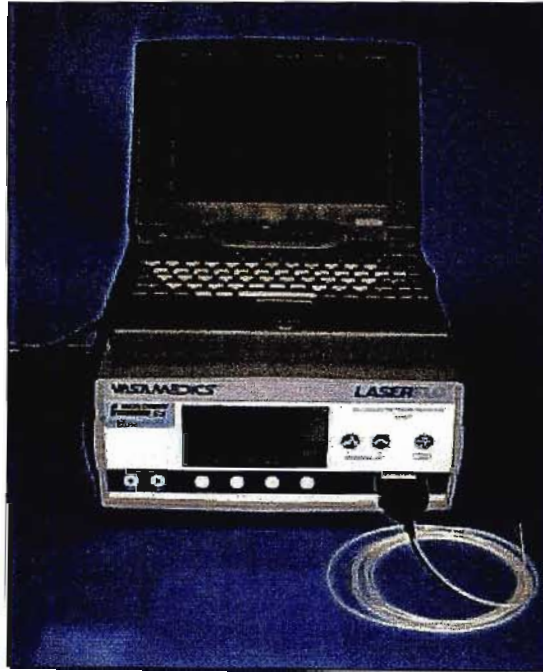
Figure 4.2   The gastroscope with the fibre-optic probe passed down the biopsy port

4.1　NORMAL STOMACHS

Patients recruited into this study had been referred for endoscopy but had no demonstrable pathology. This group included ten paid volunteers. After completing the endoscopic examination of the stomach, readings were made at single points on the antrum, incisura and fundus for one minute each after stabilisation of the readings. The laser Doppler flowmeter made two readings every second. Loss of contact between the probe and mucosa of less than 15 seconds was ignored. If un-coupling was longer than 15 seconds the initial readings were discarded and readings recommenced.

In four normal stomachs two readings were made at the antrum at sites separated by at least 2 cm. These readings were compared to establish the degree of spatial variability between readings in the same region of the stomach.

4.2　GASTRIC ULCERS

An ulcer was defined as the presence of a mucosal crater with visible loss of substance, greater than 1 cm in diameter. Measurements were made at two sites on the ulcer circumference as well as at single points on the antrum, incisura and fundus. Measurements were not made over the ulcer base. There were two reasons for this. Firstly the inflammatory tissue in the ulcer base makes measurements difficult to interpret. Secondly, healing commences at the ulcer edge and we felt that the state of mucosal blood flow at the ulcer periphery is more important than that in the ulcer crater.

All gastric ulcers were biopsied, to exclude malignancy. Evidence of *H. pylori*

infection was looked for on histo-pathological examination. All ulcer patients were empirically treated with eradication therapy and proton pump inhibitors (amoxycillin 1 g twice daily, clarithromycin 500 mg twice daily and omeprazole 40 mg daily for two weeks). Patients were re-endoscoped at approximately six weeks to assess healing and repeat the biopsy to exclude malignancy. Laser Doppler flowmetric measurements were repeated at the ulcer circumference in the unhealed ulcers. In one of the surgically treated ulcers intra-operative measurements were made. The operating surgeon fashioned a gastrotomy prior to mobilising the stomach. In this way we hoped to eliminate the possibility of mucosal blood flow being altered by division of named gastric arteries. The sterile probe was held directly on the gastric mucosa surrounding the ulcer through the gastrotomy.

If the ulcer had healed completely no further treatment was given. If the ulcer was refractory, acid suppression was continued. Eradication therapy was not recommenced unless the biopsy specimen showed persistent *H. pylori* infection. The author personally followed up all the patients. Communication between the author and the patients was actively encouraged. Patients were judged to be compliant if they attended follow-up regularly. If the author felt that the patients were non-compliant he attempted to correct this with personal interviews. The role of NSAID use, smoking and alcohol ingestion in gastric ulcer pathogenesis were emphasised in these interviews. The role of acid suppression and eradication were explained to the patients prior to commencing therapy. Patients were encouraged to contact the author at the unit if they were unsure about therapy or if they were worried about symptoms.

4.3   TECHNICAL PROBLEMS WITH MAKING THE MEASUREMENTS

During the experiment, technical problems were encountered, which were dealt with as they arose. The fact that the author performed all the endoscopies and made all the measurements allowed a considerable experience to be acquired. Most of the technical problems could be overcome with practice.

Un-coupling was a significant problem. However with practice it was possible to maintain contact between the probe and the mucosa. The site at which it was most difficult to ensure adequate mucosal probe contact was the incisura. Adequate retro-flexion of the scope was essential to ensure a view of the fundus and adequate mucosal probe contact at the fundus.

Another significant problem was the fragile nature of the fibre optic probe. This resulted in breakage, usually after much use. Great care was necessary during fundal measurements to prevent acute flexion and excessive angulation with subsequent fracturing of the fibres of the probe.

With experience and care it was possible to regularly obtain stable measurements during upper endoscopy.

4.4   GROWTH FACTOR ASSAYS

Systemic levels of the following growth factors: bFGF, EGF and TGF-β1 were measured in patients who had a gastric ulcer demonstrated on upper endoscopy. Each patient was allowed to rest for 30 minutes after the endoscopy, prior to

venesection being performed. This was in an attempt to eliminate the unpredictable response of these growth factors to the endoscopy. The author performed venesection with the patient recumbent, using a vacuum collection system. The specimens were kept refrigerated until the end of the endoscopy session when they were taken on ice to the laboratory where they were spun down. All growth factors were measured using an ELISA procedure. These assays employ the quantitative sandwich enzyme immunoassay technique. The optical density of each well was read using a micro-plate reader at 450 nm. For wavelength correction the micro-plate reader was set at 540 nm. All samples and standards were assayed in dulplicate. A thawed sample was used once and then discarded. The technique used was the same for all the growth factors measured. The technique will be described in detail for TGF-β1. Only variations in the technique will be highlighted for the remaining two growth factors.

4.4.1 TGF-β1

The blood samples were allowed to clot for one hour at room temperature. The samples were then incubated overnight at 4°C before centrifugation. This allows for the complete release of TGF-β1. After incubation, the sample was centrifuged at 1 000 x g for ten minutes. The serum was removed and aliquotted into equal volumes and stored at -70°C. TGF-β soluble receptor Type II, which binds TGF-β1, was pre-coated onto a micro-plate. A serial dilution of the standards was prepared, ranging in concentration from 1000 pg/ml to 31.2 pg/ml. Samples (diluted 4-fold) were then pipetted into the micro-plate. TGF-β1 present is bound by the immobilised receptor. After washing, to remove unbound substances, an enzyme-linked polyclonal antibody specific for TGF-β1 was added to the wells to

sandwich the TGF-β1 immobilsed during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and the colour was allowed to develop. The colour development was in proportion to the amount of TGF-β1 bound in the initial step. The colour development was stopped when the intensity of the colour was measured.

4.4.2 FGF (basic)

The blood samples were immediately placed on ice and allowed to clot at about 4°C. The samples were then centrifuged for ten minutes at 1000 x g. The serum was removed and aliquotted into equal volumes and stored at ≤-20°C until assayed. Before assay, the samples were allowed to thaw for a short period of time and immediately placed on ice until assayed.

4.4.3 EGF

The blood samples were allowed to clot for 30 minutes at room temperature. The samples were then centrifuged for ten minutes at 1 000 x g. The serum was removed and aliquotted into equal volumes and stored at -20°C until required. Before measurement for EGF, all serum samples were diluted 20-fold.

4.5 STATISTICAL ANALYSIS

A professional statistician was consulted for help regarding the presentation and statistical analysis of the results. The standard demographic data was analysed using a commercial statistics programme. The 120 readings for each one minute period were averaged. These average measurements for each patient were entered into a database for analysis. All results were expressed as a mean and

the standard deviation. Paired and unpaired Student's t test was used to compare readings and determine whether differences were statistically significant. A p value <0.05 was considered significant. Pearson's correlation test was used to assess the degree of correlation between measurements.

CHAPTER 5

RESULTS

5.1 NORMAL STOMACHS

Readings were made in 105 normal stomachs (median age 51 years, range age 16-75, 65 males and 40 females). Blood flow, volume and velocity increased as the probe ascended the stomach from antrum to fundus (Table 5.1) (Appendix 1). Blood flow and velocity were significantly higher at the fundus than the antrum. In the four stomachs in which multiple readings were made at the antrum, a close correlation was demonstrated between readings. (Table 5.2).

Table 5.1.　Blood flow, volume and velocity at the antrum, incisura and fundus.

	Antrum	Incisura	Fundus	p Value
Flow ml/min/100g	59.8 ±26.1	68.4 ± 28.9.	73.8 ± 29.9*	*<0.001
Volume by %	7.4 ± 4.4*	7.3 ± 3.7	7.8 ± 4.4*	*>0.05
Velocity mm/sec	3.8 ± 1.4*	4.6 ± 1.6	4.9 ± 1.7*	*<0.0002

Table 5.2.　Correlation of flow readings from different sites on the antrum.

	Site1	Site2	Correlation
Flow	ml/min/100g	ml/min/100g	
Patient 1	36.5 ± 3	43.2 ± 2	0.89
Patient 2	65.4 ± 3	75.6 ± 6	0.78
Patient 3	20.8 ± 3	30.7 ± 2	0.85
Patient 4	98.7 ± 3	91.5 ± 1	0.93

5.2 BENIGN GASTRIC ULCERS

5.2.1 Ulcer Demographics

A total of 26 patients with benign gastric ulcers were recruited. There were no associated duodenal ulcers. There were five incisural (lesser curve) ulcers. The remainder were pre-pyloric. Seventeen (65%) ulcers had *H. pylori* infection proven on histology.

5.2.2 Refractory Ulcers

There were six patients with refractory ulcers (median age 56 years, age range 35-76 years, five males and one female). Four of these six patients complicated and required surgery. In the remaining two patients, the ulcers never healed but did not require surgery. Both of these patients had severe co-morbid medical problems and both died of their systemic problems within six months of their presentation. They did not die from perforation or acute haemorrhage. *H. pylori,* infection was demonstrated on five of the initial biopsies. Successful eradication was documented in four of the *H. pylori* positive patients. Two of the patients who complicated were non-compliant on treatment. They both continued to smoke, take aspirin and ingest alcohol. *H. pylori,* was succesfully eradicated in one of these patients. The remaining two patients complicated whilst on therapy. The operation in all cases was an antrectomy, vagotomy and duodenostomy.

5.2.3 Healed ulcers

There were 20 patients in this group (median age of 56 years, range 33 to 73 years, 5 females and 15 males). The average size of the healed ulcers was 2 cm.

(range 1 cm to 7 cm). Twelve (60%) of the healed ulcers were *H. pylori* positive on initial biopsy. Healing was taken as evidence of effective eradication therapy. One of these ulcers was 7 cm in diameter and located on the lesser curve. Healing took 13 weeks in this patient. This patient admitted to heavy ingestion of NSAID's prior to developing her ulcer. NSAID's were discontinued successfully in this patient. In ten patients complete sets of readings were available. In six patients, follow-up readings were not made and in four patients there were no initial readings available. The reasons for this were multiple. In the latter group the initial endoscopy had been performed by clinicians, who were not involved in the study. They were subsequently referred to the author's clinic. In the group with no follow-up readings, four refused repeat measurements and two patients re-scheduled their endoscopy appointment and subsequently underwent endoscopy by a clinician not involved in the study.

5.2.4 Mucosal haemodynamics around ulcers

The healed ulcers showed significantly higher blood flow than normal gastric antrums. This persisted at follow-up. The average time of the second endoscopy was 33 days ±9 days. The refractory ulcers showed lower flows than the normal antrums and the healed ulcers (Table 5.3) (Appendix 1). The refractory ulcers demonstrated persistently lower mucosal blood flow at follow-up.

Table 5.3.   Differences in blood flow between normal and ulcerated stomachs.

	Flow ml/min/100g	Flow ml/min/100g	Flow ml/min/100g	p Value
	Normal N=105	Healed N=10	Unhealed N=6	
Initial	59.8±26.1	75.7 ± 17.5*	45.2 ± 21.9*	*< 0.003
Follow-up	-	69.8 ± 13.7*	39.2 ± 6.7*	*< 0.003

5.3   *H. pylori* AND ULCER HEALING

Repeat biopsy was used to exclude persistent *H. pylori* infection in the refractory ulcer group. In this group, five of the ulcers were positive for *H. pylori* at presentation. In only one case was *H. pylori* demonstrated on repeat biopsy. One patient developed a re-infection after eight months. The remaining three refractory ulcers which had demonstrated *H. pylori* on initial biopsy were negative three months later. In the healed group twelve (60%) patients had *H. pylori* infection proven on initial biopsy, however eradication was not confirmed in these patients.

5.4   GROWTH FACTOR LEVELS

The systemic levels of bFGF were significantly higher in the healed ulcers, compared to the refractory ulcers. TGF-β1 was higher in the healed ulcers than the refractory ulcers although this did not achieve statistical significance. EGF levels were similar in both groups (Table 5.4).

Table 5.4.   Systemic concentrations of growth factors in healed and refractory ulcers.

	FGF pg/ml	TGF pg/ml	EGF pg/ml
Healed ulcers	23.5 ± 27.8	42.3 ± 11.5	61.8 ± 30.5
Unhealed ulcers	11.3 ± 5.9	19.7 ± 32.4	55.1 ± 24.7
p Value	<0.01	<0.06	<0.2

CHAPTER 6

DISCUSSION

6.1 INTRODUCTION

In this chapter, the literature pertaining to laser Doppler, gastric mucosal blood flow and the role of growth factors in gastric healing will be discussed in the light of our own findings and those of other authors.

6.2 SPATIAL REPRODUCIBILITY

The good correlation between multiple antral readings in individual patients implies that laser Doppler flow varies little in this region during a single endoscopy session. Long-term correlation between flow measurements was not assessed in normals.

6.3 DISTRIBUTION OF MUCOSAL BLOOD FLOW IN NORMAL STOMACHS

The distribution of mucosal blood flow seen in our cohort of normal human stomachs is similar to that seen by other investigators in animal[73-75] and in human[69,76-78] stomachs. This applies to both laser Doppler flowmetry and other techniques of measurement.

6.3.1 Animal Studies

The gradient between fundal and antral blood flow is also seen in animals. Kleen et al.,[73] in splenectomized beagle dogs, found that the stomach has a regional variation in its perfusion of 26 ± 22 ml/min/100 g of tissue at the antrum and $67 \pm$

44 ml/min/100 g of tissue at the fundus. Becker *et al.*[74] reported values of 19 ml/min/100 g of tissue, and 29 ml/min/100 g of tissue moving from the antrum to the fundus. Hottenrott *et al.*[75] reported values of 12 ml/min/100 g of tissue and 35 ml/min/100 g of tissue at the antrum and fundus. These measurements were all made in canine stomachs using the radioactive microsphere technique. The trend for blood flow to increase as the stomach is ascended was confirmed in all these experiments. Kleen *et al.*[73] stated, "Gastric mucosal blood flow is most probably also heterogeneously distributed in humans". The difference in mucosal blood flow between the antrum and the fundus demonstrated in our results confirms his statement.

6.3.1 Human Studies

Investigators have demonstrated a gradient in gastric mucosal blood flow between the antrum and fundus in human stomachs by a variety of techniques.

Kawano *et al.*[79] used reflectance spectrophotometry to estimate mucosal blood volume in 55 normal controls. An image of mucosal blood volume distribution was made with a two-dimensional computer colour graphics programme. They demonstrated higher blood volume at the fundus in comparison to the antrum and a higher volume at the lesser curve than the greater curve.

Kvernebo *et al.*[77] in 34 normal human volunteers demonstrated significantly higher blood flow at the fundus than at the incisura and antrum, using laser Doppler flowmetry. They did not quantify the difference in their paper.

Allen *et al.*[76] documented a significant difference in mucosal blood flow between the antrum (44.4 ± 23.3 ml/min/100 g of tissue) and the fundus (74.9 ± 4.8 ml/min/100 g of tissue), using laser Doppler flowmetry in 38 normal patients.

Using laser Doppler flowmetry, Gana *et al.*[78] documented higher mucosal blood flow at the fundus in comparison to the antrum. Mucosal blood flow in twelve patients with either symptoms of dyspepsia or with a peptic ulcer was compared with mucosal blood flow in six asymptomatic healthy volunteers. Mucosal blood flow was measured for 1 minute at the duodenum, pre-pylorus, antrum, angularis, mid-greater curvature, fundus and the distal oesophagus. At all sites mean blood flow was 72% of the corresponding value in the volunteers. However in both groups blood flow was highest in the fundus and lowest in the antrum.

Ahn *et al.*[69] in 26 subjects made sequential laser Doppler readings at three different sites in the stomach. They documented a significant gradient between the fundal and antral readings. The difference in mucosal blood flow between the antrum and fundus in these studies is in concordance with our own findings and are documented in Table 6.1. (Some laser Doppler flowmeters give measurements in volts (V).)

Table 6.1.　A comparison of different authors results

Authors	Clarke	Ahn et al.[69]	Allen et al.[76]	Gana et al.[78]
Patient No	105	26	38	12
Laser Doppler Units	Vasamedics BPM2 ml/m/100g	Periflux PF1 and PF2 Volts	Periflux PF2 Volts	Periflux PF3 Volts
Antrum	59.8 ± 26.1	69.3 ± 21.9	44.4 ± 23.3	17.3 ± 3.3
Incisura	68.4 ± 29.9	83.6 ± 24.7	Not reported	Not reported
Fundus	73.8 ± 29.9	95.4 ± 21.3	74.9 ± 44.8	28.3 ± 0.9

The physiological explanation for the higher blood flow at the fundus and greater curvature is unclear. It may be a feature of the higher level of functional activity of the fundus, the region of acid production, in comparison to the less physiologically active antrum and most likely reflects the active neutralisation of back diffusing Hydrogen ions in the region of acid production.

6.4　AN ANATOMICAL EXPLANATION FOR THE GRADIENT IN GASTRIC MUCOSAL BLOOD FLOW

Barlow et al.[80] in 1951 demonstrated differences in the micro-vascular anatomy of the fundus and antrum, which offer some explanation for the regional perfusion differences in the gastric mucosa. Angiographic assessment of post mortem stomachs demonstrated the arteriole branches of named arteries piercing the muscle coat and forming an anastomosing network in the submucosa. The arterioles supplying the antrum and proximal duodenum are shorter and finer with fewer anastomoses compared to those supplying the rest of the stomach. In the antrum the submucosal plexus has on average a smaller diameter (100-150 µm) in comparison to that of the fundus (150-200 µm). Furthermore the mucosal

arteries are much smaller diameter or length or both in the antrum than the fundus although the arrangement is similar. In the region of the lesser curve mucosal arteries do not arise from a submucosal plexus but have their origins outside the stomach directly from the right and left gastric vessels. These slender vessels pierce the stomach wall to enter the submucosa, which they traverse obliquely without forming a submucous plexus to reach the mucosa. Here a mucosal plexus is formed. This end arteriole system may be more prone to develop reduced mucosal blood flow than the rich plexus found in the submucosa of the fundus. The difference in size of the submucosal arterioles of the fundus and antrum reflects the lower blood flow at the antrum in comparison with the fundus.

Barlow *et al.* demonstrated direct arterio-venous channels of 140 μm in diameter, commonly arising from the mucosal arteries. According to them., the distribution of mucosal blood flow depends on two types of resistance placed in parallel: the submucosal arterioles and the proximal shunts. This means that blood can be diverted from one part of the stomach to another in compliance with different functional requirements. The role of these arterio-venous shunts remains enigmatic. Gannon *et al.*[81] failed to demonstrate arterio-venous anastomosis by means of partial vascular casting and scanning electron microscopy. They went on to state, "If anyone wishes to evoke arterio-venous shunts they must provide unequivocal structural evidence of their existence".

Despite the controversy concerning the existence of the shunts, Barlow *et al.* provide evidence of regional anatomical differences which could explain the

variations in mucosal blood flow measured in this and other studies.

6.5 GASTRIC MUCOSAL BLOOD FLOW IN BENIGN GASTRIC ULCERATION

In principle, gastric ulceration results from an imbalance between the digestive action of acid and pepsin on the mucosa and the protective mechanism in place to resist mucosal digestion. Alterations in gastric mucosal haemodynamics play an important role in the pathogenesis and healing of gastric ulcers. This is well documented in the case of acute stress-related ulcers, which develop secondary to hypotension.[82] Clinically, most patients who develop stress ulcers have experienced an episode of shock from haemorrhage, sepsis, or cardiac dysfunction. Diminished mucosal blood flow is the common denominator in animal experiments that employ restraint, haemorrhage, or endotoxaemia to produce acute lesions. Gastric mucosal blood flow plays an important role in the disposal and buffering of the Hydrogen ions diffusing across the mucosa. Reduced mucosal blood flow impairs the capacity of the gastric mucosa to neutralise acid. This in turn leads to accumulation of Hydrogen ions within the mucosa and ulceration.[25]

The role of reduced mucosal blood flow in chronic ulceration is less well defined. We have documented reduced mucosal blood flow around refractory gastric ulcers, and enhanced mucosal blood flow in the case of healing ulcers. We believe this to be evidence that mucosal blood flow plays an important role in chronic as well as acute ulceration.

6.6   COMPARISON WITH THE RESULTS OF OTHER INVESTIGATORS

Other investigators have looked at the role of gastric mucosal blood flow in benign gastric ulceration in both animals and humans using a variety of techniques. Lunde and Kvernebo[83] measured mucosal blood flow in 15 patients with chronic gastric ulcers, using laser Doppler flowmetry. They demonstrated lower blood flow along the lesser curve in comparison to the greater curve. At follow-up the low blood flow persisted. At a subsequent follow-up the blood flow had significantly increased along the lesser curve, however flow at the ulcer remained low. At four to six weeks ten ulcers were healed and at four months, twelve ulcers were healed.

Kamada *et al.*[84] using reflectance spectrophotometry, measured mucosal blood volume in 42 patients with benign gastric ulceration. They repeated these measurements at regular intervals during ulcer healing. During the active phase of ulceration blood volume was diminished in all regions of the stomach, in comparison with normal stomachs. However during ulcer healing, peri-ulcer blood volume increased. In the healed ulcers, peri-ulcer blood volume increased by 33%. In the non-healed ulcers, peri-ulcer blood volume only increased by 3%. It would appear that these ulcers were treated with antacids. These results imply that reduced mucosal blood flow is important in the pathogenesis of gastric ulcers and that a local increase in mucosal blood flow plays an important role in the healing of gastric ulceration.

Sato *et al.*[85] used laser Doppler flowmetry, reflectance spectrophotometry and Hydrogen clearance to measure changes in gastric mucosal blood flow in 42

patients with ulcers. They showed that at the active ulcer stage the index for blood volume at the ulcer margin was significantly lower than in normal controls. However it increased during ulcer healing to reach a level 30% higher than normal antral flow. In the early scarring stage they found that mucosal blood flow was still elevated and only returned to normal at the late scar stage. Furthermore they showed that the mucosal blood volume index in intractable ulcers was not increased.

Results from Kamada et al.[84] and Sato et al[85] are in contrast to the findings of Lunde and Kvernebo[83] but are in agreement with ours. It would appear from our results and those of Sato et al.[85] and Kamada et al.[84] that benign gastric ulceration is associated with fluctuations in mucosal blood flow and volume during both the acute and healing stages of the disease. An understanding of mucosal defence and healing is essential for interpretation of the results of mucosal blood flow measurements.

6.7   MUCOSAL BLOOD FLOW AND THE GASTRIC MUCOSAL BARRIER

The integrity of the gut depends on preservation of existing tissue and replacement of lost tissue. Both of these functions are dependent on mucosal blood flow. Preservation of existing cells may be achieved either by enhancing the resistance of the cells or by decreasing contact time between the gastric mucosa and noxious stimuli. In the gastric mucosa the main method of preservation involves rapid clearing of the noxious substance. This is achieved by rapid gastric emptying and by maintaining adequate blood flow and mucous and bicarbonate secretion. Maintenance of gastric mucosal blood flow is essential for

these processes to occur.

Lost or damaged epithelial cells may be replaced by epithelial cells or by connective tissue. Restitution involves in-growth of cells from the edge of the lesion. In cases of superficial mucosal damage where blood flow is maintained, the partly damaged and healthy epithelial cells respond by migrating to cover the epithelial defect.[26] Deep necrosis causes cessation of blood flow in a relatively large area. This requires replacement by proliferating connective tissue cells such as fibroblasts, macrophages and collagen. Proliferation is accompanied by angiogenesis. Inflammatory cells remove the necrotic tissue. Once a new scaffold of granulation tissue has been laid down, re-epithelialisation can occur.[26]

Both gastric defence and healing are energy dependent processes, which require adequate nutrient and oxygen supply. If mucosal blood flow is reduced then mucosal defence and healing is impaired.

6.8   MUCOSAL ADAPTATION AND CYTO-PROTECTION

The gastric mucosa shows a remarkable ability to adapt to injury. The application of low doses of mild irritants significantly prevents the development of gastric ulceration produced by higher concentrations of the same or different agents. This protection is mediated by an increase in gastric mucosal blood flow in response to the initial exposure and is known as mucosal cyto-protection or adaptation.[25]

Brozozwski et al.[86] induced gastric adaptation in rats by repeated daily doses of acidified amino-salicyclic acid (ASA), given intra-gastrically. This group was

compared to control rats with intact stomachs, which were given a non-reactive vehicle only. After five days of ASA, the rats were challenged with either acidified ASA or with strong irritants such as 100% ethanol, 200 mmol acidified taurocholate, 25% NaCl for one hour, or with water immersion and restraint. The first dose of ASA produced numerous gastric lesions and deep histological necrosis. These were accompanied by a fall in the gastric blood flow, and negligible expression of EGF and TGF-α or their receptors. There was no evidence of mucosal proliferation. However, as adaptation to ASA developed the areas of gastric ulceration were reduced by more than 80%. There was a noticeable decrease in deep necrosis, a partial restoration of gastric blood flow, an approximately 4-fold increase in EGF expression and its receptors. There was an appreciable increase in mucosal cell proliferation compared with vehicle treated rats. Increases in the mucosal expression of EGF receptors and the luminal content of EGF were also found in ASA-adapted animals. When the ASA-adapted rats were challenged, the area of deep histological necrosis and gastric ulceration, was reduced in comparison to the control rats. This increased mucosal tolerance to strong irritants was also accompanied by the return of the gastric blood flow towards control levels and further significant increases in the mucosal expression of EGF receptors and mucosal cell proliferation. They concluded that gastric adaptation to ASA enhances mucosal resistance by restoring gastric blood flow and increasing cell proliferation.

Gastric mucosal adaptation is highly blood flow dependent. Svanes *et al.*[87] demonstrated an increase in mucosal blood flow in cats, when the feline stomach was pre-treated with a mild irritant. This pre-treatment reduced the damage to

the gastric mucosa caused by exposure to absolute ethanol. In a third group, the coeliac artery was partially occluded. This served to prevent an increase in mucosal blood flow following pre-treatment with the same irritant. In this group of cats, pre-treatment offered no protection against absolute ethanol. They stated that "high mucosal blood flow represents a very important factor in adaptive cyto-protection".

Once blood flow is impaired, adaptation cannot occur and mucosal injury develops. Starlinger et al.[88] showed that when the gastric mucosa of rabbits was exposed to increasing luminal acidity and increasing Hydrogen ion back diffusion, mucosal blood flow increased, whilst intra-mucosal pH remained constant. When hypovolaemic shock was induced, increasing luminal acid resulted in reduction of intra-mucosal pH and subsequent mucosal ulceration.

Ritche and Shearburn[89] showed that a large gradient of Hydrogen ion concentration between the lumen and mucosa could be tolerated provided there was adequate mucosal blood flow to sweep away the influxing Hydrogen ions. If gastric mucosal blood flow is impaired, then mucosal adaptation is inhibited and a gastric ulcer will develop.

Mucosal blood flow is an important mechanism in preventing mucosal injury. An increase in mucosal blood flow facilitates healing and restoration of mucosal integrity. Our findings of increased mucosal blood flow in healing gastric ulcers is in keeping with our understanding of the changes in mucosal blood flow in response to mucosal injury.

6.9   IMPAIRMENT OF BLOOD FLOW AND ULCER FORMATION

Impairment of gastric mucosal blood flow prevents mucosal adaptation and inhibits mucosal defence. There is experimental evidence linking impairment of mucosal blood flow with the formation of gastric ulcers. Szabo et al.[90] assessed the effect of hypotension on mucosal blood flow in anaesthetised dogs. Blood flow in the portal vein and the left gastric artery was measured electromagnetically and gastric mucosal perfusion was determined by pertechnetate clearance. Bleeding the animals to arterial pressures of 100 and 60 mmHg reduced portal venous flow and increased the mesenteric in-flow resistance. Left gastric arterial and gastric mucosal blood flow were decreased. Gastric acid secretion decreased but did not stop even at the lower level of haemorrhagic hypotension. They concluded that acid precipitated stress ulceration only in the presence of hypotension and reduced mucosal blood flow.

Sato et al.[91] investigated the gastric mucosal blood flow with an in vivo microscopic analysing system and reflectance spectrophotometry. Topical administration of either 20% ethanol, indomethacin, leukotriene C4 or platelet activating factor (PAF) decreased mucosal blood flow and mucosal blood oxygenation. The subsequent application of hydrochloric acid (HCL) induced gastric bleeding and ulceration. They concluded that gastric mucosal blood flow plays an important role in the mucosal protection. In further experiments they. administered indomethacin intravenously and a solution of ethanol and HCL topically to rats. They observed the effect on gastric mucosal blood flow with laser Doppler, Hydrogen clearance, aminopyrine clearance and in-vivo microscopy. Similar experiments were performed in mongrel dogs. Indomethacin in the dogs

decreased mucosal blood flow in the capillaries and collecting venules. However the gastric mucosal lesion one hour post treatment was small. Only petechiae were seen in the gastric fundus. In rats pre-treated with indomethacin, topical administration of HCL caused blood flow to stop and in some regions even reverse from the venules to the capillaries.

When 40% ethanol was administered to the anaesthetised rat, circulation became slow in the surface mucosa. The venules became engorged and white cells marginated, rolled and stacked the endothelium so blocking the flow of red cells. These changes were not evenly distributed throughout the stomach.

Chung et al.[92] showed that mucosal lesions occurred in rats when mucosal blood flow was reduced to 40% of baseline levels. Varhaug and Svanes[93] reduced regional mucosal blood flow by partial devascularisation of the feline stomach. He measured pentagastrin-stimulated acid secretion and gastric mucosal blood flow, by means of the microsphere distribution technique, two hours and at one week after partial gastric devascularisation. Systemic cardiac output was also determined. Devascularisation decreased the volume but not the pH of acid secreted by the stomachs. Mucosal blood flow was markedly reduced two hours post devascularisation. After one week, blood flow had improved and was not significantly different from controls. The ratio of acid secretion to mucosal blood flow was not significantly changed one week after devascularisation as compared with the acute experiments. However all cats examined one week after devascularisation had developed ulcers at the mid-portion of the greater curvature. This was the area of the gastric mucosa that had the most markedly

reduced mucosal blood flow. These ulcers could be prevented by acid inhibition. In other words the ulcers arose in a situation of normal luminal acid concentration but with inhibited mucosal defence.

Kalia *et al.*[94] and Kalia and Bardhan[95] used fluorescent *in vivo* microscopy to directly examine the response of several areas of gastric mucosa to 0.5 ml of 60% ethanol in animals. Their findings suggested a close correlation between vascular stasis and mucosal necrosis. The entire mucosal circulation showed an increase in leakage of fluoroscein-labelled albumin following exposure to 60% ethanol. This reflected extensive micro-circulatory disruption. However gross ulceration was seen in small areas only. In the peri-ulcer mucosa leakage of macro-molecules increased after exposure and remained significantly higher than normal for the duration of the experiment. In areas remote from the ulceration, macro-molecular leakage was seen to increase but declined rapidly thereafter, although it remained higher than pre-treatment levels. The areas where mucosal ulceration developed showed persistent haemostasis.

Pihan *et al.*[96] used *in vivo* microscopy and laser Doppler flowmetry to examine the effects on gastric mucosal blood flow of agents known to induce acute gastric mucosal damage. *In vivo* microscopic observation of superficial mucosal capillaries revealed vascular stasis within 54 seconds following the application of 100% ethanol, and the subsequent development of mucosal lesions. Mucosal blood flow as measured by laser Doppler flowmetry decreased by 30% at five minutes after application of 100% ethanol, and decreased further to about 40% of basal levels by 15 minutes. Decreased mucosal blood flow correlated with the

development of mucosal lesions.

All these data support the role of impaired mucosal blood flow in the pathogenesis of acute benign gastric ulceration. Decreased mucosal blood flow results in impaired buffering of Hydrogen ions and impaired mucosal defence so allowing unopposed exposure of the mucosa to gastric acid. Inhibition of mucosal blood flow disrupts the gastric mucosal barrier and inhibits mucosal cyto-protection with an end result of mucosal ulceration. Whilst the role of reduced mucosal blood flow in acute gastric lesions is well documented, its role in chronic ulceration is less clear. There is work looking at the effect of various factors which are associated with peptic ulceration on mucosal blood flow. These studies shed some light on the role of mucosal blood flow in the pathogenesis of benign chronic gastric ulceration.

6.10 THE EFFECT OF NSAID'S AND CIGARETTE SMOKE ON GASTRIC MUCOSAL BLOOD FLOW

Under pathological conditions, various stimuli or the lack of physiological regulators can disturb gastric mucosal blood flow. NSAID use and cigarette smoke are both implicated in the pathogenesis of gastric ulceration and both factors have been shown to affect mucosal blood flow.[20,97,98] NSAID's are believed to decrease mucosal prostaglandin levels by inhibiting cyclo-oxygenases. This decreases mucosal blood flow and bicarbonate secretion and this in turn impairs mucosal ability to neutralise back diffusing Hydrogen ions. NSAID use has been shown to decrease mucosal blood flow.

Ashley et al.[99] demonstrated that exposure to aspirin reduces gastric mucosal

blood flow. He compared focal gastric mucosal blood flow as measured by the Hydrogen gas clearance method, with total gastric blood flow as determined by venous outflow in an isolated segment of canine stomach before, during, and after exposure to aspirin. Despite an increase in total gastric blood flow and an increase in mucosal blood flow at non-ulcerated sites on the gastric mucosa, mucosal blood flow at the site of aspirin-induced ulceration was significantly reduced. After removal of aspirin, mucosal blood flow returned to control levels. Such a redistribution of mucosal blood flow in response to aspirin is consistent with the localised nature of acute aspirin-induced injury, and demonstrates that impaired mucosal blood flow is necessary for the development of a peptic ulcer.

Taha *et al.*[100] used laser Doppler flowmetry to measure gastric and duodenal mucosal blood flow in 70 patients who had taken NSAID's for longer than four weeks. They also studied the correlation with demographic factors, ulceration, *H. Pylori* infection and cigarette smoking. These readings were compared with readings made in 17 subjects not taking NSAID's. Both gastric and duodenal blood flow values were significantly lower in patients taking NSAID's, than in those who were not. The median duodenal mucosal blood flow was significantly lower in smokers than non-smokers. Similarly, blood flow was significantly lower in patients with duodenal ulcers in comparison to those without duodenal ulcers.

Sawant *et al.*[101] assessed the effect of a short course of indomethacin on gastric mucosal blood flow. Patients with musculo-skeletal pain of recent origin who had been prescribed a course of indomethacin for 7 days were investigated. Baseline measurements of gastric mucosal blood flow were carried out with laser Doppler

flowmetry prior to commencing indomethacin. Measurements were made at the antrum, incisura, lesser and greater curves, and fundus. These measurements were repeated after therapy was completed. The flow was measured at the same sites and at the site of any erosion. In ten out of the sixteen patients, gastric mucosal erosions developed. Mean gastric mucosal blood flow at the sites of erosion was significantly reduced. This implies that NSAID use decreases mucosal blood flow in the stomach, and that this decrease in blood flow is associated with the development of peptic ulceration. These results imply that decreased mucosal blood flow is a feature of cigarette smoke and NSAID use.

Cigarette smoking and has been shown to decrease mucosal blood flow and so predispose to gastric ulceration.[21,97,102-104] Guslandi *et al.*[21] documented decreased mucosal blood flow in the stomachs of smokers. In 20 dyspeptic patients (ten non-smokers and ten smokers) without duodenal pathology, mucosal blood flow was measured in the duodenal bulb by laser Doppler flowmetry. Basal bulbar perfusion was found to be significantly lower in smokers. This reduction in duodenal blood flow seen in smokers may promote duodenal damage secondary to an impaired ability to neutralise back-diffusing Hydrogen ions and reduced secretion of protective bicarbonate.

Battistel *et al.*[104] showed that the intake of nicotine induces vascular damage in the rat gastric mucosa. In this study rats were treated with nicotine added to their drinking water, for 50 days. They were then anaesthetised and their stomachs perfused with ASA. Basal blood flow in the gastric mucosa was unchanged by chronic nicotine intake, however the mucosal hyperaemia usually evoked by the

ASA induced acid back diffusion was averted. The concentration of sulfidoleukotrienes in the gastric wall of nicotine treated rats was significantly augmented. These data show that chronic nicotine intake causes dysregulation of the gastric mucosal blood flow, an effect that is associated with biochemical changes in the stomach. This study suggests that inappropriate regulation of gastric mucosal blood flow may inhibit recovery from gastric mucosal injury in smokers.

Both NSAID's and cigarette smoke have been shown to decrease gastric mucosal blood flow in a variety of experiments. These two factors are known to be closely associated with gastric ulceration. More importantly these factors appear to be closely linked to the problem of refractory ulceration. In our group of refractory ulcers, mucosal blood flow was significantly lower than in healing ulcers and normal stomachs. The implication of our findings and the above results is that impaired mucosal blood flow is an important factor in the pathogenesis of gastric ulceration and in the development of refractory ulceration. Furthermore, modification of lifestyle factors that are known to impair mucosal blood flow should be emphasised and addressed as part of the primary therapy for patients with gastric ulcers. This becomes even more important if the patients are part of a high-risk group, such as the group identified in this study.

6.11 THE MEDIATION OF GASTRIC MUCOSAL HYPERAEMIA AND HEALING

Mucosal defence and healing are dependent on adequate blood flow. The results from our study reflect the important role of mucosal blood flow in mucosal defence and healing. The gastric ulcers that healed all had higher mucosal blood

flow than the normal antrums. This represents gastric mucosal adaptation and cyto-protection. Experiments using a variety of mediators confirm that enhancement of mucosal blood flow is important to attenuate the extent of acute mucosal damage and to promote mucosal healing.

Mediators of enhanced mucosal blood flow include prostaglandins and Nitric Oxide. The pathways by which these effects are mediated include central vagal and local pathways. A great deal of experimental work has been undertaken using various mediators of mucosal blood flow.[31,105-106] These data provide insight into the role of mucosal blood flow in gastric mucosal healing and protection.

Brozozowski et al.[31] demonstrated an acceleration of gastric ulcer healing following administration of L-arginine, a pre-metabolite of nitric oxide, in a dose-dependent manner. The accelerated healing was accompanied by a marked increase in gastric blood flow at the ulcer margin. Furthermore, increased serum gastrin, increased mucosal DNA synthesis, and angiogenesis in the granulation tissue in the ulcer bed were also seen. A similar improvement in ulcer healing associated with enhanced mucosal blood flow at the ulcer margin and enhanced angiogenesis, was observed after treatment with the nitric oxide supplier glyceryl-trinitrate. In contrast an inhibitor of nitric oxide synthase, NG-nitro-L-arginine (L-NNA), delayed ulcer healing. This was accompanied by a reduction in mucosal blood flow at the ulcer margin. Angiogenesis in granulation tissue, serum gastrin level and mucosal growth were also decreased. The addition of L-arginine to L-NNA restored ulcer healing, and enhanced mucosal blood flow at the ulcer margin. Pre-treatment with indomethacin, which inhibits synthesis of

prostaglandins, also delayed ulcer healing. This was reversed by the co-administration of L-arginine.

Panes et al.[105] investigated the role of endogenous nitric oxide as a mediator of mucosal blood flow. The application of NG-monomethyl-L-arginine (L-NMMA), which is a specific inhibitor of nitric oxide biosynthesis, attenuated in a dose-related manner the increase in gastric mucosal blood flow, which is usually induced by haemodilution. The concurrent administration of L-arginine (the precursor of nitric oxide biosynthesis) abolished the effects of L-NMMA on gastric mucosal blood flow. L-arginine accelerates ulcer healing by enhancing gastric mucosal blood flow.

Battal et al.[107] examined the effects of beraprost sodium (a chemically stable prostaglandin I2 (PGI2) analogue) on burn-induced gastric mucosal changes in rats. Twenty male rats were burned and then divided into two equal groups. One group received beraprost sodium intra-peritoneally immediately after burn injury, while the control group received the same volume of saline. Gastric mucosal blood flow was measured with a laser Doppler flowmeter. The area of mucosal necrosis was determined macroscopically and histologically. Gastric mucosal damage was significantly reduced in the rats treated with beraprost sodium and gastric mucosal blood flow was significantly improved. These findings demonstrate that PGI2 plays an important role in the pathophysiology of burn-induced ulcers and that beraprost sodium can improve gastric mucosal blood flow and reduce mucosal damage.

Rebamipide is a prostaglandin analogue that has been approved for treatment of peptic ulcers in Japan. Arakawa et al.[108] compared male Wistar rats, which had been pre-treated with Rebamipide, to rats pre-treated with carboxy-methylcellulose. The rats were scalded and sacrificed 15 minutes after the event. The group treated with Rebamipide demonstrated a significantly lower percentage of rolling leucocytes in the mucosa than the control group. Rebamipide also decreased the amount of endothelial damage. It seems that Rebamipide preserved gastric mucosal blood flow by inhibiting leucocyte adhesion and endothelial damage in venules at an early stage of thermal-induced ulceration.

These diverse mediators all influence mucosal healing and protection by enhancing mucosal blood flow. This emphasises the importance of enhanced mucosal blood flow in mucosal protection. Enhanced mucosal blood flow supports healing. We have demonstrated enhanced mucosal blood flow in the healed ulcers in our study. The 20 healed ulcers, had significantly higher mucosal blood flow than the normal antrums. The non-healing ulcers on the other hand demonstrated reduced mucosal blood flow. This represents failure of mucosal restitution and re-epithelialisation. If angiogenesis does not takes place, new in-growth of fibroblasts and epithelial cells cannot occur. Inhibition of mucosal blood flow prevents healing. Reduced supply of bicarbonate allows accumulation of Hydrogen ions and acidification of the mucosa. This leads to mucosal ulceration. Without adequate nutrient supply, the high energy demanding process of restitution cannot take place. Inhibition of gastric mucosal blood flow was demonstrated in the group of non-healing ulcers. These six ulcers had

persistently lower mucosal blood flow than normal.

6.12   LOCATION OF GASTRIC ULCERS

The majority of human gastric ulcers occur distally in the stomach on the antrum and lesser curvature.[109]   Wright *et al.*[40] in a review of gastric ulceration, had a distribution of gastric ulcers of 61% lesser curve, 5% antral, 26% pre-pyloric and only 8% in the body. The ulcers in our series were all located in the lesser curve or pre-pyloric region. There were no greater curve ulcers and no fundal ulcers. This is consistent with a review by Wright *et al.*[40] as well as other studies.[109] Various explanations have been advanced for the distal location of gastric ulceration. Oi *et al.*[109] explained the location of gastric ulceration on the basis of the relationship between mucosa and circular muscle fibres at the border of the fundus and pylorus. They implicated kinetic strain due to gastric motility of the oblique and circular muscle at this point in gastric ulcer pathogenesis. This theory has not been confirmed by more recent investigators.

It seems more likely that lower mucosal blood flow at the antrum may contribute to the higher rate of ulceration in the distal stomach compared to the proximal stomach. This hypothesis is supported by Barlow *et al* whose anatomical data demonstrates differences between the antral and fundal mucosal blood flow.[80] The gastric antrum and incisura have relatively lower blood flow in relation to the rest of the stomach. This is the region of non-acid production. Decreased mucosal blood flow in this region may make the antrum more susceptible to mucosal damage. The lesser curve and duodenum are supplied by end arteries and it is feasible that occlusion of these arteries may lead to localised regions of

reduced mucosal blood flow, which may predispose the antrum to gastric ulceration.[80]

6.13 *H. pylori* AND MUCOSAL BLOOD FLOW

The influence of *H. pylori* on gastric mucosal blood flow is unclear. Guslandi *et al.*[110] compared gastric mucosal blood flow in ten patients with *H. pylori* positive gastritis and ten patients with *H. pylori* negative gastritis. They found that both groups had similarly decreased antral mucosal blood flow in comparison to normal stomachs. Even after eradication the blood flow did not increase to the level seen in the normal stomachs. They felt that *H. pylori* infection had no effect on gastric mucosal blood flow and that the pathogenic mechanism of *H. pylori* does not seem to be mediated by alterations in mucosal blood flow.

Atuma *et al.*[111] however came to the opposite conclusion by demonstrating that antigenic extracts from a variety of *H. pylori* sub-types lowered mucosal blood flow in rats as compared to an extract of *E. coli*. Bacterial extracts were applied to the gastric mucosa of anaesthetised rats. Blood flow was measured by means of laser Doppler flowmetry. All the *H. pylori* extracts significantly reduced gastric mucosal blood flow, whereas the *E. coli* extracts did not. They concluded that a combination of factors released from *H. pylori*, might compromise the natural defence of the gastric mucosa by reducing mucosal blood flow and so predispose to ulceration.

Taha *et al.*[100] also looked for a correlation between peptic ulceration, and *H. pylori* in his cohort of 70 patients on NSAID's. Patients infected with *H. pylori* had

significantly lower gastric and duodenal blood flow than subjects without an infection.

In our cohort, the healed group had a rate of *H. pylori* infection of 60%, and in the non-healed group the rate was 83%. However the healed ulcer group all had a higher blood flow than normal stomachs, despite the high incidence of *H. pylori* infection. It is difficult to comment on the effect of *H. pylori* on mucosal blood flow from our data. However the presence of *H. pylori* does not preclude an increase in mucosal blood flow in the injured mucosa.

6.14 REFRACTORY GASTRIC ULCERATION: THE ROLE OF LASER DOPPLER

Despite the current understanding of gastric ulcer pathogenesis and the therapeutic agents available, refractory ulceration remains a clinical problem. Despite adequate eradication and therapy, gastric ulcers remain difficult to heal.[45] In our cohort of refractory gastric ulcers eradication did not result in ulcer healing in four out of six patients. Of these four ulcers, three went on to require surgery. Refractory gastric ulceration is a cause of morbidity and mortality.

When dealing with refractory gastric ulceration a diligent search for a cause must be undertaken. Careful assessment of the efficacy of *H. pylori* eradication is essential. Absence of *H. pylori* on biopsy four to eight weeks after eradication therapy has been discontinued, reliably indicates successful eradication.[45]

NSAID use must be reliably excluded. The abuse of aspirin is a common cause of refractory and recurrent ulceration. This use is often surreptitious and therefore it

is advisable to question family members about aspirin ingestion and to assess serum salicylate levels.[112]

A higher dose of proton pump inhibitors (Omeprazole 40 mg rather than 20 mg) has been shown in uncontrolled trials to be more effective in refractory gastric ulceration.[45,113] Despite attention to these factors, refractory gastric ulcers will frequently require surgery.

The parameters commonly used to predict non-healing of gastric ulcers remain clinical and often anecdotal. These include ulcer size and position as well as the patient's compliance and ability to modify lifestyle risks. Large ulcers (>5 cm in diameter) tend to take longer to heal. Healing can however still be achieved in these patients. Size alone is therefore an inadequate predictor of healing. Ulcers in atypical positions have a higher incidence of malignancy. All gastric ulcers, but especially atypical ulcers, must be followed to healing by endoscopy and biopsy. The patients who are likely to have non-healing ulcers often have a lifestyle that prevents compliance. They are often unemployed with a strong history of alcohol and cigarette use. Attempting to alter lifestyle risk factors in these patients is difficult.

Predicting refractory ulceration may have important clinical benefits. Ulcers that are predicted to be refractory, may be placed on more intensive medical therapy at the outset (higher doses of proton pump inhibitors or combination of sucralfate with acid suppression). It may be beneficial to tailor *H. pylori* eradication therapy to the individual patient, by culturing the bacteria and determining individual

sensitivity. More intensive surveillance may be instituted (earlier follow-up endoscopy) and a greater effort may be made to encourage modification of lifestyle risks and ensure compliance. It may even be necessary to offer earlier surgery to patients who are likely to complicate.

A simple clinical tool to predict non-healing would be useful in these patients. We have identified an independent and quantifiable factor, which seems to predict non-healing of gastric ulcers. Persistently attenuated mucosal blood flow implies that the ulcer will not heal. It may be that laser Doppler has a role to play in stratifying patients with gastric ulcers into risk groups. It is too early to propose using laser Doppler measurements to make therapeutic decisions. However further prospective work to document the positive predictive value of these readings is warranted.

6.15 THE ROLE OF GROWTH FACTORS IN GASTRIC MUCOSAL HEALING

We compared systemic levels of these growth factors between patients with the non-healing gastric ulcers and those with healing gastric ulcers. This section discusses our findings with regard to the specific growth factors in light of other investigators findings.

6.16 FIBROBLAST GROWTH FACTOR

Szabo et al.[32] investigated the role of FGF in peptic ulceration. They used human bFGF, which had been stabilized to acid and pepsin by site-specific mutagenesis, to assess whether bFGF might accelerate the healing of experimental duodenal ulcers. This mutein peptide (bFGF-CS23) was administered orally to rats with

chronic cysteamine induced duodenal ulcers. This group was compared to a group with chronic cysteamine-induced ulcers which was given only cimetidine. Oral bFGF-CS23 therapy maintained for 21 days resulted in a significant acceleration of healing of the duodenal ulcers, as compared to rats undergoing therapy with cimetidine and in untreated controls. Healing of the duodenal ulcers was assessed by measuring the reduction in mean ulcer area. Complete healing with no residual ulcer was achieved in 62% of the bFGF-CS23 treated rats compared with only 7% of untreated rats. A nine-fold increase in angiogenesis in the ulcer bed was seen in the bFGF-CS23 treated group in comparison to untreated controls. A single dose of the bFGF-CS23 mutein had no effect on gastric output of hydrochloric acid or pepsin, but daily treatment for two or three weeks resulted in enhanced acid and pepsin outputs.

Folkman et al.[52] demonstrated that bFGF exists as a naturally occurring peptide in rat and human gastric and duodenal mucosa, and is also present in the bed of chronic ulcers in rats. Sucralfate binds bFGF and protects it from acid degradation. Sucralfate is angiogenic as it protects bFGF from acid breakdown so prolonging its effect. When sucralfate is administered orally to rats, it significantly elevates the level of bFGF in the ulcer bed. Cimetidine, by its capacity to reduce gastric acid, elevates bFGF in the ulcer bed by reducing breakdown of bFGF by the gastric acid.

Konutrek et al.[114] showed in rats that bFGF and the presence of an intact omentum accelerated ulcer healing and angiogenesis. Several series of rats with gastric ulcers were used: Series A with intact omentum was the control group,

Series B underwent resection of the omentum, and Series C had the omentum placed on the serosal side of the ulcer. Series A to C were divided into groups, which were treated either with a neutral vehicle, indomethacin or bFGF. Seven days post ulcer induction, the animals were anaesthetised, the gastric blood flow was determined by laser Doppler flowmetry and the ulcer area was measured. Biopsy samples of the ulcer margin were taken for determination of capillary and myofibroblast density in the granulation tissue. Attachment of omentum significantly accelerated ulcer healing, whereas omentectomy delayed this process. Laser Doppler flowmetry revealed the decrease in the gastric mucosal blood flow at the ulcer margin to be 45% and at the ulcer bed to be 18% of the value recorded in the intact adjacent mucosa. Attachment of the omentum significantly increased the blood flow at the ulcer margin and increased the number of capillaries and myofibroblasts in the granulation tissue. Indomethacin significantly delayed ulcer healing without affecting the blood flow in the ulcer area. Human recombinant bFGF did not prevent the induction of acute gastric lesions by 100% ethanol or acidified aspirin but enhanced the healing rate of acetic-acid induced gastric ulcers in rats. This enhanced healing was accompanied by an increase in the number of capillaries and myofibroblasts and in DNA synthesis in the surrounding granulation tissue.

The higher systemic concentrations of bFGF in the patients with actively healing ulcers compared to those with non-healing ulcers, probably reflects the state of mucosal blood flow in the two groups. The healing ulcers had a higher mucosal blood flow and higher systemic concentrations of bFGF than the non-healing ulcers. The high systemic concentrations of bFGF reflect the level of activity at

the gastric ulcer.

6.17 TRANSFORMING GROWTH FACTOR (TGF)

TGF-β1 is also implicated in ulcer healing.[37] Our own finding that TGF-β1 was elevated systemically in patients with healing ulcers in comparison to non-healing ulcers supports our current understanding of its role in promoting ulcer healing. TGF stimulates cell DNA synthesis, and epithelial cell migration and reduces the effect of ethanol on the gastric mucosa. Relatively higher systemic concentrations of TGF-β1 in patients with healing gastric ulcers compared to patients with non-healing ulcers can be taken as evidence that TGF-β1 plays a role in healing gastric ulcers.

6.18 EPIDERMAL GROWTH FACTOR (EGF)

Konturek *et al.* assessed the rate of cell proliferation, gastric secretion and gene expression of mRNA for EGF and TGF during ulcer healing.[27,38] They induced gastric ulcers in rats by serosal application of 100% acetic acid. In some of the rats, a gastric fistula was fashioned to enable assessment of acid secretion during ulcer healing. The animals were killed at different times after ulcer induction, and the ulcer area was determined.

The mucosal sections with gastric ulcers were immuno-stained for Proliferating Cell Nuclear Antigen (PCNA) and for immuno-expression of EGF, TGF-α, and EGFR. The expression of mRNA EGF and mRNA TGF-α was also determined in the ulcer margin by Reverse Transcriptase (RT) Polymerase Chain Reaction (PCR) using specific primers. Two, four, six, and eight days after ulcer induction

the gastric ulcer area was gradually reduced from the initial size (day 0) by 47%, 70%, 80%, and 87% respectively, and this was accompanied by an increase in PCNA with its maximum on day four.

Konturek et al.[27] showed that gastric, acid and pepsin secretion was significantly reduced two days post ulcer induction. Secretion had returned to normal by the eighth day. The expression of EGF, TGF, and EGFR was negligible initially, but increased significantly during the healing. Cell proliferation during ulcer healing seems to be mediated by an increased release of EGF and TGF. The expression of EGF and TGF and mRNA precedes the over-expression of these growth factors at the ulcer margin during ulcer healing. The over-expression of growth factors coincides with the inhibition of gastric secretion and increased blood flow at the ulcer margin. These factors all affect gastric secretion and blood flow in the course of ulcer healing. EGF is believed to inhibit gastric acid secretion by a separate mechanism to its mitogenic mechanism.

Hui et al.[115] has assessed the effect of oral EGF on gastric mucosal blood flow in rats following exposure to ulcerogenic stimulants. In this experiment blood flow, was measured by laser Doppler flowmetry. Male rats were anaesthetised and then subjected to a midline laparotomy. The stomach was exposed and the pylorus was ligated. The stomach was drawn up through an aperture in a plexiglass platform and opened. In effect this created an ex vivo stomach whilst leaving the vascular supply intact. Mucosal damage was induced by the application of pure ethanol.

Blood flow was determined at 0 and 15 minutes post exposure to ethanol. EGF or normal saline was applied to the mucosa after 30 minutes and blood flow was again determined at 45 minutes. Following the third blood flow measurement either normal saline or ethanol was applied to the mucosa. The authors showed that EGF at a dose greater than 6.25 µg decreased the size of the ethanol induced ulcer. Blood flow was significantly higher in the mucosa exposed to EGF than the controls and the ulcer size was inversely related to the level of mucosal blood flow. The authors concluded that EGF increased mucosal blood flow and that this accounted for its early gastro-protective effect.

Tarnawski et al.[116] demonstrated a 75-fold increase in the number of cells expressing EGFR in ulcerated rat gastric mucosa when compared to normals. This increase was specifically marked at the ulcer circumference. This indicated that the major target for EGF action was the ulcerated gastric mucosa.

The above data indicates that EGF plays an important role in mucosal defence and mucosal healing. We could not demonstrate a significant difference in systemic levels of EGF in the healing ulcers in comparison to the non-healing ulcers. In light of the evidence this is surprising. It may be that at the early stage at which we collected the samples, EGF expression had not been sufficiently stimulated. It may well be that an increase in EGF levels would only be detectable later in the healing process.

It seems reasonable to ascribe the elevation in the systemic concentrations of these peptides to the increased level of activity and increased gastric mucosal

blood flow which accompanies mucosal healing. The next avenue of investigation would be to compare the systemic levels of growth factors with the levels at the ulcer site.

CHAPTER 7

CONCLUSION

There is a significant regional variation in the distribution of gastric mucosal blood flow in the human stomach, with blood flow in the antrum being lower than in the fundus. This reflects the lower functional state of the antrum when compared to the fundus and corresponds with described anatomical differences in the micro-circulatory anatomy of the antrum and fundus. The lower blood flow in the antrum may contribute to the predilection of gastric ulceration in the distal stomach. The normal response of the gastric mucosa to injury is one of enhanced mucosal blood flow. We have demonstrated this in the setting of benign ulceration. The increase in gastric mucosal blood flow supports the high energy demanding process of re-epithelialisation. Failure of this response seems to inhibit mucosal healing. Low peri-ulcer blood flow implies that an ulcer will prove refractory to standard medical therapy. The elevated systemic concentrations of bFGF and TGF-β1 in patients with healing gastric ulcers in comparison to those in patients with non healing ulcers reflects the state of angiogenesis and active healing at the ulcer site. Both arms of the study enhance our understanding of gastric ulceration and healing. The rationale behind the standard approach to gastric ulcer therapy, the reduction of acid and the eradication of *H. pylori*, have been shown to be based on the concept of gastric ulceration being due exclusively to noxious luminal substances. Understanding the importance of mucosal blood flow in gastric ulceration, alerts clinicians and investigators to other potential forms of therapy such as angiogenic promoting substances. Whilst the standard therapies

for gastric ulceration are highly effective, the novel therapeutic modalities currently under investigation may in the future complement them. This may result in improved quality of ulcer healing, and succeed in eliminating the problem of refractory and complicated gastric ulceration, which is still a cause of significant morbidity and even mortality.

Our results have clinical implications in predicting healing and non-healing of gastric ulceration. It would seem that non-healing of gastric ulcers can be predicted on the basis of reduced peri-ulcer mucosal blood flow, as measured by laser Doppler flowmetry and low systemic levels of bFGF. Further work is required to determine the positive predictive value of these two factors.

CHAPTER 8

REFERENCES

1. Celsus AC. Chapter 5. In: *De Medicina*. Book IV Chapter 5 : Of the disorders of the stomach and their cure. Translated by Grieve, London: 1756;198:201.

2. Donatus M. *De medica historia mirabili*. Book 6 1586;195:8.

3. Baillie M. *The morbid anatomy of some of the most important parts of the human body.* London: J Johnson, St Paul's Churchyard. G Nichol, Pall-Mall, 1793.

4. Cruvellhier J. *Anatomie pathogique du corps humain.* Paris: JB Balliere, 1829-35.

5. Rydygier L. Ueber magen Resection mit demonstration von praparaten. *Arch Klin Chir* 1881;**26**:171.

6. Johnson HD. Aetiology and classification of gastric ulcers. *Gastroenterol* 1957;**33**:121.

7. Andersen S, Hestrup H. The Aarhus County vagotomy trial II. an interim report on reduction of acid secretion and ulcer recurrence rate following parietal cell vagotomy and selective gastric vagotomy. *World J Surg* 1978;**2**:91.

8. Madsen P, Schousen P. Long term results of truncal vagotomy and pyloroplasty for gastric ulcer. *Br J Surg* 1982;**69**:39.

9. Marks IN. Observations on the pathogenesis of gastric ulcer. *Lancet* 1959;**1**:1107-1111.

10. Du Plessis DJ. Pathogenesis of gastric ulcer. *Lancet* 1965;**1**:974.

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

12. Marshall BJ. Campylobacter pylori: its link to gastritis and peptic ulcer disease. *Rev Infect Dis* 1990;**12 Suppl 1**:S87-S93.

13. Marshall BJ. Campylobacter pyloridis and gastritis. *J Infect Dis* 1986;**153**:650-657.

14. Summary of the NIH consensus. Helicobacter pylori in peptic ulcer disease. *Md Med J* 1994;**43**:923-924.

15. NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease *JAMA* 1994;**272**:65-69.

16. Panel endorses antimicrobial therapy for ulcer disease. *Am J Hosp Pharm* 1994;**51**:1126 -1129.

17. DeCross AJ, Marshall BJ. The role of Helicobacter pylori in acid-peptic disease *Am J Med Sci* 1993;**306**:381-392.

18. Forbes GM, Glaser ME, Cullen DJ, *et al*. Duodenal ulcer treated with Helicobacter pylori eradication: seven-year follow-up. *Lancet* 1994;**343**:258-260.

19. Laine L. Helicobacter pylori, gastric ulcer, and agents noxious to the gastric mucosa. *Gastroenterol Clin North Am* 1993;**22**:117-125.

20. Borody TJ, Brandl S, Andrews P, Jankiewicz E *et al.* Helicobacter pylori-negative gastric ulcer. *Am J Gastroenterol* 1992;**87**:1403-1406.

21. Guslandi M, Sorghi M, Foppa L, Tittobello A. Brief communication: smoking and duodenal blood flow. *J Gastroenterol Hepatol* 1997;**12**:347-348.

22. Quimby GF, Bonnice CA, Burstein SH, Eastwood GL. Active smoking depresses prostaglandin synthesis in human gastric mucosa. *Ann Intern Med* 1986;**104**:616-619.

23. Sawant P, Bhatia R, Kulhalli P, Mahajani SS *et al.* Effect of a short course of indomethacin on gastric mucosal blood flow using endoscopic laser-Doppler velocimetry. *Indian Journal of Gastroenterology* 1995;**14**:137-138.

24. Vigneri S, Scialabba A, Termini R *et al.* Pathophysiology of the gastric microcirculation. *Ital J Gastroenterol* 1992;**24**:22-30.

25. Sorbye H, Svanes K. The role of blood flow in gastric mucosal defence, damage and healing. *Dig Dis* 1994;**12**:305-317.

26. Schmassmann A. Mechanisms of ulcer healing and effects of nonsteroidal anti-inflammatory drugs. *Am J Med* 1998;**104**:43S-51S.

27. Konturek PC, Brzozowski T, Konturek SJ.. Expression of epidermal growth factor and transforming growth factor alpha during ulcer healing. Time sequence study. *Scand J Gastroenterol* 1997;**32**:6-15.

28. Tepperman BL, Soper BD. Effect of epidermal growth factor, transforming growth factor alpha and nerve growth factor on gastric mucosal integrity and microcirculation in the rat. *Regul Pept* 1994;**50**:13-21.

29. Brzozowski T, Konturek SJ, Majka J, Dembinski A *et al.* Epidermal growth factor, polyamines, and prostaglandins in healing of stress-induced gastric lesions in rats. *Dig Dis Sci* 1993;**38**:276-283.

30. Goodlad RA, Wright NA. Epidermal growth factor (EGF). *Baillieres Clin Gastroenterol* 1996;**10**:33-47.

31. Brzozowski T, Konturek SJ, Drozdowicz D, Dembinski A *et al.* Healing of chronic gastric ulcerations by L-arginine. Role of nitric oxide, prostaglandins, gastrin and polyamines. *Digestion* 1995;**56**:463-471.

32. Szabo S, Folkman J, Vattay P, Morales RE *et al.* Accelerated healing of duodenal ulcers by oral administration of a mutein of basic fibroblast growth factor in rats. *Gastroenterology* 1994;**106**:1106-1111.

33. Satoh H, Shino A, Sato F *et al.* Role of endogenous basic fibroblast growth factor in the healing of gastric ulcers in rats. *Jpn J Pharmacol* 1997;**73**:59-71.

34. Szabo S, Kusstatscher S, Sakoulas G, Sandor Z *et al.* Growth factors: new 'endogenous drugs' for ulcer healing. *Scand J Gastroenterol Suppl* 1995;**210**:15-18.

35. Podolsky RS, Grabowski M, Milner R, Ritchie WP *et al.* Capsaicin-induced gastric hyperemia and protection are NO- dependent. *J Surg Res* 1994;**57**:438-442.

36. Baird A, Klagsbrun M. The fibroblast growth factor family. *Cancer Cells* 1991;**3**:239-243.

37. Gangarosa LM, Dempsey PJ, Damstrup L, Barnard JA *et al.* Transforming growth factor. *Baillieres Clin Gastroenterol* 1996;**10**:49-63.

38. Konturek PC, Ernst H, Brzozowski T, Ihlm A *et al.* Expression of epidermal growth factor and transforming growth factor- alpha after exposure of rat gastric mucosa to stress. *Scand J Gastroenterol* 1996;**31**:209-216.

39. Tepperman BL, Soper BD. Interaction of nitric oxide and salivary gland epidermal growth factor in the modulation of rat gastric mucosal integrity. *Br J Pharmacol* 1993;**110**:229-234.

40. Wright JP, Kahanovitz C, Marks IN. Gastric ulceration - a follow-up study. *S Afr Med J* 1981;**60**:611-612.

41. Wright JP, Marks IN, Mee AS *et al.* Ranitidine in the treatment of gastric ulceration. *S Afr Med J* 1982; **61**:155-158.

42. Jones DB, Howden CW, Burget DW, Kerr GD *et al.* Acid suppression in duodenal ulcer: a meta-analysis to define optimal dosing with antisecretory drugs. *Gut* 1987;**28**:1120-1127.

43. Howden CW, Jones DB, Peace KE, Burget DW *et al.* The treatment of gastric ulcer with antisecretory drugs. Relationship of pharmacological effect to healing rates. *Dig Dis Sci* 1988;**33**:619-624.

44. Yeomans ND. New data on healing of nonsteroidal anti-inflammatory drug-associated ulcers and erosions. Omeprazole NSAID Steering Committee. *Am J Med* 1998;**104**:56S-61S.

45. Soll AH. Consensus conference. Medical treatment of peptic ulcer disease. Practice guidelines. Practice Parameters Committee of the American College of Gastroenterology *JAMA* 1996;**275**:622-629.

46. Marks IN, Girdwood AH, Wright JP *et al.* Nocturnal dosage regimen of sucralfate in maintenance treatment of gastric ulcer. *Am J Med* 1987;**83**:95-98.

47. Marks IN, Wright JP, Girdwood AH, Gilinsky NH *et al.* Maintenance therapy with sucralfate reduces rate of gastric ulcer recurrence. *Am J Med* 1985;**79**:32-35.

48. Marks IN, Wright JP, Lucke W, Girdwood AH. Relapse rates after initial ulcer healing with sucralfate and cimetidine. *Scand J Gastroenterol* 1982;**17**:429-432.

49. Szabo S. The mode of action of sucralfate: the 1 x 1 x 1 mechanism of action. *Scand J Gastroenterol Suppl* 1991;**185**:7-12.

50. Korman MG, Bolin TD, Szabo S, Hunt RH *et al.* Sucralfate: the Bangkok review. *J Gastroenterol Hepatol* 1994;**9**:412-415.

51. Chen BW, Hiu WM, Lam SK, Cho CH *et al.* Effect of sucralfate on gastric mucosal blood flow in rats. *Gut* 1989;**30**:1544-1551.

52. Folkman J, Szabo S, Stovroff M, McNeil P *et al.* Duodenal ulcer. Discovery of a new mechanism and development of angiogenic therapy that accelerates healing. *Ann Surg* 1991;**214**:414-425.

53. Zoorob RJ. NIH consensus on Helicobacter pylori in peptic ulcer disease. *J Am Board Fam Pract* 1996;**9**:392.

54. Varhaug, J. E., Svanes, K., Svanes C. Gastric blood flow determination: Intramural distribution and arteriovenous shunting of microspheres. *Am J Physiol* 1984;247,G468-G479.

55. Jacobson ED, Linford RH, Grossman MI. Gastric secretion in relation to mucosal blood flow studied by a clearance technic. *J Clin Invest* 1966;**45**:1-13.

56. Jacobson ED, Swan KG, Grossman MI. Blood flow and secretion in the stomach. *Gastroenterology* 1967;**52**:414-420.

57. Tague LL, Jacobson ED. Evaluation of [^{14}C] aminopyrine clearance for determination of gastric mucosal blood flow. *Exp Biol Med* 1976;**151**:707-710.

58. Guth PH, Baumann H, Grossman MI, Aures D *et al.* Measurement of gastric mucosal blood flow in man. *Gastroenterology* 1978;**74**:831-834.

59. Archibald LH, Moody FG, Simons MA. Comparison of gastric mucosal blood flow as determined by aminopyrine clearance and gamma-labeled microspheres. *Gastroenterology* 1975;**69**:630-635.

60. Murakami M, Moriga M, Miyake T, Uchino H. Contact electrode method in hydrogen gas clearance technique: a new method for determination of regional gastric mucosal blood flow in animals and humans. *Gastroenterology* 1982;**82**:457-467.

61. Soybel DI, Ashley SW, Yan ZY, Cheung LY. Comparison of gastric mucosal blood flow as measured by H2 gas clearance and microspheres during secretory stimulation and inhibition. *Surgery* 1985;**98**:174-182.

62. Stern MD, Lappe DL, Bowen PD *et al.* Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. *Am J Physiol* 1977;**232**:H441-H448.

63. Bonner RF, Nossal R. Model for laser doppler measurement of blood flow in tissue. *Applied Optics* 1981;**20**:2097.

64. Bonner RF, Nossal R, Havlin S, Weiss GH. Model for photon migration in turbid biological media. *J Opt Soc Am [A]* 1987;**4**:423-432.

65. Kvietys PR, Shepherd AP, Granger DN. Laser-Doppler, H2 clearance, and microsphere estimates of mucosal blood flow. *Am J Physiol* 1985;**249**:G221-G227.

66. Kiel JW, Riedel GL, DiResta GR, Shepherd AP. Gastric mucosal blood flow measured by laser-Doppler velocimetry. *Am J Physiol* 1985;**249**:G539-G545.

67. Feld AD, Fondacaro JD, Holloway GAJ, Jacobson ED. Laser Doppler velocimetry: a new technique for the measurement of intestinal mucosal blood flow. *Gastrointest Endosc* 1984;**30**:225-230.

68. Gana TJ, Huhlewych R, Koo J. Focal gastric mucosal blood flow by laser-Doppler and hydrogen gas clearance: a comparative study. *J Surg Res* 1987;**43**:337-343.

69. Ahn H, Ivarsson LE, Johansson K, Lindhagen J *et al.* Assessment of gastric blood flow with laser Doppler flowmetry. *Scand J Gastroenterol* 1988;**23**:1203-1210.

70. Johansson K, Ahn H, Lindhagen J, Lundgren O. Tissue penetration and measuring depth of laser doppler flowmetry in the gastrointestinal application. *Scand J Gastroenterol* 1987;**22**:1081-1088.

71. Shepherd AP, Riedel GL. Continuous measurement of intestinal mucosal blood flow by laser doppler velocimetry. *Am J Physiol* 1982;**242** G617-G622.

72. Casadevall M, Panes J, Pique JM, Bosch J *et al.* Limitations of laser-Doppler velocimetry and reflectance spectrophotometry in estimating gastric mucosal blood flow. *Am J Physiol* 1992;**263**:G810-G815.

73. Kleen M, Habler O, Hutter J *et al.* Effects of hemodilution on gastric regional perfusion and intramucosal pH. *Am J Physiol* 1996;**271**:H1849-H1855.

74. Becker H, Hottenrott C, Seufert R, von Gerstenbergk L. Gastric blood flow in animals. Normal blood flow. *Exp Med Berlin* 1979;**176**:31-36.

75. Hottenrott C, Seufert R, Doertenbach J, Buckberg G. The influence of autonomous nervous activity on total and regional gastric blood flow. *Scand J Gastroenterol Suppl* 1984;**89**:37-39.

76. Allen PI, Chesner I, Wheatley K, Goldman M. Human gastric perfusion: evidence for non-uniformity of blood flow. *Br J Surg* 1988;**75**:741-742.

77. Kvernebo K, Lunde OC, Stranden E, Larsen S. Human gastric blood circulation evaluated by endoscopic laser Doppler flowmetry. *Scand J Gastroenterol* 1986;**21**:685-692.

78. Gana TJ, Soenen GM, Koo J. A controlled study of human resting gastric mucosal blood flow by endoscopic laser-Doppler flowmetry. *Gastrointest Endosc* 1990;**36**:264-267.

79. Kawano S, Sato N, Tsuji S *et al.* Two-dimensional computer color graphics of gastric mucosal blood distribution in normal subjects and ulcer patients *Endoscopy* 1991;**23**:317-320.

80. Barlow TE, Bentley FH, Walder DN. Arteries,veins, and arteriovenous anastomoses in the human stomach. *Surg Gynecol Obstet* 1951;**93**:657-671.

81. Gannon B, Browning J, O'Brien P, Rogers P. Mucosal microvascular architecture of the fundus and body of human stomach. *Gastroenterology* 1984;**86**:866-875.

82. Cheung LY, Ashley SW. Gastric blood flow and mucosal defense mechanisms. *Clin Invest Med* 1987;**10**:201-208.

83. Lunde OC, Kvernebo K. Gastric blood flow in patients with gastric ulcer measured by endoscopic laser Doppler flowmetry. *Scand J Gastroenterol* 1988;**23**:546-550.

84. Kamada T, Kawano S, Sato N, Fukuda M *et al.* Gastric mucosal blood distribution and its changes in the healing process of gastric ulcer. *Gastroenterology* 1983;**84**:1541-1546.

85. Sato N, Kawano S, Tsuji S, Ogihara T *et al.* Gastric blood flow in ulcer diseases. *Scand J Gastroenterol Suppl* 1995;**208**:14-20.

86. Brzozowski T, Konturek PC, Konturek SJ, Ernst H *et al.* Gastric adaptation to injury by repeated doses of aspirin strengthens mucosal defence against subsequent exposure to various strong irritants in rats. *Gut* 1995;**37**:749-757.

87. Svanes K, Gislason H, Guttu K, Herfjord JK *et al.* Role of blood flow in adaptive protection of the cat gastric mucosa. *Gastroenterology* 1991;**100**:1249-1258.

88. Starlinger M, Schiessel R, Hung CR, Silen W. Hydrogen ion back diffusion stimulating gastric mucosal blood flow in the rabbit fundus. *Surgery* 1981;**89**:232-236.

89. Ritchie WPJ, Shearburn EW. Acute gastric mucosal ulcerogenesis is dependent on the concentration of bile salt. *Surgery* 1976;**80**:98-105.

90. Szabo G, Benyo I, Sandor J. The effect of haemorrhage on gastric circulation and acid output in the dog. *Injury* 1979;**10**:190-193.

91. Sato N, Kawano S, Tsuji S, Kamada T. Microvascular basis of gastric mucosal protection. *J Clin Gastroenterol* 1988;**10 Suppl 1**:S13-S18.

92. Chung SC, Leung JW, Leung FW. Effect of submucosal epinephrine injection on local gastric blood flow. A study using laser Doppler flowmetry and reflectance spectrophotometry. *Dig Dis Sci* 1990;**35**:1008-1011.

93. Varhaug JE, Svanes K. Gastric ulceration and changes in acid secretion and mucosal blood flow after partial gastric devascularization in cats. *Acta Chir Scand* 1979;**145**:313-319.

94. Kalia N, Brown NJ, Jacob S, Reed MW *et al.* Studies on gastric mucosal microcirculation. 1. The nature of regional variations induced by ethanol injury. *Gut* 1997;**40**:31-35.

95. Kalia N, Bardhan KD. Mast cells and gastric mucosal damage: yet another mechanism of injury? *J Gastroenterol Hepatol* 1998;**13**:1081-1083.

96. Pihan G, Majzoubi D, Haudenschild C, Trier JS *et al.* Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16,16-dimethyl prostaglandin E2 or sodium thiosulfate. *Gastroenterology* 1986;**91**:1415-1426.

97. Eastwood GL. Is smoking still important in the pathogenesis of peptic ulcer disease? *J Clin Gastroenterol* 1997;**25** Suppl 1:S1-S7.

98. Hirose H, Takeuchi K, Okabe S. Effect of indomethacin on gastric mucosal blood flow around acetic acid- induced gastric ulcers in rats *Gastroenterology* 1991;**100**:1259-1265.

99. Ashley SW, Sonnenschein LA, Cheung LY. Focal gastric mucosal blood flow at the site of aspirin-induced ulceration. *Am J Surg* 1985;**149**:53-59.

100. Taha AS, Angerson W, Nakshabendi I *et al.* Gastric and duodenal mucosal blood flow in patients receiving non-steroidal anti-inflammatory drugs-influence of age, smoking, ulceration and Helicobacter pylori. *Aliment Pharmacol Ther* 1993;**7**:41-45.

101. Sawant P, Bhatia R, Kulhalli P, Mahajani SS *et al.* Effect of a short course of indomethacin on gastric mucosal blood flow using endoscopic laser-Doppler velocimetry. *Indian J Gastroenterol* 1995;**14**:137-138.

102. Iwao T, Toyonaga A, Ikegami M *et al.* Gastric mucosal blood flow after smoking in healthy human beings assessed by laser Doppler flowmetry. *Gastrointest Endosc* 1993;**39**:400-403.

103. Guslandi M, Sorghi M, Pontikaki I, Tittobello A. Gastric microcirculation and bicarbonate production in heavy smokers. *European J Gastroenterology and Hepatology* 1995;**7**:985-987.

104. Battistel M, Plebani M, Di Mario F, Jocic M *et al.* Chronic nicotine intake causes vascular dysregulation in the rat gastric mucosa. *Gut* 1993;**34**:1688-1692.

105. Panes J, Casadevall M, Pique JM, Bosch J *et al.* Effects of acute normovolemic anemia on gastric mucosal blood flow in rats: role of nitric oxide. *Gastroenterology* 1992;**103**:407-413.

106. Raybould HE, Holzer P, Thiefin G, Holzer HH *et al.* Vagal afferent innervation and regulation of gastric function. *Adv Exp Med Biol* 1991;**298**:109-127.

107. Battal MN, Hata Y, Matsuka K *et al.* Effect of a prostaglandin I2 analogue, beraprost sodium, on burn-induced gastric mucosal injury in rats. *Burns* 1997;**23**:232-237.

108. Arakawa T, Kobayashi H, Yoshikawa I, Taranawski A. Rebamipide: Overview of its mechanisms of action and efficacy in mucosal protection and ulcer healing. *Dig Dis Sci* 1998;**43**:5s-13s.

109. Oi M, Oshida K, Sugimura S. The location of gastric ulcer. *Gastroenterology* 1959;**36**:45-56.

110. Guslandi M, Sorghi M, Tittobello A. Does Helicobacter pylori affect gastric microcirculation? *Ital J Gastroenterol* 1994;**26**:383-384.

111. Atuma C, Engstrand L, Holm L. Extracts of Helicobacter pylori reduce gastric mucosal blood flow through a VacA- and CagA-independent pathway in rats. *Scand J Gastroenterol* 1998;**33**:1256-1261.

112. Hirschowitz BI, Lanas A. Intractable upper gastrointestinal ulceration due to aspirin in patients who have undergone surgery for peptic ulcer. *Gastroenterology* 1998;**114**:883-892.

113. Bardhan KD, Naesdal J, Bianchi PG *et al*. Treatment of refractory peptic ulcer with omeprazole or continued H2 receptor antagonists: a controlled clinical trial *Gut* 1991;**32**:435-438.

114. Konturek SJ, Brzozowski T, Majka I, Pawlik W *et al*. Omentum and basic fibroblast growth factor in healing of chronic gastric ulcerations in rats. *Dig Dis Sci* 1994;**39** :1064-1071.

115. Hui WM, Chen BW, Kung AWC, Cho CH *et al*. Effect of epidermal Growth Factor on gastric blood flow in rats: Possible role in mucosal protection. *Gastroenterology* 1993;**104**:1605-1610.

116. Tarnawski A, Stachura J, Durbin T, Sarfeh IJ *et al*. Increased expression of epidermal growth factor receptor during gastric ulcer healing in rats. *Gastroenterology* 1992;**102**:695-698.

117. Malan. The application of laser velocimetry and hot-wire anemometry to phase-locked flows in turbomachinery.1984 *Dissertation for degree of Master of Science: University of Natal.*

118. Loubser R. Comparison of laser Doppler velocimetry and pitot-static flow measurement techniques used in turbomachines.1987 *Dissertation for degree of Master of Science: University of Natal.*

119. Belcaro GV, Labropoulos N, Nicolaides AN. Appendix 1 Technical characteristics of laser Doppler flowmeters. In: *Laser Doppler.* London, Los Angeles, Nicosia: Med-Orion Publishing Company. 1994;272-274.

APPENDIX 1

AVERAGE DATA

MINUTE AVERAGES FOR FLOW IN NORMAL STOMACHS

NORMAL STOMACHS
FlOW ml/min/100g

NORMAL STOMACHS
FLOW ml/min/100g

ANTRUM	INCISURA	FUNDUS	ANTRUM	INCISURA	FUNDUS
19.7	109.4	35.3	88.7	89.9	80
13.9	3.2	91.2	62.1	95.6	43.3
59.7	75	42.6	86.4	87.3	61.1
14.9	61	61.8	61.3	86	52.6
38.9	26.9	99	38.4	63	30
58.8	50.9	122	80	58.6	109.7
40.3	75.1	122	83.3	64.1	97.5
37.8	26	47.9	88.7	41	52.2
35.8	73.8	77.3	62.1	46.2	56.7
16.8	49.7	142.3	86.4	51.5	74.4
42.4	113.1	3.1	74.6	38.1	85.1
86.2	111.6	76.8	47	1.9	126.8
31.1	81	110.9	76.6	46.5	101.8
21.7	37.6	56.2	44.3	82.7	67
45.4	131.1	56.7	64.4	93.1	100.2
31.9	73.7	74.4	54.8	111.4	77.1
39.3	84.1	62.4	53.9	60	91.6
96.8	113.1	91	36.6	85.4	67.1
57.8	91.6	42.9	14.9	97.8	78.6
32.8	120.9	87.9	44.3	72.7	80
13.9	30.3	25.1	35.1	97.5	19.7
59.7	87.6	61.2	15.7	102.4	95.5
14.9	23	66.1	57.1	81.8	37.8
38.9	87.7	62.5	93.4	80.3	65.8
86.7	51.5	51.9	53.2	67.9	51.4
96	1.9	64.2	118.8	64.3	2.8
55.2	60	117.2	80.7	85.6	108.9
89.4	127	108.5	66.5	99	99
60.4	54.7	99	29.7	77.8	68.2
52.4	14.9	106.2	74.3	26.6	113.4
80	64.3	79.6	88.3	97.2	92.2
40.7	85.6	122	110.1	61	45.7
81.2	75.3	111.6	60	50.2	45.2
68	62.6	76	100.5	16.5	80.4
49.4	90	34.4	80.4	1	111.9
123.1	31.6	32	34.3	56.2	117.6

MINUTE AVERAGES FOR FLOW IN NORMAL STOMACHS contd

NORMAL STOMACHS FlOW ml/min/100g			NORMAL STOMACHS FLOW ml/min/100g		
ANTRUM	INCISURA	FUNDUS	ANTRUM	INCISURA	FUNDUS
25	34.4	68.2	66.1	68.7	76.8
29.1	68.5	92.2	68.1	88.5	111.6
40.7	75	1.9	35.4	87.7	95.1
104.2	63.2	76.8	85.1	51.5	58.1
61.8	83.5	88	62	93.1	79.9
105.1	62.5	63.8	64.6	111.4	58.8
34.7	75.7	63.5	74.9	60	63.8
41.3	113.8	69.5	66.5	73.6	25.1
32.9	73.2	56.6	81.3	31.2	78
68.8	73.6	92.1	91	98.6	99.4
35	71.5	74.2	65	91.5	84.5
50.4	38.1	17.6	97.9	71.5	72.1
52	1.9	116.5	64.3	75.9	56.2
52	46.5	38.1	74.3	71.7	72.1
56.7	82.7	94.6	97.7	64.4	50.3
111.8	44.1	3.1	61.8	83.5	88

MINUTE AVERAGES FOR VELOCITY IN NORMAL STOMACHS

NORMAL STOMACHS VELOCITY mm/sec			NORMAL STOMACHS VELOCITY mm/sec		
ANTRUM	INCISURA	FUNDUS	ANTRUM	INCISURA	FUNDUS
2.4	5.5	5.0	6.6	5.6	6.5
1.5	3.9	3.8	3.7	5.6	5.1
2.0	4.5	3.6	4.6	8.2	4.5
3.9	5.6	4.2	4.5	3.4	4.8
1.7	4.4	4.3	1.9	5.6	1.8
3.3	4.1	10.7	5.8	5.4	4.1
3.4	4.5	10.7	6.1	5.3	5.3
4.2	3.7	3.6	6.6	3.7	3.2
4.7	6.0	7.0	3.7	4.7	3.8
4.9	3.9	6.2	4.6	3.1	5.6
2.5	6.2	0.5	2.9	3.3	6.1
3.2	5.8	3.3	3.1	0.5	5.0
6.0	4.7	6.0	7.0	2.7	3.4
3.3	3.7	4.6	2.2	6.3	4.3
1.9	5.6	3.8	4.5	7.8	5.0
2.6	3.2	5.6	5.3	5.9	4.9
4.0	5.0	4.7	4.7	4.0	4.1
2.2	6.2	5.3	3.1	5.5	3.7
5.1	5.6	3.2	1.2	4.1	4.2
4.6	7.1	5.6	2.2	4.9	6.5
1.8	2.7	2.9	3.0	4.4	3.2
2.0	5.8	3.3	0.8	5.2	6.2
3.9	3.0	3.8	3.2	4.7	6.1
1.7	5.9	4.7	5.6	3.7	5.0
3.3	3.1	3.1	3.9	2.8	4.1
5.3	0.5	3.2	7.4	4.1	1.6
6.8	4.0	6.9	4.1	7.6	4.8
3.8	8.7	6.5	4.1	5.4	7.3
5.6	4.4	4.3	1.9	5.8	4.2
4.5	1.4	6.5	5.8	5.0	7.1
3.1	4.1	6.4	4.5	5.0	3.7
5.8	7.6	10.7	3.4	4.3	3.4
4.1	4.1	5.8	5.6	4.0	3.5
2.7	3.7	3.7	5.4	0.7	5.6
2.6	6.0	2.8	4.4	1.7	5.3
2.8	3.2	2.7	2.1	5.4	7.5
4.2	3.6	4.2	3.5	4.7	3.3
2.8	4.7	3.7	4.2	6.6	7.1
2.4	4.5	1.0	3.7	5.9	7.7
2.1	2.2	0.5	3.7	6.4	5.5

MINUTE AVERAGES FOR VELOCITY IN NORMAL STOMACHS CONTD

NORMAL STOMACHS VELOCITY mm/sec			NORMAL STOMACHS VELOCITY mm/sec		
ANTRUM	INCISURA	FUNDUS	ANTRUM	INCISURA	FUNDUS
5.5	4.8	3.3	6.5	3.1	4.3
5.1	4.5	4.7	3.2	3.3	3.6
3.9	5.0	3.2	4.2	0.5	5.6
5.0	5.2	4.7	4.2	2.7	3.6
4.9	4.6	3.8	2.0	6.3	5.9
4.8	4.7	4.1	4.2	7.8	4.8
2.6	3.7	6.8	1.3	5.9	3.1
3.8	5.3	4.5	3.6	4.0	5.4
2.9	9.8	3.8	5.0	3.9	2.8
2.9	4.2	3.2	4.6	4.8	6.2
1.4	4.1	5.6	4.6	4.5	5.9
5.2	4.5	6.2	6.0	3.4	3.4
3.1	4.0	3.5			

MINUTE AVERAGES FOR VOLUME IN NORMAL STOMACHS

NORMAL STOMACHS VOLUME BY %			NORMAL STOMACHS VOLUME BY %		
ANTRUM	INCISURA	FUNDUS	ANTRUM	INCISURA	FUNDUS
7.5	11.3	5.7	4.9	9.0	5.7
4.7	4.8	8.3	6.1	10.1	5.3
2.9	7.2	4.5	12.5	6.3	11.6
8.9	3.9	11.2	7.9	20.4	7.8
2.3	3.5	23.1	6.1	4.0	7.8
10.2	3.2	6.5	12.5	3.7	12.0
16.9	7.2	6.5	12.6	5.1	9.3
3.6	3.7	4.3	4.0	9.7	6.6
5.4	3.9	4.6	14.5	6.5	8.2
1.1	8.4	12.1	3.4	6.8	5.0
7.9	16.7	10.2	4.8	6.8	6.4
1.6	8.5	5.3	7.3	0.7	4.8
4.7	7.7	5.7	1.8	7.7	15.2
5.8	3.8	5.2	14.5	4.9	9.1
4.3	11.5	4.8	5.3	6.1	14.9
5.3	15.3	12.3	3.4	9.3	6.8
4.2	7.4	4.4	6.2	5.9	26.9
4.0	16.7	8.2	8.1	13.5	9.6
8.7	13.7	9.1	2.7	6.3	11.3
3.8	13.6	7.4	11.8	9.6	5.7
20.9	3.9	7.7	13.8	7.0	3.0
6.8	10.6	11.8	7.1	13.5	7.6
23.0	2.8	7.6	10.4	7.5	1.8
2.4	7.6	7.9	7.8	17.7	5.3
8.5	6.8	23.1	6.5	10.3	3.8
13.9	5.9	8.3	8.6	6.8	0.4
12.6	8.2	8.2	4.2	5.1	4.2
14.2	3.3	6.5	9.6	8.2	5.0
2.9	2.9	8.5	5.6	5.2	8.1
6.4	5.1	17.0	5.6	3.0	9.0
17.4	8.7	4.2	10.5	12.1	4.5
17.8	9.0	3.8	10.5	6.4	10.9
11.2	7.7	8.1	15.5	4.3	7.8
3.7	5.0	0.6	4.5	2.6	5.2
4.8	3.5	0.5	2.3	0.1	19.9
10.6	6.0	10.2	9.0	5.4	6.3
4.0	7.2	5.7	8.0	5.0	10.2
4.1	5.5	10.2	5.8	8.7	11.4
4.8	9.0	5.7	9.9	7.6	7.6
13.8	6.6	5.3	9.6	8.6	7.5

MINUTE AVERAGES FOR VOLUME IN NORMAL STOMACHS contd

NORMAL STOMACHS VOLUME BY %			NORMAL STOMACHS VOLUME BY %		
ANTRUM	INCISURA	FUNDUS	ANTRUM	INCISURA	FUNDUS
7.3	7.7	5.6	7.9	7.7	6.0
4.2	5.4	7.7	1.6	4.9	6.0
4.4	4.4	5.5	4.7	6.1	8.7
4.5	6.9	4.8	11.1	9.3	9.0
5.3	8.8	9.8	2.5	5.9	4.1
3.3	9.0	6.5	4.1	10.8	8.0
9.5	5.4	10.2	6.2	2.2	3.7
9.5	5.6	4.5	3.6	16.3	9.8
6.5	12.1	8.0	2.9	9.0	8.0
6.2	5.5	5.7	6.5	3.0	6.7
7.4	6.8	4.6	8.0	5.0	10.3
4.7	6.8	2.0	7.5	10.1	4.4
1.1	6.1	11.1			

GASTRIC ULCER MUCOSAL HAEMODYNAMIC MEASUREMENTS

	Unhealed Ulcers: Initial Readings				Unhealed Ulcers: Follow-up Readings		
	Flow	Volume	Velocity		Flow	Volume	Velocity
	ml/m/100 g	By %	mm/sec		ml/m/100g	By %	mm/sec
Patient 1	45.7	11.5	2.3	Patient 1	46.5	6.8	3.1
Patient 2	20.9	4.8	1.1	Patient 2	41.0	6.9	2.3
Patient 3	55.3	5.7	4.0	Patient 3	45.7	7.0	2.7
Patient 4	81.0	6.9	5.6	Patient 4	35.4	7.5	2.1
Patient 5	24.5	2.3	3.3	Patient 5	28.7	5.0	1.9
Patient 6	44.0	6.0	2.0	Patient 6	38.5	3.6	3.9
Average	45.2	6.2	3	Average	39.2	6.1	2.6
SD	21.9	3	1.6	SD	6.7	1.4	1

	Healed Ulcers: Initial Readings				Healed Ulcers: Follow-up readings		
	Flow	Volume	Velocity		Flow	Volume	Velocity
	ml/m/100g	By %	mm/sec		ml/m/100g	By %	mm/sec
Patient 1	77.1	11.9	4.5	Patient 1	90.4	8.3	5.0
Patient 2	93.7	11.5	4.1	Patient 2	54.0	7.9	2.6
Patient 3	53.7	7.0	3.7	Patient 3	58.6	7.2	2.7
Patient 4	54.1	6.9	3.4	Patient 4	67.3	9.9	4.2
Patient 5	84.6	6.3	5.8	Patient 5	86.7	8.4	4.9
Patient 6	98.3	9.6	6.2	Patient 6	67.4	6.4	4.6
Patient 7	77.0	16.0	3.0	Patient 7	73.9	6.5	5.1
Patient 8	97.2	5.0	5.5	Patient 8	58.3	5.4	5.4
Patient 9	60.1	9.0	3.5	Patient 9	55.4	6.4	3.8
Patient 10	61.3	12.6	2.8	Patient 10	85.4	4.8	5.9
Average	75.7	9.6	4.2	Average	69.8	7.1	4.4
SD	17.5	3.4	1.2	SD	13.7	1.5	1

	Healed Ulcers: Initial readings only				Healed Ulcers: Follow-up Readings only		
	Flow	Volume	Velocity		Flow	Volume	Velocity
	ml/m/100g	By %	mm/sec		ml/m/100g	By %	mm/sec
Patient 1	61.2	7.7	3.8	Patient 1	59.4	5.9	3.2
Patient 2	73.6	11.6	3.9	Patient 2	55.4	6.4	3.9
Patient 3	70.7		4.5	Patient 3	61.2	4.0	4.3
Patient 4	77.2	11.9	4.5	Patient 4	113.4	4.3	5.3
Patient 5	71.2	8.1	4.1				
Patient 6	82.7	9.0	3.9				
Average	72.8	9.7	4.1	Average	72.4	5.2	4.2
SD	7.2	1.9	0.3	SD	27.4	1.2	0.8

APPENDIX 2
LASER DOPPLER

THE PRINCIPLES AND THEORY OF LASER DOPPLER

Johann Christiaan Doppler in 1843 predicted that the pitch of sound of an approaching vehicle would be higher than that of a retreating vehicle. This effect was christened the Doppler shift in his honour. Any particles with a wave motion will exhibit this phenomenon. Laser produces intense, concentrated and highly parallel beams of coherent light and allows for the detection of velocities that would be too small for detection by white light. Laser Doppler was initially applied to the field of fluid mechanics.[117,118] Realising, the usefulness of laser Doppler in measuring fluid flow involving particles suspended in a flow-stream, Stern *et al.*[62] and Bonner and Nossal[63] applied it to the study of moving red cells in tissue. Laser Doppler can derive a value for blood flow, based on the detected reflected laser spectrum.

A laser source generates a laser beam, which irradiates the mucosal surface under investigation. The tissue matrix surrounding the blood vessels is considered to be stationary and is a strong diffuser of light. This ensures that the moving red cells are illuminated by a spatially distributed source. The Doppler shift arises only from interactions between red cells and photons. The reflected light consists of Doppler shifted light and unshifted light. The randomisation of the incident light prior to interaction with the red cells and the effect of multiple scatterings with red cells, preclude an exact calculation of the shape of the Doppler spectrum.

The final spectrum is a superimposition of contributions from red cells moving with multiple velocities, and light which has been randomly scattered by stationary tissue. The wavelength of this spectrum is proportional to the velocity of the red cell and the cosine of the angle between the direction of the red cell motion and the scattering vector. For viscous flow in a fine network, this wavelength should scale in proportion to red cell flow. The amplitude of the Doppler signal will scale in proportion to the number of red cells in the flow field. As multiple collisions are expected, this is not a linear relationship. The major assumption made in applying laser Doppler to biological models is that red cell flow is laminar and that vascular geometry remains constant. It is generally accepted that these variations are not significant enough to affect the readings.

The reflected photocurrent is passed through a low-pass filter, which is just wide enough to pass the entire Doppler spectrum. This signal consists of the Doppler signal and a noise component. Limiting the wavelength of the filter reduces the noise component without affecting the signal. Doppler flow (F) can be defined as the root-mean-square of the wave-length of the Doppler signal.

$$F = \sqrt{\int f\omega^2 P(\omega)d\omega}$$

In this equation, $P(\omega)$ is the power spectrum of the Doppler frequency. The Doppler signal is proportional to F^2.

DERIVATION OF THE DOPPLER EQUATION

The signal consists of a Dopper shifted component and a noise component. The noise component is proportional to the mean photocurrent and is white noise with an infinite wavelength and is independent of flow.

Thus $I = id+in$

I is the combined Doppler signal intensity.

Id = Doppler signal

In = noise component

The spectral power of the combined signal is the sum of the Doppler signal and the noise.

Thus $S(\omega) = p(\omega)+\alpha I; \omega < B$

In this equation, α is a constant, that depends on the gain of the phototube, I is the mean photocurrent and B is the wavelength of the filter. By differentiating the signal at this point, the power spectrum $S(\omega)$, is multiplied by ω^2. The output of the root-mean-square detector, applied to this spectrum is the square root of the total integrated power in the spectrum.

Thus, $R = \sqrt{\int \omega^2 S(\omega)d\omega} = \sqrt{\int \omega P(\omega)d\omega + I\alpha \int \omega^2 d\omega}$

The first term under the second square root sign is the flow parameter defined by $F = \sqrt{\int \omega^2 P(\omega)d\omega}$, whilst the second term can be integrated directly to give sI,

where s is a constant dependent on the gain of the photodetector and its processing bandwidth. The final result is

$R = \sqrt{F^2} + sl$

This can be re-arranged to

$F = \sqrt{R} - sl$

Dividing the flow parameter by the mean photocurrent will normalize the unit intensity and give the final equation.

$F = \sqrt{(R^2 - sl)}\backslash l$

F = Flow

R = output of the root-mean-square detector, which captures the combined signal.

I = mean photocurrent.

S is a constant, which depends on the gain of the photomultiplier.

Division by I normalizes the flow parameter. This ensures that it is independent of the intensity of the laser and total reflectivity of the tissue.

The frequency of the spectrum is proportional to the velocity of the scattering particles (red cells) and amplitude is proportional to the number of the scattering particles.

LASERFLO BLOOD PERFUSION MONITOR®

The LASERFLO Blood Perfusion Monitor® is unique in that it measures the three parameters of blood volume, blood velocity and blood flow.[119]   Blood volume is derived from the mean number of Doppler scatterings per photon, which is derived from the proportion of detected photons that have undergone a Doppler shift. The instruments signal processor scales this result to provide an indication of blood volume in units of percent tissue haematocrit. Velocity is calculated by computing the first moment of the Doppler spectrum and then scaling this spectral moment to give an average velocity in mm/sec. The signal processor computes the product of the velocity and volume parameters to indicate local tissue blood flow in units of ml/minute/100g.