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Studies Of The Luminal Environment
Of The Gastro-Oesophageal Junction

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April 2011
DECLARATION

I declare that the work contained within this thesis is my own original work unless stated otherwise.

Chapters 4, 5 and portions of chapter 6 have been published as papers.

Sister Wirz assisted me during my clinical studies in her role as research sister in the GI investigation laboratory

Dr Manning and Dr Gillen provided technical advice on aspects of oesophageal manometry and acid secretion while I was setting up my studies.

Dr Seenan provided assistance in extracting numerical data from the pH analysis software in our laboratory.

Dr Alcorn and Dr Ballantyne gave radiological advice and performed our radiology studies.

Professor McColl provided expert advice and guidance in writing the published manuscripts in his role as my research supervisor.

This work has not been previously submitted for a higher degree.
ACKNOWLEDGEMENTS

I would like to thank a number of people who made it possible for me to complete this body of work.

I would like to thank all the staff at Gartnavel and the Western who directly helped me carry out the work reported in the thesis. This includes Sister Angela Wirz who provided a combination of experience and enthusiasm in supporting me in my research. I would like to thank all the staff at the endoscopy unit who helped me to carry out the procedures required for the studies and also the team at the radiology department who were vital in providing the radiographs and barium studies required.

No study can be successful without administrative support and this was provided by Mrs Janice Arnott and Mrs Dorothy Ronney, both of whom helped me enormously.

I would like to give particular thanks to my clinical collaborators in the radiology department, Dr Stuart Ballantyne and Dr Des Alcorn who were always available for advice regarding radiological issues and performed the barium studies for this thesis.

Commercial support was also appreciated through frequent discussions with the team at Synectics in designing the bespoke catheter and also financial support in the form of an unrestricted grant from Astra-Zeneca, Sweden.

I would like to reserve my greatest appreciation to Professor K.E.L. McColl who was a constant source of inspiration and provided unlimited support in my efforts to complete these studies.

I would also like to thank the members of Professor McColl’s research team and collaborators who provided me with invaluable advice and support including Dr Jonathan
Manning, Dr Jack Winter, Dr Mohammed Derkashan, Dr Jonathan Fletcher, Dr Derek
Gillen and Dr John Paul Seenan.

I would finally like to thank my wife, Rachel and my children, Ben, Rebecca and Emily
for supporting me through my studies.
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GORD  Gastro-oesophageal reflux disease
BMI   Body mass index
TLOSR Transient lower oesophageal sphincter relaxation
N2O3 Dinitrogen trioxide
NO+ Nitrosyl cation
NOSCN Nitrosyl thiocyanate
L-NMMA NG-monomethyl-L-arginine
NO Nitric oxide
5-HT3 5-hydroxytryptamine
HPZ High pressure zone
SCJ Squamo-columnar junction
LIST OF PUBLICATIONS

Papers

Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KE.

Severe reflux disease is associated with an enlarged unbuffered proximal gastric acid pocket. *Gut* 2008;57:292-297

Clarke AT, Wirz AA, Seenan JP, Manning JJ, Gillen D, McColl KEL.

Paradox of gastric cardia: it becomes more acidic following meals while the rest of stomach becomes less acidic. *Gut* 2009;58(7):904-9

Abstracts

Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KE.

Oesophagitis Is Associated with Enlarged Unbuffered Postprandial Acid Pocket. *Gut* 2006;55:A19

Clarke AT, Wirz AA, Manning JJ, Ballantyne SA, Alcorn DJ, McColl KE.

Esophagitis Is Associated with Enlarged Unbuffered Postprandial Acid Pocket.

*Gastroenterology* 2006;130(4); Supplement 2;M2025
Clarke AT, Wirz AA, Seenan JP, Manning JJ, Gillen D, McColl KEL.

Dynamics of Unbuffered Postprandial Acid Pocket and Role in Acidic Gastro-oesophageal Reflux. *Gut* 2007;56;A9

Clarke AT, Wirz AA, Seenan JP, Manning JJ, Gillen D, McColl KEL.

Dynamics of Unbuffered Postprandial Acid Pocket and Role in Acidic Gastroesophageal Reflux. *Gastroenterology* 2007;132(4); Supplement S1; S1906
SUMMARY

The first chapter of my thesis “The Gastro-oesophageal Junction” discusses the histology of the gastro-oesophageal junction and the components integral to the function of the anti-reflux barrier. It also discusses the pathology found at the gastro-oesophageal junction and describes the nitrite chemistry in this region thought to contribute to this pathology.

The second chapter “Mechanisms of Gastro-oesophageal Reflux” discusses the mechanisms of gastro-oesophageal reflux. This includes factors that reduce lower oesophageal pressure, the role of the hiatal hernia and the contribution of transient lower oesophageal sphincter relaxations. The relationship between obesity and reflux disease is also discussed.

The third chapter “Research into the Nature of the Acid Pocket” details previous research into the nature of the acid pocket, the primary focus of my own studies.

The fourth chapter “Severe Reflux Disease is Associated with Enlarged Unbuffered Proximal Gastric Acid Pocket” details my studies comparing the postprandial acid pocket in healthy subjects and patients with severe reflux disease and my attempt to define its position relative to anatomical and manometric landmarks. 12 healthy subjects and 16 patients with severe reflux disease were studied. While fasted, a station pull-through was performed using a combined dual pH and manometry catheter. Position was confirmed by radiological visualisation of endoscopically-placed radio-opaque clips. The pull-through study was repeated 15 minutes after a standardized fatty meal. Barium meal examination was performed before and following the meal. A region of unbuffered acid (pH≤2) immediately distal to the proximal gastric folds was more frequent in reflux
patients (23/32 studies) than in healthy subjects (11/24) (p<0.05). This unbuffered acid pocket was longer in the reflux patients versus healthy subjects (median length 3cm, range 1cm to 15cm vs. 2 cm, range 1cm to 5cm; p<0.05). The acid pocket extended proximally as far as the proximal gastric folds in the patients but stopped a median of 1.1cm distal in healthy subjects (p=0.005). In healthy subjects the acid pocket occupied the distal portion of the sphincter which opened postprandially, whereas in reflux patients it corresponded to the proximal displacement of the gastric folds i.e. hiatus hernia.

The fifth chapter “Paradox Of Gastric Cardia – It Becomes More Acidic Following Meals While The Rest Of Stomach Becomes Less Acidic” details stationary pH studies of the cardia in healthy subjects. The proximal cardia region of the stomach has a high incidence of inflammation, metaplasia and neoplasia. It demonstrates less acid buffering following meals than the more distal stomach. I employed novel high definition pHmetry to investigate acidity at the cardia under fasting conditions and in response to a meal. 15 healthy subjects were studied. A custom made 12 electrode pH catheter was clipped at the squamo-columnar junction with 4 electrodes recording proximal to and 8 distal to the squamo-columnar junction. The most distal pH electrode was located at the catheter tip and 9 electrodes in the region of the squamo-columnar junction were 11mm apart. The electrode situated in the cardia 5.5mm distal to the squamo-columnar junction differed from all other intragastric electrodes during fasting in recording minimal acidity (pH<4 = 2.2%) while all other intragastric electrodes recorded high intragastric acidity (pH<4 = >39.%) (p<0.05). The cardia also differed from the rest of the stomach showing a marked increase in acidity in response to the meal (from 2.2% fasting to 58.4% at 60-70min after meal; p<0.05) while the electrodes distal to the cardia all showed a marked
decrease in acidity (p<0.05). These changes in acidity at the cardia following the meal caused the gastric acidity to extend 10mm closer to the squamo-columnar junction.

The final discussion chapter discusses the results of our studies and my conclusions. Papers concerning the acid pocket since my own work are also discussed.

My studies had full approval from the West Ethics Committee and North Glasgow Trust (COREC Reference 04/50709/26).
Chapter 1

The Gastro-oesophageal Junction
Histology of the Gastro-oesophageal Junction

The oesophagus is a muscular tube of variable length (18 to 26cm) extending from the pharyngo-oesophageal junction at C5/C6 down through the posterior mediastinum to the gastro-oesophageal junction at T10. The oesophagus at the gastro-oesophageal junction passes through an elliptical opening in the diaphragm called the hiatus and the oesophagus also has a short intra-abdominal component of variable length (0.5 to 2.5cm).

The hiatus through which the oesophagus passes has muscular borders formed by the diaphragmatic crura. The diaphragm has two components of separate embryological origin – the costal diaphragm, originating from myoblasts in the lateral body wall and the crural portion which develops from the dorsal mesentery of the oesophagus (1). The left and right crural fibres arise from the first 4 lumbar vertebrae, intervertebral discs and anterior longitudinal ligament. They pass upward and anteriorly around either side of the oesophagus to from the muscular borders of the hiatus and insert into the transverse ligament of the central tendon of the diaphragm. A membrane of collagen and elastic fibres extends from the hiatal border to the circumference of the oesophagus at points above and below the hiatal orifice (the phreno-oesophageal ligament)(2;3).

The oesophagus is lined by stratified squamous epithelium and contains mucous glands in the lamina propria and mixed mucous/serous glands in the submucosa. The gastric mucosa is characterised by mucinous columnar epithelium and the type of gland found in the deep mucosa depends on the portion of the stomach. Pure oxyntic glands are found in the corpus region of the stomach and pure mucous glands found in the antrum and pyloric region. A mixture of oxyntic and mucous glands are found in the region between the corpus and antrum (4). The anatomical transition from oesophageal squamous-type
mucosa to gastric type mucosa is defined by an irregular line termed the Z-line or squamo-columnar junction. Immediately distal to the Z-line is a region of the gastro-oesophageal junction called the cardia.

There has been recent interest in the extent and histological nature of the gastric cardia. Its historical definition has been a zone of mucous gland containing epithelium (similar to antral mucosa) in the distal 2 cm of the oesophagus and the proximal stomach between squamous epithelium and acid-producing oxyntic mucosa (5). The term has also been applied to the anatomical area at the most proximal extent of the stomach distal to the z-line without explicit histological definition. There is controversy over the origin of cardia mucosa with some authors believing this is an acquired type of mucosa whereas other groups maintain that it is present from birth and have used foetal/paediatric studies to support this hypothesis. Derdoy et al (6) reported the results of 100 paediatric autopsy studies of the gastro-oesophageal junction. Cardia mucosa was identified in all 100 samples with a mean length of 1mm (range 0.1mm to 3mm) and they suggested that the normal gastro-oesophageal junction has a short cardia-type mucosal region present from birth. Kilgore et al (7) reported on 30 consecutive paediatric autopsies and found cardia mucosa present in all samples on the gastric side of the gastro-oesophageal junction (mean length 1.8mm; range 1 to 4mm) and studies by Glickman et al (8) found either pure mucous glands (81%) or mixed mucous/oxyntic glands (19%) within 1mm of the squamo-columnar junction in 74 paediatric autopsy cases. Interestingly, the Glickman study also found that the presence of active oesophagitis and cardia inflammation correlated with the length of pure mucous gland-type mucosa measured suggesting a role for acid-mediated damage in propagating the length of cardia observed. De Hertogh et al
reported on their series of embryonic, foetal and neonatal autopsies demonstrating the development of cardia mucosa during pregnancy although they found that this region was very short at birth (0.3 to 0.6mm in length). An excellent paper by Chandrasoma et al (10) studied the hypothesis that the cardia mucosa may be acquired. In their adult autopsy studies of gastro-oesophageal junction mucosa, they classified mucosa into five types (i) stratified squamous epithelium, (ii) pure oxyntic mucosa characterized by glands composed only of parietal and chief cells, (iii) pure cardiac mucosa characterized by glands composed only of mucous cells, (iv) oxyntocardiac mucosa characterized by glands containing a mixture of mucous cells and parietal cells, and (v) intestinal mucosa characterized by goblet cells. They found that pure cardia mucosa was absent in 56% of cases studied. All cases had oxyntocardiac mucosa present at some point of the circumference of the junction but in 50% of cases at least one longitudinal section showed absence of both cardia and oxyntocardia mucosa, with the squamous epithelium transitioning directly to pure oxyntic mucosa. The length of cardia plus oxyntocardia mucosa was less than 5mm in 76% of cases with only 4% of cases measuring over 10mm (far shorter than the previous definition of 2 cm by Hayward et al (5). The also noted a tendency for cardia presence and extent to correlate with increasing age and the finding of associated cardia inflammation led them to postulate the role of gastro-oesophageal reflux in the histological manifestation of acquired cardia mucosa.

The role of inflammation in the development of cardia mucosa, carditis and intestinal metaplasia were studied in a paper by Oberg et al (11). They studied biopsies of the gastro-oesophageal junction from 344 consecutive patients with foregut symptoms in conjunction with oesophageal 24 hour pH and manometry studies. They found cardiac
epithelium in 73.7% of patients and its presence was strongly associated with hallmarks of gastro-oesophageal reflux disease i.e. increased oesophageal acid exposure, presence of hiatal hernia, structural abnormality in the lower oesophageal sphincter and erosive oesophagitis. When cardia epithelium was present, evidence of inflammation was present in 96% of these patients and carditis was associated with reduced pressure and length of the high pressure zone. Specialized intestinal metaplasia was found in 11.7% of patients with cardia epithelium and always occurred in the presence of cardia inflammation. They postulated that the development of cardia mucosa, carditis and intestinal metaplasia are early manifestations of gastro-oesophageal reflux disease in a healthy appearing oesophagus. This mechanism has been postulated by several other groups suggesting that the repeated and frequent exposure of squamous epithelium of the distal oesophagus to acid gastric juice leads to damage, metaplasia and transformation to columnar-type mucosa with proximal extension of the cardia region (4;12-14).

A second mechanism for development of cardia mucosa has also been postulated through extension of its distal margin secondary to H.pylori mediated gastritis. H.pylori gastritis produces progressive atrophy of the gastric mucosa with loss of parietal and chief cells and replacement with “antral-type” mucosa which resembles that of the cardia. It has been postulated that such atrophy of oxyntic mucosa immediately distal to established cardia mucosa could explain progressive enlargement of the cardia region with increasing age (15-17).
The Structure And Physiology Of The Anti-Reflux Barrier

The primary role of the gastro-oesophageal junction is to facilitate the passage of ingested boluses during digestion in coordination with peristalsis but at other times to maintain an effective barrier to reflux of potentially harmful gastric contents (an anti-reflux barrier). This antireflux barrier has six components integral to its function i) the intrinsic lower oesophageal sphincter/high pressure zone ii) the extrinsic crural sphincter iii) the intra-abdominal location of the high pressure zone iv) integrity of the phrenoesophageal ligament v) maintenance of the acute angle of His and vi) the gastrooesophageal “flap valve”(18;19).

The studies reported in this thesis report the lower oesophageal sphincter as the high pressure zone although the two terms are interchangeable.

The high pressure zone, as mentioned above, comprises two components: the true intrinsic sphincter in the distal oesophagus and the crural portion of the diaphragm which has extrinsic sphincteric properties (20). The high pressure zone has dynamic properties to prevent reflux in a variety of situations including swallowing, recumbency and abdominal straining (21).

i) Intrinsic Lower Oesophageal Sphincter

The intrinsic lower oesophageal sphincter is situated in the distal oesophagus and measures approximately 3-4cm in length with around 1cm situated above the squamo-columnar junction and approximately 2cm distal to the squamo-columnar junction (22). The intrinsic sphincter is tonically contracted in its resting state with pressure ranging from 10 to 30 mmHg relative to intra-gastric pressure and peak pressure corresponding
closely to the position of the squamo-columnar junction (18). The intrinsic sphincter is asymmetrical in both axial and radial orientations with higher pressure recorded on the left side. This corresponds to the position of gastric sling fibres and to the fibres which demonstrate greatest cholinergic input in the resting state (23-25). Muscle tone in the intrinsic sphincter is due to myogenic and neurogenic mechanisms. In vivo animal studies of lower oesophageal sphincter function demonstrated persistent lower oesophageal pressure despite abolition of neural mechanisms with the administration of tetradotoxin suggesting myogenic tone is a major factor in sphincter integrity (26). The major contribution of neurogenic tone is due to cholinergic innervation whereas myogenic mechanisms rely on shifts in intracellular calcium in the sphincter muscle (1). Large fluctuations in intrinsic lower oesophageal sphincter pressure have been demonstrated in several physiological conditions. Dent et al (27) demonstrated significant tonic contraction of the lower oesophageal sphincter during (late) phase 2 and phase 3 of the gastric interdigestive migratory motor complex, presumably a mechanism to prevent significant gastro-oesophageal reflux during a period of vigorous gastric contraction. The pressure of the lower oesophageal sphincter may exceed 80mmHg and this usually reaches its maximum prior to gastric contraction.

Swallowing initiates relaxation of the lower oesophageal sphincter within 1 to 2 seconds of onset and the sphincter remains relaxed until bolus transit is completed. Sensory afferents from the pharynx during swallowing (as well as vagal afferents triggered by gastric distension) stimulate the nucleus solitarius leading to stimulation of inhibitory preganglionic neurons in the dorsal motor nucleus (26). Inhibitory preganglionic neurons originate in the caudal portion of the dorsal motor nucleus of the
vagus whereas excitatory preganglionic neurons originate in the rostral part of the dorsal motor nucleus (28). Vagal efferent neurons activate inhibitory neurons found within the myenteric plexus leading to nitric oxide release which induces relaxation of circular smooth muscle (25;29;30). Oesophageal distension also induces lower sphincter relaxation by intramural neural pathways whereas vomiting/belching reduces sphincter pressure via a central vagal reflex (25)

### ii) Crural Component of High Pressure Zone

The crural diaphragm also plays an important role in augmenting pressure measured at the lower oesophageal sphincter. Boyle et al (31) studied the effect of crural augmentation on anesthetized cats. They demonstrated that peak lower oesophageal pressure corresponded to end-inspiration and that “intrinsic” lower oesophageal pressure best corresponded to pressure measured at end-expiration during spontaneous respiration. Active diaphragmatic contraction was responsible for these respiration-induced oscillations in pressure and diaphragmatic electromyogram activity directly correlated with degree of variation in lower oesophageal pressure. These findings have confirmed in human studies which demonstrated that during inspiration, the end-expiratory lower oesophageal pressure rise exceeds the change in pressure between stomach and oesophagus (the transdiaphragmatic pressure;Pdi), thereby contributing significantly to the antireflux barrier (20). Klein et al (32) reported the presence of a sphincter-like mechanism at the thoraco-abdominal junction in patients who had undergone distal oesophagectomy. In their study, upper gastrointestinal endoscopy and station manometry
was performed on ten distal oesophagectomy patients and ten controls. They demonstrated a definite high pressure zone at a similar position and of similar length in all the controls and 9/10 of the oesophagectomy patients. The average mid-inspiratory (13.7mmHg vs. 23.5mmHg) and end-expiratory pressures (6mmHg vs. 16.3mmHg) were significantly lower in the patient group compared to controls but the phasic component of the high pressure zone was similar in both groups (approximately 7mmHg) suggesting a tonic component of the crural diaphragm. Increased intra-abdominal pressure in both groups resulted in a greater rise in high pressure zone pressure, suggesting that the crural diaphragm has an important adaptive mechanism in preventing reflux of gastric contents during periods of raised intra-abdominal pressure. Mittal et al (33) demonstrated that increased lower oesophageal pressure during periods of increased intra-abdominal pressure is associated with tonic contraction of the crural diaphragm demonstrated by electromyography. The administration of atropine to healthy subjects reduced resting lower oesophageal pressure by 50 to 70% but did not alter the peak lower oesophageal pressure attained in response to raised intra-abdominal pressure and had no affect on diaphragmatic electrical activity. They postulated that tonic diaphragmatic contraction is an important adaptive response to physiological conditions (ie. raised intra-abdominal pressure) that would favour gastro-oesophageal reflux.

The tone of the crural diaphragm is also influenced by several physiological processes involving bolus transit across the gastro-oesophageal junction. Transient lower oesophageal relaxations, swallowing and oesophageal distension are accompanied by reflex inhibition of the crural diaphragm thereby reducing junctional tone and allowing the propelled bolus to overcome the anti-reflux barrier (34;35).
iii) Other Factors Integral to Gastro-oesophageal Junction Competence

The oesophagus enters the stomach at an oblique angle (the Angle of His) on the greater curve of the stomach and it has been suggested that restoration of this acute angle during anti-reflux surgery is essential to junctional competence (36). This angle is thought to be important in creating a flap valve which has been demonstrated in cadaver studies to consist of a musculo-mucosal fold which could exert a pressure gradient that could be increased with surgical lengthening of the valve (37). Sling fibres of the stomach are arranged in a C-shaped manner with the open side orientated toward the lesser curve and this flap of fibres presses against the lower portion of the oesophagus during periods of increased pressure within the fundus (1;22;38). Hill et al (37) went on to classify four grade of gastro-oesophageal flap valve (I-IV) based on evaluation of the gastro-oesophageal junction with a retroflexed endoscope and demonstrated a correlation between flap valve grade and presence of regurgitation. Further studies have also demonstrated that Hill’s classification may be useful in predicting risk of newly developing reflux oesophagitis after H.pylori eradication (39) suggesting integrity of the flap valve is important in the maintenance of the anti-reflux barrier.

The distal 2 cm of the lower oesophageal sphincter (the intra-abdominal high pressure zone) lies below the hiatus and within the abdominal cavity. It is postulated that exposure of this segment augments lower oesophageal pressure and is an important mechanism for maintaining the anti-reflux barrier during straining (18). Fluoroscopic studies of the gastro-oesophageal junction by Palmer et al (40) in 1953 described a tubular gastric segment of the oesophagus which could be up to 3cm in length in the resting healthy adult. They made the interesting observation that this intra-abdominal portion could be
obliterated ie. incorporated in the wall of the stomach with fundal distension and the squamo-columnar junction seemed to move caudally to “the brink of the stomach proper”.

This would suggest that fundal distension could be a mechanism by which the squamous epithelium of the oesophagus is brought into close proximity to the highly acidic environment of the proximal stomach which could be potentially damaging to the distal squamous oesophageal mucosa.

The phrenico-oesophageal ligament is thought to be of importance in maintaining the integrity of the gastro-oesophageal junction. Michelson et al (2) performed canine studies demonstrating that division of the ligament led to a marked reduction in the resting pressure of the lower oesophageal sphincter and subsequent surgical restoration of the ligament reversed this effect. They postulated the formation of hiatal hernia involved stretching and atrophy of the phrenico-oesophageal ligament. Cadaveric studies by Higgs et al (41) described the phrenico-oesophageal ligament as a fibroelastic sleeve in which the fibres of the ligament penetrated the muscular oesophageal wall and postulated that changes in ligament tension might lead to variations in sphincter tone.

Clearly, several factors are critical in maintaining the integrity of the gastro-oesophageal junction and understanding the mechanisms by which the anti-reflux barrier is breached by gastric contents should lead to a clearer understanding of the pathophysiology of gastro-oesophageal reflux disease.
Figure 1.1

*The anatomy of the lower oesophageal sphincter and neural mechanisms involved in the physiological relaxation of the sphincter.*

*Figure modified on an illustration by Van Herwaarden et al (42)*
Pathology at the Gastro-oesophageal Junction

The gastro-oesophageal junction is a site that exhibits a high prevalence of mucosal pathology ranging from columnar metaplasia, carditis, intestinal metaplasia, oesophagitis and adenocarcinoma. It has been shown that the symptomatic patients with gastro-oesophageal reflux disease have an increased risk of Barrett’s oesophagus (43) and gastro-oesophageal adenocarcinoma (44). Studies of incidence of adenocarcinoma in Barrett’s oesophagus have shown an incidence of 1 per 208 to 285 patient years of follow-up (45;46).

During the last 25 years there has been five- to six-fold increase in the incidence of adenocarcinoma of the gastric cardia and a seven-fold increase in the incidence of oesophageal adenocarcinoma in the US (47). Interestingly, there has been a significant decline in the incidence of more distal cancers with a 70% reduction in North America since the 1930s as an example (15). This suggests a distinct mechanism in the aetiology of cardia/junctional adenocarcinoma to that of more distal gastric adenocarcinoma.

Studies by Botterweck et al (48) demonstrated a rising incidence of adenocarcinoma of the oesophagus and the gastric cardia in the majority of the 11 European cancer registries analysed over the period 1968 to 1995. This increase was accompanied by a decrease in non-cardia gastric cancer. The highest age-standardised incidence of cardia-oesophageal cancer was in Scotland (9.7 and 2.9 per 100 000 person-years for males and females respectively). The rising incidence of oesophageal and junctional adenocarcinoma in both Scottish male and female populations has recently been reported in a paper by Brewster et al (49). They also reported a simultaneous decline in incidence of non-cardia gastric cancer (Figure 1.1).
FIGURE 1.2

Age-standardized incidence of oesophageal and gastric adenocarcinoma in male and female Scottish population between 1977 and 1996 (data from Brewster et al.)(50).
Recent data from ISD Scotland for the period 1985 to 2008 shows a rise in oesophageal cancer particularly in males with a 36% rise in all types of oesophageal in this group. The incidence of gastric cancer (all types) has fallen in both male and female groups during this period (Figure 1.2).
Figure 1.3

Age-standardized incidence of oesophageal and gastric cancer (all types) in male and female Scottish population between 1985 and 2008 (data from www.isdscotland.org/isd/1493.html#Summary statistics for oesophagus cancer (51))
It has been postulated that cardia cancer may have two different aetiological pathways. Lagergren et al (44) performed a population-based case-control study to examine the relationship between gastro-oesophageal reflux and cardia/oesophageal adenocarcinoma. They found that subjects with recurrent symptoms of reflux, compared with asymptomatic subjects demonstrated an odds ratio of 7.7 for oesophageal adenocarcinoma and an odds ration of 2.0 for cardia cancer. They found that the more frequent, more severe, and longer-lasting the symptoms of reflux were, the greater the risk of adenocarcinoma. This study would suggest that only a partial contribution to the development of cardia cancer is due to traditional, symptomatic oesophageal reflux.

Another risk factor for cardia adenocarcinoma is Helicobacter pylori. This organism is well established as a risk factor for gastric adenocarcinoma distal to the cardia due to its role in causing atrophic gastritis/hypochlorhydria (52-54). A meta-analysis reported in *Gut* reported no association between Helicobacter pylori infection and cardia adenocarcinoma. However, a recent study by Derakhshan et al (55) found cardia cancer to be strongly associated with both severe gastric atrophy (quantified by serum pepsinogen I/II ratios) and with frequent reflux symptoms. The association with reflux symptoms was only apparent in the non-atrophic subgroup and in the intestinal subtype. Non-cardia gastric cancer was associated with atrophic gastritis but not reflux symptoms. The association of cardia cancer with atrophy was stronger for the diffuse versus intestinal subtype and this was the converse of the association observed with non-cardia cancer. This led them to postulate two distinct aetiologies of cardia cancer, one arising from severe atrophic gastritis and being of intestinal or diffuse subtype similar to non-
cardia cancer, and one related to gastro-oesophageal reflux disease (intestinal in subtype, similar to oesophageal adenocarcinoma).

Genetic influence has also been postulated as being potentially relevant in the predisposition of adenocarcinoma (56) and environmental factors have also been extensively studied (ie. smoking, alcohol, dietary intake of fruit and vegetables) with particular recent interest in the influence of obesity (57-61).

The changes seen at the gastro-oesophageal junction are not always associated with symptoms of reflux. Gerson et al (62) performed upper gastrointestinal endoscopy on 110 asymptomatic subjects attending for sigmoidoscopy for colorectal cancer screening. They found a high incidence of junctional pathology with intestinal metaplasia in 25%, short segment Barrett’s oesophagus in 17% and long segment Barrett’s oesophagus in 7%. This raises interesting questions as to the pathophysiology of such changes in asymptomatic patients.
Nitrite Chemistry at the Gastro-oesophageal Junction

There has been much recent interest in the role of ingested dietary nitrate in the pathogenesis of disease at the gastro-oesophageal junction. Ingested nitrate is absorbed by the small bowel and 25% of this circulating nitrate is then taken up into the salivary glands with subsequent secretion into the oral cavity in saliva (63-65). Commensal bacteria at the base of the tongue convert this secreted nitrate into salivary nitrite which is then swallowed and passes into the oesophagus (66-68). Ingested salivary nitrite undergoes conversion to nitrous acid and nitrosating species (N2O3, NO+) on encountering gastric acid. NO+ has been shown to react with thiocyanate found in saliva to create the nitrosating species, NOSCN, which reacts with secondary amines/amides creating potentially carcinogenic N-nitroso compounds (69;70). Ascorbic acid is present in gastric juice and competes with secondary amides/amines to react with NOSCN producing dehydroascorbic acid and nitric oxide, rather than carcinogenic N-nitroso compounds (71-73). It was initially postulated that this acid-catalysed nitrosative chemistry occurred equally throughout all regions of the stomach. Studies using in-situ microdialysis probes have demonstrated that the highest ratios of nitrite:ascorbic acid occur in the most proximal stomach favouring production of N-nitroso compounds in this region and has been postulated as a reason for the cardia/gastro-oesophageal junction displaying such high incidence of pathology (15;74;75). A highly acidic environment is also required for this chemistry to occur and previous demonstration of unbuffered postprandial acid (the acid pocket) in this region suggests that the cardia is an optimal site for the generation of these potentially carcinogenic nitrosating species (15;75).
Chapter 2

Mechanisms of Gastro-oesophageal Reflux
**Introduction**

Gastro-oesophageal reflux is the process by which gastric contents pass across the gastro-oesophageal junction from the proximal stomach to the distal oesophagus. This is a physiological process but gastric refluxate can be potentially damaging to the distal oesophageal mucosa with the region of the gastro-oesophageal junction displaying a high incidence of pathology (15;74;75). Disease due to gastro-oesophageal reflux is a common problem and is defined as reflux of gastric contents into the oesophagus that causes either oesophagitis, reflux symptoms sufficient to impair quality of life or risk of long-term complications (76-78). Three mechanisms lead to conditions by which reflux across the gastro-oesophageal junction is facilitated i) reduced lower oesophageal sphincter tone ii) anatomical disruption of the anti-reflux barrier ie. hiatal hernia and iii) transient lower oesophageal sphincter relaxations. The relationship between obesity and reflux disease has been the subject of recent interest and is also discussed in this chapter.

**Reduced Lower Oesophageal Sphincter Pressure**

Lower oesophageal sphincter pressure can be augmented by various neuroendocrine factors, drugs and foodstuffs.

Gastrin (79;80), motilin and substance P (81) increase the lower oesophageal sphincter pressure. A number of hormones reduce lower oesophageal sphincter pressure including secretin (82;83), cholecystokinin (83;84), glucagons (82;83), gastric inhibitory polypeptide (85), vasoactive intestinal polypeptide (82), and progesterone (86). Neural mechanisms also have an important role in determining tone at the lower oesophageal sphincter with a substantial part due to cholinergic innervation (1;87) and beta-adrenergic
agonists decreasing sphincter pressure (88;89). Various medications can also alter sphincter pressure with the prokinetics/antiemetics metoclopramide (90-93), cisapride (94) and domperidone (95-97) increasing sphincter pressure. A number of medications can reduce sphincteric pressure including nitrates (98), calcium channel blockers (99;100), theophylline (101;102), atropine (103;104) and diazepam (105).

Ingested foodstuff has also been shown to have an effect on lower oesophageal pressure with protein increasing lower oesophageal sphincter pressure and fat, chocolate, ethanol and peppermint reducing lower oesophageal sphincter tone (106-110). Luminal fat has also been shown to increase the incidence of reflux episodes associated with transient lower oesophageal sphincter relaxations (107).

**The Hiatus Hernia – Definition and Historical Perspective**

Anatomical disruption of the gastro-oesophageal junction is commonplace with historical reports of up to a third of asymptomatic subjects having hiatal hernia on radiological study (111). There is progressive cephalic migration of the squamo-columnar junction and intrinsic lower oesophageal sphincter from the crural diaphragmatic component of the gastro-oesophageal junction. This is termed hiatal herniation. The mechanisms by which this occurs are still unclear although, as stated in the previous chapter, functional integrity of the phrenico-oesophageal ligament appears to be important (2;41).

Diagnosis of hiatal herniation can be made on the basis of endoscopic studies, radiological contrast studies or manometric testing (the relatively recent introduction of high resolution manometry has allowed more detailed measurement of the separation of
the crural component and the intrinsic sphincter. Radiological definition is based on identifying the gastro-oesophageal junction seen as a thin transverse mucosal fold (the B-ring). The intrinsic sphincter produces a thicker ring (the A-ring) approximately 2-4 cm above the B-ring. The distensible portion of the oesophagus between these two rings is known as the vestibule or phrenic ampulla. If the B-ring is measured more than 2 cm proximal to the crural impression then this signifies laxity of the phreno-oesophageal ligaments and allows the radiological diagnosis of hiatal hernia (112). Endoscopic diagnosis of hiatal hernia is made on the basis of observing the gastro-oesophageal junction 2 cm proximal to the diaphragmatic pinch. This can be difficult in the presence of columnar metaplasia of the distal oesophagus as the z-line migrates proximally thus potentially over-estimating the proximal extent of the junction. The use of the proximal margin of the gastric folds rather than the z-line reduces the risk of this error. The distal oesophagus during endoscopy is subject to air insufflation and retching by the patient can lead to a great deal of movement in this region. Further studies into accuracy of endoscopy in diagnosing hiatus hernia using high resolution manometry as the gold standard would be useful in clarifying whether we over or under-report hiatal herniation endoscopically.

The hiatus hernia has been the subject of scientific study since the mid-19th century with the possibility of in-vivo research arising with the introduction of radiology techniques at the turn of the 19th century. In 1853, Henry Ingersoll Bowditch published *A Treatise on Diaphragmatic Hernia* with details of 88 post-mortems on patients with diaphragmatic herniation since the early 1600s. Three of these examinations revealed “dilatation of the esophageal opening,” with details suggesting he was describing what
would now be termed a para-oesophageal hiatal hernia. WV. B. Cannon and R. Moser, 1st and 2nd year medical students at Harvard University respectively, were the first to perform contrast studies to examine the anatomy and physiology of the cardia. Their studies, performed at the end of the 19th century, involved patients swallowing capsules filled with bismuth. The capsules were attached to strings that permitted extraction of the capsules once they had passed the cardia. They noted that the capsules were temporarily arrested as they passed immediately above the cardia i.e. the location of the lower oesophageal sphincter, and presented their findings to the American Society of Physiology in 1898. In 1900 an American radiologist Hirsch diagnosed the first hiatus hernia using a combination of x-ray studies and a mercury-filled balloon prior to performing autopsy. Hiatal hernia was later classified into three types, by Ake Akerlund in 1926, in an article entitled “Hernia diaphragmatica hiatus oesophagei vom anastomischen und roentgenologischen gesichtspunkt”. He described either a) a hernia due to shortened oesophagus b) a para-oesophageal type hernia or c) neither type a or type b. Interestingly he also observed the relation between food ingestion and symptoms consistent with reflux in the patients (113;114).

Subsequent surgical research was based on anatomical restoration of the gastro-oesophageal junction rather than aiming to understand and improve physiological function of the junction. Phillip Allison in the early 1950’s published a seminal article “Reflux esophagitis, sliding hiatal hernia, and the anatomy of repair” (115) describing a 59 year old women with reflux symptoms. He postulated the cause of the patient’s reflux symptoms to be related to incompetence of the gastro-oesophageal junction.
Several repair techniques were developed over the later half of the 20th century, most notably by Nissen and Belsley (113). Lucious Hill studied the role of the gastro-oesophageal flap valve which he felt vital to junctional integrity (116). His later work developed a grading system for this flap valve according to morphological appearance of the gastro-oesophageal junction (37).

**Role of Hiatal Hernia in Reflux Disease**

Several groups have examined the relationship of hiatal hernia to reflux oesophagitis with 60 to 84% of patients with oesophagitis having a hiatus hernia and 50 to 58% of patients with hiatal hernia having oesophagitis (117-119). Wright et al (117) reported no evidence of hiatal hernia in 229 of 293 patients endoscoped over an 18 month period and only 6 of the 229 patients without hiatal hernia (2.6%) had endoscopic evidence of reflux oesophagitis. Berstad et al (118) demonstrated that the size of hiatal hernia is related to the severity of oesophagitis with significantly greater hernia size in patients with Grade 2/3 versus Grade 1 oesophagitis (4.17cm versus 3.15cm; p<0.05). Several other studies have also shown that patients with hiatus hernia are more likely to have severe oesophagitis (119-122) and greater oesophageal acid exposure (121;123;124). The prevalence of oesophagitis in the absence of hiatal hernia is 6-7% (118;119) and the prevalence of Barrett’s oesophagus in the absence of hiatal hernia is also low (3-4%) (125;126).

The role of the hiatal hernia in reflux disease is still incompletely understood. Several mechanisms have been postulated for its contribution to gastro-oesophageal reflux disease. Separation of the crural component and intrinsic sphincter has been shown to be associated with alteration of the pressure topography of the gastro-oesophageal junction.
Kahrilas et al (127) studied the axial and radial features of the gastro-oesophageal junction using combined manometry/fluoroscopy with identification of the squamo-columnar junction using radio-opaque endoscopic clips defining the hiatus hernia. They demonstrated a double high pressure zone in hiatal hernia patients with reduction of maximal lower oesophageal sphincter pressure (28mmHg in normal subjects vs. 17mmHg in hiatal hernia patients), reduction of the length of sub-hiatal pressure zone and alteration of its radial asymmetry (which is thought to be primarily attributable to the crural component)(127).

It has also been postulated that hiatal hernias compromise acid clearance, an important mechanism in minimising mucosal exposure to potentially damaging gastric refluxate. Johnson et al (128) reported on 24 hour pH monitoring in 100 individuals and observed that the presence of hiatal hernia was associated with increased reflux frequency and prolonged recumbent acid clearance. Sloan et al (19) investigated oesophageal emptying during barium swallows whilst performing simultaneous videofluoroscopy and manometry in patients with hiatal hernias and controls. The patients with hiatal hernias were subdivided into those with reducible and those with irreducible hernias. They demonstrated that complete emptying, without retrograde flow was seen in 86% of controls, 66% of those with reducible hernias and 32% in the irreducible hernia group. They also demonstrated that the patients with irreducible hernias had significantly longer acid clearance times than controls (624 seconds vs. 346 seconds). The increase in acid clearance time in the irreducible hernia group was attributed to loss of the intra-abdominal portion of the sphincter which could allow early retrograde reflux at the time of lower oesophageal relaxation with backwash of gastric contents. In normal subjects,
complete lower oesophageal relaxation was seen within 3 seconds of the swallow but ampullary opening was not seen until 5 to 10 seconds due to the distending effect of the propelled barium bolus. It was postulated that the distal intra-abdominal portion of the sphincter is critical in this mechanism as extrasphincteric intra-abdominal pressure prevents opening until distended by the bolus and prevents the early retrograde reflux seen in patients with fixed hernias (19). Mittal et al (129) studied the phenomenon of delayed acid clearance in patients with gastro-oesophageal reflux by performing pH and radionuclide studies while injecting radiolabelled hydrochloric acid. They observed in subjects without hiatal hernia, a step-wise monophasic increase in pH after acid installation in response to swallows. In patients with hiatal hernia, they observed a biphasic pH response with an initial fall in pH in response to swallowing followed by an increase in pH due to clearance. They attributed this biphasic response to reflux of infused acid which had been retained within the hiatal sac and eventual clearance of acid from the hiatal sac due to the neutralizing washout effect of swallowed saliva. They postulated that acid could become trapped within the hiatal sac due to the absence of swallow-induced peristalsis within this region and also the presence of the crural component of the high pressure zone at the diaphragmatic hiatus. Interestingly they speculated that if any acid were secreted within the hiatal sac by oxyntic cells located there, then this could potentially accumulate and reflux during swallow-induced lower oesophageal sphincter relaxation (129).

Jones et al (130) investigated the characteristics of oesophageal acid exposure in asymptomatic controls and gastro-oesophageal reflux patients with and without oesophagitis. Patients with oesophagitis were found to have significantly larger hiatal
hernias, greater oesophageal acid exposure, more prolonged episodes of reflux and longer acid clearance times compared to controls and those with non-erosive disease. There was a significant association between increasing hernia size and increasing oesophageal acid exposure (R=0.5), number of prolonged acid reflux events (R=0.58) and prolonged acid clearance times (R=0.46). The most pronounced effect of increased acid clearance time was seen in patients with non-reducing hernias. The authors postulated that increasing hernia size has an important role in determining efficacy of acid clearance and thereby implicating hiatus herniation as an important factor in the pathophysiology of erosive oesophagitis.

**Transient Lower Oesophageal Sphincter Relaxations (TLOSRs)**

Transient lower oesophageal sphincter relaxations are brief episodes (10 to 60 seconds) of lower oesophageal relaxation to intragastric pressure unrelated to prior swallowing or oesophageal peristalsis (1;21). They are manometrically defined by i) the absence of swallowing for 4 seconds before to 2 seconds after the onset of lower oesophageal relaxation, ii) relaxation rate of ≥ 1mm/Hg per second, iii) time from onset to complete relaxation of ≤ 10 seconds, and iv) nadir pressure of ≤ 2mmHg (131). The efferent pathway for these episodes is the vagus nerve with nitric oxide acting as the postganglionic neurotransmitter (1). It is postulated that gastric distension, particularly in gastric cardia, activates mechanoreceptors which then send signals to brainstem centres via vagal afferent pathways. The motor arm is also thought to be the vagus nerve and the complex coordination of lower oesophageal relaxation and crural inhibition is under the control of a pattern generator in the brainstem vagal nuclei which is also thought to have
a role in swallow induced relaxation mechanisms (18). Canine gastric distension studies by Boulant et al (132) demonstrated an increased frequency of transient lower oesophageal relaxations in response to infusion of cholecystokinin and this effect was negated by the infusion of a cholecystokinin-A antagonist and nitric oxide synthase inhibitor. They postulated a role for peripheral cholecystokinin-A receptors and nitric oxide in the neurohumoral control of transient lower oesophageal relaxations. Further work by Hirsch et al (133) demonstrated that NG-monomethyl-L-arginine (L-NMMA), a specific NO synthase inhibitor, significantly inhibited the increase in TLOSRs seen in human subjects during gastric distension. They postulated that nitric oxide is one of the neurotransmitters involved in the reflex arc mediating the triggering of TLOSRs. In addition to cholecystokinin-A antagonists and nitric-oxide synthase inhibitors, a number of other neurohumoral agents have been identified as reducing the rate of TLOSRs including anti-cholinergics, morphine, somatostatin, 5-hydroxytryptamine (5HT3) antagonists and gamma amino butyric acid agonists (18).

**Role of Transient Lower Oesophageal Sphincter Relaxations in Gastro-oesophageal Reflux Disease**

Studies by Dent et al demonstrated that transient lower oesophageal sphincter relaxations were the most common mechanism of reflux episodes in patients. They studied 644 reflux events in 67 patients and found transient lower oesophageal sphincter relaxations responsible for 82% of episodes. The contributing role of absent basal lower oesophageal tone increased with increasing severity of reflux oesophagitis and this accounted for 23% of episodes in patients with severe reflux disease. More than one
mechanism of reflux was commonly seen in patients suggesting multi-factorial pathophysiology but transient relaxations appeared to be the predominant factor (134).

Studies by Mittal et al (135) looked at the frequency of acidic reflux during transient relaxation of the lower oesophageal sphincter in reflux patients and healthy controls. They performed submental electromyography, pHmetry and manometry during a 1 hour fasting period and 3 hour postprandial period. They found that an equal number of transient relaxations were measured in the reflux and control group. Transient relaxation of the lower oesophageal sphincter was the only mechanism of reflux in the asymptomatic group whereas it accounted for 73% of reflux events in the reflux group. 36% of transient relaxations in the control group where accompanied by pH evidence of acid reflux whereas a greater proportion of transients in the reflux group (65%) had acid reflux demonstrable on pHmetry. This suggested that transient relaxation of the lower oesophageal sphincter had a role in the pathophysiology of reflux disease but other factors were important in determining whether pathological acid reflux occurred.

These studies would suggest that reflux in healthy subjects and non-erosive reflux disease predominantly occur by a mechanism of transient sphincter relaxation although other factors such as lower oesophageal sphincter integrity become more important with increasing severity of erosive reflux oesophagitis (135-137).

There has been speculation as to whether hiatal herniation and disruption of the anatomy of the gastro-oesophageal junction itself predisposes to gastro-oesophageal reflux through increased frequency of transient lower oesophageal sphincter relaxation. It is well established that transient lower oesophageal sphincter relaxation is an important mechanism of reflux, triggered by gastric distension through the action of proximal
gastric tension receptors in the subcardiac region (138-140). Kahrilas et al (141) investigated whether hiatal hernia influences vulnerability to reflux by increasing susceptibility to transient lower oesophageal sphincter relaxation in response to gastric air distension. They observed that intragastric air infusion increased the frequency of acidic reflux through transient relaxations in both control and gastro-oesophageal reflux patients. The frequency of transient lower oesophageal sphincter relaxations recorded correlated significantly with measured separation of the squamo-columnar junction and diaphragmatic hiatus which was a measure of hiatal hernia size. They also found that the threshold increase in intragastric pressure for triggering transient lower oesophageal relaxations was similar in all subject groups (4mmHg) suggesting the phenomenon was due to the disturbed anatomy of the oesophago-gastric junction rather than increased sensitivity of gastric tension receptors in the hiatal hernia patients.

While transient relaxations appear to be more frequent in response to infused air-distension (in supine subjects with hiatal hernia), twenty-four hour ambulatory pH studies have suggested that reduced lower oesophageal sphincter pressure is the more critical factor in determining excess reflux in gastro-oesophageal reflux patients with hiatus hernia. Van Herwaarden et al (124) demonstrated that mean lower oesophageal sphincter pressure, number of transient lower oesophageal sphincter relaxations and number of transient relaxations associated with acid reflux were similar in gastro-oesophageal reflux patients with and without hiatal hernia. In reflux patients without hiatal hernia, 60.2% of reflux episodes were associated with a transient lower oesophageal sphincter relaxation, significantly more than the 32.8% of reflux events associated with transient relaxations in the hiatal hernia group. The absolute number of reflux episodes associated with transient
sphincter relaxations was similar in both groups. Reflux during reduced lower oesophageal pressure (22.5% of reflux events), swallow-associated lower associated relaxation (14% of reflux events) and straining plus reduced lower oesophageal pressure (15.5%) were mechanisms responsible for reflux in the hiatal hernia group but less commonly seen in the reflux patients without hiatal hernia. The authors suggested that these mechanisms rather than increased transient lower oesophageal sphincter relaxations could explain the increased reflux observed in gastro-oesophageal reflux patients with hiatal hernia.

In summary, it would appear that in patients with gastro-oesophageal reflux disease without hiatal hernia the primary mechanism of reflux is due to transient lower oesophageal sphincter relaxations. The evidence also suggests little difference in the frequency of transient relaxations in studies comparing healthy subjects and patients with gastro-oesophageal reflux disease. However, it appears that in the subset of patients with severe erosive oesophagitis/hiatal hernia, other factors relating to the disrupted anatomy of the anti-reflux barrier contribute to pathogenesis of their severe acid reflux. The paper by Mittal et al (135) leads one to postulate that in reflux patients a greater reservoir of acidic gastric juice available for reflux may be located in the proximal stomach/cardia compared to healthy subjects and this may be influenced/exaggerated by the anatomical disruption associated with hiatal herniation. This hypothesis would explain the difference in acidic reflux between healthy subjects and reflux patients despite similar frequency of transient relaxations. With increasing severity of reflux disease, reduced gastro-oesophageal junction integrity (and its associated hiatal herniation) would then supercede transient relaxations as the major factor in acid reflux as lower oesophageal sphincter
tone and impaired oesophageal clearance allow this proximal pool of gastric acid to collect in the distal oesophagus leading to acid-mediated damage. Studies (as described in my thesis) to investigate the proximal stomach for differences in acidity in healthy and reflux patients are required to investigate this hypothesis further.

Studies by Beaumont et al (142) have recently demonstrated the influence of hiatal hernia and acid pocket on determining the acidity of refluxate associated with transient relaxations and further details of this work are given in the Discussion section of my thesis.

**Gastro-oesophageal Reflux Disease and Obesity**

The rising incidence of oesophageal cancer (48;51), Barrett’s (143) and gastro-oesophageal reflux disease (144) has been postulated to be related to several factors but the coincident rise of obesity in the Western world has raised much interest in whether this is a causative factor.

Stene-Larsen et al (145) reported an association between “weight-for-height index” and both hiatal hernia and reflux oesophagitis although this association was most marked in lower grades of severity of oesophagitis. Wilson et al (146) also reported an association between excessive body weight and the probability of both hiatal hernia and oesophagitis. The association between body mass index and oesophagitis was still evident when controlled for the effect of the hiatal hernia. Both these authors suggested the link between obesity, hiatal hernia formation and reflux oesophagitis.

High-resolution manometry studies by Pandolfino et al (147) have demonstrated significant association between increased body mass index/increased waist circumference
and disruption of the lower oesophageal sphincter components mirroring previous work postulating the role of obesity in hiatal hernia formation.

Wu et al (148) studied the role of transient lower oesophageal sphincter relaxations and obesity performing postprandial manometry and ambulatory pH studies in obese, overweight and normal weight subjects without gastro-oesophageal reflux disease. They demonstrated all three groups had comparable mean lower oesophageal sphincter pressure and length. However both the overweight and obese group demonstrated a significant rise in the number of postprandial transient relaxations and also the proportion associated with acid reflux. They postulated these findings may give some insight into the early stages of development of obesity related acid reflux disease.

A cross-sectional study of 206 patients undergoing 24 hour pH studies demonstrated significant association between body mass index and oesophageal acid exposure with the effect attenuated when controlled for waist circumference suggesting that waist circumference/central adiposity plays some part in this relationship (149).

Studies into the association of reflux symptoms and obesity have yielded mixed results. Lagergren et al (150) performed a population-based cross-sectional interview study reporting no association between body mass and the severity, duration or presence of recurrent reflux symptoms. A meta-analysis by Hampel et al (151) reported on nine cross-sectional studies examining the association between body mass index and reflux symptoms. Six studies demonstrated a statistically significant association. They reported a trend in eight of the studies for a dose-response relationship with an increase in pooled adjusted odds ratios for reflux symptoms of 1.43 for BMI >25 kg/m2 and 1.94 for BMI >30 kg/m2. Nilson et al (152) also reported a dose-response relationship with an increase
in BMI of over 3.5 units yielding about a three-fold risk of developing new reflux symptoms.

Complications of gastro-oesophageal reflux have been studied for their association with body mass index. The meta-analysis by Hampel et al (151) reported significant associations between body mass index and both erosive oesophagitis and oesophageal adenocarcinoma. They found adjusted odds ratios for oesophageal adenocarcinoma of 1.52 for those subjects with BMI between 25 kg/m2 and 30 kg/m2, and 2.78 for those with a BMI greater than 30 kg/m2. The ProGERD prospective study reported on a multivariate risk factor analysis of 5289 patients with troublesome heartburn. Obesity was identified as an independent predictor of severe erosive oesophagitis with a body mass index between 25 and 30 kg/m2 yielding an odds ratio 1.7 and a body mass index greater than 30 giving an odds ration of 1.97 (153).

The evidence for the link between obesity and Barrett’s oesophagus is less clear. Corley et al (154) reported on a case-control study within the Kaiser Permanente Northern California population. Interestingly they did not find an association between body mass index and increased body mass index. There was, however, an association between waist circumference and risk of Barrett’s oesophagus suggesting distribution of body fat the more important factor. There was also an association between waist circumference and reflux symptom severity and adjustment for reflux symptoms partially attenuated the association seen between central adiposity and Barrett’s. Edelstein et al (155) reported similar findings with waist-to hip ratio rather than body mass index being the greater predictor of Barrett’s in their case-control study. They did not see any attenuation of association when adjusted for frequency of heartburn symptoms.
The association between body mass index and oesophageal adenocarcinoma has been reported in several studies (156-158). Studies by Steffen et al (159) demonstrated high BMI and central adiposity (waist circumference/waist-to-hip ratio) appear to be risk factors for oesophageal adenocarcinoma. Interestingly, when adjusted for body mass index they found positive associations for between central obesity and oesophageal squamous carcinoma in both smokers and non-smokers.
Chapter 3

Research into the Nature of the Acid

Pocket
Previous Work

Previous work by my research group has demonstrated the presence of highly acidic unbuffered gastric juice in the region of the gastro-oesophageal junction after eating and this has been confirmed by several other groups (160-164). Although the ingestion of a meal stimulates secretion of gastric acid, the buffering effects of the ingested food have previously been demonstrated as causing a postprandial elevation in intragastric pH to >2.5 (165). This presents one with a seemingly paradoxical situation as the postprandial period is a period when reflux symptoms are commonly prevalent. The presence of this unbuffered acid pocket at the gastro-oesophageal junction has been postulated as a potential source of reflux although has never been demonstrated in vivo.

Fletcher et al (160) performed dual pH electrode pull-through studies in ten healthy subjects before and after a standardised fatty meal. These were performed in the semi-recumbent position with the dual pH catheter withdrawn by 1cm increments every minute. Endoscopic clips were also employed to mark the position of the squamo-columnar junction thus allowing radiological localisation of the acid pocket relative to the anatomy of the gastro-oesophageal junction. A region of unbuffered acidity was demonstrated at the gastro-oesophageal junction (median pH 1.6) in comparison to the body of the stomach (pH 4.7). This region of acidity seemed to extend from the gastric cardia and 1.8cm proximal to the squamo-columnar junction ie. onto distal oesophageal squamous epithelium. The fasting pH step-up point had migrated proximally to a point situated above the squamo-columnar junction (Figure 3.1).
Figure 3.1

Unbuffered postprandial acid pocket is demonstrated in the proximal stomach on pH pull through study (1 cm intervals) with proximal migration of the pH step-up point. Median pH minimum for each electrode position in stomach and oesophagus after the meal.

* pH greater than the electrode position marked † (p<0.01)

Reproduced from Fletcher et al (160)
It was postulated that this acid pocket could contribute to the high prevalence of disease in the region of the gastro-oesophageal junction. Previous magnetic resonance imaging studies had shown partitioning of food and its constituents in the stomach with a lipid layer floating on top of the more aqueous phase (166). It was hypothesised that gastric juice secreted by the proximal stomach might be trapped above the lipid layer and separated from the buffering effect of the food in the more distal stomach. They performed in vitro studies using a homogenized meal confirming that acidic human gastric juice added on top of the homogenized meal escaped the buffering effect of the food even after agitation of the mixture. This phenomenon was postulated as an explanation for the persistence of an acid pocket at this site.

Further studies by Fletcher et al (167) demonstrated the presence of substantial short segment reflux, even in the absence of conventional reflux using a 24-hour dual pH catheter clipped to the oesophageal mucosa with electrodes positioned 5mm and 55mm above the squamo-columnar junction. The frequency of these short segment events was greater than the expected frequency of transient lower oesophageal sphincter relaxations (188 versus 72 expected transient relaxations) suggesting another aetiology for short segment reflux. It was postulated that distal shortening of the high pressure zone due to postprandial distension could allow the unbuffered acid pocket to intermittently extend onto squamous epithelium and acidify the adjacent pH electrode (short segment reflux). This mechanism of postprandial distension and distal shortening of the sphincter had previously been postulated by Oberg et al (11). Their group had performed studies to test the hypothesis that cardiac mucosa, carditis, and intestinal metaplasia at the gastro-
oesophageal junction is caused by injury of the squamous epithelium within the sphincter by gastric juice. They suggested that fundic distension occurs due to a combination of overeating and delayed gastric emptying secondary to a high-fat diet. The distension causes the sphincter to be "taken up" by the expanding fundus and this exposes the distal squamous epithelium of the sphincter to gastric juice. They suggested that repeated exposure leads to inflammation of the squamous epithelium and could subsequently cause columnar metaplasia and carditis.

Pandolfino et al (164) studied the feasibility of using a Bravo capsule for proximal intra-gastric pH recording in 9 healthy subjects and 9 symptomatic reflux patients. A Bravo capsule was placed endoscopically at the gastro-oesophageal junction with the pH electrode positioned 1.5-2cm distal to the squamo-columnar junction (within the cardia region). A second Bravo capsule was placed 6cm proximal to the squamo-columnar junction to detect reflux events. Interpretable recording for 24 hours from both pH capsules was achieved in 16 of the 18 subjects. They found no consistent difference in the cardia luminal environment (the integrated acidity) between healthy controls and symptomatic patients over the study period. However there was a discordant pattern in which the integrated acidity was higher for symptomatic patients compared to controls during periods when meals were most frequently consumed. They postulated that this may be due to abnormal pooling of gastric juice in the proximal stomach but strong conclusions could not be reached as dietary intake was not standardised in the study. They suggested further study using standard meals at predetermined time points would be helpful. They found that there was some degree of postprandial buffering at the cardia but this was substantially less than the buffering measured in the gastric body in previous
work by Fletcher et al (160). They found that the nadir cardia pH was 0.5-0.7 pH units lower than the nadir oesophageal pH during postprandial and upright reflux events and the median cardia pH in symptomatic patients was significantly lower than the median cardia pH value in controls during postprandial reflux events. As there was no pH measurement distal to the cardia during the study, no conclusion could be drawn as to whether these reflux events originated from the highly acidic cardia region or from a more distal intra-gastric source.

Studies by Vo et al (162) confirmed the presence of the postprandial acid pocket in healthy subjects. They were interested in examining regional differences in the effects of rabeprazole on gastric acidity but included a placebo group who underwent 24 hour pH analysis and pH catheter pull-through studies fasting and 15, 45, 90 and 150 min after completion of lunch, dinner and breakfast on two separate days. An acid pocket was defined during the pull-through as a drop in intraluminal pH > 2 pH units to a pH of <4. They detected an acid pocket in 112 of 300 pull-through studies performed on 10 healthy subjects prior to and following six meals. Acid pockets were usually detected in the postprandial period extending from 3.1 to 5.8cm below the proximal LES border with a mean length of 2.7 cm. The pH of the acid pockets ranged from 1.5 to 2.2. This region was primarily the cardia of the stomach and, in some cases, the acid pocket extended through the intra-sphincteric lumen into the distal oesophagus. Meal composition seemed to have some significance with a greater number of postprandial acid pockets seen after the spicy/fatty meals compared with breakfast which had a bland composition. They also found that the number, length and magnitude of proximal acid pockets were reduced by the administration of rabeprazole.
A similar study by Simonian et al (163) employed a four-probe pH catheter to study 10 healthy subjects over a 27 hour period. Pull-through pH readings were taken at similar intervals as the study by Vo et al (162) ie. fasting and 15, 45, 90 and 150 minutes after the meal. All subjects had a spicy lunch, high-fat dinner and bland breakfast. The fatty meal had the highest postprandial buffering effect (mean proximal gastric pH 4.9 and mean mid/distal gastric pH 4.0) and this was significantly higher than the buffering of the spicy or bland meals. The buffering achieved by the fatty meal was also more prolonged (150 minutes) in comparison to that achieved with the spicy lunch (45 minutes only). The bland breakfast did not achieve a mean pH > 4 at any time. They postulate that this may be partly due to greater intragastric acidity in the morning after an overnight fast. In vitro studies showed that meal composition was also an important factor as each meal was homogenized in a beaker with 50ml of homogenate taken and titrated to a pH of 2 using 1.07 pH solution. The quantity of titratable acid added to reduce the pH to 2.0 represented the measure of in vitro buffering capacity of each meal which was then corrected for meal volume. The fatty dinner required 90ml, the spicy lunch 80ml and bland breakfast 60ml of acid solution and when corrected for meal volume, the fatty meal had greatest total buffering capacity followed by the spicy meal then the bland breakfast. The degree of buffering capacity seemed to correlate better with protein content and volume than with fat or calorific content. They reported 51/120 (43%) postprandial pH pull-through studies detected an acid pocket with the majority seen between 15 and 90 minutes after completion of the meal. The proximal extent of acid pocket was a mean 3.4cm from the proximal border of the high pressure zone and a mean 2.3cm in length. Many acid pockets extended into the intraluminal high pressure zone and some into the distal
oesophagus. The frequency of acid pocket was similar after the spicy and fatty meals but less so after the bland breakfast meal.

Further studies by Pandolfino et al (161) have examined the position of the gastric-to-oesophageal pH transition point relative to the squamo-columnar junction and manometric landmarks in healthy subjects and patients with gastro-oesophageal reflux disease. They employed similar techniques previously described by myself in the paper published in Gut (168) (methods included in this thesis) and they reported that the acid pocket was located at or distal to the squamo-columnar junction in healthy subjects. They found that the unbuffered acid pocket was longer in reflux patients than healthy subjects, a finding that mirrored my previous work described in this thesis. Their analysis did not account for oesophageal reflux in the reflux patients which could lead to over-estimation of the proximal extent of the acid pocket in this group. They reported that the acid pocket extended through the high-pressure zone in these reflux patients in the context of an intact lower oesophageal sphincter and postulated that what was previously described an acid pocket may in fact be merely a thin “acid film”.

If one postulates that the acid pocket may have a role in pathogenesis of reflux oesophagitis, then one would expect to see differences in the characteristics of the acid pocket in these patients when compared to healthy subjects. It was also unclear as to whether the acid pocket in healthy subjects was a transient phenomenon as previous studies had relied on pull-through techniques which provided information at set time-points after the meal. A system which provided continuous pH analysis throughout the fasting period, meal-time and postprandial period would give greater insight into the nature of acidification of the proximal stomach/cardia in response to meal ingestion.
Methodological Issues To be Considered In Future Studies

These papers raise several issues which should be considered when designing studies looking at the luminal characteristics of the gastro-oesophageal junction.

The paper by Fletcher et al (167) suggests that standard pH analysis i.e. electrode 5cm above the gastro-oesophageal junction, may be inadequate if trying to detect differences between subject groups with varying degrees/risk of junctional pathology. If one agrees that short segment reflux, as demonstrated in their study, contributes to acid-related damage to the distal oesophagus then a system allowing measurement of pH very close to the squamo-columnar junction is required. If standard pH electrode placement is utilised then subtle differences in frequency of short segment reflux may be missed.

The work by Fletcher et al (160) utilised radio-opaque clips to mark the position of the squamo-columnar junction. There was no correction for respiratory excursion during x-ray acquisition and my own studies reported in this thesis demonstrate significant movement of these clips in healthy subjects. This is worth consideration in study design of future projects using radio-opaque markers as radiological definition of junctional anatomy will need to take this into account.

The development of wireless pH electrode systems has allowed very precise placement of electrodes at the site of anatomical interest and constant measurement of pH over long periods in study subjects (164). This may seem an attractive option when designing studies looking at intra-luminal pH over prolonged periods of time but may also have drawbacks. The single electrode only allows pH measurement at one anatomical location (which may be entirely appropriate if this is the aim of the study) and does not give insight into simultaneous changes at other anatomical locations. To do this with wireless
electrodes, multiple Bravo capsules would have to be deployed at different anatomical sites or a standard multi-electrode pH catheter would have to be deployed in addition to the wireless Bravo capsule to measure pH at distal and proximal sites. My initial studies described in this thesis were aimed at comparing differences between healthy subjects and reflux patients with respect to pHmetry, manometry and anatomical landmarks. I then went on to design a bespoke high-resolution pH which could be clipped to the mucosa of the gastro-oesophageal junction. The high-resolution pH catheter allowed continuous pH measurement but also yielded novel information regarding the location/extent of acidification of the both stomach, junction and oesophagus and provided evidence that the proximal acid pocket was of sufficient volume that it could be a potential source of acidic refluxate. This information would be difficult to collect using single wireless electrodes.

The studies by Vo (162) and Simonian (163) highlighted the influence of meal composition on degree of buffering and would suggest that standardisation of meals in any pH study with regards fat/protein content should be considered.

The study by Pandolfino et al (161) highlights the issue of defining the proximal extent of the acid pocket appropriately. Their analysis of acid pocket length did not account for the influence of acid reflux as they measured acidity above the squamo-columnar junction. The presence of acid reflux, if not accounted for, will lead to over-estimation of the proximal extent of the acid pocket and therefore over-estimation of its total length. Future studies designed to measure acid pocket length should ensure that this effect is considered.
Chapter 4

Severe Reflux Disease Is Associated With Enlarged Unbuffered Proximal Gastric Acid Pocket
**Introduction**

Acidic gastro-oesophageal reflux is common following meals and this may be attributed partly to the increased frequency of transient lower oesophageal sphincter relaxations during the postprandial period (136;169-171). However, the source of the postprandial acidic refluxate has been unclear. During periods of fasting the gastric juice is highly acidic with a pH of less than 2. However, on eating, the intragastric pH rises due to the buffering effect of the food and this persists during the postprandial period. Acidic gastro-oesophageal reflux occurring during periods of postprandial gastric buffering presents a paradox.

Fletcher et al recently reported the presence of an unbuffered acidic region in the gastric cardia during the postprandial period which often traversed the squamo-columnar junction (160). This small acid pocket has been postulated to be the source of the acidic refluxate occurring after a meal. Further work by Pandolfino et al has confirmed less meal-related buffering in the gastric cardia of both normal controls and symptomatic gastro-oesophageal reflux disease patients (164). Both groups propose that this acidified zone could be the source of postprandial acid reflux events as the nadir cardia pH is similar to the nadir pH of acidic refluxate. The existence of a postprandial acid pocket has also been confirmed in studies by other groups (162;163).

Several questions regarding this unbuffered acidic zone and its possible role in gastro-oesophageal reflux disease remain to be answered. It is unclear whether the postprandial acid pocket extends across the squamo-columnar junction or is confined to the gastric cardia region. The relation of the acid pocket to the high pressure zone (HPZ) of the lower oesophagus is also unknown. The most important question, however, is whether
the acid pocket contributes to the aetiology of reflux disease and/or to the high prevalence of carditis and intestinal metaplasia of the gastro-oesophageal junction. If the acid pocket is of pathological significance then it should be different in subjects with versus without gastro-oesophageal reflux disease.
Aims

1. To define the location of the acid pocket in healthy subjects relative to the squamo-columnar junction and HPZ.
2. To compare the acid pocket in patients with severe reflux disease versus healthy subjects.

Subjects

The study population consisted of 12 healthy subjects (3 females) with no history of gastro-oesophageal reflux symptoms and 16 patients (7 females) with ≥ grade 3 reflux oesophagitis (n=4) or Barrett’s oesophagus (n=12). Patients and subjects were similar with respect to intragastric acidity (Table 4.1) and thus the mean age of the healthy subjects was lower than the reflux group (30.9 years; range 21 to 58 years versus 62.6; range 28 to 74 years). Helicobacter pylori status was documented in all patients prior to the study (one healthy volunteer and one reflux patient were found to be helicobacter positive). 14 of the reflux patients were on proton pump inhibitors at enrolment and the medication was discontinued a mean of 8 days prior to the study.

Study Design

Each subject had two combined pH/manometric station pull-through studies, one fasted and one after a meal. The pH and manometric recordings were related to the anatomy by the use of endoscopically-placed radio-opaque clips visualised with lateral chest x-ray. Fasting and postprandial barium studies were also performed to delineate the anatomy of the gastro-oesophageal junction.
**Study Protocol**

The study took place over two consecutive days. On day 1 the subjects reported fasted and underwent upper gastrointestinal endoscopy. This was performed either with Xylocaine throat spray or under conscious sedation with intravenous midazolam. Two standard haemostatic metal clips (HX-600-090, Olympus, UK) were deployed, using an endoscopic clip-fixing device (HX-5LR-1, Olympus, UK), 180 degrees apart at the proximal margin of the gastric folds (which in volunteers equated to the squamo-columnar junction) (Figure 4.1). In patients with endoscopic appearances consistent with a hiatus hernia, 2 further clips were placed at the diaphragmatic hiatus (Figure 4.2). One hour after the endoscopy, a barium swallow was performed to delineate the fasting morphology of the gastro-oesophageal junction and the position of the clips relative to the gastro-oesophageal junction and hiatal hernia sac if present. Endoscopic definition of hiatal hernia can be difficult unless the subject has a large fixed hiatus hernia. The fasting barium swallow was performed to confirm the absence of hiatus hernia in healthy subjects and reflux patients deemed not to have a hiatal hernia endoscopically. An example of a barium study in a healthy subject is given in Figure 4.3.
Figure 4.1

Endoclips deployed at the proximal margin of the gastric folds.

Picture courtesy of Dr Jonathan Fletcher
Figure 4.2

Position of endoclips deployed in a normal healthy subject and in a reflux oesophagitis patient with hiatal hernia.

Figure modified on an illustration by Van Herwaarden et al (42)
Fig 4.3 a-d

Barium swallow (3 seconds) in a healthy subject. Endoclips circled prior to being obscured by passage of barium through gastro-oesophageal junction.
On the following day the subjects again reported fasted from midnight and had a combined apparatus, consisting of an antimony dual-channel pH catheter (Synectics Medical, Enfield, England, UK) and solid state manometer (Gaeltec CTO-3, Isle of Skye, Scotland, UK) (Figure 4.4), passed nasally into the stomach. The pH catheter had two electrodes 7 cm apart with an external reference electrode. A dual pH electrode was employed to examine whether any region of acidity was transient in nature or whether this “acid pocket” could be reproduced in the same individual as the two electrodes passed through the same region of the stomach several minutes apart. The electrodes were calibrated in buffer (Synectics, UK) before the procedure and subsequent data collected using Polygram Net software (Medtronic, Denmark). Calibration at room temperature was corrected by the computer software for pH measurements at body temperature. The solid state manometer had 3 sensors arranged 120 degrees radially (Figure 4.5) and was calibrated before the procedure and subsequent data collected using Polygram Net software (Synectics). The pH catheter was attached to the manometer so that the distal pH electrode was 1 cm proximal to the 3 radial manometry sensors (and the proximal pH electrode was 8 cm proximal to the manometry sensors).
Figure 4.4

Combined pH and manometry apparatus.

Dual electrode pH catheter with electrodes 7cm apart.

Solid state manometry catheter.
Figure 4.5

Solid state manometry catheter with 3 manometry sensors at positioned 120 degrees to each other.

Distal pH electrode from pH catheter also seen in figure.
Position in the stomach was confirmed by acidic gastric pH recorded by both pH electrodes and an increased pressure measured by the manometer on deep inspiration. The patient was placed in a semi-recumbent position and, after 15 minutes with the apparatus in the stomach, the apparatus was withdrawn at 1 cm intervals every 1 minute. This pull-through continued until the manometry sensors recorded oesophageal pressure i.e. when the end-expiratory pressure in all three sensors were lower than the intragastric pressure. At this point, the apparatus was immediately taped to the nose and lateral X-rays were performed immediately in the semi-recumbent study position in an adjoining room. To minimise patient movement, the subjects were wheeled over to the x-ray area on the trolley used for the pull-through study thus maintaining the position held during the pull-through study. These semi-recumbent lateral chest x-rays were performed to establish the position of the clips relative to the pH/manometry apparatus. X-rays were taken in inspiration and expiration and mid-respiratory position of the clips calculated. The measured distance between the pH electrodes (known to be 7 cm apart) on x-ray was used as an internal scale for other radiographic measurements (Figure 4.6).
Figure 4.6

Radiograph demonstrating proximal and distal pH electrodes after pull-through study and endoscopically placed clips situated at the gastro-oesophageal junction.
The apparatus was then re-introduced into the stomach and the position confirmed as before. The subjects were then given a meal of fried fish and french fried potatoes. A second pull-through study was started 15 minutes after finishing the meal, again withdrawing 1cm intervals every 1 minute. As before, the apparatus was fixed to the nose and the patient underwent a lateral chest x-ray to establish the position of the clips relative to the apparatus. The pH/manometry apparatus was then removed and the patient underwent a second barium swallow to delineate the morphology of the postprandial gastro-oesophageal junction (Figure 4.7) and to determine whether there was evidence of food debris in the region of the gastro-oesophageal junction/hiatal hernia that could account for buffering observed on pH studies.
Figure 4.7

*Hiatus hernia seen on postprandial barium swallow in reflux patient.*
Analyses

For each pull-through study, the pH was recorded at each position of both the proximal and distal pH electrode. The mean pH for the time interval at each catheter position was then calculated. The fasting pH step-up point was defined as the most distal catheter position where mean pH was >4. In the postprandial state, the pH step-up point could only be identified if there was an unbuffered acidified zone in the proximal stomach and the pH step-up point was the catheter position where pH rose above 4 proximal to this acidic region.

The lower border of the HPZ was defined as the first point where there was an increase of >2 mmHg in end-expiratory pressure above gastric baseline measured in one of the manometry sensors. The proximal border of the HPZ was identified by a step-down in the recorded end-expiratory pressure to the intra-thoracic pressure.

The position of the proximal margin of the gastric folds was calculated by measuring the distance between the end of the manometry sensor and the midpoint of the 2 endoclips placed at the proximal gastric folds on lateral chest x-ray. This distance was then added to the distance the manometry sensor was from the nares, allowing calculation of the position of the proximal gastric folds from the nares.

To correct for error in measuring the position of the pH step-up and lower border of the HPZ using 1cm increments, 0.5cm was added to the distance from the nares for the position of each mean pH and mean end-expiratory pressure measured.

The postprandial maximal intragastric pH was defined as the highest mean pH recorded distal to the position of the proximal gastric fold clips after the meal and reflected postprandial buffering.
A region of highly acidic pH at the gastro-oesophageal junction was observed in both normal and reflux patients after the meal. To compare these two groups directly, the number of consecutive 1cm increments (from the pull-through study), where the mean pH was less than 2 immediately distal to the location of the proximal gastric folds was recorded. For each patient there were two pH pull-through recordings available for analysis (one from the proximal pH electrode and one from the distal pH electrode).

**Statistical Analysis**

Statistical analysis was performed using one-sample Wilcoxon test or Mann-Whitney-U test unless specified otherwise. Results are given as medians and ranges unless otherwise specified.

**Ethics**

The study was approved by the North Glasgow University NHS Trust Ethics Committee. All subjects participating gave written informed consent.
Results

Movement of Radio-opaque Clips with Respiration

Distal movement of the endoscopically-placed clips was seen radiologically with inspiration in both groups. In the healthy subjects, the respiratory movement of the clips at the proximal gastric folds relative to the pH/manometry apparatus was 2.5cm (range 0.2 to 4.6). The proximal gastric folds moved 0.2cm (range -0.2 to 2.2) distally with inspiration in the reflux group. There was a non-significant trend for increased movement with respiration in the healthy subjects compared to reflux patients (p=0.08) (Figure 4.8). There was no significant respiratory movement of the diaphragmatic clips between the inspiratory and expiratory phases of respiration measured in the reflux group.
Figure 4.8

The respiratory excursion of the clips situated at the proximal margin of the gastric folds (PGF) in healthy subjects and patients with reflux oesophagitis.

There was a non-significant trend towards increased movement with respiration in healthy subjects versus reflux patients (p=0.08)
**Healthy Subjects - Fasting**

In the healthy subjects the lower oesophageal HPZ had its proximal border at 44.5cm (range 39.5 to 45.5cm) from the nares and it extended 4cm (range 3 to 5cm) distal to this. The proximal margin of the gastric folds was 2.8cm (range -0.1 to 4.6; p=0.005) distal to the proximal border of the HPZ.

The maximal fasting intragastric pH for the 12 normal subjects was 1.8 (range 1.0 to 6.0). The fasting pH step-up occurred 3.5cm (range 2 to 5cm) distal to the proximal border of the HPZ. The fasting pH step-up point was thus slightly distal to the proximal gastric folds (median 0.8cm; range 3.0 cm to -1.5cm; p=0.08), and 1cm (range -1 to 2cm; p=0.08) proximal to the lower border of the HPZ (Table 4.1).
<table>
<thead>
<tr>
<th></th>
<th>Fasted Healthy subjects</th>
<th>Postprandial Healthy subjects</th>
<th>Fasted Reflux Patients</th>
<th>Postprandial Reflux Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximal intragastric pH</strong></td>
<td>1.8 (1.0, 6.0)</td>
<td>6.1** (2.4, 7.0)</td>
<td>1.6ªª (0.9, 6.9)</td>
<td>4.8 (1.1, 7.8)</td>
</tr>
<tr>
<td><strong>Position of proximal border of HPZ (cm)</strong></td>
<td>44.5 (39.5, 45.5)</td>
<td>44.5ª (38.5, 45.5)</td>
<td>41.0ª (35.5, 44.5)</td>
<td>41.5 (34.5, 44.5)</td>
</tr>
<tr>
<td><strong>Position of distal border of HPZ (cm)</strong></td>
<td>48.5 (42.5, 50.5)</td>
<td>47.5ª (40.5, 49.5)</td>
<td>46.5ªª (37.5, 48.5)</td>
<td>43.5 (36.5, 48.5)</td>
</tr>
<tr>
<td><strong>Length of HPZ (cm)</strong></td>
<td>4 (3, 5)</td>
<td>2** (1, 4)</td>
<td>4 (2, 6)</td>
<td>3 (1, 6)</td>
</tr>
<tr>
<td><strong>Position of proximal gastric folds (cm)</strong></td>
<td>47.2 (42.9, 49.1)</td>
<td>47.1ªª (41.6, 48.1)</td>
<td>43.2ª (39.2, 48.1)</td>
<td>43.2 (38.0, 45.6)</td>
</tr>
<tr>
<td><strong>Position of diaphragmatic clips (cm)</strong></td>
<td>-</td>
<td>-</td>
<td>44.6</td>
<td>45.4 (41.0, 49.1)</td>
</tr>
<tr>
<td><strong>Length of hiatus hernia (cm)</strong></td>
<td>-</td>
<td>-</td>
<td>1.6</td>
<td>2.3 (0.9, 4.1)</td>
</tr>
<tr>
<td><strong>Respiratory inversion point (cm)</strong></td>
<td>45.5 (40.5, 47.5)</td>
<td>45.5 (39.5, 47.5)</td>
<td>42.5ª (39.5, 46.5)</td>
<td>42.5 (38.5, 48.5)</td>
</tr>
<tr>
<td><strong>Maximal mean end-expiratory pressure of HPZ (mmHg)</strong></td>
<td>27.5 (11.9, 65.7)</td>
<td>17.7ª§ (0.6, 35.5)</td>
<td>12.7ª§ (4.4, 27.9)</td>
<td>6.1§ (6.7, 39.2)</td>
</tr>
</tbody>
</table>

**Table 4.1**

All values are medians (range) for all subjects in each group.

HPZ = high pressure zone

* signifies p ≤ 0.05  versus fasting healthy subjects

** signifies p ≤ 0.005 versus fasting healthy subjects

ª signifies p ≤ 0.05  versus postprandial reflux patients

ªª signifies p ≤ 0.005 versus postprandial reflux patients

§ signifies difference relative to fasting pressures on 1-sample Wilcoxon testing versus no difference (p ≤ 0.05)
Healthy subjects – After Meal

Following the meal, the proximal border of the HPZ was unchanged from its fasting position but the length of the HPZ was 1.5cm shorter than its fasting length (p=0.006) (Table 4.1). Following the meal there was therefore loss of the distal 1.5cm portion of the HPZ. The proximal margin of the gastric folds was 2.6cm (range -1.1 to 4.3) distal to the proximal border of the HPZ, which was not significantly different from its fasting position.

Following the meal the maximal intragastric pH was increased at 6.1 (range 2.4 to 7.0) compared to the fasting maximal pH of 1.8 (Table 4.1) (Figure 4.9). An unbuffered acid pocket (a region of intra-gastric pH of 2 or less, distal to the clips at the proximal margin of the gastric folds) was observed in 8 subjects with the distal pH electrode and in three of these subjects also by the proximal electrode. In no subjects was an acid pocket detected only by the proximal pH electrode. The 8 acid pockets detected by the distal electrode had a median length of 1.5cm (range 1 to 5cm) and the 3 acid pockets detected by the proximal electrode had a median length of 3cm (range 1 to 4cm).

Of the 11 acid pockets detected by either electrode in 8 healthy subjects, the lowest intragastric pH detected by the respective pH electrode was within the acid pocket. The median postprandial intragastric pH nadir was 1.1 (range 0.7 to 2.0) and was located 1.3cm (range 0 to 4.5cm) distal to the proximal margin of the gastric folds. The proximal extent of the acid pocket was 1.1cm distal to the proximal margin of the gastric folds (range -1.7cm to 4.5cm; p=0.07 versus 0cm).

Seven of the 8 healthy subjects, with acid pockets, had adequate manometry to localise the acid pocket relative to the lower border of the HPZ. Ten acid pockets from the
proximal (n=3) and distal electrodes (n=7) combined exhibited a median distance of 0cm (range -2 to 3cm) between their proximal extent and the lower border of the HPZ. The postprandial acid pocket was thus occupying the fasting location of the distal HPZ that opened following the meal (Fig.4.10). An example of an acid pocket in a healthy subject is demonstrated in Figure 4.11.
Figure 4.9

Maximal intragastric pH measured in the fasting and postprandial phase for both healthy subjects and reflux patients. There was no significant difference for fasting and postprandial medians between the two populations.
Figure 4.10

Location of fasting pH step-up and postprandial acid pocket relative to manometric landmarks in 11 studies with acid pockets in healthy subjects.

All values are medians of the results obtained in the 11 studies in healthy subjects that exhibited an acid pocket.

* significant shortening of the high pressure zone seen after the meal (p<0.05)

The respiratory inversion point was a median 1cm distal to the proximal border of the high pressure zone in both the fasting and postprandial state.

The high pressure zone is the region filled by oblique lines.
Figure 4.11

An example of an acid pocket detected by both proximal and distal electrodes in a healthy subject and their position relative to high pressure zone demonstrated on pull-through manometry.
Reflux Patients – Pre and Postprandial Anatomy

In the reflux patients, the postprandial position of the proximal border of the HPZ was 41.5 cm (range 34.5 to 44.5), from the nares, which was similar to its fasting position (Table 4.1). The HPZ extended 3 cm (range 1 to 6 cm) distal from its proximal border after the meal. There was a strong trend for the HPZ to shorten after the meal (median difference 1 cm; range -1 to 3; p=0.06) due to the loss of its distal portion.

Postprandially, the proximal margin of the gastric folds was 1.6 cm (range -0.4 to 4.5 cm) distal to the proximal border of the HPZ, which was similar to its fasting position. The diaphragmatic clips were 4.7 cm (range 1.9 to 7.3 cm) distal to the proximal border of the HPZ after the meal which was similar to their fasting position. The length of the hiatus hernia, as determined by the distance between the clips at the proximal margin of the gastric folds and the diaphragmatic impression, was 2.3 cm (range 0.6 to 4.7) after the meal which was similar to its fasting length (1.6 cm; range 0.9 to 4.1 cm; p=0.8) (Figure 4.12).
Figure 4.12

The length of the hiatus hernia before and after the meal in reflux oesophagitis patients.

There was no significant difference in response to the meal.
**Postprandial Acid Pockets in Reflux Patients Versus Controls**

In the 16 reflux patients the fasting maximal intragastric pH was 1.6 (range 0.9 to 6.9) and was similar to that seen in healthy subjects (Table 4.1). Following the meal the maximal intragastric pH was increased to 4.8 (range 1.1 to 7.8) which, again, was similar to the healthy subjects (Table 4.1) (Figure 4.9).

An unbuffered acid pocket (a region of high acidity of pH≤ 2 distal to the clips at the proximal gastric folds) was observed by the distal pH electrode in 13/16 reflux patients. Ten of the 16 reflux patients also had an acid pocket detected by the proximal electrode. In no patient was an acid pocket detected by only the proximal pH electrode. There was a significantly higher frequency of acid pockets in the reflux patients (23/32 studies) than in the healthy subjects (11/24) (p<0.05). This difference in frequency was predominantly due to a higher incidence of acid pockets detected by the proximal electrode in the reflux patients (10/16) compared to healthy volunteers (3/12) (p=0.049). The frequency of acid pockets detected by the distal electrode was similar in the reflux patients (13/16) and healthy volunteers (8/12) (p=0.378) (Figure 4.13). A typical tracing of an acid pocket in a reflux patient is shown in Figure 4.14.
Figure 4.13

*Frequency of postprandial acid pockets detected – proximal and distal electrode studies combined.*

*There was a significantly higher frequency of acid pockets in the reflux patients (23/32) than in healthy subjects (11/24) (p<0.05).*
**Figure 4.14**

Example of pH and manometric study obtained from a reflux patient during station pull-through at 1cm intervals each minute.

*Tracing at top is from proximal pH electrode demonstrating a region of low acidity at 43 and 42cm from the nares.*

*Middle 3 tracings are from the radially positioned manometry sensors demonstrating the position of the high pressure zone.*

*Bottom tracing is from distal pH electrode again demonstrating a large acid pocket extending from 44cm to 40cm.*
The median length distal to the proximal margin of the gastric folds of the 23 acid pockets from both electrodes combined in the reflux group was 3cm (range 1 to 15cm) and this was significantly longer than the median length of the 11 acid pockets seen by both electrodes in the healthy subjects (median length =2cm; range 1 to 5cm; p<0.05) (Figure 4.15). The length of acidified zone (pH<2), that extended distal to the proximal margin of the gastric folds, for the proximal and distal electrodes for both healthy subjects and reflux patients is given in Fig 4.16.
Figure 4.15

The length of acid pocket (pH≤2) distal to the clips at the proximal margin of the gastric folds in 12 healthy subjects and 15 reflux patients.

The acid pocket was significantly longer in reflux patients when compared to healthy subjects.
Figure 4.16

The length of acid pocket (\(pH \leq 2\)) distal to the clips at the proximal margin of the gastric folds in 12 healthy subjects and 15 reflux patients measured by the proximal and distal pH electrode.

The acid pocket was significantly longer in reflux patients than healthy subjects for both proximal and distal electrodes.

One reflux patient is not included as unable to adequately visualize radio-opaque clips.

Shaded boxes indicate interquartile ranges.

All studies in which there was no acid pocket distal to the clips at the proximal margin of the gastric folds plotted as a value of 0 cm.
Of the 23 acid pockets detected by either electrode, the lowest postprandial intragastric pH was within the acid pocket in 21 of the 23 recordings. The median postprandial intragastric pH nadir within the acid pockets was 1.1 (range 0.7 to 1.8) and was located 1.3cm (range 0.3 to 7.3cm) distal to the proximal margin of the gastric folds. Both acid pocket pH nadir and location of the nadir within the acid pocket was similar to that seen in healthy subjects.

The distance between the proximal margin of the gastric folds and the proximal extent of the intragastric acid pocket was calculated for both electrodes in each subject group. If the acid pocket extended to or above the level of the proximal gastric folds, a value of 0cm was recorded. Combined analysis of acid pockets from both electrodes revealed the proximal extent of 11 acid pockets (found in 8 healthy subjects) to be 1.1cm (range 0 to 4.5cm) distal to the proximal margin of the gastric folds whereas 23 acid pockets (from 13 reflux patients) had a median distance of 0cm (range 0 to 1.5cm) between the proximal extent of the acid pocket and the proximal margin of the gastric folds (p=0.005) (Figure 4.17).
Figure 4.17

Distance between the proximal extent of the postprandial acid pocket distal to the proximal margin of the gastric folds in healthy subjects and reflux patients.

Combined studies from proximal and distal pH electrode and only included if acid pocket demonstrated distal to the proximal margin of the gastric folds.

11 studies in healthy subject group and 23 studies in reflux group analysed.

Shaded boxes indicate interquartile ranges.

If proximal edge of acid pocket located above the proximal margin of the gastric folds then a value of 0cm reported.
The length of extension of the acid pocket distal to the diaphragmatic impression was similar in the reflux patients (median 2cm; range -2 to 9cm) and healthy subjects (median 2cm; range 1 to 5cm) (Figure 4.18). The longer acid pocket seen in the reflux patients thus corresponded to the proximal migration of their proximal gastric folds above the diaphragmatic hiatus ie. their hiatus hernia. This location of the extended acid pocket in the reflux patients is seen when the median pH at each catheter station is plotted against the position of the anatomical and manometric landmarks (Figure 4.19). Correlation of individual postprandial hiatal hernia length and length of acid pocket measured by the distal pH electrode was not found to be statistically significant (R=0.212).
Figure 4.18

The length of extension of the acid pocket distal to the diaphragmatic impression.

There was no significant difference in the length of the “intragastric” acid pocket extended in healthy subjects and reflux oesophagitis patients.
Figure 4.19

The median of mean pH measurements by proximal electrode at each catheter position relative to the nares in 12 healthy subjects and 16 reflux patients.

The position of the high pressure zone for each subject group represented by boxed area and represents the median positions of the proximal and distal borders of the high pressure zone.

Median position of clips at proximal margin of gastric folds for each group represented by * symbol.

Vertical dotted line represents median position of clips at the diaphragmatic impression in the reflux group.

Respiratory inversion point represented by downward pointing arrow
**Radiological studies**

Comparison of fasting and postprandial barium swallows revealed no radiological alteration in the morphology of the hiatal sac in response to the meal. In addition there was no evidence of food debris seen within the hiatal sac after the meal. More detailed analysis of the barium swallows was not performed as the dynamic properties of this region during swallowing i.e. variable oesophageal shortening due to persitalsis, made accurate measurement of the radiological landmarks difficult on review of the films. The measurement of the radiological landmarks (utilising the radio-opaque clips) was based on the plain x-rays taken with the ph/manometry apparatus in-situ as this was felt to be less prone to movement artefact (as stated previously we corrected our measurements for the effects of respiratory movement).
Chapter 5

Paradox Of Gastric Cardia – It Becomes More Acidic Following Meals While The Rest Of Stomach Becomes Less Acidic
**Introduction**

The gastro-oesophageal junction and gastric cardia is a complex region with respect to its anatomy, physiology and luminal chemistry. At this location, the squamous epithelium of the distal oesophagus transforms into columnar cardia-type epithelium which then becomes oxyntic epithelium of the body of the stomach. The cardia-type epithelium is characterised by branched mucus-secreting glands but absent or very few specialised parietal or chief cells. The length of this cardia-type epithelium is highly variable, ranging from 0-15mm (4;10).

The gastro-oesophageal junction and adjacent gastric cardia is also a region of high prevalence of epithelial pathology including inflammation (carditis), intestinal metaplasia and adenocarcinoma and the aetiology of this pathology is unclear. In the developed world 30-50% of all upper gastrointestinal tract adenocarcinomas occur at this location (172;173). Unlike oesophageal adenocarcinoma, the association between cardia adenocarcinoma and reflux is weak (44). Intestinal metaplasia of the epithelium in the cardia region is reported in 12-16% of the general population undergoing screening colonoscopy and shows no association with reflux symptoms (62;174).

Cardia-type epithelium may itself be pathological. It has been postulated that it might be mainly the result of columnar metaplasia of the most distal oesophageal squamous epithelium and arising from exposure to gastric acid (10). Evidence in support of this is the near absence of cardia epithelium in neonates and its increase in extent with age (9;10).

We, and others, have demonstrated that the proximal cardia region of the stomach differs from the more distal stomach in escaping the buffering effect of food and
remaining acidic during the postprandial period (acid pocket). It has been postulated that
this could be a source of acid exposure of the most distal oesophagus and contribute to
the high prevalence of epithelial pathology at this site (160-164;168;175).

Several characteristics of the unbuffered acid pocket remain unclear and may be
relevant to its potential role in disease at this site. Little is known regarding its length at
different times following commencement of a meal and there is also controversy
regarding its proximity to the squamo-columnar junction throughout the postprandial
period. In addition, it has been suggested that the acid pocket is merely a thin film of
acid with no potential for producing significant oesophageal acid exposure (161). The
lack of information regarding these issues is largely related to the limitations of methods
used so far to study it.

In order to address the above issues, I have developed and employed for the first time a
high definition probe allowing constant and simultaneous recording of pH at twelve sites.
Nine of these electrodes are in the region of the squamo-columnar junction and cardia
and spaced only 11mm apart. The catheter is clipped to the epithelium at the squamo-
columnar junction to allow accurate documentation of the pH environment in this
dynamic and important region.
Aims

The aims of my study were to investigate (i) the length of the acid pocket throughout the postprandial period; (ii) its location relative to the squamo-columnar junction and (iii) whether the acid pocket contains sufficient acid to produce distal oesophageal acidification.

Subjects

The study population consisted of 15 healthy subjects (mean age 40.5 years, range 24 to 57, 6 male/ 9 female). Helicobacter pylori status was documented in all patients prior to the study by either breath test or endoscopic urease-based test with 12 testing negative and 3 positive.

Study Design

The high definition 12 electrode pH catheter was placed in the upper gastrointestinal tract and endoscopically clipped to the mucosa at the squamo-columnar junction. After a 2 hour rest period, the pH at each of the 12 sites was recorded at a frequency of 8 Hertz. The patients then underwent pH recording during a 15 min fasting period, during ingestion of a standardized meal and during a 90 min postprandial period.
Study Protocol

High Definition pH Catheter

The pH catheter was specifically constructed for this study (Synectics Medical, Enfield, UK). It was 2.1mm in diameter, flexible and had 12 antimony electrodes along its distal end and an external reference electrode for application to the upper arm. The most distal pH electrode was located at the tip of the catheter and the other 11 electrodes were 30, 50, 61, 72, 83, 94, 105, 116, 127, 138 and 172 mm proximal it (Figure 5.1). Two loops, of 3mm diameter, were tied to the catheter using 2-0 prolene suture material (Ethicon, Somervile, USA). These loops were tied between the electrodes No.4 and No.5 which was 110.5mm proximal to the catheter tip (Figure 5.2).
Figure 5.1

The position of the 12 pH catheter electrodes relative to the squamo-columnar junction. The catheter was clipped to the squamo-columnar junction with endoclips through a loop tied between electrodes 4 and 5 110.5mm proximal to electrode 12 at the catheter tip.
Figure 5.2

12 electrode pH catheter with two loops, of 3mm diameter, tied to the catheter using 2-0 prolene suture material.

The prolene loops were tied between the electrodes No.4 and No.5 which was 110.5mm proximal to the catheter tip.
Prior to nasal intubation, individual electrodes were calibrated in buffer pH 1.07 and pH 7 with data collected using Polygram Net software (Synectics Medical, Enfield, UK). Calibration at room temperature was corrected by the computer software for pH measurements at body temperature.

Procedure

On the day of the study, the subjects reported fasted to the gastrointestinal unit. The pH catheter was passed nasogastrically. Once the catheter was passed, the subject then underwent upper gastrointestinal endoscopy. This was performed either with Xylocaine throat spray or under conscious sedation with intravenous midazolam. Under direct endoscopic vision, the prolene loop, attached to the nasogastric pH catheter, was fixed to the squamo-columnar junction using standard haemostatic metal clips (HX-600-090, Olympus), deployed by an endoscopic clip-fixing device (HX-5LR-1, Olympus) (Figure 5.3). None of the healthy subjects had endoscopic evidence of columnar-lined oesophagus. The endoscope was then removed and the patient allowed to rest for 2 hours following the procedure.

Once the 2 hour rest period was completed, the pH was measured at all 12 electrodes using the Polygram Net software for a 15 min fasting period. The subject was then fed a standardised fish and french fries meal (the patients were asked to eat until the sensation of fullness). Once the meal was completed, the pH recording continued for a further 90 min. The pH catheter was then removed with simple traction, dislodging the endoclip in the process. All subjects were studied in a semi-recumbent position on an examination couch with the headrest at 45 degrees for the duration of both fasting and postprandial pH recording.
Figure 5.3

Position of 12 electrode pH catheter relative to the gastro-oesophageal junction.

The catheter was clipped to the mucosa with electrode no.4 situated 5.5mm above the squamo-columnar junction and electrode no.5 situated 5.5mm below the squamo-columnar junction.

Figure modified on an illustration by Van Herwaarden et al (42).
Analysis

The dynamic nature of the postprandial intragastric luminal environment was examined by subdividing the 90 min postprandial period into six consecutive 15 min intervals and the % time pH<4 calculated for each electrode. The difference between the %time pH<4 in the first 15 min after the meal and subsequent postprandial 15 min intervals (15 to 30 min, 30 to 45 min, 45 to 60 min, 60 to 75 min and 75 to 90 min) was calculated in each subject and median differences for the whole group determined. The %time pH<4 for the 15 min immediately prior to meal ingestion was also calculated for each electrode in each subject to allow direct comparison with the 6 consecutive postprandial 15 min intervals and the total 90 minute postprandial period.

The design of the catheter with its 12 continuous readings from the pH electrodes led to the decision to measure %time pH<4 prior to commencing the study. It was felt that this measure of acidity rather than pH at specific time intervals would give a more consistent representation of acid exposure in this dynamic region. When designing the study I decided upon a 90 minute postprandial period to allow adequate time after the meal to assess the effect of meal buffering and no further readings were taken after this 90 minute postprandial period had elapsed in my study subjects. I also decided upon a 15 minute fasting period during study design as this would allow direct comparison with six 15 minute sub-sections of the 90 minute postprandial period as previously stated above.

Gastro-oesophageal reflux events in the postprandial period were defined as a fall in pH to less than 4 in one or more of the three most proximal oesophageal electrodes (at least 16.5mm above the squamo-columnar junction). Simultaneous intragastric pH measured in the more distal catheter electrodes during the episodes of acidic reflux was
also noted. A reflux event was defined as attributable only to the proximal acid pocket if
i) the pH recorded simultaneously in one of the 3 most proximal intragastric electrodes
(electrode No.s 5,6,7 i.e. within 2.75cm of the squamo-columnar junction) or electrode
No.4 immediately above (5.5mm) the squamo-columnar junction was less than or equal
to the minimum pH recorded in the 3 most proximal oesophageal electrodes and ii) the
pH recorded simultaneously in the 4 more distal intragastric electrodes (electrodes No.s
8,9,10,11 i.e. 3.85cm to 8.05cm distal to the squamo-columnar junction) was greater than
or equal to the minimum pH of the acidic oesophageal refluxate. Reflux that did not fulfil
both the above criteria was deemed to be of indeterminate intragastric source.

**Statistical Analysis**

Statistical analysis was performed using one-sample Wilcoxon test, Mann-Whitney-U
test or Krukal-Wallis Analysis of Variance unless specified otherwise. Results are given
as medians and ranges unless otherwise specified.

**Ethics**

The study was approved by the North Glasgow University NHS Trust Ethics
Committee. All subjects participating gave written informed consent.
Results

Regional Intragastric Acidity During Fasting

Intragastric acidity during the fasting period was found to be high except for the electrodes situated within the cardia. The median %time pH<4 recorded by the electrodes 27 to 110.5mm distal to the squamo-columnar junction was high (94-100%). Electrode No.5 situated 5.5mm distal to the squamo-columnar junction recorded minimal acidity (median %time pH<4 2.25%) which was significantly lower than the acidity recorded in all other intragastric electrodes (p<0.05). Electrode No.6 situated 16.5mm distal to the squamo-columnar junction recorded significantly less acidity (median %time pH<4 39%) than more distally situated electrodes No.s 8-12 (median %time pH<4 98.85% to 100%) (p<0.05).
Regional Intragastric Acidity Following Meal

In the first 15 min after completion of the meal the greatest acidity (median %time pH<4) was at electrode No.6 (7.8%) located 16.5mm distal to the squamo-columnar junction (Table 5.1). The electrodes immediately proximal and distal to electrode No.6 also showed a small amount of acidity at this time; this included electrode No.5 closest to the squamo-columnar junction (median =2.5%) and electrode No.7 located 27.5mm distal to the squamo-columnar junction (median = 1.7%). The other intragastric electrodes showed a median %time pH<4 = 0 during the first 15 min period except for the most distal intragastric electrode (5.1%).

Over the following five 15 min periods, the acidity at electrode No.6 located 16.5mm distal to the squamo-columnar junction increased progressively to reach 91.4% at 75-90 min. Electrode No.7 located 27.5mm distal to the squamo-columnar junction showed a similar progressive increase reaching 79% at 75-90 min. Electrode No.5 located 5.5mm distal to the squamo-columnar junction also showed an increase reaching a maximum of 58% at 60-75min. The most distal electrode (No. 12) also recorded increasing acidity giving a high reading at 30-45min (42%) and at 75-90 min (38%). In contrast, very little acidity was recorded by the intermediate electrodes (No.s 8, 9, and 10) throughout the entire 90 min postprandial period, never recording a median acidity greater than 3% (Table 5.1).
Table 5.1

Regional differences in acidity during the fasting period and in response to the meal.

Values are median percentage time at pH<4.

The median duration of the meal was 15 minutes.

For clarity, shaded figures indicate a median percentage time at pH<4 > 5%.

Bold horizontal line indicates position of squamocolumnar junction.

*Different from value recorded at the same site at 0-15 min after meal (p<0.05).

**In the first 15 min after the meal, different from value recorded by electrode 6 (p<0.05).

<table>
<thead>
<tr>
<th>Electrode</th>
<th>15 min fasting</th>
<th>Meal</th>
<th>Time following completion of meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5 min</td>
<td>15-30 min</td>
<td>30-45 min</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>0 0</td>
<td>0 0.3*</td>
<td>0 0.51*</td>
</tr>
<tr>
<td>Squamocolumnar Junction</td>
<td>0.03</td>
<td>0.07</td>
<td>0.14</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.25 0.98</td>
<td>2.46 2.875</td>
<td>37.01*</td>
</tr>
<tr>
<td>Stomach</td>
<td>39.0 7.35</td>
<td>7.85 9.25</td>
<td>75.44*</td>
</tr>
<tr>
<td>Stomach</td>
<td>94 8.52</td>
<td>1.74 7.57</td>
<td>18.46*</td>
</tr>
<tr>
<td>Stomach</td>
<td>98.85 14.51</td>
<td>0**</td>
<td>0.94*</td>
</tr>
<tr>
<td>Stomach</td>
<td>99.6 19.72</td>
<td>0**</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>100 23.56</td>
<td>0**</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>99.7 45.83</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>100 75.78</td>
<td>5.125</td>
<td>14.375</td>
</tr>
</tbody>
</table>
Unique Changes Occurring at the Cardia in Response to the Meal

The electrode (No. 5) situated in the cardia 5.5mm distal to the squamo-columnar junction differed from all the other intragastric electrodes both during fasting and in its response to the meal. During fasting, minimal acidity (pH<4 = 2.2%) was recorded by electrode No.5 whereas high intragastric acidity (39-100%) was recorded by each more distal electrode (Table 5.1). Electrode No.5 also differed from all other intragastric electrodes in recording a marked increase in acidity in response to the meal with median %time pH<4 being 37%, 30%, 58%, 25% during the 15 min periods from 30-90 min following the meal (p<0.038 versus fasting). In contrast, all of the other intragastric electrodes demonstrated a decrease in acidity relative to their fasting state (p<0.05 electrodes No.7 to No.12) except for electrode No.6 which showed no significant change in acidity in response to the meal.
Proximal Acid Pocket as Source of Postprandial Acid Reflux

Oesophageal acidity was greater during the total 90 min postprandial period than fasting period for each of the oesophageal electrodes. The median % time pH<4 for fasting versus postprandial was 0% versus 2.5%, p<0.005; 0% versus 1.2%, p<0.005; 0% versus 0.6%, p<0.005; 0% versus 0.1%, p<0.05 for the electrodes situated at 5.5mm, 16.5mm, 27.5mm and 61.5mm above the squamo-columnar junction respectively. In order to determine whether the proximal acid pocket was a source of significant postprandial acid reflux, the pH of oesophageal refluxate was compared with simultaneous recordings from different regions of the stomach. For this I only included the three more proximal oesophageal electrodes which were all at least 16.5mm proximal to the squamo-columnar junction. A reflux event was defined as a pH<4 recorded by any of these electrodes. 84 postprandial reflux episodes in total were identified in the 15 healthy subjects. 51.1% (range 0-100) of postprandial reflux occurred at a time when the only region of the stomach of equivalent or lower pH was in the cardia within 27.5mm of the squamo-columnar junction (Table 5.2). This demonstrates that a minimum of 51.1% of the reflux observed had originated entirely from the acidic cardia region. My methodology does not allow us to determine the intragastric source of the other 48.9% of reflux observed. Reflux recorded from both of these sources extended at least 16.5mm above the squamo-columnar junction.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Acidic Oesophageal Reflux (% time pH&lt;4)</th>
<th>% reflux attributable only to the acid pocket</th>
<th>Helicobacter status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.14</td>
<td>100</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>9.73</td>
<td>87.86</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>0.48</td>
<td>83.73</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>0.27</td>
<td>77.39</td>
<td>negative</td>
</tr>
<tr>
<td>5</td>
<td>14.01</td>
<td>63.32</td>
<td>negative</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>51.05</td>
<td>negative</td>
</tr>
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<td>7</td>
<td>1.29</td>
<td>47.04</td>
<td>negative</td>
</tr>
<tr>
<td>8</td>
<td>8.39</td>
<td>31</td>
<td>negative</td>
</tr>
<tr>
<td>9</td>
<td>8.18</td>
<td>26.16</td>
<td>negative</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>23.26</td>
<td>positive</td>
</tr>
<tr>
<td>11</td>
<td>3.81</td>
<td>7.1</td>
<td>negative</td>
</tr>
<tr>
<td>12</td>
<td>64.39</td>
<td>0.12</td>
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</tr>
<tr>
<td>13</td>
<td>0</td>
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<td>negative</td>
</tr>
<tr>
<td>14</td>
<td>1.24</td>
<td>65.18</td>
<td>positive</td>
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<td>negative</td>
</tr>
<tr>
<td>median</td>
<td></td>
<td>51.05</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.2**

*Oesophageal reflux was assessed by the recordings from the 3 electrodes which were ≥16.5mm proximal to the squamo-columnar junction over the 90 min postprandial period.*

*For definitions of % reflux attributable only to the acid pocket please refer to “Analysis” in the study methods section.*
Reflux events that could only be attributed to the acid pocket were a median 9.8 seconds with a nadir pH of 1.99. Reflux events that could not be solely attributed to the acid pocket were of longer duration (median=17.7 seconds; p=0.011) and lower pH nadir (median pH 1.58; p<0.005).

We compared the characteristics of the reflux events which could only be attributed to the acid pocket with those of undetermined origin. The median nadir pH of the former (pH 1.99) was higher than the latter (pH 1.58) (p<0.005) and the median duration of the former (9.8 s) was shorter than the latter (17.7 s) (p<0.05) (Table 5.3). There was a trend for the former to extend less proximally up the oesophagus, but this did not reach statistical significance (Table 5.3).

The real-time recording of luminal pH over the fasting and postprandial period allowed close observation of dynamic changes in both the oesophagus and stomach (Figure 5.4a-f).

Figure 5.4e demonstrates oesophageal acid reflux originating from the acid pocket in one of the subjects during the postprandial period. Figure 5.5 shows an example of a 12 pH electrode study demonstrating postprandial acidification of the cardia.
Reflux that could only be attributed to acid pocket

<table>
<thead>
<tr>
<th></th>
<th>Reflux that could only be attributed to acid pocket</th>
<th>Reflux events of undetermined origin</th>
<th>p&lt;0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Nadir pH</td>
<td>1.99</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.18 to 3.77)</td>
<td>(0.69 to 3)</td>
<td></td>
</tr>
<tr>
<td>Median Duration of Reflux Events</td>
<td>9.8 seconds</td>
<td>17.7 seconds</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>(3.1 to 128.5)</td>
<td>(3.1 to 384.1)</td>
<td></td>
</tr>
<tr>
<td>Percentage of Reflux Events Recorded by Electrode</td>
<td>• 16.5mm 52.4%, 38%</td>
<td>• 27.5mm 28.6%, 31%</td>
<td>• 61.5mm 19%, 31%</td>
</tr>
</tbody>
</table>

above the squamo-columnar junction

Table 5.3

*Characteristics of reflux events only attributable to the acid pocket versus those of undetermined origin.*
Figures 5.4 a-f

Surface contour plots (each 120 seconds duration) of high resolution 12 electrode pHmetry.

Figure 5.4a.

The surface contour plot demonstrates the fasting state with marked intragastric acidity.

SCJ = squamo-columnar junction
Figure 5.4b

3 minutes after completion of the meal shows intragastric buffering by the ingested meal.

SCJ = squamo-columnar junction
Figure 5.4c

17 minutes after the meal the emergence of the acid pocket at the gastro-oesophageal junction is seen.

SCJ = squamo-columnar junction
Figure 5.4d

43 ½ minutes after the meal the enlarging acid pocket is demonstrated.

SCJ = squamo-columnar junction
Figure 5.4e

47 ½ minutes after the meal an acidic reflux episode (circled) from the acid pocket is seen with simultaneous distal intragastric buffering.

SCJ = squamo-columnar junction
Figure 5.4f

73 ½ minutes after the meal both the proximal acid pocket and distal acidity is recorded simultaneously

SCJ = squamo-columnar junction
Figure 5.5

An example of a 12 pH study demonstrating postprandial acidification of the cardia. In this particular example the earliest evidence of postprandial acidification is in the electrode 16.5mm distal to the proximal margin of the gastric folds (No.5) rather than the electrode immediately distal (electrode No.6).
Chapter 6

Discussion
Severe Reflux Disease Is Associated With Enlarged Unbuffered Proximal Gastric Acid Pocket

This study confirms the frequent presence of a region of unbuffered acidity at the gastro-oesophageal junction in healthy subjects following a meal. Previous work by Fletcher et al (160) first described the presence of an unbuffered postprandial acid pocket defined as a minimum pH <2 using a station pull-through technique. Using the more strict criteria of a mean pH ≤ 2, I identified an acid pocket in 46% (11/24) of studies in healthy subjects. The term unbuffered is justified as the pH was similar to fasting pH at a time when the pH in the main body of the stomach was substantially higher due to the effects of the meal. The frequency of acid pockets detected was higher with the distal pH electrode than with the proximal electrode. This may reflect elongation/evolution of the acid pocket with time as the distal electrode traversed the proximal stomach several minutes after the passage of the proximal electrode. My subsequent studies employing the static 12 electrode pH catheter further clarified the differences in measurement by the proximal and distal electrodes in this study as we observed progressive acidification of the cardia region with time after the meal. Therefore, any pH electrode passing through this region at a later time (i.e. the distal electrode) would be more likely to detect an acidic pH due to the progressive postprandial acidification of this region.

In the present study were able to document the location of the unbuffered region relative to the HPZ in the healthy subjects. The acid pocket extended proximally as far as the distal end of the postprandial HPZ. I observed that the HPZ was 1.5cm shorter after the meal than when fasted and that this was due to loss of its distal segment as previously observed by Manning et al (176). The median length of the acid pockets
was 2cm in healthy subjects and thus they were located mainly within the abdominal portion of the HPZ that “opens” after ingesting a meal (Figure 4.10).

In the original report of the unbuffered acid pocket in healthy subjects, Fletcher et al (160) observed that it extended across the squamo-columnar junction onto the distal oesophagus. In the current study I also studied the proximal extent of the acid pockets relative to the radio-opaque clips applied to the proximal margin of the gastric folds and which corresponded to the squamo-columnar junction in the healthy subjects. I observed that the median proximal margin of acid pockets was 1.1cm distal to the clips and only 2 of 11 extended proximal to the clips. I believe the discrepancy between the current study and the earlier report of Fletcher et al (160) can be explained by the movement with respiration of the clips attached at the squamo-columnar junction relative to the pH probe. I observed in the current study that the clips moved a median of 2.5cm during the respiratory cycle and therefore I recorded the pH and pressure relative to the clips at the mid-respiratory cycle. In the earlier study by Fletcher et al (160), the clip position was measured only on inspiration which would tend to over-estimate the proximal extent of the acid pocket relative to the squamo-columnar junction (Figure 6.1)
Figure 6.1

The position of the clips in healthy subjects at the proximal margin of the folds (which approximated the squamo-columnar junction) demonstrated excursion of 2.5 cm due to respiration. This may explain differences in measurement of the proximal extent of the acid pocket relative to the proximal margin of the folds seen between my study and previous work by Fletcher et al (160).
In addition, in the study by Fletcher et al (160) the minimum pH at each site was analysed rather than the mean pH in the current study. The proximal movement of the squamo-columnar junction with expiration relative to the pH probe will result in it being intermittently acidified even when at a mean position 1cm above the squamo-columnar junction and the effect will be much more apparent when employing minimal pH rather than mean pH at each station.

Our observation that the acid pocket rarely extends across the squamo-columnar junction in healthy subjects is consistent with the findings of Fletcher et al (167) when a pH electrode was clipped within the HPZ 5mm proximal to the squamo-columnar junction. At that location intermittent acidification was observed and interpreted as short segment reflux but there was no evidence of prolonged acidification following a meal as would be expected from the presence of an acid pocket extending proximal to the squamo-columnar junction.

The location of the unbuffered acid pocket relative to the HPZ in the healthy subjects provides information regarding its nature and origin. If the region of unbuffered pH had been confined to the distal region of the sphincter it could have been explained by the pH probe being encircled by acid secreting mucosa and thus recording mucosal surface pH. However, the fact that the unbuffered region extended more than 1cm distal to the HPZ supports the existence of a finite amount of unbuffered gastric secretions.

In the present study I investigated whether healthy subjects and patients with severe reflux disease differed with respect to proximal postprandial unbuffered acid pockets. The great majority of the reflux patients had evidence of hiatus hernia, representing a well recognised association (117;118;120;121;124). Reflux patients on regular acid inhibitory therapy discontinued it one week prior to the studies, a time-point at which
our research group have previously shown that gastric acid secretion is similar to pre-treatment levels (177). The reflux patients and healthy subjects were also confirmed to be similar with respect to maximal fasting and postprandial intragastric pH (Table 4.1/Figure 4.9).

Three differences were noted with respect to the postprandial unbuffered acid pockets between the reflux patients and healthy subjects. Firstly, the reflux patients had a significantly higher incidence of unbuffered acid pockets detected distal to the proximal margin of the gastric folds than the healthy subjects. Secondly, the acid pockets in the reflux patients extended a greater distance distal to the proximal gastric folds than the healthy subjects. Comparing acid pockets seen in reflux patients with those in the healthy subjects showed that the median length of postprandial pH \( \leq 2 \) extending distal to the proximal margin of the gastric folds was 3cm compared to 2cm respectively \((p<0.05)\). The third difference between the reflux patients and healthy subjects was that the acid pockets extended closer to the proximal margin of the gastric folds in the former. In the healthy subjects the proximal extent of the acid pocket was 1.1cm distal to the proximal gastric folds whereas it was 0cm in the reflux patients.

The current studies do not allow us to explain the aetiology of the extended acid pocket in the reflux patients, but it may be related to their abnormal anatomy in the region of the gastro-oesophageal junction. In the reflux patients the proximal margin of the gastric folds was displaced proximally due to herniation of the proximal stomach into the chest. The location and median length of the extended region of unbuffered acidity both corresponded to those of the hiatus hernia (Figure 6.2).
The increased length of the acid pocket in reflux oesophagitis patients corresponded to the proximal migration of their proximal gastric folds above the diaphragmatic hiatus ie. their hiatus hernia.

The yellow arrows in the healthy subject correspond to the position of the clips at the proximal margin of the gastric folds.

The yellow arrows in the reflux oesophagitis patient correspond to clips placed at both the proximal margin of the folds which have migrated proximally due to hiatal herniation and the diaphragmatic hiatus.
We could not demonstrate a significant correlation between the length of the hiatus hernia and length of acid pocket in the reflux patients but that may have been due to the fact that the hiatus hernias were of similar size. A hiatus hernia would provide a physiologically plausible unbuffered acid pocket provided it is lined by healthy acid secreting gastric mucosa and provided food does not reside within it. My postprandial barium studies showed no evidence of food within the hernias. There is little information on the parietal cell density of the mucosa lining hiatus hernias but it is a topic worthy of study.

Mittal et al demonstrated in 1987 that gastro-oesophageal refluxate in patients with hiatus hernia originates from the hernial sac (129). They proposed that the hernial sac provided a reservoir of gastric juice readily available for refluxing into the oesophagus. My current studies indicate that the gastric juice at this anatomical site following a meal is highly acidic due to escaping the buffering effect of the meal and potentially highly damaging to the oesophageal mucosa.

In summary, my study demonstrates that postprandial unbuffered acid pockets are larger, more frequent and closer to the gastro-oesophageal junction after meals in reflux patients than healthy subjects. The extended acid pocket in reflux patients corresponds to the extension of the gastric mucosa above the diaphragmatic hiatus and may be explained by it. This region of unbuffered postprandial acidic juice just below the gastro-oesophageal junction may contribute to severe reflux disease.
Paradox Of Gastric Cardia – It Becomes More Acidic Following Meals While The Rest Of Stomach Becomes Less Acidic

This study, employing high definition oesophago-gastric pHmetry, demonstrates for the first time that the cardia region closest to the squamo-columnar junction is a unique intragastric region in recording minimal acidity under fasting conditions but becoming highly acidic following the meal. This acidification of the cardia is a source of postprandial acidic reflux.

During fasting, the acidity (median %time pH<4) recorded by the 6 more distal intragastric electrodes was high (94-100%). However, the degree of acidity was less in the cardia being 39% at 16.5mm and 2.2% at 5.5mm distal to the squamo-columnar junction. Several mechanisms may explain this less marked acidity immediately distal to the squamo-columnar junction under fasting conditions. The gastric cardia mucosa extends a variable distance (0 – 15mm) from the squamo-columnar junction to the oxyntic mucosa of the body of the stomach and it contains few or no acid secreting parietal cells (10). In addition, the lumen of the cardia is usually closed as it is contained within the abdominal segment of the high pressure zone. Consequently, the pH of the cardia mucosa will be near neutral as its mucosa does not secrete acid and the sphincter will occlude the acid secreted by the adjacent oxyntic mucosa. The neutralizing effect of swallowed saliva may also lessen acidity in the proximal stomach.

The ingestion of the meal produced a rapid and marked reduction of intragastric acidity consistent with the buffering effect of the food. During the first 15 min following the meal, intragastric acidity was greatest at electrode No. 6 situated 16.5mm distal to the squamo-columnar junction and next highest at the most distal intragastric electrode. Over the subsequent 15 min periods, the acidity observed at the
3 most proximal intragastric electrodes and the most distal intragastric electrode showed a progressive rise in pH. In contrast, very little acidity (<3%) was recorded by the intermediate electrodes No.s 8, 9, 10 through the entire 90 min postprandial period. These observations confirm that the proximal stomach largely escapes the buffering effects of ingested food and remains highly acidic during the postprandial period (160-164;168;175).

The fact that the most distal intragastric electrode (No. 12) also showed relatively little buffering has been previously reported by our research group and others (160;175). The explanation for the high postprandial acidity recorded by the most distal electrode is unclear but might be related to its location relative to the oxyntic mucosa of the stomach. During fluoroscopic screening I have observed that the distal tip of this probe usually lies in close proximity to the wall of the greater curve of the stomach (unpublished observation). The two electrodes showing little buffering are thus both near the wall of the stomach: electrode No.6 near the wall of the proximal stomach and electrode No.12 near the wall of the opposite side of the stomach. As acid is secreted by the wall of the stomach, with the food occupying the lumen, the electrodes nearest the wall are likely to be exposed to most acid and least buffering. This effect would also be consistent with the change in intragastric acidity over the 90 min postprandial period when the acidity progressively increased at electrodes No.s 6 and 12 and with time also increased at electrodes No.s 7 and 11 which would be the next electrodes nearest the acid secreting wall of the stomach. The unbuffered acid pocket might therefore be to some extent an unbuffered “acid coat” if viewed in three dimensions (Figure 6.3).
Figure 6.3

We postulate that the “acid coat” gradually overcomes the buffering of the ingested meal over the postprandial period. This would explain the finding that the most proximal and most distal intra-gastric electrodes exhibit the lowest pH initially (electrodes 5 and 12) and this gradually involves the electrodes that are next nearest the gastric wall as intra-gastric buffering is overcome.
A paradoxical acid response to the ingestion of the meal was observed in the gastric cardia. The electrode 5.5mm distal to the squamo-columnar junction recorded minimal acidity during fasting (2.2%) and showed a marked increase in acidity in response to the meal (58% at 60-75min). The electrode in the distal cardia 16.5mm distal to the squamo-columnar junction showed a tendency to increased acidity following the meal but this did not reach statistical significance. In contrast, all the other intragastric electrodes showed an initial and persisting reduced acidity throughout the 90 min postprandial period. An example of cardia acidification following the meal is shown in Figure 5.5.

What is the explanation for the electrode located in the cardia, 5.5mm distal to the squamo-columnar junction, changing from reading minimal acidity under fasting conditions to reading high acidity during the postprandial period? The lack of acidity under fasting conditions may be explained by being within the distal segment of the high pressure zone which is covered by non-acid secreting cardia mucosa and where the pressure of the sphincter occludes the highly acidic gastric juice secreted immediately distal to it. The marked increase in acidity following the meal might be explained by opening of this distal segment of the sphincter allowing acid to move proximally onto the mucosa of the cardia region. Our research group has previously observed loss of the distal segment of the high pressure zone following a meal using a manometry pull-through technique (168;178) and the pH step-up point moving closer to and even across the squamo-columnar junction with the pH pull-through technique following meals in healthy subjects and reflux patients (160;168). Such a phenomenon was postulated some years ago by Oberg and DeMeester (17,18). An additional or alternative explanation for the apparent rise in acidity in the cardia following a meal relates to previous reports that this region largely escapes the
buffering effect of the meal. If the cardia is stimulated to secrete acid by ingestion of a meal but is not buffered by the meal, then its acidity will increase. In contrast, in the rest of the stomach, its buffering effect more than counteracts the increased acid secretion causing a reduction in the acidity.

It should be emphasized that this acidification of the cardia following the meal extends the concept of the uniqueness of the cardia. It has been previously reported that the cardia differs from the rest of the stomach by not becoming less acidic following a meal and this attributed to it escaping the buffering effect of the meal. However, the current studies extend the previous work by showing for the first time that the cardia actually becomes markedly more acidic following a meal.

Though the subjects studied were healthy volunteers, they showed variable degrees of gastro-oesophageal reflux during the study. I defined oesophageal acid reflux as that reaching the electrodes at least “16.5mm” proximal to the squamo-columnar junction so as to exclude any effects of proximal migration of the pH step-up as discussed above. Oesophageal acid reflux was greater during the postprandial versus fasting period consistent with previous observations of provocation of reflux by meal ingestion (108;139;169;170;179-183). I assessed whether the region of acidity close to the squamo-columnar junction might be a source of this postprandial acid reflux. My analysis indicated that 51.1% of the reflux episodes occurred at a time when the only recorded region of the stomach with a pH as low as that of the oesophageal refluxate was the gastric cardia. This is consistent with this region containing a finite amount of acid able to travel up the oesophagus and change the pH several centimetres above it. Previous work by Pandolfino et al (164) had suggested that the cardia may be a source of postprandial reflux using Bravo capsules clipped just distal to the squamo-
columnar junction. I have demonstrated this phenomenon in real time using the static high definition pH catheter (Figure 5.4e).

There were no obvious differences in the studies performed on the helicobacter positive subjects and I only included the helicobacter status for completeness of data. The study was not designed to examine differences between these two groups and therefore statistical comparison between Helicobacter positive and negative patients cannot be made in the paper but may warrant future study.

As stated in the methods section, all the studies were performed in the semi-recumbent position to ensure uniformity of recordings between subjects. One potential limitation of this technique is that it does not explore the effects of postural change on these regional changes in pH after the meal and may require further studies to clarify this point in future.

In conclusion, the major new finding of the study is that the cardia shows a paradoxical response to a meal in becoming more acidic while the rest of the stomach becomes less acidic. In addition, the acidic region within the cardia extends towards the squamo-columnar junction during the postprandial period. This acidification of the cardia during the postprandial period may be relevant to the high incidence of inflammation and intestinal metaplasia noted of the cardia in asymptomatic subjects and to the etiology of junctional adenocarcinoma. It might also be relevant to the hypothesis that cardia mucosa is largely acquired due to gastric acid exposure producing columnar metaplasia of the squamous mucosa of the most distal oesophagus (10).
**Summary and Concluding Remarks**

My studies of the luminal environment of the gastro-oesophageal junction have provided some novel insights into the nature of the acid pocket in both healthy subjects and reflux oesophagitis patients.

I have confirmed in healthy volunteers that following a meal the distal portion of the high pressure zone is “lost” in keeping with a mechanism previously postulated by Oberg (11) and more recently confirmed by studies by my own research group (176). The acid pocket occupies this region of reduced pressure at the distal aspect of the postprandial sphincter and this explains the proximal migration of the pH step-up point noted in studies by Fletcher (160).

I have demonstrated three major differences in the nature of the acid pocket in reflux patients when compared to healthy subjects. The postprandial acid pocket was demonstrated more frequently in reflux patients, was longer and extended proximally to the proximal margin of the gastric folds whereas it was found a median 1.1cm distal to the squamo-columnar junction in healthy subjects.

My work has demonstrated that caution has to be taken in interpretation of studies employing radio-opaque clips to mark out the position of the squamo-columnar junction as this may lead to false localisation of the junction due to respiratory excursion. Development of novel techniques to monitor the position of the squamo-columnar junction in real time will allow more accurate analysis of the complex region and may be a subject of future study.

My further studies employed a novel bespoke high resolution 12 electrode pH catheter clipped to the mucosa in healthy subjects. The catheter was clipped to the mucosa to minimise the impact of movement of adjacent structures (ie. oesophageal shortening due to swallowing) which could lead to false location of the acid pocket.
I have demonstrated that the gastric cardia exhibits paradoxical “behaviour” compared to the rest of the stomach. The cardia was a region of low acidity fasting when the rest of the stomach was highly acidic. In response to the meal the cardia region underwent a significant rise in acidity whereas the rest of the stomach demonstrated falling acidity. I postulated that this unique environment of the cardia may give some clues as to why a high degree of pathology is found here in asymptomatic healthy subjects. The combination of high postprandial acidity, swallowed salivary nitrite and close proximity to the adjacent mucosa would make this an optimal site for pathological change due to generation of potentially carcinogenic nitrosating species.

The use of the 12 electrode catheter also revealed acidification of the most distal sensors and led us to postulate that the acid pocket may in fact represent an “acid coat” surrounding the ingested food bolus rather than an isolated pocket of acid sitting on top of the food bolus. The relevance of this finding (ie. whether the observed postprandial proximal region of subcardial acidification is due to an “acid pocket” or “acid coat”) to the pathogenesis of gastro-oesophageal reflux and pathology at the junction is currently uncertain and the studies I undertook were not designed to look at this particular point. It is, however, an interesting observation that should be borne in mind when designing future studies investigating regional intra-gastric acidity, particularly if employing wireless electrodes clipped close to the mucosal surface. If there is a postprandial acid coat as postulated, then it would seem likely that wireless pH electrodes clipped to the gastric mucosal surface would be bathed in gastric acid throughout most of the postprandial period and underestimate the degree of intraluminal buffering seen by unattached pH catheter electrodes.

Finally, I have demonstrated for the first time that postprandial reflux events can originate from the cardia acid pocket in healthy subjects. This suggests that the acid
pocket is a finite volume of acid capable of causing reflux up into the distal oesophagus rather than a thin film as suggested by other investigators (161).

My studies suggest several further avenues of research which may be fruitful. Novel techniques to localise the exact location of the squamo-columnar junction would be helpful to further study the location of the acid pocket in real-time and this could be employed with high-resolution manometry which has now become widely available since the time these studies. The combination of high resolution pHmetry, manometry and more accurate real-time localisation of squamo-columnar junction position would gave further insights into the dynamic physiological responses to meal ingestion. I have also postulated the hiatus hernia as a source of acid production in reflux patients and studies into the acid-secreting potential of the hernia would be valuable with particular attention to the histology of this region. Finally development of techniques to study the intra-gastric and oesophageal acid environment in real time and 3-dimensions would help us to map the true nature of the postprandial acidified region, establishing whether it is a true acid pocket or acid coat.
Papers Published Since Completion of My Own Studies Relating To the Postprandial Acid Pocket

Since completion of my own experimental work there have been some further papers relating to the acid pocket published which are of note.

Beaumont et al (142) utilised combined manometry/impedance, 4-channel pHmetry and scintigraphy to establish whether the position of the postprandial acid pocket relative to the gastro-oesophageal junction, and in particular the hiatal hernia, influenced the degree of acid reflux associated with transient lower oesophageal sphincter relaxations. They studied ten healthy volunteers and twenty two patients with gastro-oesophageal reflux disease (12 had a small hiatus hernia under 3cm and 10 with large hiatus hernia over 3cm). Anatomical landmarks were marked with radio-opaque clips. They found acid pocket length increased in reflux patients. The frequency of transient relaxations was similar in all groups but the degree of acid reflux was greater in reflux patients, particularly those with a large hiatus hernia. Their scintigraphy studies demonstrated the acid pocket located within the hiatal hernia sac in 40% of patients with large hiatal hernias for the entire duration of the study. Intermittent migration of the acid pocket into the hiatus hernia was seen in 90% of the other patients with large hiatal hernia. They demonstrated that the proportion of transient relaxations accompanied with acid reflux was significantly greater in the large hiatal hernia group compared to healthy subjects and reflux patients with small hernias. The location of the acid pocket relative to the anatomical landmarks appeared critical in determining whether transient relaxations where accompanied with acid reflux. When the acid pocket was located below the diaphragm, only 7 to 20% of transients were accompanied with acid reflux across the 3 groups. When the acid
pocket was in a supra-diaphragmatic position, this frequency of acid reflux with transient relaxations increased to 74 to 85%. These studies confirm our finding of an enlarged acid pocket in reflux patients compared to healthy subjects. Their study also highlights the integral role of the hiatal hernia in determining whether reflux from this proximal acid pocket is acidic and therefore stresses the integral role of hiatus hernia (particularly over 3cm) in the pathogenesis of acid reflux. The role of transient relaxations of the lower oesophageal sphincter is also highlighted in their study and may be a potential therapeutic target, particularly in patients with large hiatal hernias, as these relaxations appear to be highly correlated with acid reflux (184).

A study utilising 24 hour wireless pHmetry by Grigolon et al (185) evaluated subcardial pH in 14 healthy subjects and 22 reflux patients (10 with no hiatus hernia and 12 with hiatus hernia greater than 3cm). They found no significant difference in median 24 hour pH measured 2cm below the squamo-columnar junction (median 24 hour pH 1.4, 1.5 and 1.4 in healthy subjects, reflux patients without and with hiatus hernia respectively). Median pH after a standardized meal was highly acidic (median pH 2.7, 1.9 and 2.5 in healthy subjects, reflux patients without and with hiatus hernia respectively) consistent with postprandial acidification of the subcardia region but not significantly different between subject groups. High acidity (pH<2) was first seen between 14 and 20 minutes after the meal. They could not however comment on the proximal or distal extent of the acidified zone due to their use of just one wireless pH electrode and this acidified zone cannot be truly deemed an acid pocket as no measurement of more distal buffering was made. The study does again, like my own studies, confirm the presence of a highly acidic region just distal to the squamo-columnar junction which appears to escape the buffering of the ingested meal and could be a potential source of post-prandial acidic refluxate.
Two studies by Herbella et al (186;187) examined the influence of upper gastrointestinal surgery on the presence of a postprandial acid pocket. They reported that an acid pocket was only seen in 3 of 15 patients who had undergone Roux-En-Y gastric bypass surgery and on the basis of this, postulated a role for the fundus in generation of acid pockets when present. Their second study described a heterogeneous population of surgical patients (8 Roux-En-Y procedures, 6 laparoscopic Nissen fundoplications and 7 open subtotal open gastrectomy procedures) as well as 5 healthy subjects as controls. They did not perform pH studies on their healthy controls and rarely found acid pockets in their post-surgical patients. They postulated rapid movement of food from the subcardial region led to observation of an acid pocket in the 3 of 8 Roux-En-Y patients found to have an acid pocket. Given the design of these studies and the differing nature of their subjects, it is difficult to comment on these two papers in relation to my own work.
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