

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

THE DEVELOPMENT AND USE
OF RADIOPAQUE MARKERS FOR
THE ASSESSMENT OF GASTRIC
EMPTYING IN DOGS

A THESIS PRESENTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF VETERINARY SCIENCE
AT MASSEY UNIVERSITY

FRAZER JAMES ALLAN

1995

Abstract

Currently, there is no suitable technique that can be used by veterinarians in private practice to assess the rate of emptying of solids from the stomach of dogs. Radiographic studies using barium sulphate suspension are commonly employed by veterinarians to assess gastric emptying. However, these methods are qualitative and assess the gastric emptying of liquids not solids. Diseases which affect gastric emptying are more likely to affect the emptying of solids rather than liquids. The objective of this study was to develop a technique that the practising veterinarian could use to assess the rate of gastric emptying of solids in dogs.

The study was divided into two parts, the development of radiopaque markers and the development of a method utilising these radiopaque markers that could be used in veterinary practice to assess the gastric emptying of solids.

A 1.5 mm diameter (small) marker and a 5.0 mm diameter (large) marker were developed based on studies by other investigators. It was anticipated that the small marker would empty from the stomach with food and the large marker would empty with the onset of the migrating motility complex. Both markers were made from a compound containing high density polyethylene and barium sulphate.

The gastric emptying of both sizes of marker was then assessed in 20 healthy, mixed breed dogs. Studies were performed on days one, six and nine of the investigation. After a 24 hour fast, thirty small and ten large markers were placed into a standard meal comprising of canned Prescription Diet® d/d. With the dogs restrained in ventrodorsal and left lateral recumbency, radiographs were taken hourly until all, or most of, the markers had emptied from the stomach. Percent gastric emptying of the markers versus time curves (GEvT curves) were then generated from this data. The time taken to reach the point of inflection on the GEvT curve (the lag phase), and the times taken to empty 25%, 50% and 75% of the markers (T_{25} , T_{50} and T_{75} respectively) were calculated from the GEvT curves. The sex and age of the dogs and training the dogs to the radiographic procedure did not have a significant effect on the gastric emptying parameters. There was a weak but significant positive correlation between dog weight and the T_{50} .

There were no significant differences in the T_{25} , T_{50} and T_{75} between the large and small markers. Contrary to their anticipated behaviour, the large markers left the stomach during the fed motility pattern. A larger, 7 mm diameter marker, may be required to mark the onset of the MMC in dogs.

The mean GEvT curve of the small markers on day one (with 95% confidence intervals) was considered to represent the most appropriate gastric emptying reference curve for clinical use. The lag phase of the small markers on day one was 2.45 ± 2.04 hours, the T_{25} was 4.85 ± 2.15 hours, the T_{50} was 6.05 ± 2.99 hours and the T_{75} was 8.32 ± 2.72 hours. If delayed gastric emptying is suspected, taking two or three sets of radiographs at regular intervals from 6-16 hours after feeding and comparing the results with the reference curve is probably the most appropriate method of assessing gastric emptying in a patient. Conversely, if excessively rapid gastric emptying is suspected, taking two or three sets of radiographs at regular intervals from 0-5 hours after feeding and comparing the results with the reference curve is most appropriate.

In conclusion, radiopaque markers provide a simple quantitative method of evaluating the gastric emptying rate of dogs. However, the results of this study have not established that the gastric emptying of the small markers occurs at the same rate as the gastric emptying of food. In addition, the sensitivity and specificity of this diagnostic procedure still needs to be determined. These steps in the validation process are currently being carried out at the Department of Veterinary Clinical Sciences at Massey University.

Acknowledgements

I would like to thank my supervisors, Grant Guilford and Boyd Jones, for their guidance, patience and support. In particular, Boyd's constructive comments on the finer points of the English language and Grant's endless enthusiasm and optimism were of great help to me.

To Ian Robertson, thank you for your expert advice and for the many hours you spent poring over radiographs on my behalf. Special thanks also to Marge Chandler and Phillip Judge for sacrificing many nights of sleep to help me collect data through the "graveyard shift".

This thesis would not have been possible without the loving support of my wife, Rebecca, who helped me collect data, proof-read the text and encouraged me through this project.

Thank-you to Fiona Dickinson for assisting me with the graphics and the trickier aspects of word-processing. I extend my appreciation to the staff of the small animal hospital, in particular, Jill Hogan and Pauline Gordon for their expert attention to the dogs during the study. Thank-you to the radiology staff, Hilary Burbidge, Nicki Moffat and Su Jenkins for their professional advice and for sharing the radiology suite with me during the data collection.

I acknowledge the generous support of the Comparative Gastroenterology Society for funding this study, Stallion Plastics who supplied the radiopaque markers at cost price and Friskies Pet Care for providing the maintenance dog food ration.

Table of Contents

Chapter One

Introduction

1.1	General Introduction	1
1.2	Physical Obstructions	1
1.3	Gastric Motility Disorders	2
	<i>Gastric motility disorders resulting in delayed gastric emptying</i>	4
	<i>Gastric motility disorders resulting in excessively rapid gastric emptying</i>	9
1.4	Diagnosis of Gastric Emptying Disorders	10
1.5	Gastric Emptying Studies- The ideal technique?	12
1.6	A Veterinary Perspective on Gastric Emptying Studies- Justification for the study	14
1.7	Radiopaque Markers	15
1.8	Study Objectives	16

Chapter Two

Anatomy of the Canine Stomach

2.1	General Description	18
2.2	Nerves of the Stomach	20
2.3	Layers of the Stomach	21
	<i>The serosa</i>	21
	<i>The muscularis</i>	23
	<i>The submucosa</i>	24
	<i>The mucosa</i>	24

**Chapter Three Physiology of Gastric Emptying
in Humans and Monogastric Animals**

3.1	General Introduction	25
3.2	Electromechanical Properties of Gastric Smooth Muscle	26
	<i>The isolated gastric smooth muscle cell</i>	26
	<i>The gastric pacemaker and the gastric slow wave</i>	28
	<i>Electromechanical properties of the proximal stomach</i>	32
3.3	Gastric Accommodation and Receptive Relaxation	33
3.4	Gastric Emptying of Liquids	35
	<i>Role of the proximal stomach</i>	35
	<i>Role of the distal stomach and small intestine</i>	37
3.5	Intestinal Feedback Inhibition	40
	<i>"The intestinal brake"</i>	40
	<i>Neurohormonal control of intestinal feedback inhibition</i>	45
	<i>Intestinal feedback inhibition- Summary</i>	48
3.6	Gastric Emptying of Solids	49
3.7	Neural Control of Distal Stomach Motility	54
3.8	The Pylorus and Gastric Emptying	56
3.9	Control of Pyloric Contractility	58
3.10	The Hydrodynamic Model of Gastric Emptying	61
	<i>Preliminary observations</i>	61
	<i>Hydrodynamic theory</i>	63
3.11	The Migrating Motility Complex	66
	<i>Introduction</i>	66
	<i>Description of the migrating motility complex</i>	67
	<i>Function of the migrating motility complex</i>	68
	<i>Control of the migrating motility complex</i>	69
	<i>The migrating motility complex- Summary</i>	73
3.12	Converting from the MMC to the Fed Pattern	73
3.13	Control of the Fed Pattern	76
	<i>Hormonal control</i>	76
	<i>Neural control</i>	77
	<i>Control of the Fed Pattern- Summary</i>	78

Chapter Six**Materials and Methods****Phase Two- Gastric Emptying Studies
Using Radiopaque Markers in Healthy Dogs**

6.1	Radiopaque Markers	122
6.2	The Test Meal	123
6.3	Prescription Diet® canine/feline d/d Formulation Change	124
6.4	Study Animals	126
6.5	Experimental Method	128
6.6	Radiographic Equipment	131
6.7	Radiographic Interpretation	132
6.8	Observer Variation	133
6.9	Statistical Methods	134

Chapter Seven**Results**

7.1	Experimental Protocol	135
7.2	Observer Variation and Radiograph Interpretation	136
7.3	Effects of Variables on Gastric Emptying Parameters	138
7.4	Individual GEvT Curves and Lag Phases	141
	<i>Small markers</i>	141
	<i>Large markers</i>	142
7.5	Clinical Data	144

Chapter Eight**Discussion**

8.1	Experimental Protocol	145
8.2	Observer Variation and Radiograph Interpretation	146
8.3	Effects of Variables on Gastric Emptying Parameters	152
8.4	Gastric Emptying of the Small Markers	154
8.5	Gastric Emptying of the Large Markers	156
8.6	Clinical Implications	158
8.7	Validation of the Technique	160
8.8	Summary	161

Bibliography	164
---------------------------	-----

List of Tables

Table 1.1	Conditions which can cause abnormal gastric emptying in humans and animals	3
Table 1.2	Features of the ideal gastric emptying study	12
Table 4.1	Characteristics of the ideal radioisotope	86
Table 4.2	Summary of the advantages and disadvantages of the techniques used to determine the rate of gastric emptying	110
Table 5.1	Summary of the physical properties of an indigestible solid and their relative importance to the rate of gastric emptying of indigestible solids	114
Table 6.1	Summary of the physical properties of the radiopaque markers used in this study	123
Table 6.2	A comparison of the nutritional analyses of the old canned Prescription Diet® canine/feline d/d and the new canned Prescription Diet® canine d/d diets	125
Table 6.3	Summary of the sexes, ages and weights of the dogs used in the study and the type of test meal fed	127
Table 6.4	Typical Proximate Analysis of Friskies Go Dog®	128
Table 7.1	Summary of the mean T_{25s} , T_{50s} and T_{75s} for the small and large markers on days 1, 6 and 9	140
Table 7.2	Lag phases for the small and large markers on days 1, 6 and 9	142
Table 8.1	An example of how a large number of markers of indeterminate location can cause substantial errors in gastric emptying calculations	150

List of Figures

Figure 2.1.	Diagram showing the important anatomical landmarks of the stomach	19
Figure 2.2.	Diagram showing the layers of the stomach	21
Figure 2.3.	Musculature of the stomach	22
Figure 3.1.	The electrical activity recorded from individual gastric smooth muscle cells from nine regions of the canine stomach	27
Figure 5.1.	Diagram representing how prospective marker material was assessed for radiopacity	116
Figure 6.1.	Photograph showing the two sizes of radiopaque marker used in the study	122
Figure 6.2.	Photographs of the ventrodorsal radiograph and the left lateral radiograph of Dog E on day 1 at 5 hours	130
Figure 7.1	Histograms showing the observer variation between two radiologists for the small and large markers	137
Figure 7.2	Mean percent gastric emptying versus time curves for the male and female dogs	138
Figure 7.3	Mean percent gastric emptying versus time curves for the old and new diets	138
Figure 7.4.	Scattergram and line of best fit showing the effect of age on T_{50} for the small and large markers	139
Figure 7.5	Scattergram and line of best fit showing the effect of weight on T_{50} for the small and large markers	139
Figure 7.6	Mean percent gastric emptying versus time curves for days 1, 6 and 9	140
Figure 7.7	Percent gastric emptying versus time curves for the small markers in dog Q on days 1, 6 and 9	141
Figure 7.8	Percent gastric emptying versus time curves for the large markers in dog N on day 9 and dog F on day 1	143
Figure 7.9	Mean percent gastric emptying versus time curves for the small markers and large markers on day one	144
Figure 8.1	The four overlay regions where interpretive errors can be made	148

Chapter One

Introduction

1.1 General Introduction

Abnormal gastric emptying may result from either a physical obstruction of the gastric outlet or disordered gastric motility. Both can result in a wide range of clinical signs although vomiting, abdominal distention and abdominal pain are most commonly recognised.

In veterinary practice, gastric obstructions are readily diagnosed by common diagnostic techniques such as radiography, gastroscopy or exploratory celiotomy. The advent of scintigraphy has greatly aided the diagnosis of gastric motility disorders in human medicine but, primarily due to the expense of this technique, its use is limited to large veterinary referral institutions. Veterinarians in private practice must rely on insensitive and cumbersome radiographic techniques for diagnosing gastric emptying disorders. This study was undertaken to develop a simple, reliable, inexpensive method for diagnosing gastric emptying disorders in private practice.

1.2 Physical Obstructions

Physical obstructions of the stomach result in food being retained for abnormally long periods of time. Prolonged gastric retention can cause vomiting, abdominal distention, abdominal discomfort, anorexia and altered drug and nutrient absorption. Generally, the degree of obstruction of the gastric outlet is proportional to the severity of the clinical signs observed. Dogs with partial gastric obstructions may have waxing and waning clinical signs over a long period before presentation to a veterinarian.

Obstructions usually occur in the antrum and the pylorus of the stomach because these areas have the narrowest luminal diameter. However, it should be noted that the lumen of the small intestine is also small and if it is obstructed, the clinical signs will often resemble those of gastric outlet obstruction.

Common causes of gastric outlet obstruction include the presence of intraluminal foreign bodies, polyps, neoplastic masses and granulomas (Strombeck and Guilford, 1990). Hyperplasia of the gastric mucosa surrounding the pylorus (chronic hypertrophic pyloric gastropathy) and hypertrophy of the circular muscle of the pylorus (congenital pyloric stenosis) may also cause gastric outlet obstruction in the dog (Strombeck and Guilford, 1990). Occasionally, masses outside the stomach involving other abdominal organs may reduce the diameter of the antrum and pylorus and cause partial or complete obstruction.

1.3 Gastric Motility Disorders

Gastric motility disorders result from a functional disturbance of the normal mechanisms which control gastric motility. Normal gastric motility relies on the constant rhythmic depolarisation and repolarisation of gastric smooth muscle which gives rise to the gastric slow wave (described in detail in chapter three). The gastric slow wave is modified by a complex interaction between the autonomic nervous system (sympathetic, parasympathetic and enteric), the central nervous system, gut hormones and exogenously administered drugs. A disturbance to any of these mechanisms can interfere with normal gastric emptying.

Table 1.1 Conditions which can cause abnormal gastric emptying in humans and animals^a.

Mucosa	Gastric ulceration ↓ (h) Duodenal ulceration ↓ (h) <i>H. pylori</i> gastritis ↓ (h) Inflammatory bowel disease ↓ (c,f)
Muscle	Progressive systemic sclerosis ↓ (h) Polymyositis ↓ (h) Dermatomyositis ↓ (h) Myotonic dystrophy ↓ (h) Duchenne muscular dystrophy ↓ (h) Carnitine deficiency ↓ (h) Gastric dysrhythmias ↓ (h,c)
Endocrine	Hypoadrenocorticism ↓ (h,c) Diabetes mellitus ↓ (h) Hypothyroidism ↓ (h,c) Hyperparathyroidism ↓ (h,f,c)
Metabolic	Acid-base imbalance ↓ Electrolyte imbalance ↓ Uraemia ↓ Hepatic failure ↓ Malnutrition ↓
Iatrogenic	Abdominal surgery ↓ Drugs ↓
Idiopathic	Idiopathic gastroparesis ↓ (h) Chronic idiopathic intestinal pseudoobstruction ↓ (h)
Neurological	
Enteric	Myenteric ganglionitis ↑ (c)
Autonomic	Dysautonomia ↓ (h,c,f,e) Alcoholism ↓ (h)
Central	Anorexia nervosa ↓ (h) Bulimia nervosa ↓ (h) Morbid obesity ↑ (h) Pain ↓

^a ↓ indicates that the condition may cause delayed gastric emptying; ↑ indicates that the condition may result in an increased rate of gastric emptying; and ↓ indicates that the condition may cause both delayed and rapid gastric emptying. Bracketed letters refer to species in which the condition has previously been reported: h- human; c- canine; f- feline; e- equine. No bracketed letter indicates that the condition may affect all species.

Gastric motility disorders resulting in delayed gastric emptying

Gastric motility disorders which result in delayed gastric emptying can be due to either hypomotility of the stomach or a derangement of the normal gastric motility pattern. The former state is termed *gastroparesis* and the latter state is termed a *gastric dysrhythmia*. *Ileus*, correctly called *adynamic ileus*, also refers to hypomotility of the stomach but it can also refer to hypomotility of intestinal segments or the entire gastrointestinal tract. To avoid confusion, ileus will be reserved for hypomotility of the entire gastrointestinal tract in this thesis.

Patients with ileus experience the same range of clinical signs as a patient with a physical obstruction because digesta cannot move at its normal rate through the parietic segment of the gastrointestinal tract. The severity of the clinical signs is proportional to the degree of hypomotility and the length of gastrointestinal tract involved. Consequently, the term *functional obstruction* has been coined to describe severe gastrointestinal hypomotility.

Causes of gastroparesis

Inflammatory and neoplastic conditions affecting the gastric mucosa have been associated with delayed gastric emptying in man and animals through an as yet poorly understood mechanism. *Helicobacter pylori*-induced chronic gastritis delayed gastric emptying in humans but the rate of gastric emptying returned to normal once the infection was eliminated and the gastritis had resolved (Chaudhuri and Fink, 1991). Tatsuta *et al* (1990) have shown that the rate of gastric emptying is significantly longer in humans with severe fundal gastritis and differentiated gastric adenocarcinoma. They speculated that gastric neoplasia may be the result of delayed gastric emptying and prolonged contact between the gastric mucosa and carcinogens rather than the neoplasia resulting in delayed gastric emptying. Using the gastric emptying technique described in this thesis, we have been able to demonstrate delayed gastric emptying in cats and dogs with chronic gastritis and inflammatory bowel disease (Guilford and Allan, unpublished observations, 1994). Humans with gastric or duodenal ulceration have also been shown to have delayed gastric emptying but this is not a consistent finding as some patients have normal or rapid rates of gastric emptying (Chaudhuri and Fink, 1991).

Any condition which can result in disease of gastrointestinal smooth muscle may cause gastroparesis and delayed gastric emptying. Fatigue, weakness and dysphagia are common in patients with the inflammatory myopathies such as dermatomyositis and polymyositis since these conditions predominantly affect skeletal muscle. However, in humans afflicted with these myopathies, Horowitz *et al* (1986) have observed a delay in the gastric emptying of solids and liquids which parallels the severity of their oesophageal motility disorder.

Duchenne muscular dystrophy is an X-linked recessive disease of humans which is characterised by muscle necrosis and the progressive replacement of normal muscle with fat and fibrous tissue. When gastrointestinal smooth muscle is affected, patients may show signs of acute gastric dilation and abdominal pain associated with delayed gastric emptying (Hyser and Mendell, 1988).

Endocrine disorders may induce gastroparesis through a number of pathological processes. Abnormalities in serum electrolyte concentrations may contribute to gastroparesis in many endocrinopathies. In patients with hypoadrenocorticism, rises in serum potassium concentrations result in an increasingly positive resting membrane potential of gastrointestinal smooth muscle. As the resting membrane potential approaches the threshold potential, a depolarisation block is induced which leads to gastroparesis. Conversely, in hypokalaemic endocrinopathies (ketoacidosis associated with diabetes mellitus) falling serum potassium concentrations decrease the resting membrane potential and a hyperpolarisation block ensues. Increases in parathyroid hormone (primary hyperparathyroidism) or parathyroid-like hormone (hypercalcaemia of malignancy) can induce gastroparesis by raising serum calcium concentrations which decreases the excitability of the gastric smooth muscle.

Vagal neuropathy has been demonstrated in humans with diabetes mellitus and this can result in delayed gastric emptying of solids (Feldman *et al* 1984; Malagelada *et al*, 1980a; Fox and Behar, 1980). Neuropathy and myopathy may also occur with hypothyroidism in humans and dogs (Chaudhuri and Fink, 1991; Chastain, 1992). Although no clear association has been made between hypothyroidism and delayed gastric emptying, constipation, megaesophagus and diarrhoea have been reported in hypothyroid dogs suggesting that neuromuscular dysfunction of the alimentary tract may occur in this condition (Chastain, 1992).

Numerous drugs have the potential to cause delayed gastric emptying. Most notably, the administration of anticholinergic drugs, such as atropine, inhibit acetylcholine mediated contractility of gastrointestinal smooth muscle. Xylazine hydrochloride administration in the horse and the long-term administration of opioids such as diphenoxylate hydrochloride in dogs have also been reported to induce ileus (Kohn, 1992). Alcoholics have delayed gastric emptying principally caused by vagal nerve degeneration (Chaudhuri and Fink, 1991).

Ileus is a common sequelae to abdominal surgery and abdominal pain, particularly in humans and horses. Increased adrenergic tone is partly responsible for post-operative ileus, however, other factors must play a role because alpha and beta blockade does not prevent the development of ileus (Strombeck and Guilford, 1990). Increases in circulating inhibitory humoral factors such as dopamine and catecholamines and impaired release of the prokinetic hormones neurotensin and motilin may also play a role in post-operative ileus (Strombeck and Guilford, 1990).

Dysfunction of the sympathetic and parasympathetic nervous systems is referred to as dysautonomia and has been described in dogs, cats, horses and humans (Wise and Lappin, 1991). Gastrointestinal signs are primarily referable to failure of the parasympathetic nervous systems and include regurgitation, vomiting and constipation. Wise and Lappin (1991) observed delayed gastric emptying of a barium sulphate suspension from a 1-year-old Labrador with dysautonomia. In this dog, fluoroscopy revealed an immotile stomach.

Gastric dysrhythmia

Gastric dysrhythmias result in delayed gastric emptying because of uncoordinated gastric contractions rather than a paresis of gastric smooth muscle (Code and Marlett, 1974). Gastric dysrhythmias can take the form of tachygastrias and bradygastrias. In tachygastria, an ectopic gastric pacemaker situated in the gastric antrum discharges 8-14 pacesetter potentials per minute, which is twice as fast as the rate of discharge from the normal pacemaker (Kim *et al*, 1986). Rather than sweep distally, as normal pacesetter potentials do, the pacesetter potentials induced in tachygastria are propagated orally (Kim *et al*, 1986). However, tachygastric pacesetter potentials generally remain confined to the antrum and rarely involve the corpus (Kim *et al*, 1986). This retrograde pacing has been reported to delay gastric emptying of liquids and solids (Sarna *et al*, 1976).

Unlike tachygastria, bradygastria involves both the antrum and the body and the pacesetter potentials are propagated aborally (Kim *et al*, 1986). The underlying pathophysiological process is a prolonged isopotential segment (Kim *et al*, 1986). It has been suggested by Kim *et al* (1986) that bradygastria may result from dysfunction of the normal gastric pacemaker, analogous to the cardiac condition, sick sinus syndrome.

Gastric dysrhythmias may occur spontaneously or they can be induced by the administration of drugs (Code and Marlett, 1974; Kim *et al*, 1986). Although gastric dysrhythmia has been reported to occur in apparently healthy dogs (Code and Marlett, 1974) and humans (Stoddard *et al*, 1981), it has also been associated with gastric stasis, nausea, vomiting and abdominal pain in humans (You *et al*, 1980; Telander *et al*, 1978).

Persistent tachygastria and a continuously irregular pacesetter potential was recorded from patients suffering from chronic idiopathic intestinal pseudoobstruction (Devane *et al*, 1992). This condition primarily affects children and is characterised by recurrent episodes of intestinal obstruction, vomiting and an inability to tolerate meals. Chronic idiopathic intestinal pseudoobstruction is usually caused by diseases of the enteric nerves or gastrointestinal smooth muscle.

Gastric motility disorders resulting in excessively rapid gastric emptying

Physicians more often perform gastric emptying studies to confirm delayed gastric emptying than to confirm excessively rapid gastric emptying (Galil *et al*, 1993). Excessively rapid gastric emptying can result in a rapid shift of electrolytes and water into the intestinal lumen causing haemoconcentration and gut distention (Chaudhuri and Fink, 1991). This may result in nausea, palpitations, dizziness and syncope and when these symptoms appear, the condition is referred to as *gastric dumping*. In humans, gastric dumping is generally seen following vagotomy and pyloroplasty for the treatment of peptic ulcers (Galil *et al*, 1993). Vagotomy abolishes vagally mediated receptive relaxation and the initial rate of emptying of liquids from the stomach is excessively fast. Gastric dumping has also been associated with malabsorption and maldigestion of food and duodenal ulcers (Chaudhuri and Fink, 1991).

Excessively rapid gastrointestinal transit has been shown to occur in a dog with diarrhoea and weight loss as a consequence of myenteric ganglionitis (Willard *et al*, 1988). The oro-colic and oro-rectal transit times of a suspension of barium sulphate were 3 and 15 minutes respectively. In this condition, mononuclear cells infiltrate Auerbach's plexi in the oesophagus, stomach, small intestine and the colon. Willard *et al* (1988) proposed that myenteric ganglionitis in this dog resulted in the loss of segmental contractility without affecting peristaltic activity. Consequently, the propulsive force of peristalsis was not countered by resistance normally provided by segmental contractility. However, this explanation does not adequately explain the gastric dumping exhibited by this dog.

1.4 Diagnosis of Gastric Emptying Disorders

Vomiting, anorexia and abdominal discomfort are clinical signs which are not unique to disorders of gastric emptying. For example, patients with food allergy, pancreatitis, food intolerance or inflammatory bowel disease may have a similar range of clinical signs and although they may have delayed gastric emptying as a secondary complication, some have normal rates of gastric emptying. How the clinician determines the cause of the clinical signs will generally depend on the patient's age, breed and sex and the history and severity or chronicity of the clinical signs.

When there is a history of ingestion of a foreign body or when the clinical signs are acute or severe (marked abdominal pain or distention, continual emesis), the clinician will often choose a diagnostic procedure to initially rule out an obstruction. The diagnosis of gastric outlet obstruction requires the use of techniques which aid in viewing the antrum and pylorus.

Plain radiography is usually diagnostic when gastric dilation-volvulus or the ingestion of a radiopaque foreign body is suspected. Plain radiography may also detect some large masses which are obstructing gastric outflow. Positive or double contrast radiography may outline radiolucent foreign bodies or masses. Nowadays, the advent of gastroduodenoscopy has replaced many of the indications for radiography. Gastroduodenoscopy offers the advantage of not only being able to see a foreign body or mass but foreign bodies can be retrieved or biopsies of the gastric mucosa can be taken at the same time.

Where the clinical signs are more chronic or less severe, techniques which aid in viewing the gastrointestinal tract rarely result in a diagnosis. Other diagnostic techniques such as gastric emptying studies, food elimination trials, gastrointestinal biopsy and blood and urine tests are usually performed first.

Gastric emptying studies are primarily performed when a partial gastric obstruction or a gastric motility disorder is suspected. Gastric emptying studies essentially act as "screening tests" allowing the clinician to answer the question, "Is the patient unwell because of delayed gastric emptying or gastric dumping?". If the gastric emptying study is abnormal, the clinician can either try to determine the primary cause of the abnormality or alternatively, the patient can be treated symptomatically.

In some circumstances, the cause is suspected prior to the gastric emptying study being performed and the study is undertaken to confirm that the clinical signs are indeed due to a particular disorder. For example, humans with diabetes mellitus often experience a prolonged feeling of fullness, epigastric pain and they may vomit. The clinician may attribute these symptoms to gastroparesis secondary to diabetes mellitus-induced vagal neuropathy. Delayed gastric emptying in these patients is confirmed by a gastric emptying study and prokinetic drugs, such as erythromycin (Tack *et al*, 1992) and cisapride (Feldman and Smith, 1987), may then be prescribed to improve gastric motility.

Recently, a review by Galil *et al* (1993) retrospectively assessed the reasons why gastric emptying studies were performed at the Royal Liverpool University Hospital. Approximately 70% of the patients referred for gastric emptying studies had previous gastric or upper intestinal surgery. One hundred and fifty-four patients were suspected of having delayed gastric emptying and 102 patients suspected of having gastric dumping and diarrhoea after surgery. Fifteen patients with peptic ulcer were referred for gastric emptying studies to help determine the most appropriate surgical procedure for this condition. Eighteen diabetic patients were referred with symptoms suggestive of gastroparesis, 72 patients were referred with unexplained dyspeptic symptoms not associated with peptic ulcers and a few patients were referred because of suspected delayed gastric emptying due to systemic sclerosis, gastro-oesophageal reflux, adult onset hypertrophic pyloric stenosis and chronic renal failure.

1.5 Gastric Emptying Studies - The ideal technique?

A large number of techniques have been employed to assess the rate of gastric emptying. Chapter four provides a comprehensive review of the gastric emptying studies which are currently used in veterinary and human medicine. The utilisation of such a vast number of techniques is testament to the fact that there is no "ideal" method of assessing gastric emptying. To some extent, the reason for performing a gastric emptying study will determine which technique is chosen. Clinicians have developed techniques which are non-invasive, safe, technically easy to perform and relatively inexpensive whereas gastrointestinal physiologists have developed highly accurate techniques which may forfeit some of these qualities. Table 1.2 highlights some of the main qualities expected in the ideal gastric emptying study.

Table 1.2. Features of the ideal gastric emptying study.

Inexpensive
 Non-invasive
 Easy to Perform
 Safe for Operator and Patient
 Reflects Physiological Function
 Provides Quantitative Information
 Assesses Emptying of Both Solids and Liquids

Radiographic and scintigraphic techniques expose the patient to radiation. Safety considerations limit the number of times these techniques can be used on an individual. Moreover, these techniques should not be used in young or pregnant subjects due to the adverse effects of radiation on dividing cells.

The cost and technical ease of a procedure are major practical considerations, particularly in the veterinary field. The use of techniques which involve specialised equipment and/or computers are not only costly but also require a high level of technical expertise.

Scintigraphy requires the use of an expensive gamma camera and an on-line computer or data storage facility. The capital investment in such a system requires a high workload in order to justify this expense. Clearly, this limits the use of scintigraphy to large human and veterinary hospitals.

Intubation methods, although very accurate, are also invasive as they require the passage of a nasogastric or nasoduodenal catheter. Poor subject compliance associated with invasive techniques means that they are not used for routine clinical investigation. However, their accuracy does make them suitable for research investigation where precision is crucial. Also, invasive methods may interfere with gastric emptying either directly or indirectly by delaying gastric emptying through stress (Gué *et al*, 1989).

A large number of techniques provide only qualitative information about gastric emptying. The time taken to initiate and/or complete gastric emptying may be determined by such studies but intermediate rates of gastric emptying and the shape of the gastric emptying versus time curve cannot be established. Ideally, gastric emptying techniques should provide quantitative information. Furthermore, gastric emptying techniques should employ a marker which is a normal food source for the target species. In humans, [¹¹¹In]DTPA labelled orange juice is sometimes utilised in scintigraphy as a marker for the gastric emptying of liquids. Assessing the gastric emptying of this marker is more "physiological" than assessing the gastric emptying of a barium sulphate suspension, which is used in radiographic studies.

Most techniques can assess the gastric emptying of either liquids or solids only. These are termed monophasic studies. It is generally believed that most disorders which affect gastric motility in humans result in delayed gastric emptying of solids rather than liquids (Chaudhuri and Fink, 1991; Colmer *et al*, 1973). Consequently, techniques which investigate the gastric emptying of solids are probably more useful to the clinician than techniques which examine the gastric emptying of liquids. Scintigraphy, however is unique in that it not only allows assessment of gastric emptying of both liquids and solids but it can also be used to assess the gastric emptying of both phases simultaneously (biphasic gastric emptying study). Since most meals have a solid and a liquid component, the ability to perform biphasic gastric emptying studies allows the clinician to assess gastric emptying in the "normal" situation.

1.6 A Veterinary Perspective on Gastric Emptying Studies

- Justification for the study.

Scintigraphy is the gold standard method for assessing gastric emptying in veterinary medicine as it is in human medicine but due to the considerable expense of this procedure, its use is limited to large referral institutions.

In veterinary practice, the most commonly performed gastric emptying study is positive contrast radiography which assesses the gastric emptying of either a barium sulphate suspension or barium sulphate coated food. This method is utilised because standard x-ray equipment is used, it is inexpensive to perform and special skills are not required to interpret the radiographs. However, this technique has a number of serious shortcomings.

Barium sulphate studies provide very little quantitative information about gastric emptying. The initiation and termination of gastric emptying can be assessed but other values, such as the time taken to empty 50% of gastric content, cannot be determined. Furthermore, the merit of this procedure is questionable because of the wide range of "normal" emptying values cited in the literature. Barium sulphate studies only assess the gastric emptying of liquids (barium sulphate suspension) or suspended solids (food ground to a very small particle size and suspended in barium sulphate). Consequently, if most gastric motility disorders primarily affect the gastric emptying of solids, then the sensitivity of this technique is poor. Finally, veterinarians are reluctant to use barium sulphate because it is messy and only occasionally will dogs eat barium sulphate test meals voluntarily. Usually the meal has to be administered via a stomach tube.

Due to these shortcomings, gastric emptying studies are rarely performed in veterinary practice and veterinarians often prefer to treat animals symptomatically. Consequently, gastric emptying disorders are often not confirmed or are misdiagnosed.

1.7 Radiopaque Markers

Over the last decade, a number of workers have used radiopaque markers to measure gastric emptying times in humans. Reece *et al* (1982) used radiopaque capsules to quantitate gastric emptying and small intestinal transit times in healthy people. They also demonstrated altered transit times after subcutaneous injections of atropine, bethanecol and 16,16-dimethyl prostaglandin E₂. Feldman *et al* (1984) measured gastric emptying times of 30 healthy individuals and 20 insulin dependent diabetics utilising radiopaque markers made of barium sulphate impregnated polyvinyl tubing. Diabetic people were found to empty liquids and digestible solids at rates similar to normal subjects but the clearance of indigestible solids from the stomach was significantly delayed. This correlated with the documentation of an absence or reduction in number of phase III migrating motor complexes in diabetic subjects (Malagelada *et al*, 1980a; Fox and Behar, 1980). In this instance, radiopaque marker studies proved to be a more sensitive indicator of gastroparesis diabetorum than scintigraphy.

Since Feldman's study (1984) radiopaque markers have been used to demonstrate: delayed gastric emptying in people on continuous ambulatory dialysis (Brown-Cartwright *et al*, 1980); faster gastric emptying times in gastroparetic diabetics after treatment with cisapride (Feldman and Smith, 1987); and to more clearly elucidate the mechanisms surrounding gastroparesis diabetorum in people (Pozzi *et al*, 1988). Radiopaque markers have also been used to demonstrate beneficial effects of naloxone in people with intestinal pseudoobstruction (Schang and Devroede, 1985).

We considered that the assessment of gastric emptying using radiopaque markers may have a number of important advantages over the assessment of gastric emptying using barium sulphate. Firstly, if markers could be made sufficiently radiopaque so that they could be seen radiographically, their passage through the gastrointestinal tract could be assessed and quantitative data on gastric emptying obtained. Secondly, since markers are solid, their rate of gastric emptying may reflect the gastric emptying of solids ingested in a normal meal and consequently, they may be more sensitive at diagnosing gastric emptying disorders. Thirdly, if the markers were small enough, they could be disguised in dog food and eaten voluntarily, thereby avoiding the inconvenience of stomach tubing dogs. Furthermore, all these potential advantages could be capitalised upon without losing any of the advantages of the currently utilised barium sulphate suspension techniques.

1.8 Study Objectives

Bearing in mind the potential advantages outlined above and the precedent use of radiopaque markers in humans, the specific objectives of this study were:

- a) to find an inexpensive, inert, non-toxic material of sufficient radiopacity to be visible in plain radiographs of the abdomen of dogs without being so dense as to separate from a test meal;

- b) to utilise previously published information to design, develop and manufacture markers of specific physical characteristics (eg. size, shape and surface energy) which would favour one marker type leaving the stomach proportionately with food and a second marker type being retained by the stomach until all digestible food had entered the duodenum;
- c) to select a food which was available worldwide, with minimal batch to batch variation in composition and which contained very little radiopaque material so it could be fed to dogs simultaneously with the markers without obscuring them;
- d) to develop a protocol which could be used with ease by veterinarians in private practice to assess the gastric emptying times of the markers in dogs;
- e) to utilise the protocol developed above in healthy dogs to
 - i) determine the normal gastric emptying rate of the radiopaque markers in dogs;
 - ii) assess the effect of variables such as age, weight and sex of the dogs on the gastric emptying of the markers;
 - iii) assess the effect of stress on the gastric emptying of the markers;
 - iv) assess whether the two types of marker designed in objective b) fulfill their goal;
 - v) assess the extent of observer variation in interpreting the radiographs;

Chapter Two

Anatomy of the Canine Stomach

2.1 General Description

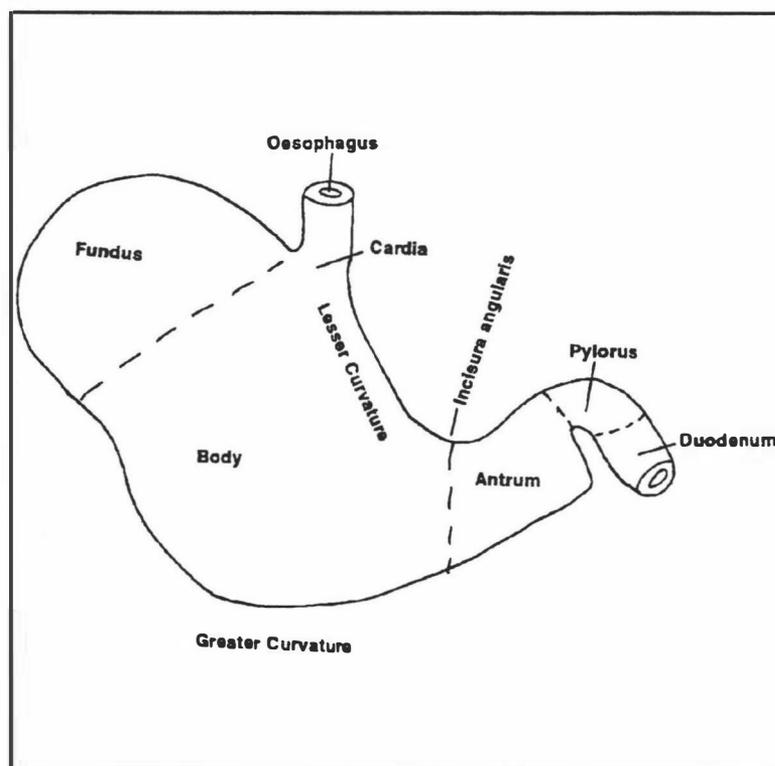
The stomach is located between the oesophagus proximally and the duodenum distally. Most of the empty stomach is located in the left cranial quadrant of the abdominal cavity but part of the distal stomach is situated in the right cranial quadrant. The size and shape of the "U"-shaped stomach varies considerably depending on the volume of ingesta it contains. The stomach is bound by the *cardia* proximally and the *pylorus* distally (Ellenport, 1975).

The convex parietal surface of the stomach, the *greater curvature*, faces partly cranial but mainly ventrally and to the left of midline. When empty, the greater curvature lies in the gastric impression of the left lobe of the liver and does not contact the ventral abdominal wall. However, as the stomach distends, the greater curvature extends ventrally and to the left as far caudally as a transverse plane through the second or third lumbar vertebrae. The distended stomach will come in contact with the ventral abdominal wall (Ellenport, 1975).

The less extensive visceral surface of the stomach, the *lesser curvature*, represents the shortest distance between the cardia and the pylorus. It faces dorsally and to the right of midline and is adjacent to the intestine, pancreas and the left kidney. It forms a deep narrow angle, the *incisura angularis*, in which the papillary process of the liver lies (Ellenport, 1975).

The stomach has four regions: the fundus, the corpus (also called the body), the antrum and the pylorus (Figure 2.1). The *fundus* is the saccular, dorsal left extremity of the stomach. It lies ventrally to the dorsal aspect of ribs 11 and 12. The *corpus* is the large middle portion of the stomach. It extends from the fundus on the left to the antrum on the right. The distal one-third of the stomach comprises of the *antrum* and *pylorus*. This part of the stomach is small and cylindrical in comparison to the large, rounded fundus and body. The antrum and pylorus are directed dorsocaudally and form an irregular funnel with the pylorus being narrower than the antrum. The proximal two-thirds of the small, cylindrical part of the stomach is the thin-walled antrum. The distal one-third is called the pylorus and is bent so that the caudal side is three to four times longer than the cranial side. The pylorus has a large muscular wall and forms the narrowest part of the stomach (Ellenport, 1975).

Figure 2.1. Diagram showing the important anatomical landmarks of the stomach.



2.2 Nerves of the Stomach

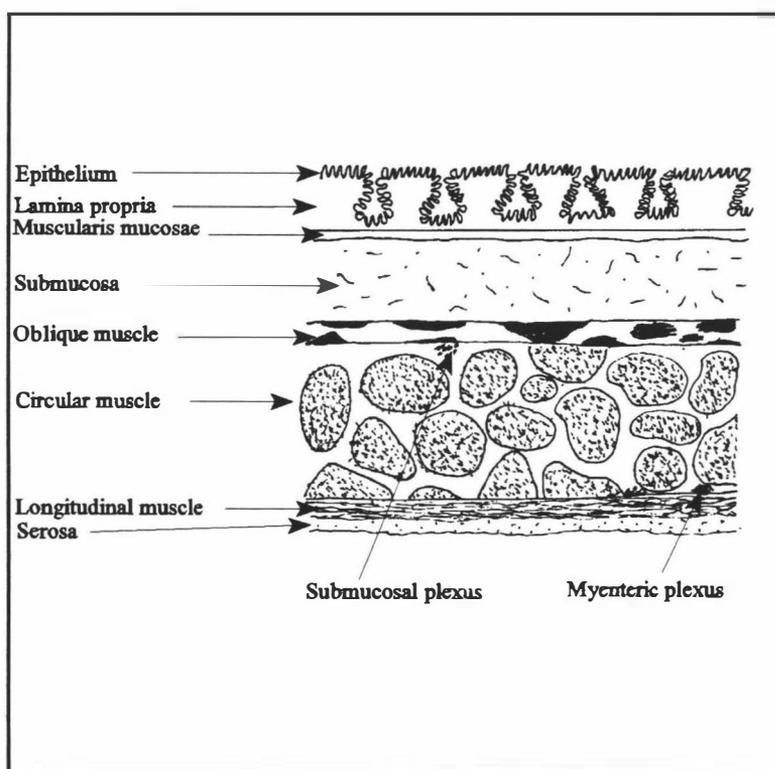
The extrinsic nerve supply to the stomach is supplied by parasympathetic and sympathetic nerves. Parasympathetic nerve fibres originate from the vagus. The ventral vagal trunk passes through the oesophageal hiatus and divides sending two to four small branches to the pylorus and the liver. Other branches supply the lesser curvature of the stomach. The dorsal vagal trunk has branches supplying the lesser curvature and the ventral wall of the stomach. Sympathetic nerve fibres originate from the celiac plexus and nerve fibres pass adjacent to the gastric arteries (Dyce *et al*, 1987).

The gastrointestinal tract also has its own intrinsic nervous system, the enteric nervous system. The nerve cell bodies of the enteric nervous system are located within the myenteric (Auerbach's) plexus which is situated between the longitudinal and the circular muscle layers of the stomach wall and within the submucosal (Meissner's) plexus. Axons from these ganglionated plexi form nonganglionated plexi within the longitudinal and circular muscle layers, the muscularis mucosae, the centres of the villi and around blood vessels and mucosal glands. (Guilford, 1990).

2.3 Layers of the stomach

The stomach wall has four distinct layers when viewed in cross section; the serosa, the muscularis, the submucosa and the mucosa (Figure 2.2) (Dyce *et al*, 1987).

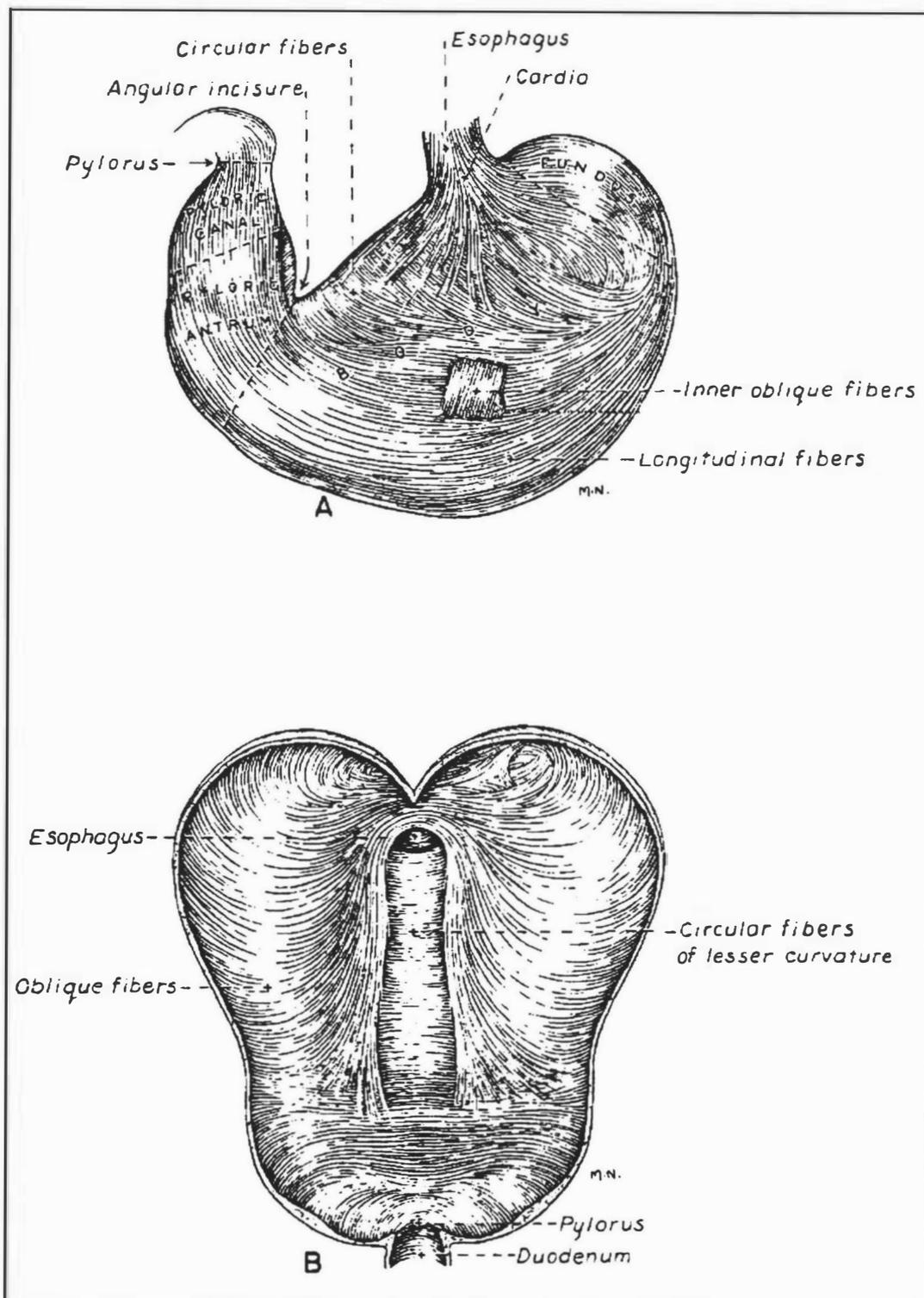
Figure 2.2. Diagram showing the layers of the stomach.



The serosa

The *serosa*, the outermost layer, consists of loose connective tissue covered by visceral peritoneum. Visceral peritoneum completely covers the stomach except for two extremely narrow lines, one on the distal half of the greater curvature and the other along the lesser curvature from the cardia to the duodenum. These areas are where the dorsal and ventral peritoneal sheets fuse to form the greater and lesser omenta. (Dyce *et al*, 1987)

Figure 2.3. Musculature of the stomach (from Evans and Christensen (1979), with permission).



The muscularis

The *muscularis* consists of an outer longitudinal layer, a middle circular layer, and an inner oblique layer of smooth muscle fibres (Figure 2.3) (Evans and Christensen, 1979).

The outer longitudinal layer is continuous with the longitudinal layers of the oesophagus proximally and the duodenum distally. The longitudinal fibres of the lesser curvature fan out as they course distally and end before reaching the *incisura angularis*.

Consequently, the inner layer of circular fibres become superficial at this point. The longitudinal fibres of the dorsal and ventral walls of the body also end before reaching the middle of the body. The longitudinal fibres of the greater curvature are continuous from the oesophagus to the duodenum. At the junction of the fundus and the body, a whorl is formed as muscle bundles change direction. In the antrum and pylorus, the longitudinal layer is particularly dense between the greater and lesser curvatures (Evans and Christensen, 1979).

The circular layer, which is more complete than the longitudinal layer, is thickened at the cardia where it forms the *lower oesophageal sphincter*. An inner layer of transversely running oblique fibres on the greater curvature also contributes to the lower oesophageal sphincter. A muscular groove approximately 2 cm wide runs from the cardia to the pyloric antrum along the lesser curvature. The circular muscle layer forms the floor of the groove and the parallel, longitudinal parts of the oblique layer form the walls of the groove. The circular muscle is well developed at the pylorus and is thickest as the muscle fibres cross the greater curvature. The circular muscle forms a sphincter at the distal extremity of the stomach, the *pyloric sphincter* (Evans and Christensen, 1979).

The oblique muscle layer is adjacent to the submucosa. The oblique fibres which form the wall of the groove in the lesser curvature are essentially longitudinal and parallel but they progressively fan out the further they are from the lesser curvature. At the level of the cardia they are in a transverse plane to the stomach and contribute to the muscle mass of the lower oesophageal sphincter (Evans and Christensen, 1979).

The submucosa

The *submucosa* is a loose connective tissue layer between the tunica muscularis and the tunica mucosa. It is closely adhered to the tunica mucosa but is easily detached from the tunica muscularis except at the pylorus. This layer contains relatively large blood vessels and lymphatics. When the stomach is contracted, the lumen is thrown into folds or *rugae*, and the tela submucosa occupies the centre of the rugae. The submucosal plexus is located deep within the tela submucosa. The postganglionic fibres of this plexus innervate the muscularis mucosa and the mucosal glands (Cormack, 1984).

The mucosa

The *mucosa* is composed of a luminal epithelial membrane, an underlying loose connective tissue layer (the *lamina propria*) and a thin smooth muscle layer (the *lamina muscularis mucosae*) (Cormack, 1984).

The epithelial lining of the stomach is comprised of mucus-secreting, simple columnar, epithelial cells. The epithelial lining is thrown into folds and the resulting numerous invaginations are termed gastric pits (Cormack, 1984).

The *lamina propria* is a loose connective tissue layer, rich in blood vessels, lymphatics and gastric glands. The gastric glands vary in their anatomy and function depending on their location within the stomach. All gastric glands however, open into the base of gastric pits (Cormack, 1984).

The deepest layer of the mucosa, the *muscularis mucosae*, is a thin band of smooth muscle interposed between the lamina propria and the submucosa. This muscular layer is partly responsible for creating the gastric rugae (Dyce *et al*, 1987).

Chapter Three

Physiology of Gastric Emptying in Humans and Monogastric Animals

3.1 General Introduction

At the turn of the century Cannon (1898) fed bismuth subnitrate mixed with food to cats and observed their gastric movements fluoroscopically. He observed that the fundus and proximal corpus relaxed to receive boluses of food from the oesophagus. Slow sustained contractions of the fundus then passed the ingesta into more distal regions of the stomach. Peristaltic waves arising from the distal stomach pushed the gastric content towards the duodenum and in doing so the food was mixed with gastric juices and was broken down into small particles. From these observations, Cannon determined that the stomach is comprised of two distinct motor areas; the proximal stomach which consists of the fundus and the oral corpus; and the distal stomach comprising of the distal two-thirds of the corpus and the antrum and pylorus. A number of questions about gastric motility arose from these original observations. What causes the stomach to relax in response to a meal? How are gastric movements coordinated? What causes the food to be broken down into smaller particles? And what controls the rate of gastric emptying? Since this pioneering study these questions have been addressed and this chapter represents a summary of these findings.

3.2 Electromechanical Properties of Gastric Smooth Muscle

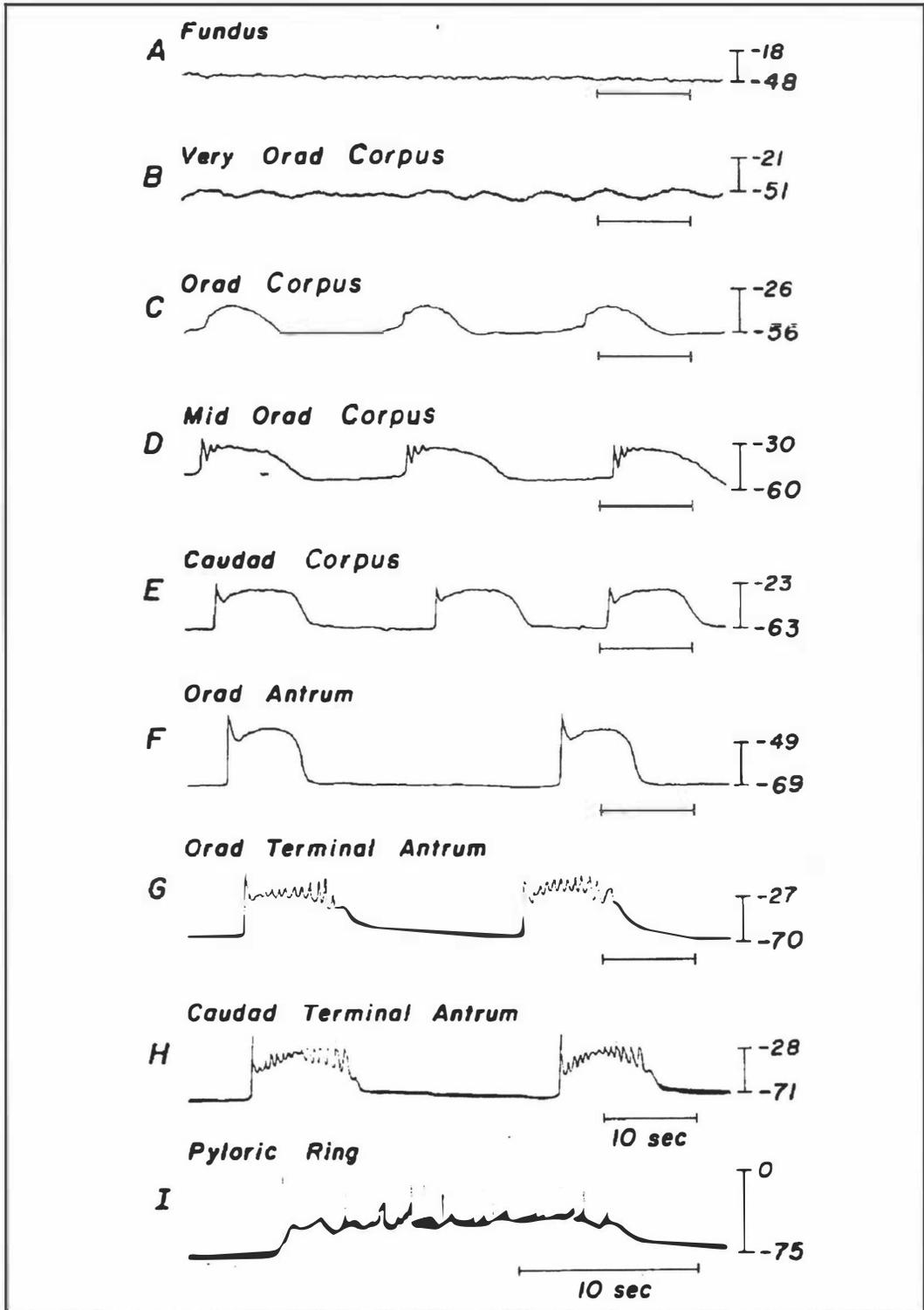
The isolated gastric smooth muscle cell

The basic unit of gastric motility is the gastric smooth muscle cell. The electrical events which occur in isolated gastric smooth muscle cells are ultimately responsible for gastric contractility and it is therefore appropriate to firstly describe the electromechanical events which occur in these cells.

Szurszewski (1981) recorded the electrical activity of individual gastric smooth muscle cells by implanting canine gastric myocytes with intracellular electrodes. He recorded the electrical activity of cells from nine different regions of the stomach from the fundus to the pyloric ring (Figure 3.1).

Myocytes from the distal two-thirds of the stomach exhibited *spontaneous depolarisation*, a characteristic that is shared with cardiac muscle. Without external stimulation, the cell membrane potential of gastric myocytes slowly depolarises from their resting negative membrane potential and rapid depolarisation occurs once a critical membrane voltage has been reached. This sudden depolarisation precedes muscular contraction and is termed a *pacesetter potential* or *electrical control potential* (Szurszewski, 1981).

Figure 3.1. The electrical activity recorded from individual gastric smooth muscle cells from nine regions of the canine stomach (from Szurszewski (1981), with permission).



Following the pacesetter potential, there is a period of rapid and partial repolarisation which is followed by a sustained, more positive membrane potential which slowly repolarises the muscle cell membrane back to the resting membrane potential (Szurszewski, 1981). This sustained plateau potential occurs because of an inward flow of calcium ions through voltage dependent calcium channels (Szurszewski, 1981). It coincides with smooth muscle contraction and is termed an *action potential*. The action potential is monophasic in the middle one-third of the stomach but consists of several oscillating spikes in the distal one-third of the stomach. The strength and duration of muscle contraction is directly related to the amplitude and duration of the action potential (Szurszewski, 1981). Cells from the fundus, the very orad corpus and the orad corpus do not spontaneously depolarise (Szurszewski, 1981).

The gastric pacemaker and the gastric slow wave

Szurszewski's (1981) electrical recordings from individual gastric myocytes help to explain Cannon's fluoroscopic observations that peristaltic waves originate in the corpus and sweep aborally. Szurszewski (1981) found that as intracellular recordings were taken progressively from cells from the mid-corpus to the caudad terminal antrum, the frequency of pacesetter potentials decreased. From this finding it follows that cells from the most active area in the stomach, the orad corpus, act as the "gastric pacemaker", entraining the slower areas and driving them to its own frequency. This is analogous to the way that the sinoatrial node acts as the pacemaker of the heart. Furthermore, in order for the peristaltic wave to be propagated distally, cells from distal gastric regions must be inherently more excitable than those proximally; a property which was confirmed by Szurszewski's study.

In a two part study in live dogs, Kelly and Code (1971) experimentally determined the location of the canine gastric pacemaker. Firstly, they disrupted the neural pathways between the greater and lesser curvatures of the stomach by bisecting the stomach along its longitudinal axis from the fundus to the pylorus. The stomach was immediately sutured and electrical activity was recorded from each half using surgically implanted serosal silver wire monopolar electrodes. Electrical recordings obtained from the greater curvature after bisection showed that the pacesetter potential had a regular rhythm and was propagated caudally with a mean frequency of 5.2 cycles per minute. In contrast, the pacesetter potential of the lesser curvature arose from multiple sites and had an irregular rhythm with a slower mean frequency. Kelly and Code (1971) concluded that the pacemaker was situated in the greater curvature. Three to six months after the gastric bisection study they resected the oral one-fourth of the stomach on the greater curvature side and remeasured the electrical activity from surgically implanted gastric electrodes. They found that the pacesetter potential decreased from 5.1 cycles per minute to 4.2 cycles per minute, but neither the electrical coupling between the greater and lesser curvature nor the propagation of the pacesetter potential was affected. Overall, their data indicated that the gastric pacemaker is in the oral one-fourth of the stomach along the greater curvature.

Weber and Kohatsu (1970) also established that the gastric pacemaker was situated in the oral corpus of the greater curvature. They too completely sectioned and immediately reanastomosed dogs' stomachs in order to disrupt the normal neural pathways between gastric regions. However, this study involved a larger number of incisions and consequently they were able to define the site of gastric pacing more accurately.

Weber and Kohatsu (1970) noted that the longitudinal smooth muscle of the stomach radiated out from the proximal greater curvature to the antrum and the lesser curvature. Based on this anatomical observation they hypothesised that the longitudinal muscle was responsible for propagating the pacesetter potential. Their hypothesis was proved to be correct by Balwinder *et al* (1972) who performed longitudinal and circular/oblique myomectomies in dogs and implanted monopolar silver-silver chloride serosal electrodes orad and aborad to the surgical sites. The longitudinal myomectomy interrupted propagation of the pacesetter potential permanently whereas the pacesetter potential was interrupted for only 14 days after the circular/oblique myomectomy.

The spread of the gastric pacesetter potential from the pacemaker region in the orad corpus is faster along the greater curvature than along the lesser curvature (Szurszewski, 1981). This ensures that the propagated wave arrives at the pylorus simultaneously. Also, as the wave moves aborally, it accelerates from a velocity of 0.5 cm/s in the corpus to 4 cm/s in the terminal antrum (Szurszewski, 1981).

The gastric pacemaker and the spread of electrical activity in the stomach of humans is identical to that in dogs (Hinder and Kelly, 1977a). However, the frequency of the gastric electrical cycle in humans is three per minute, approximately two cycles per minute less than the recorded frequency in the dog (Hinder and Kelly, 1977a).

Extracellular, serosal electrical recordings obtained from the studies above differ in appearance from Szurszewski's intracellular, single cell recordings because extracellular recordings average the voltage changes from many smooth muscle cells (Meyer, 1987). Pacesetter potentials appear as triphasic complexes of 2 to 3 seconds duration. An initial positive deflection is followed by a larger negative deflection which then returns to baseline after a small positive overshoot. As with the intracellular recordings, pacesetter potentials seen in extracellular recordings are found only along the distal two-thirds of the stomach. The amplitude of the triphasic complex increases from 2 mV in the proximal stomach to 4 mV at the pylorus. Similarly, action potentials in serosal electrical recordings follow pacesetter potentials. Recordings of action potentials from the gastric serosa appear as either slow, monophasic, sustained negative potentials of 0.5 to 2.0 mV, or they are a series of rapid, negative oscillations. Action potentials mark the occurrence of smooth muscle contraction, however, in serosal recordings, not every pacesetter potential is followed by an action potential (Meyer, 1987). Therefore, the maximum rate of gastric contraction is governed by the rate of pacesetter potentials which is approximately five per minute in the dog and three per minute in humans. Also, the magnitude and duration of the action potential parallels the intensity and duration of smooth muscle contraction at any particular locus (Meyer, 1987).

Since action potentials, which trigger gastric contractility, are temporally related to the pacesetter potential, the origin and spread of the pacesetter potential parallels the origin and spread of gastric muscle contractility (Meyer, 1987). Thus, gastric contractions originate in the orad corpus and move aborally in a ring-like fashion. The wave of contraction accelerates as it moves from the corpus to the pyloric ring (Meyer, 1987). This peristaltic wave is termed the *gastric slow wave*. Since action potentials do not always follow a pacesetter potential, the gastric slow wave may fade out before it reaches the pyloric ring (Meyer, 1987). Alternatively, there may be no gastric contractions after a pacesetter potential in the orad stomach, but the slow wave may suddenly appear as the pacesetter potential reaches more distal sites.

Electromechanical properties of the proximal stomach

The proximal stomach consists of the fundus and oral one-third of the corpus. It differs from the distal stomach as it manifests neither pacesetter potentials nor action potentials. Consequently, peristalsis does not occur in the proximal stomach but two types of tonic contractions occur (Lind *et al*, 1961). Type III contractions are of low amplitude (10 to 25 cmH₂O) and long duration (1-6 minutes) and they are not readily visible fluoroscopically. Type I contractions are weaker (5 cmH₂O) and shorter (10-15 seconds). Both types of contraction are superimposed on a tonic contraction of about 10 cmH₂O. Type II contractions are of high amplitude and short duration and are produced by the gastric slow wave of the distal stomach. They frequently elevate intragastric pressures by more than 20 cmH₂O for up to 10 seconds. The increased intensity of the type II contraction makes them easy to see by fluoroscopy.

The electrical events underlying the contractions of the proximal stomach are poorly understood. Bursts of rapid spikes occur just before, or during, type I contractions but the electrical events responsible for type III contractions or the tonic contractions have not been identified (Okike and Kelly, 1977).

3.3 Gastric Accommodation and Receptive Relaxation

One of the stomach's functions is to act as a reservoir for ingesta. A reflex, called *receptive relaxation*, allows the gastric fundus and corpus to relax during swallowing. Also, when the fundus senses an increase in intragastric pressure a second reflex, termed *gastric accommodation*, causes the stomach to relax (Cannon and Leib, 1911). Receptive relaxation and gastric accommodation are extrinsic, vagally mediated reflexes. They are abolished by bilateral vagotomy at the level of the vagosympathetic trunk (truncal vagotomy) or at the level of the fundus and body (proximal vagotomy) (Wilbur and Kelly, 1973). Miolan and Roman (1974) determined that during receptive relaxation some vagal efferent preganglionic fibres have an increased rate of discharge whereas the discharge of other vagal efferent preganglionic fibres are abolished or markedly inhibited. They concluded that receptive relaxation results from either stimulation of inhibitory postganglionic myenteric neurons or inhibition of excitatory postganglionic neurons.

Ganglion blocking agents such as hexamethonium block vagally mediated receptive relaxation suggesting that the preganglionic neurotransmitter stimulates nicotinic acetylcholine receptors (Beani *et al*, 1971). However, the postganglionic neurotransmitter is neither adrenergic nor cholinergic as sympathetic antagonists and atropine do not block vagal inhibitory activity (Meyer, 1987).

There is strong evidence to suggest that the neurotransmitter, vasoactive intestinal polypeptide (VIP), may be partly responsible for receptive relaxation and gastric accommodation. VIP directly relaxed antral and fundic smooth muscle cells in vitro (Morgan *et al*, 1978; Bitar and Makhlouf, 1982), it is released following oesophageal distention (Fahrenkrug *et al*, 1978) and VIP-mediated gastric relaxation is inhibited by VIP antibodies (Grider *et al*, 1985). Very recently, Grundy *et al* (1992) have shown that immunisation against VIP in ferrets results in a significant increase in the spontaneous motility of the corpus. However, following VIP immunisation, the proximal stomach was still able to accommodate fluid. Based on these findings, these workers concluded that there are different noncholinergic, nonadrenergic inhibitory mechanisms for phasic and tonic proximal gastric contractions and that VIP acts primarily to regulate phasic contractile activity. Gastric tone may be regulated by other neurotransmitters such as nitric oxide (Boeckxstaens *et al*, 1991) and peptide histidine isoleucine (PHI) (Lefebvre *et al*, 1991).

However, it is interesting to note that in the cat, experimentally induced inhibition of gastric tone by intestinal nociceptive stimulation and chemical peritoneal stimulation involves a major sympathetic component (Glise *et al*, 1980). This helps to explain why gastric motility is suppressed after abdominal surgery and why some humans with postoperative ileus respond to sympatholytic agents (Dubois, 1988).

In summary, gastric accommodation and receptive relaxation allow the proximal stomach to fulfil one of its major functions, a reservoir for ingesta. Gastric accommodation and receptive relaxation are vagally mediated reflexes but the post-ganglionic neurotransmitter is neither adrenergic nor cholinergic. Vasoactive intestinal polypeptide, nitric oxide and PHI have been suggested as possible neurotransmitters.

3.4. Gastric Emptying of Liquids

Role of the Proximal Stomach

Hinder and Kelly (1977a) demonstrated that the stomach can discriminate between various components of a meal. They determined the gastric emptying rates of 400 mls of a 1% dextrose solution, 50 g of liver and forty 7 mm plastic spheres when given as one meal to dogs. Ninety percent of the dextrose solution left the stomach within the first hour, whereas four hours was required for 90% of the liver to empty. The majority of the plastic spheres did not leave the stomach until the food had entered the duodenum.

Subsequent to this study, Kelly (1980) proposed that the proximal stomach (the fundus and proximal one-third of the corpus) is responsible for the emptying of liquids whereas the distal stomach (the distal two-thirds of the corpus and the antrum) is responsible for the emptying of solids. The rate of gastric emptying of any component of a meal is given by the equation (Nelson and Kohatsu, 1971)

$$dv/dt = (P_s - P_d)/R_p$$

where dv/dt is the rate of gastric emptying; $P_s - P_d$ is the difference in pressure between the stomach (P_s) and the duodenum (P_d); and R_p is the resistance to flow across the pylorus.

Kelly (1980) hypothesised that, in the case of liquids, the resistance to flow across the pylorus is minimal, therefore, the rate of gastric emptying of liquids is governed by the pressure gradient, $P_s - P_d$. Since intragastric pressure is regulated mainly by fundic tone (Strunz and Grossman, 1980), Kelly suggested that the proximal stomach has the major role in determining the rate of emptying of liquids. On the other hand, the antrum and pylorus offer a large resistance to flow for solids and consequently this gastric region governs the emptying of solid particles.

A number of studies support Kelly's "two compartment model" for the role of the proximal stomach in the gastric emptying of liquids. When gastric fundectomy was performed on dogs, intragastric pressures increased which in turn lead to an increased rate of gastric emptying of saline (Wilbur *et al*, 1974). There was no difference in the gastric emptying rate of plastic spheres before and after fundectomy. Conversely, when the distal antrum was resected, the plastic spheres emptied faster but the emptying of the saline was not affected (Wilbur and Kelly, 1973).

Further evidence is provided by studies which have investigated gastric emptying following operations which vagally denervate the fundus and corpus without disrupting the innervation of the antrum. These procedures increased intragastric pressure and hastened the gastric emptying of solutions of isotonic saline and hypertonic glucose (Wilbur and Kelly, 1973). However, the operation did not affect the gastric emptying of solid spheres.

The exogenous administration of gastrointestinal hormones which affect fundic tone and intragastric pressure also alter the gastric emptying rate of liquids. Gastrin, when given in pharmacological doses^a, profoundly inhibits the contractions of the proximal stomach and reduces intragastric pressure (Okike and Kelly, 1977; Wilbur and Kelly, 1976). The intravenous administration of gastrin to human subjects (Hunt and Ramsbottom, 1967) and to dogs (Dozois and Kelly, 1971) slowed the gastric emptying of liquids in both species. Cholecystokinin (CCK) administration, reduced contractility in the proximal stomach and intragastric pressure (Valenzuela, 1976) which consequently slowed the gastric emptying rate of liquids (Debas *et al*, 1975). Unlike gastrin however, this action of CCK occurs at physiological doses^b.

^aA pharmacological dose of a hormone is defined as one that is greater than that producing a half-maximal response of the hormone's major physiological activity. The major physiological role of gastrin is the stimulation of hydrochloric acid secretion from the stomach.

^bA physiological dose of a hormone is a dose not greater than that producing a half-maximal response for that hormone's major physiological role. The major physiological role of cholecystokinin is the stimulation of pancreatic enzyme secretion.

Convincing evidence for the role of the proximal stomach in the regulation of gastric emptying of liquids is provided by Strunz and Grossman (1980). By controlling intragastric pressures with barostats they showed that there was a linear relationship between gastric pressure and gastric emptying rate. There also appears to be a linear relationship between the volume of solution emptied from the stomach per unit time and the total volume ingested. In the dog, this linear relationship occurred for ingested volumes less than or equal to 66 ml/kg (Leib *et al*, 1986), a range that encompasses quantities that a dog would normally drink. However, for larger ingested volumes, the rate of gastric emptying appears to approach a maximum value. Factors which may inhibit gastric emptying at high volumes include decreased gastroduodenal pressure gradients, increased duodenal muscular activity (Strunz and Grossman, 1980), or excessive stretching of gastric and duodenal smooth muscle beyond their physiological limits (Leib *et al*, 1986).

Role of the Distal Stomach and Small Intestine

In order for Kelly's two compartment model to be correct, any differences in gastric emptying rate of liquids must result from changes in proximal gastric pressure. However, recent evidence suggests that the two compartment model may oversimplify the gastric emptying of liquids.

This was illustrated by Miller *et al* (1981) who controlled fundic and duodenal pressures with barostats. They determined that when the average gastroduodenal pressure gradient was maintained at 0 cmH₂O ($P_s - P_d = 0$ cmH₂O, see discussion above) fluid left the stomach rapidly. Even when the average fundic pressure was 2 cmH₂O lower than that in the duodenum ($P_s - P_d = -2$ cmH₂O) fluid was still able to leave the stomach. Both these findings are discordant with the two compartment model since this requires $P_s - P_d$ to be always greater than 0 cmH₂O for fluids to pass into the duodenum. Furthermore, these workers found that there was no change in intragastric pressure in dogs after the infusion of saline, glucose or oleate liquid meals although the saline meal emptied twice as fast as the glucose or oleate meals. Clearly, there must be other mechanisms which also regulate the gastric emptying of liquids.

In 1969, Weisbrodt *et al* showed that a trisodium citrate/glucose solution stimulated almost continuous antral contractions without stimulating duodenal contractions. A solution containing trisodium citrate and oleic acid however, stimulated strong duodenal contractions and weak antral contractions. The citrate/glucose solution emptied at a much faster rate than the citrate/oleic acid solution. Since neither of these solutions stimulated contractility of the corpus, these authors concluded that the gastric emptying rate of the citrate/glucose solution was faster because it induced a higher ratio of antral contractility to duodenal contractility than the citrate/oleic acid solution. From this study it was evident that changes in antroduodenal contractility affected the rate of emptying of liquids from the stomach.

The application of a new imaging device, called a dynamic spatial reconstructor (DSP), has allowed researchers to non-invasively determine the movement of ingesta within the stomach. This device allowed for accurate quantification of regional changes in gastric volume following the ingestion of a meal. Kumar *et al* (1993) recently applied this technology to show that in dogs, the presence of nutrients in the upper small intestine determined the antral volume of a liquid meal. Perfusion of the small intestine with fat (oleic acid) resulted in a significantly smaller volume within the antrum compared to the volume after perfusion of the intestine with isotonic, equicaloric, isomolar volumes of carbohydrate (maltose) and protein (casein hydrolysate). Although these workers did not measure proximal gastric pressures concomitantly, it is reasonable to assume, based on earlier work (Aspiroz and Malagelada, 1985), that a fat meal lowers proximal gastric tone and results in smaller volumes of ingesta being delivered to the antrum. However, these workers also showed that changes in antral tone can regulate the emptying of liquids. Intestinal perfusion with fat not only resulted in a reduction in antral volume but also, with each peristaltic cycle, the volume of liquid propelled back into the corpus was approximately four times greater than the volume propelled aborally into the duodenum. This compared with a propulsion:retropulsion ratio of approximately 3:1 for saline and glucose instillates and a ratio of approximately 2:1 for the protein instillate.

The above studies have focused on the antrum as a possible controller of the gastric emptying of liquids. However, there is evidence to suggest that the intestinal tract itself may offer resistance to the gastric emptying of liquids. When truncal vagotomy and pyloroplasty were used to render the antrum and pylorus non-functional, there was no alteration in the selective resistance to outflow of a glucose or oleate solution compared with saline (Miller *et al*, 1981). Williams *et al* (1986) also observed in dogs that resistance to outflow of a glucose or oleate meal still persisted after antropylorectomy and a duodenal bypass. This indicated that even the small intestine, distal to the duodenum, could control gastric emptying of nutrient containing liquids. Resistance to gastric emptying also persisted in people after antrectomy (Berger, 1969).

Recently, in humans, the pylorus has been shown to provide resistance to the gastric emptying of liquids. However, the role of the pylorus in the gastric emptying of liquids is discussed further in section 3.8.

Prove and Ehrlein (1982) demonstrated that liquid meals of differing viscosity elicited different gastric motor responses in dogs. Gastric emptying of low, medium and high viscosity meals was measured via a duodenal cannula and observed simultaneously by fluoroscopy. Gastric motility was recorded from strain gauge transducers and induction coils surgically implanted in the serosa. They determined that gastric peristaltic waves propagated in the antrum were significantly stronger after the ingestion of a low viscosity meal than after the consumption of medium and high viscosity meals. Gastric emptying was fastest with the low viscosity meal and slowest with the high viscosity meal. They concluded that gastric emptying was regulated by the strength of the peristaltic wave which depended on the viscosity of the solution. Very viscous solutions induce weak antral contractions which result in a greater volume of ingesta being retropulsed back into the corpus. These workers suggested that stimulation of the slowly adapting in-series tension receptors of the stomach (Iggo, 1955; Paintal, 1954) may incite an inhibitory reflex which may decrease the depth of the peristaltic wave. Wall tension is a function of resistance to flow and since viscous solutions are more resistant to flow than non-viscous solutions, they produce the greatest stimulation of tension receptors.

More recently, Russell and Bass (1985a) have challenged the conclusions of Prove and Ehrlein (1982). Although both groups found that gastric emptying rate decreased as meal viscosity increased, Russell and Bass (1985a) determined that viscous fibre meals of psyllium and guar gum did not stimulate antral or duodenal motility relative to saline control meals. Low concentration guar and psyllium meals emptied from the stomach with the same 50% emptying time and exponential pattern as the saline control meal. Conversely, high concentration psyllium and guar meals emptied slowly with a constant amount being emptied per unit time. They concluded that antral and duodenal motor activity does not influence the gastric emptying of viscous fibre meals but rather the hydrodynamic flow properties of the liquid itself or tonal changes in the gastric body may be the major influence on the evacuation of viscous meals.

3.5 Intestinal Feedback Inhibition

"The Intestinal Brake"

Many of the studies discussed in the previous section have shown that liquids comprised of different nutrients leave the stomach at different rates. Therefore, there must be a gastrointestinal mechanism capable of firstly recognising the nutritional composition of ingesta and then altering gastric emptying rate accordingly. In 1979, McHugh and Moran showed that in rhesus monkeys, a constant rate of energy delivery to the duodenum determines the rate of gastric emptying of liquids. This calorie-dependent control of gastric emptying applied to a range of different nutrients over a range of concentrations. These workers also determined that regardless of the type of nutrient solution given and its concentration, the stomach emptied a 20 to 30 ml bolus rapidly before the calorie dependent regulation of emptying took place. They proposed that receptors in the small intestine "sampled" the fluid before providing feedback inhibition on the rate of gastric emptying. These findings suggested that the stimulation of nutrient receptors in the intestinal tract may be responsible for inhibiting the rate of gastric emptying. Recently, researchers have attempted to determine the location and the mechanism of action of these intestinal nutrient receptors.

Two studies have determined that glucose infused into the distal small intestine is more potent at inhibiting the rate of gastric emptying of liquids than the infusion of glucose into the proximal small intestine (Aspiroz and Malagelada, 1985; Lin *et al*, 1993). Read *et al* (1984) also found that perfusion of the ileum with fat inhibited gastric emptying of liquids. Intestinal feedback inhibition is not only confined to calorie containing nutrients. As intestinal acidity increases, gastric emptying rate decreases, a reflex induced more strongly by hydrochloric acid than by citric acid (Hunt and Knox, 1969). Acid receptors are found only in the proximal bowel (Lin *et al*, 1993). Furthermore, the feedback inhibition of gastric emptying is not confined exclusively to the gastric emptying of liquids. The perfusion of the ileum with glucose solutions was approximately three times more effective at delaying the gastric emptying of solids than an equivalent glucose load in the jejunum (Lin *et al*, 1993).

Lin *et al* (1993) determined that gastric emptying of solids and liquids was delayed when potato starch, which had been hydrolysed by incubation in pancreatic juice, was perfused into the distal ileum. Delayed gastric emptying was not observed however when unhydrolysed potato starch was perfused into the distal ileum. Clearly, the hydrolytic products of starch, which include maltose, maltotriose, and alpha-limit dextrans, are effective at delaying gastric emptying whereas more complex carbohydrates are ineffective. Since the products of starch hydrolysis are themselves hydrolysed further to glucose at the brushborder of the enterocyte it is not surprising that equicaloric amounts of glucose and these hydrolytic products had equivalent effects (Lin *et al*, 1993). Normally however, free glucose is absorbed before it reaches the distal jejunum (Gray, 1970) but in humans, an estimated 10-20% of ingested carbohydrate reaches the distal small intestine unabsorbed (Stephen *et al*, 1983). Recent work has shown that poorly digestible carbohydrates, which escape proximal absorption, delay the gastric emptying of a meal ingested at a later time (Lin *et al*, 1992). The gastric emptying of a solid meal was significantly slower in humans and dogs who were fed, four hours earlier, lentils compared with bread.

In an earlier study, Lin *et al* (1989) also determined that the chemical structure of the carbohydrate molecule, rather than its osmolarity, initiated feedback inhibition. A hypotonic solution of hydrolysed potato starch at 140 mOsm/l induced a delay in gastric emptying equivalent to a glucose solution at 500 mOsm/l. They also showed that for full expression of dose-responsive inhibition, at least 65 cm of small intestine needed to be exposed to the glucose solution. Recently, Melone and Mei (1991) have shown in cats that there are two types of lipid receptors responsible for negative feedback inhibition. One receptor was sensitive to long chain fatty acids and the other was sensitive to short chain fatty acids and glycerol. These workers demonstrated that medium chain fatty acids induced only slight changes in gastric motor activity whereas short chain fatty acids induced no change in gastric motor activity.

Previous dietary experience appears to modulate intestinal feedback inhibition. In humans, gastric emptying of glucose is faster when the diet had been supplemented with glucose for the previous three days (Cunningham *et al*, 1991a). Similarly, the gastric emptying of a fatty test meal was faster when human subjects had consumed a high fat diet in the preceding 14 days (Cunningham *et al*, 1991b). These findings may explain why many patients with anorexia nervosa have delayed gastric emptying (Holt *et al*, 1981) and why many obese people have rapid gastric emptying (Wright *et al*, 1983).

In a study designed to determine how prior exposure to nutrients may reduce intestinal feedback inhibition, Edelbroek *et al* (1992) assessed antral, pyloric and duodenal contractility after continuous long-term (0 to 120 minutes) intraduodenal infusions of dextrose. Their results showed that the infusion of intraduodenal dextrose initially increased basal pyloric pressure and increased the frequency of isolated pyloric pressure waves^c but each parameter returned to baseline values after 80 and 30 minutes respectively. However, antral and duodenal pressure waves remained suppressed during the long-term intraduodenal infusion of dextrose. These workers hypothesised that the decrease in basal pyloric tone and the reduction in isolated pyloric pressure waves is responsible for the reduction in intestinal feedback inhibition with chronic exposure.

^cFor a more detailed discussion on isolated pyloric pressure waves refer to section 3.8.

In 1993, Nightingale *et al* showed that intestinal feedback inhibition was provided by the colon as well as the small intestine. They studied the gastric emptying of a meal containing radiolabelled orange juice and radiolabelled pancakes in three groups of humans. Group one subjects had the majority of their small intestine resected but a normal colon (jejunectomy alone), group two subjects had their entire colon and the majority of their small intestine resected (jejunectomy and colonectomy) and group three were healthy, non-operated subjects. They found that in patients with a jejunectomy and colonectomy, the time taken to empty 25% of the orange juice was significantly faster than that in non-operated subjects and in subjects with a jejunectomy alone. In two out of seven patients with a jejunectomy and colonectomy, the time taken to empty 25% of the pancakes was significantly faster than non-operated subjects. The emptying of both solids and liquids was normal for the first three hours in patients with a jejunectomy alone. However, at six hours, both operated groups had retained significantly more liquid and solid than the non-operated group. From these results, Nightingale *et al* (1993) concluded that preservation of the colon, after a major small intestinal resection, exerts a braking effect on the rate of gastric emptying of liquid. They also proposed that the retention of solids and liquids after six hours in both operated groups may be due to a disturbance of the migrating motility complex, although this has yet to be confirmed.

Balloon distension of the colon and rectum in animals caused a rapid inhibition of gastric and intestinal contractions and tone (Youmans and Meek, 1937; Percy and van Liere, 1926). In humans, tonic or phasic rectal distension caused delayed gastric emptying and intestinal transit of a solid meal (Youle and Read, 1984). Therefore, visceral distension, as well as the presence of intraluminal nutrients, can cause intestinal feedback inhibition.

What changes in gastric motor activity are associated with feedback inhibition? The observation that glucose in the ileum and fat in the ileum, reduced fundic tone (Aspiroz and Malagelada, 1985) suggested that intestinal feedback inhibition of gastric emptying may be partly mediated by changes in pressure in the proximal stomach.

In humans, Fone *et al* (1990) simultaneously measured intraluminal antral, pyloric and duodenal pressures and gastric emptying of a ground beef meal after infusion of the terminal ileum with saline and a commercial triglyceride emulsion (Intralipid 20%, Pharmacia Pty. Ltd., Australia). Gastrointestinal pressures were measured by a manometric assembly which consisted of a sleeve sensor for measurement of pyloric pressures and a series of side holes to record pressures in the antrum and duodenum. Gastric emptying was assessed by scintigraphy. These workers found a decrease in antral, duodenal and propagated antropyloroduodenal pressure waves and an increase in isolated pyloric pressure waves in response to infusion of the ileum with lipid compared with a saline infusion. The rate of gastric emptying correlated significantly with all these measurements. They also found that following the ileal infusion of lipid, there was a retrograde movement of gastric content from the antrum to the fundus. These workers suggested that the retrograde movement of ingesta may have been due to either alterations in fundic tone or due to the occurrence of a mid-gastric contraction band which they observed in some subjects.

Neurohormonal Control of Intestinal Feedback Inhibition

In 1886, Ewald and Boas noted that the ingestion of fat induced inhibition of gastric acid secretion. Forty years later, a group of Chinese physiologists proposed that gastric acid secretion was inhibited by a humoral agent produced by the small intestine (Feng *et al*, 1929). This humoral agent was named *enterogastrone* (Kosaka and Lim, 1930). Since the 1930s, considerable research effort has gone into isolating enterogastrone without success. Instead, a number of hormones which can inhibit gastric secretion and motility have been isolated. Today, gut physiologists believe that not one single hormone functions as enterogastrone, but rather, negative feedback inhibition results from a combination of control mechanisms. This section is a synopsis of the current theory on intestinal feedback inhibition of gastric motility.

Many studies provide evidence for the role of cholecystokinin (CCK) as a possible mediator of intestinal feedback inhibition. Cholecystokinin is released in response to meals containing fat or protein (Meyer and Jones, 1974). Intravenous infusions of exogenous cholecystokinin octapeptide (CCK-8) or CCK-33 to humans and dogs in physiological doses have been shown to delay gastric emptying (Liddle *et al*, 1986; Debas *et al*, 1975; Kleibecker *et al*, 1988). The CCK antagonist, loxiglumide, significantly accelerated gastric emptying of a liquid test meal (Meyer *et al*, 1989a), however, accelerated gastric emptying was not noted when another CCK antagonist, MK-39, was used (Liddle *et al*, 1989).

Recent studies in humans have shown that an intravenous infusion of cholecystokinin octapeptide (CCK-8) stimulated isolated pyloric pressure waves and inhibited antral and duodenal pressure waves in a dose dependent fashion (Fraser *et al*, 1993). This combination of pyloric stimulation and antroduodenal inhibition was identical to that previously reported in response to intraduodenal lipid and dextrose infusion (Heddle *et al*, 1988; Houghton *et al*, 1988a). These results suggested that CCK may act as an enterogastrone in humans. However, the doses of CCK used in this study were considered to be pharmacological rather than physiological.

The polypeptide hormone somatostatin is released after the ingestion of fat (Kihl, 1980) and the exogenous administration of somatostatin delayed gastric emptying (Bloom *et al*, 1975). Consequently, it too has been considered a possible hormonal messenger for feedback inhibition of gastric motility. Somatostatin has been shown to be an important enterogastrone in rats (Seal *et al*, 1987). The inhibitory effect of intraintestinal fat on gastric acid secretion was not present when rats were immunised with anti-somatostatin antibodies.

Recently however, Mogard *et al* (1988) have rejected somatostatin as an enterogastrone in humans because intragastric administration of vegetable oil did not increase plasma somatostatin-like immunoreactivity. Moreover, gastric emptying rate increased, rather than decreased, in response to exogenous administration of somatostatin.

In dogs, plasma somatostatin concentrations increased significantly following duodenal and jejunal perfusion with a 20% lipid solution (Lloyd *et al*, 1992). Also, somatostatin administered intravenously caused a dose-dependent inhibition of gastric emptying similar to that seen after intestinal perfusion with lipid (Lloyd *et al*, 1992). However, the administration of anti-somatostatin antibodies did not prevent the delay in gastric emptying induced by the presence of lipid in the intestinal lumen. Therefore, somatostatin does not appear to be an important enterogastrone in dogs.

Gastric inhibitory polypeptide (GIP) was released by the ingestion of fat (Falko *et al*, 1975) and glucose (Cataland *et al*, 1974) in man and therefore, it too has been considered a potential enterogastrone. Edelbroek *et al* (1992) have shown that although intraduodenal dextrose infusion significantly increased plasma GIP concentrations in humans, there was no correlation between changes in plasma GIP concentration and antral, pyloric or duodenal pressures. However, these workers did not measure changes in fundic pressure which would also alter the rate of gastric emptying, particularly of liquid meals.

Intestinal feedback inhibition is also mediated by intrinsic and extrinsic neural pathways. Allescher *et al* (1989) have shown that pyloric contractions induced by acidification of the duodenum are mediated by both intrinsic duodenal neurons and the vagus nerves. Melone and Mei (1991) demonstrated lipid-sensitive intestinal receptors present in the cat which mediate feedback inhibition via the vagus nerves. Bilateral cervical vagotomy abolished intestinal feedback inhibition due to intrainestinal lipid infusion. Excision of the splanchnic nerves in dogs abolished the inhibition of gastric and intestinal contractions associated with balloon dilation of the colon and rectum (Youmans and Meek, 1937).

Intestinal feedback inhibition- Summary

Intestinal feedback inhibition of gastric motility regulates the supply of nutrients to the intestinal tract thereby allowing the intestine to maximise its digestive and absorptive functions. Intestinal receptors for lipids, carbohydrates and acids are concentrated in specific areas along the length of the intestinal tract. It appears to be the chemical structure of a nutrient, rather than its osmolality, that activates these nutrient receptors. Negative feedback inhibition is also affected by the length of bowel exposed to a particular nutrient and prior intestinal exposure to nutrients. Intestinal feedback inhibition slows gastric emptying by reducing the tone of the fundus, decreasing antral and duodenal peristalsis and increasing the number of isolated pyloric pressure waves. Intestinal feedback inhibition is mediated by a number of, as yet, poorly understood neural and hormonal mechanisms.

3.6 Gastric Emptying of Solids

In the two-compartment model of gastric emptying, Kelly (1980) proposed that the gastric slow wave of the distal stomach is well designed to break down solids into smaller particles before allowing digesta to pass into the duodenum. Kelly supported his hypothesis by citing the work of Carlson *et al* (1966) who observed canine gastric motility fluoroscopically. They observed that the gastric slow wave propelled solid food towards the pylorus and duodenum. However, because of the large size of recently ingested food, it was not able to pass into the duodenum but it was trapped in the distal antrum. As the advancing peristaltic wave passed over the food it provided the energy necessary to grind the food into smaller particles. Finally, because food cannot pass forward through the closed pylorus, it is propelled back into the corpus. Kelly (1980) stated that the repetitive propulsion, grinding and retropulsion of food is responsible for food being broken down into particles small enough for them to pass into the duodenum.

Other workers have added to Carlson's observations by noting that a weak peristaltic wave of gastric contraction is followed two to three seconds later by a much stronger peristaltic wave (Ehrlein and Heisenger, 1982; King *et al*, 1984). Both waves started in the oral corpus and moved distally to the pyloric ring. The initial weak wave was insufficient to obliterate the lumen of the antrum but it obliterated the lumen of the pylorus. The weak peristaltic wave closed the pylorus at the time when the strong peristaltic wave was half way through the antrum. The temporal relationship between pyloric closure and the advancing peristaltic wave was constant regardless of meal constituents and the speed of gastric emptying (Ehrlein and Heisenger, 1982). Szurszewski (1981) has suggested that the weak peristaltic wave is generated by the early, rapid depolarisation of the pacesetter potential and the strong peristaltic wave is due to the occurrence of the action potential which occurs two to three seconds later. Therefore, the gastric slow wave is entirely driven by the intrinsic nervous system of the stomach although neural and hormonal influences can modify this basic motility pattern.

There is considerable evidence to support Kelly's hypothesis that the distal stomach functions as a mill to grind food into small particles. Hinder and Kelly (1977b) determined that radiolabelled chicken liver left the canine stomach much more quickly when it was homogenised rather than when it was fed as 1 cm cubes. Moreover, the emptying rate of the homogenised liver was identical to the emptying rate of the dextrose solution in which it was suspended. These workers concluded that the stomach retains solids for reduction to a smaller size before the solids are discharged into the duodenum. More recently, Houghton *et al* (1988a) observed gastric emptying in humans fed a mixed solid/liquid meal and found that solids remained in the fundus where they were not exposed to antral contractions until most of the liquid had left the stomach. Solids then enter the antrum and gastric emptying of solids was immediately initiated. These workers concluded that grinding of solids by antral contractions occurred simultaneously with the gastric emptying of solids. The lag period allowed the stomach to empty excess liquid before the solid phase entered the antrum.

In the first part of an intricate two-part experiment, Meyer *et al* (1979) determined the size of food particles passed from the normal canine stomach. Chronic duodenal fistulas were created by placing a Thomas cannula in the duodenum opposite the main pancreatic duct. A Foley catheter inflated distal to the Thomas catheter provided a water tight seal so that all digesta leaving the stomach could be collected. Dogs were then fed radiolabelled chicken liver and broiled steak cut into 10 mm cubes then all the chyme leaving the stomach was collected and filtered through a stack of sieves with pore sizes ranging from 9.5 mm to 0.063 mm every 15 minutes, for a total of five hours. A strong positive correlation existed between the gamma count of the liver collected on each of the sieves and liver weight. Once the chyme was gamma counted it was either rediverted back into the small intestine through the Foley catheter or it was discarded. These workers determined that almost all (>99%) of the liver emptied as particles sizes less than 2.0 mm in diameter. Approximately 70% of the liver left as particles less than 0.063 mm in diameter. Moreover, there was a linear, zero order relationship between percent liver emptied and time. The gastric emptying rate was significantly faster when the chyme was discarded compared with returning the chyme to the small intestine. They proposed that the intestinal feedback inhibition was responsible for the difference in the rate of gastric emptying. However, diverting the chyme did not result in the recovery of significantly different particle sizes.

In the second part of the above study, Meyer *et al* (1979) helped to clarify which areas in the stomach are required for normal trituration and sieving of food by determining the effects that antrectomy, truncal vagotomy and pyloroplasty have on particle size. Although antrectomy resulted in significantly more liver leaving the stomach as particles of 2.0 mm diameter or greater, most of the liver recovered was still in particles sizes less than 2 mm diameter. However, if the gastric emptying rate was increased by discarding the chyme rather than returning it to the small intestine, the stomach emptied even larger particles of liver. These findings suggested that the antrum is probably responsible for the normal trituration and/or sieving of food, however, following antrectomy or antral failure, another area(s) can adopt this role, albeit less effectively. There was no difference in the particle size emptied from the stomach in dogs which underwent a pyloroplasty, an operation designed to increase the size of the pyloric opening, and non-operated dogs. However, following both truncal vagotomy (which disturbs normal antral motility) and pyloroplasty, the particle size selectivity of the stomach was greatly affected. Only 70% of the liver particles left the stomach in sizes less than 0.25 mm in diameter compared with 94% in controls. Truncal vagotomy alone did not result in a slowing of gastric emptying of radiolabeled liver, however, there was a slight but statistically significant effect on particle size. Considered together, these results suggested that in the presence of a functionally normal antrum, the sieving function of the pylorus is not required but when the antrum has been rendered non-functional by vagal denervation, then ablation of the supplementary pyloric mechanism results in markedly disturbed sieving.

Recently, antroduodenal motility has been the focus of considerable attention. In humans, Hausken *et al* (1992) used duplex sonography to simultaneously study antroduodenal motility and the flow of chyme across the pylorus. Duplex sonography utilises a pulsed Doppler for quantitative velocity measurements and colour Doppler to show direction of flow across the pylorus. These workers noted that the forward flow of chyme began when the pylorus was opening, approximately 6 seconds after the preceding terminal antral contraction. The period of forward flow lasted approximately 8.5 seconds. Forward flow through the pylorus ended when the pylorus was still open, 3.5 seconds before the next terminal antral contraction. In 50% of all peristaltic cycles the end of the forward flow period was immediately followed by duodenogastric reflux which lasted approximately two seconds. Duodenogastric reflux occurred most commonly after a coordinated antroduodenal contraction. Coordinated antroduodenal contractions occurred in two-thirds of the peristaltic cycles and are characterised by a contraction in the proximal duodenum occurring 2.5 seconds before the terminal antral contraction. In 50% of coordinated antroduodenal contractions, duodenal contraction was preceded by duodenal dilation. A gush of duodenogastric reflux occurring between two antral cycles but unrelated to coordinated antroduodenal contractions, termed a mid-cycle reflux, was seen in 45% of all peristaltic cycles. These workers also described a to-fro movement of chyme across the pylorus which was synchronous with the pulse in the aorta. This pulse-related flow was superimposed on forward transpyloric flow and duodenogastric reflux and occurred in all the subjects studied.

Hausken *et al* (1992) proposed that coordinated antroduodenal contractions are the consequence of distal duodenal contractions. Distal duodenal contractions result in the passive reflux of chyme into the proximal duodenum which in turn results in duodenal dilation. Passive reflux into the proximal duodenum is followed by contraction of the proximal duodenum and duodenogastric reflux.

Considerable insight into the events surrounding the gastric and duodenal emptying of solids in dogs has been provided by Haba and Sarna (1993). These workers simultaneously recorded the total gastroduodenal transit time of a meal of canned dog food with the frequency, amplitude and duration of fundic, corporeal, antral, pyloric, and duodenal contractions. Gastroduodenal transit time was defined as the time taken from the ingestion of the food to the time when no food particles could be seen in the effluent of a sample leaving a jejunal catheter. Gastroduodenal contractility was recorded with the aid of surgically implanted serosal strain-gauge transducers.

This study determined that only two parameters of postprandial gastric contractility had a significant positive correlation with gastroduodenal emptying rate: the mean frequency of corporeal, antral and pyloric contractility and the percentage of contractions that propagate past two or more of the recording sites in the stomach (which includes the pylorus). Although, the amplitude and duration of gastric contractions were positively associated with gastric emptying, the correlation was not statistically significant. They showed that the mean postprandial frequency of corporeal and antral contractions was only 3.5 per minute, which is only 75% of the maximum frequency at which the canine stomach can contract. Furthermore, although the mean frequency of contractions in the corpus, antrum, and the pylorus were nearly the same, only 60% of the contractions propagated over two or more recording sites. These comparisons suggested that there is considerable gastric "reserve" for increasing emptying rate and an external stimulus provided by drugs or the extrinsic nerves may increase gastric emptying by either increasing the mean frequency of postprandial contractions or by increasing the percentage of contractions that propagate through the stomach.

Haba and Sarna (1993) also determined that the rate of gastric emptying of solids was directly proportional to the mean distance of propagation of contractions and the percentage of propagated contractions in the distal duodenum. The transit rate in the small intestine is proportional to both these parameters (Cowles and Sarna, 1990). Rapid transit rates in the distal duodenum produce a more rapid rate of emptying of solids from the stomach. Rapid transit through the duodenum may cause a more rapid rate of gastric emptying either by reducing neurohormonal feedback or by removing chyme from the proximal duodenum where it may cause mechanical resistance to further gastric emptying.

This study also illustrated that the total number of duodenal contractions (the sum of all non-propagated and propagated contractions) was inversely proportional to the rate of gastric emptying of solids. This suggested that the non-propagated duodenal contractions resist gastric emptying. Non-propagated duodenal contractions are not propulsive but they mix and stir the chyme (Cowles and Sarna, 1990).

3.7 Neural Control of Distal Stomach Motility

Vagal denervation or inhibition of the vagal supply to the distal stomach decreased the rate of emptying of solids (Mroz and Kelly, 1977) whereas stimulation of the vagus nerves supplying the distal stomach increased the rate of gastric emptying of solids. Vagal stimulation in the dog caused an increase in the amplitude and duration of action potentials recorded from the antrum which resulted in an increase in pressure in the distal antrum (Miolan and Roman, 1971). These effects are completely blocked by atropine and almost completely blocked by the ganglionic blocking agent hexamethonium (Miolan and Roman, 1971; Sarna and Daniel, 1975). This observation suggested that vagal stimulation of antral smooth muscle is from pre- and postganglionic cholinergic fibres. Delbro *et al* (1984) have recently suggested that some of the vagal excitatory responses which are resistant to hexamethonium may be due to vagal afferent stimulation. Mucosal nociceptor stimulation may result in the release of substance P from vagal afferent fibres which form intramural synapses with postganglionic cholinergic neurons, the net effect being a hexamethonium-resistant excitation of the postganglionic cholinergic neurons.

Bilateral truncal vagotomy and total gastric vagotomy decrease the force of antral peristalsis (Wilbur and Kelly, 1973). Electrical recordings taken from the serosa of the antrum immediately after vagotomy show marked alterations in the gastric slow wave (Kelly and Code, 1969). The configuration of some pacesetter potentials became variable and arrhythmic. Abnormal pacesetter potentials were not followed by action potentials and therefore muscle contraction was not initiated. In most dogs, a normal slow wave was restored in the gastric antrum approximately one week later. In addition, a gastric slow wave arrhythmia resembling that seen in the immediate post-vagotomy period can be induced by the administration of atropine. This suggested that a decrease in vagal cholinergic tone was responsible for the arrhythmia induced by vagotomy.

There also appears to be a vagally mediated excitatory reflex of the antrum. In ferrets, when the proximal stomach was distended after it had been sectioned so the antrum was separated from the remainder of the stomach, action potentials increased with a concomitant increase in antral contractility (Scatcherd and Grundy, 1982; Andrews *et al*, 1980). In ferrets, dogs and humans this *antral reflex* is abolished by vagotomy (Kelly and Code, 1969; Staadas and Aune, 1970).

Splanchnic denervation of the stomach results in accelerated gastric emptying (McSwiney, 1931) and stimulation of the splanchnic nerves results in smooth muscle relaxation in the proximal stomach and an inhibition of peristalsis in the distal stomach (Miolan and Roman, 1971). These effects are mediated by adrenergic agents as they are blocked by guanethidine and bretylium (Beani *et al*, 1971; Campbell, 1966). Adrenergic nerves appear to inhibit gastric motility by acting directly on gastric smooth muscle and indirectly by synapsing with the myenteric neurons of Auerbach's plexus. The evidence for direct sympathetic innervation is best provided by the observations that the administration of tetrodotoxin does not alter the activity of noradrenaline on the action potential of antral smooth muscle and via fluorescent histochemical observations of adrenergic innervation of gastric smooth muscle (El Sharkawy and Szurszewski, 1978; Furness and Costa, 1974). It appears that stimulation of α -adrenergic receptors inhibit acetylcholine release from intramural cholinergic neurons, whereas stimulation of β -adrenergic receptors directly inhibits smooth muscle (Furness and Costa, 1974).

A number of workers have reported that stimulation of the splanchnic nerves at low frequencies can result in the contraction of the body and the fundus (Semba and Hiraoka, 1957; Nakazato *et al*, 1970). These excitatory effects are partly mediated by cholinergic nerve fibres since atropine effectively blocks contraction. However, Nakazato *et al* (1970) were able to show that splanchnic-stimulated or noradrenaline-stimulated gastric contractions could still exist in the presence of atropine. These noncholinergic excitatory effects were blocked by the α -antagonist phenoxybenzamine which resulted in gastric relaxation. The ensuing gastric relaxation was then blocked by the β -antagonist pronethalol.

In summary, stimulation of the vagus nerves increases the emptying of solids from the stomach by increasing contractility in the distal stomach. The increase in contractility is predominately mediated through cholinergic pathways. Stimulation of the splanchnic nerves usually delays the emptying of solids from the stomach by decreasing distal gastric motor activity. Post-ganglionic adrenergic nerves inhibit contractility either by inhibiting acetylcholine release from intramural cholinergic neurons (alpha receptors) or by directly inhibiting gastric smooth muscle (beta receptors).

3.8 The Pylorus and Gastric Emptying

The anatomy of the pylorus, with its thick wall of circular smooth muscle and narrow lumen, and its position at the gastric outlet suggests that it functions as a sphincter. Physiologically, a sphincter should act as "the keeper of the gate", allowing luminal content to pass through when it is open and inhibiting flow when it is closed. But does the pylorus act in this manner?

Certainly, emptying begins when the pylorus opens but emptying stops while the pylorus is still open rather than when the pylorus constricts (Hausken *et al*, 1992). Crider and Thomas (1937) found that there was no difference in gastric emptying rates when the pylorus was held open by an intraluminal stent, suggesting that the pylorus was not a true sphincter. Their observations were later confirmed by Stemper (1976). Prove and Ehrlein (1982) also showed that there was no difference in the phasic contractions of the canine pylorus when a high and low viscosity meal was fed despite the low viscosity meal emptying significantly faster.

Conversely, Houghton *et al* (1988a, 1988b) showed that after humans drink liquids, pyloric contractions occurred which were not associated with either antral or proximal duodenal contractions. They proposed that these so-called isolated pyloric pressure waves (IPPWs) may prevent the gastric emptying of liquids. They supported this hypothesis by noting that the occurrence of IPPWs correlated with a reduction in transpyloric flow during duodenal infusion of a lipid emulsion (Touqas *et al*, 1987). Moreover, the increased resistance to gastric emptying observed during gastric infusion of lipid solutions occurred when intragastric pressure was kept constant indicating that a decrease in fundic tone was not the only mechanism responsible for delaying the gastric emptying of solutions (Miller *et al*, 1981). Since solids start to leave the stomach after most of the liquids have emptied, the above observations may explain why pyloroplasty abolished the normal delay in gastric emptying after the ingestion of calorie-rich meals in humans (Clarke and Alexander-Williams, 1973).

Haba and Sarna (1993) have recently provided data which showed that pyloric contractions do not inhibit gastric emptying, rather, they help to promote the gastric emptying of solids. They established that the mean frequency, amplitude and duration of pyloric contractions has a statistically significant positive correlation with the gastric emptying of solids. In addition, the proportion of contractions which were propagated from the antrum or pylorus to the proximal duodenum also showed a significant positive correlation with the rate of emptying of solids. These data suggest that the pylorus, in association with the antrum and proximal duodenum, acted as a "peristaltic pump". Haba and Sarna (1993) did not observe

IPPWs in the fasting or postprandial state as described by Houghton *et al* (1988a, 1988b). However, when they perfused a liquid nutrient meal directly into the proximal duodenum they did observe some IPPW activity. The discrepancy between the findings of Haba and Sarna and the findings of Houghton *et al* may be due to species difference (dogs vs. humans), differences in type of meal (mixed vs. single nutrient), or differences in recording methods (strain gauge transducers vs. sleeve sensor).

In summary, the role of the pylorus in gastric emptying is still controversial although most investigators would agree that the pylorus is not a true sphincter. Some investigators have shown that the pylorus is an integral part of an "antropyloroduodenal pump" whereas others believe it is a "resistor" of gastric emptying. It may be that the function of the pylorus may change depending on the phase of gastric emptying. In fact, there are significantly fewer IPPWs during the gastric emptying of solids than in the preceding liquid emptying phase (Houghton *et al*, 1988b). Conversely, the antropyloroduodenal pump model of Haba and Sarna (1993) was only shown to promote the gastric emptying of solids. Clearly, more work is required to define the exact role of the pylorus in gastric emptying.

3.9. Control of Pyloric Contractility

Intracellular electrophysiological studies have shown that gastric slow waves propagate into the circular and longitudinal layers of the pylorus (Bass *et al*, 1961). However, slow waves appear to be restricted to a layer of circular muscle adjacent to the myenteric plexus (Sanders and Vogalis, 1989). The luminal layer of circular muscle, which is adjacent to the submucosa, is electrically quiescent and appears to be regulated by neurotransmitters and hormones (Sanders and Vogalis, 1989; Vogalis and Sanders, 1991). An excitatory and inhibitory nerve supply are also important for regulating the amplitude and duration of slow waves and phasic contractions in the myenteric portion of the circular muscle layer (Vogalis and Sanders, 1991).

A predominant part of the excitatory innervation of the pylorus is cholinergic (Allescher *et al*, 1988; Mir *et al*, 1979), arising from the vagus and intrinsic neurons which have sensory inputs from the duodenum (Allescher *et al*, 1989). Cholecystokinin and secretin (Fisher *et al*, 1973) and histamine (Biancani *et al*, 1981) (through H₁ receptors) have also been shown to contract the pylorus.

There is also inhibitory innervation (Anuras *et al*, 1974) which appears to be tonically active *in vivo* because after the intravenous administration of tetrodotoxin there was significant enhancement in pyloric pressure in the dog (Allescher *et al*, 1988). Vagal stimulation and direct stimulation of the gastric antrum also produced inhibition of pyloric contractions suggesting that the inhibitory innervation arises from extrinsic and intrinsic pathways respectively (Allescher *et al*, 1988). These effects are not blocked by atropine, phentolamine, or propranolol (Anuras *et al*, 1974; Mir *et al*, 1979) suggesting that the inhibitory effects are mediated by nonadrenergic noncholinergic enteric nerves.

Several studies of other regions of the gastrointestinal tract have suggested that nitric oxide (NO) might serve as an enteric inhibitory neurotransmitter (Bult *et al*, 1990; Dalziel *et al*, 1991; Stark *et al*, 1991). Recent studies have suggested that NO may also mediate enteric inhibitory reflexes in the pyloric region of the dog (Allescher *et al*, 1992; Bayguinov and Sanders, 1993). Bayguinov and Sanders (1993) provided the following data to show that NO, or a related compound, served as a pyloric neurotransmitter.

- 1) Arginine analogues, which inhibit NO synthesis, reduced the amplitude of circular muscle inhibitory junction potentials (IJPs).
- 2) Oxyhaemoglobin, which sequesters NO in extracellular fluid, also reduced circular muscle IJPs.
- 3) The combination of arginine analogues and oxyhaemoglobin completely abolished IJPs.

- 4) Exogenous NO mimicked the hyperpolarisation responses and the disruption in rhythm caused by enteric inhibitory nerve stimulation.

These data suggested that inhibitory neurotransmission in the pylorus depended completely on NO synthesis. However, it does not rule out the possibility that other neurotransmitters are involved. For example, vasoactive intestinal polypeptide (VIP) has been shown to stimulate NO synthesis in muscle strips and preparations of dispersed smooth muscle cells (Chakder and Ratan, 1993; Grider *et al*, 1992) and the mechanical response to VIP can be inhibited by arginine analogues in some preparations. Additionally, the hormone gastric inhibitory polypeptide (GIP), has been considered a possible candidate for regulation of IPPWs because it is released by small intestinal exposure to nutrients. However, a recent study has shown no correlation between plasma concentrations of both GIP and insulin and IPPWs (Edelbroek *et al*, 1992).

3.10 The Hydrodynamic Model of Gastric Emptying

Preliminary Observations

Kelly's (1980) archetype two compartment model of gastric emptying assumes that gastric emptying is governed by two motor areas; the distal stomach and the proximal stomach. The motor activity of the proximal stomach is responsible for the gastric emptying of liquids whereas the distal stomach is responsible for the gastric emptying of solids. However, this model gives very little consideration to the physical characteristics of the ingesta and the way in which these may affect gastric emptying. In the mid to late 1980s, a number of studies in dogs and humans determined how the physical characteristics of indigestible solids affected their gastric emptying rate. Meyer *et al* (1985, 1988) demonstrated in these species that the rate of gastric emptying of indigestible solids with a diameter between one and five millimetres was inversely proportional to their density and size. All small solids less than 1 mm in diameter however emptied at a similar rate, irrespective of density. For solids greater than 7 mm in diameter the major determinant of gastric emptying time was the duration of the fed state, which in turn was a function of meal size. Particle shape and surface energy were unimportant determinants of gastric emptying in dogs. In humans, object shape had no influence on gastric emptying rate but soft particles left significantly faster than hard particles (Meyer *et al*, 1989b).

It is also apparent that the physical properties of liquids affects gastric emptying rate. Liquids with a high viscosity have a slower rate of emptying than do liquids with a low viscosity (Prove and Ehrlein, 1982; Russel and Bass, 1985a). Moreover, the gastric emptying of indigestible solids is affected by the viscosity and the flow rate of the solution in which they are suspended. Meyer *et al* (1986) found a significantly greater number of 3.2 mm Teflon particles (density = 2 g/ml) emptied from the stomach when they were suspended in 1.5% guar gum than when they were suspended in water.

Very recently, Horowitz *et al* (1993) demonstrated that the specific gravity has a major effect on the emptying of solutions from the stomach. They used scintigraphy to determine the gastric emptying times of the aqueous and oil components of a mixture of olive oil and beef consomme in humans who were either lying in left lateral recumbency or sitting. The rate of gastric emptying of the aqueous component was significantly faster when the subjects were sitting compared to when they were lying down. Gastric emptying of the oil was not affected by posture. They concluded that in the sitting position the less dense oil "floats" on the aqueous component and consequently the aqueous component empties first. However, in left lateral recumbency the pylorus is uppermost and the oil empties first. This results in negative feedback inhibition and a delay in the gastric emptying of the aqueous component. When oil is administered in an homogenised form the aqueous component and the oil empty simultaneously (Cortot *et al*, 1979).

The studies outlined above have demonstrated that many of the physical properties of solids and the fluid in which they are suspended influence the rate of gastric emptying. In 1990, Sirois *et al*, realising that there is a codependence between solid (particle size and density) and fluid (viscosity and fluid flow rates), integrated these variables into the hydrodynamic model of gastric emptying.

Hydrodynamic theory

The hydrodynamic model is based on two main assumptions: that the antrum and pylorus are predominantly responsible for the gastric emptying of solids; and the passage of fluid and solid through the antrum and pylorus is akin to the laminar flow of liquid through a pipe.

Fluid close to the walls of a pipe moves slower than the fluid moving along the pipe axis. In cross-section, the fluid develops a parabolic velocity profile which is represented by the equation

$$V_r = 2V_{av}(1 - [r/R]^2) \quad (1)$$

where V_r is the fluid velocity at a given distance r from the pipe axis; V_{av} is the average linear fluid velocity; and R is the pipe radius.

Now consider a particle moving in fluid. A particle moving along the pipe axis will exit the pipe before a particle moving near the walls. However, particles will only maintain their position in the central region of flow if they do not float or sink. The rate at which a particle sediments (floats or sinks) is given by Stokes' law

$$V_t = g(\rho_f - \rho_p)D_p^2/\partial \quad (2)$$

where V_t is the sedimentation velocity; g is the gravitational constant, 9.8 m/s^2 ; ρ_f and ρ_p are the fluid density and particle density respectively; D_p is the particle diameter; and ∂ is the viscosity of the fluid.

Stokes' law incorporates the equation for buoyancy, $g(\rho_f - \rho_p)$. A buoyant particle, where $\rho_f - \rho_p = 0$, will not float or sink but it will remain suspended within a fluid.

From Stokes' law it follows that the distance a particle flows in a stream is dependent on how quickly it floats or settles out of the centrally moving stream in relation to the linear velocity of that stream. Sirois *et al* (1990) described this ratio as the particle emptying coefficient (PEC)

$$V_t/V_{av} = \text{PEC} = g(\rho_f - \rho_p)D_p^2/\partial(v) \quad (3)$$

The smaller the PEC, the further along the pipe the particle will travel per unit time. It should be noted however that the above equations apply to laminar flow. The stomach is not a rigid tube because its luminal diameter changes as the stomach wall contracts and relaxes with each peristaltic wave. Consequently, eddies occur and flow becomes non-laminar. Moreover, sedimentation, V_t , is impaired when the ratio of pipe diameter to particle diameter falls below a critical value. The hydrodynamic model of Sirois *et al* (1990) has attempted to account for some of these "wall factors" by modifying equation three above with the ratio of the average estimated pyloric diameter to the particle diameter. They termed this new equation the gastric emptying coefficient (GEC)

$$\text{GEC} = (D_{py}/D_p) \cdot [g(\rho_f - \rho_p)D_p^2/\partial(v)] \quad (4)$$

where D_{py} is the pyloric diameter.

Experimentally, Sirois *et al* (1990) showed that individual parameters such as particle size and density and the viscosity and flow rate of fluid from the stomach were poorly correlated with the emptying of nondigestible solids. However, emptying was highly correlated to both the PEC and the GEC which suggested that gastric emptying of nondigestible solids is determined, in part, by hydrodynamics. Furthermore, the GEC is better correlated to gastric emptying than the PEC which suggested that the gut wall effects discussed above are also important.

Very recently, real-time ultrasound images of the gastroduodenal junction of humans has provided visual evidence for the hydrodynamic model. Brown *et al* (1993) showed that after ingestion of a meal consisting of garbanzo beans and chicken broth, the beans, which are more dense than the broth, settled out in an area they termed the gastric sinus. The gastric sinus, the most gravity dependent part of the human stomach, is situated along the greater curvature directly opposite the incisura angularis. As the stomach filled with ingesta, the gastric sinus became deeper and progressively more displaced from the pylorus. Early in gastric emptying, the distal antrum assumed a funnel shape and since the pylorus is more dorsal than the corpus and antrum, fluids were "decanted" through the pylorus into the duodenum. However, with each peristaltic wave, solids were propelled from the gastric sinus for a short distance before they fell back into the gastric sinus. As the stomach emptied, the greater curvature moved closer to the lesser curvature and the distal antrum became tubular. Peristaltic waves could now triturate solids because they were pressed against the contracting walls of the antrum. Small solid particles, suspended in solution then passed through the pylorus. These findings help explain the lag period between the emptying of solids and liquids observed by Houghton *et al* (1988a). They also give credence to the hydrodynamic model of Sirois *et al* (1990) by illustrating the importance of sedimentation in gastric emptying.

3.11 The Migrating Motility Complex

Introduction

After the advent of x-rays and fluoroscopy at the turn of the century, Cannon (1898) observed that in fasting cats the stomach had periods of inactivity which were replaced by either weak contractions which waned before they reached the pylorus or frequent, powerful contractions which involved the whole distal stomach. In 1969, Szurszewski recorded from intestinal implants a recurring band of spike activity which progressed along the entire small intestine of the fasting dog which he termed an *electric complex*. A few years later Code and Schlegel (1973) demonstrated that this migrating electric complex was temporally related to a migrating series of peristaltic contractions, the *migrating motor complex*. In 1975, Code and Marlett showed that the majority of the motor complexes began in the stomach.

Today the term "migrating motor complex", which describes the muscular activity of the stomach and intestine, and the term "migrating myoelectric complex", which describes the electrical events responsible for the muscular activity, are used interchangeably, although they are not synonymous. For this reason, the term "migrating motility complex (MMC)" is used by this author to describe both the myoelectric activity and the motor activity. The following sections provide a description of the MMC, propose a function for the MMC and discuss the neurohormonal control of the MMC.

Description of the migrating motility complex

In fasting dogs, Code and Marlett (1975) showed that every 90 to 114 minutes an MMC migrated aborally from the stomach or proximal small intestine to the terminal ileum with each complex taking approximately 105 to 134 minutes to traverse the small intestine. In over half of the dogs studied, a new MMC began just before its predecessor had terminated in the distal ileum. These authors also showed that greater than 95% of the MMCs began in the distal stomach and terminated in the terminal ileum. However, other authors have recorded that 81% (Szurszewski, 1969) and less than 70% (Grivel and Ruckebusch, 1972) of MMCs reached the distal ileum. The disparity in these findings is probably a reflection of differences in recording techniques and experimental design. Work by Itoh *et al* (1978) demonstrated that the fundus and proximal corpus, as well as the distal stomach, contracted with MMC activity.

The velocity of MMC migration through the small intestine is not constant. It is difficult to accurately assess migration velocities in the intestine because the distance between two points is dynamic depending on whether the longitudinal smooth muscle is contracted or relaxed. Based on observations from three papers, the propagation velocity in the first 10% of the small intestine was between 5 and 12 cm/min whereas in the last 20% of the small intestine is between 1 and 2 cm/min (Code and Marlett, 1975; Grivel and Ruckebusch, 1972; Carlson *et al*, 1972). Overall, these observations suggested that the propagation velocity in the proximal intestine was many times faster than that in the distal intestine.

Code and Marlett (1975) determined that for each MMC cycle there are four separate motor and myoelectric phases. Phase I is characterised by an omnipresent cycle of pacesetter potentials which are not associated with action potentials and consequently muscular contractility is minimal. The mean duration of phase I activity of four different dogs ranged between 39 and 48 minutes in the stomach and 31 to 64 minutes in the distal ileum.

The onset of phase II is characterised by persistent but random action potentials. The ratio of action potentials (and hence muscle contractions) to pacesetter potentials increases steadily from 1:10 to a ratio of 1:1 which indicates the onset of phase III activity. The mean duration of phase II activity of four different dogs ranged between 31 and 48 minutes in the stomach and 26 and 62 minutes in the ileum.

Phase III activity is a short period of intense smooth muscle contraction. Phase III, otherwise known as the *activity front*, sweeps aborally obliterating the lumen of the antrum. During phase III activity, the ratio of pacesetter potentials to action potentials is approximately 1:1. Phase III activity lasts for approximately 12 minutes in the antrum and it is significantly shorter in the duodenum (~8 minutes) and jejunum (~6 minutes).

Phase IV activity is characterised by a rapid decrease in the incidence and activity of action potentials and terminates with a new phase I period when the ratio of action potentials to pacesetter potentials is less than 1:10. Phase IV activity is longest in the stomach and duodenum (6-16 minutes) and becomes progressively shorter as the complexes pass along the jejunum (2-4 minutes).

Function of the migrating motility complex

There is almost unanimous agreement among gastrointestinal physiologists that the function of the MMC is to clear large, indigestible debris from the stomach and small intestine. For this reason the MMC has been colloquially termed the "housekeeper wave" (Kelly, 1980). Hinder and Kelly (1977b) found that 7 mm diameter, indigestible, plastic spheres with a specific gravity near gastric juice only left the canine stomach after all digestible food had emptied. Later, Mroz and Kelly (1977) demonstrated that these spheres left the stomach during late phase II and early phase III of the MMC.

In humans, indigestible solids emptied rapidly when no food was present in the stomach (Mojaverain *et al*, 1985). In the presence of food, there was a delay in the emptying of the indigestible solids which was proportional to the volume of food ingested (Mojaverain *et al*, 1985; Smith and Feldman, 1986). This is understandable given that food prolongs the digestive period proportionally to meal size which in turn delays the onset of the MMC and the emptying of indigestible solids (Mojaverain *et al*, 1985).

Not all indigestible solids leave during the activity front of the MMC however. Russell and Bass (1985b) determined that 1-3 mm diameter polycarbophil particles induce a postprandial motility pattern when fed to dogs and leave the stomach independently of the activity front of the MMC. Therefore, the time when indigestible solids leave the stomach may be affected by the particle size and possibly by other physical characteristics (see section 3.10).

Control of the migrating motility complex

Motilin

Considerable debate exists as to which factors control MMC activity. There is strong evidence to suggest that the polypeptide hormone, motilin, is involved in the initiation of phase III MMC activity in the stomach and duodenum. Peak plasma concentrations of motilin occur just before, or during, phase III activity in dogs (Lee *et al*, 1978; Itoh *et al*, 1978a; Thomas *et al*, 1979; Poitras *et al*, 1980) and humans (Vantrappen *et al*, 1979). However, the simultaneous occurrence of peak plasma concentrations of motilin and phase III activity does not necessarily imply a cause and effect relationship since phase III activity may release motilin rather than motilin inducing phase III activity. A number of other studies have partly addressed this issue.

In fasting dogs, exogenous administration of motilin induced ectopic phase III activity (Poitras *et al*, 1980). The doses which produced peak serum motilin concentrations were similar to those observed with naturally occurring phase III activity indicating a physiological role. However, in humans, the role of motilin in inducing phase III activity is less certain because exogenous serum motilin concentrations higher than those observed with naturally occurring phase III activity must be administered before phase III activity is initiated (Vantrappen *et al*, 1979).

The most convincing evidence for the role of motilin in phase III activity is provided by the observation that intravenous administration of motilin-specific antibodies abolished activity fronts arising from the stomach and duodenum (Lee *et al*, 1983; Poitras, 1984). However, motilin antiserum did not affect activity fronts arising from the jejunum and ileum.

In 1993, Mizumoto *et al* showed that physiological doses of synthetic motilin can induce phase III-like contractions in the extrinsically denervated, perfused canine stomach.

Pharmacological agents

A number of exogenously administered drugs can induce phase III-like activity. These drugs include morphine (Sarna *et al*, 1983a) and erythromycin (Itoh *et al*, 1984; Tomomasa *et al*, 1986) in dogs and humans; metoclopramide (Achem-Karam *et al*, 1985) and intraduodenal acid (Collins *et al*, 1978) in humans; and in sheep, the serotonin antagonist, methysergide (Ruckebusch and Bardon, 1984). It is reasonable to assume that these drugs either released motilin or acted as motilin receptor agonists. Recent evidence suggests that erythromycin and some of its derivatives were motilin receptor agonists (Peeters *et al*, 1989; Depoortere *et al*, 1989; Depoortere *et al*, 1990). However, the presence of acid within the duodenum of humans caused phase III-like activity without causing a predictable release of motilin (Collins *et al*, 1978).

Neural control

Does motilin affect smooth muscle directly or are nervous pathways involved? One *in vitro* study has shown that motilin-induced smooth muscle contractility was not blocked by various neural antagonists indicating a direct effect (Strunz *et al*, 1975). However, Ormsbee and Mir (1978) found that, *in vivo*, the activity of motilin was antagonised by hexamethonium and atropine which suggested the involvement of both postganglionic and preganglionic cholinergic neurons. Adrenergic inhibition was probably involved as well. Evidence for a neural mediator was also provided by work which has shown that the MMC is modified by both surgical denervation of a length of small intestine (Aeberhard *et al*, 1980) and by direct arterial injection of drugs which block nerve conduction (Sarna *et al*, 1981).

The intrinsic nerves are required for the normal propagation of the MMC through the intestinal tract. When the intrinsic nerves of the small intestine were disrupted by intestinal transection at several locations followed by immediate reanastomosis the activity fronts occurred asynchronously in each segment (Sarna *et al*, 1983b).

Studies which have examined myoelectric activity immediately after vagal denervation have shown marked changes in MMC activity (Marik and Code, 1975; Hall *et al*, 1982). Hall *et al* (1982) blocked vagal nerve transmission by cooling the surgically exteriorised vagosympathetic trunks of dogs and immediately recorded motor activity from the lower oesophageal sphincter, stomach, duodenum and two sites in the jejunum. During vagal blockade, there was no contractility in the lower oesophageal sphincter or stomach. In the intestine, there were bursts of muscular activity resembling phase III activity and periods of motor quiescence (phase I) but there was no phase II activity. These results suggested that either the vagus nerves play a dominant role in mediating MMC activity in the stomach or that the disappearance of the gastric MMC during vagal cooling is caused by unopposed adrenergic inhibition of the stomach via sympathetic nerves entering the vagal trunks distal to the cooling site. However, Chung *et al* (1992) showed that simultaneously cooling the vagosympathetic trunk and blocking adrenergic transmission with phentolamine and propranolol did not restore the gastric MMC in dogs. These results suggested that the vagus nerves are the most important pathways for central control of the appearance of the gastric MMC.

However, other studies which have examined myoelectric activity two to eight weeks after bilateral truncal vagotomy have failed to show any abolition of MMC activity (Itoh *Z et al*, 1978b; Weisbrodt *et al*, 1975; Thomas *et al*, 1979). Furthermore, Mizumoto *et al* (1993) showed that physiological doses of synthetic motilin can induce phase III-like contractions in the extrinsically denervated, perfused canine stomach which suggested that the vagus nerves are not essential for motilin's activity. These workers also demonstrated that atropine and hexamethonium almost completely inhibited motilin-induced contractions indicating that motilin exerted its action through myenteric cholinergic neurons. Moreover, two different types of 5-HT₃ receptor antagonists and the α_2 -antagonist, yohimbine, inhibited motilin-induced contractions. However, yohimbine has been shown to have an antagonistic effect on peripheral 5-HT receptors (Lambert *et al*, 1978) therefore further studies are required to determine the role of α_2 receptors in motilin-induced contractions.

Extrinsic innervation also appears to carry afferent information from the small intestine which may modify MMC activity. In dogs, Melo *et al* (1981) created Thiry-Vela loops which had lost their intrinsic nervous nexus with the rest of the small intestine but had retained their extrinsic nervous connections. They found that distension of the loops inhibited phase III activity not only in the loops themselves but also in the rest of the bowel. Sympathetic denervation did not abolish phase III activity although it may modify activity front formation (Marlett and Code, 1979). Moreover, a number of surgical procedures and anaesthetic agents have been found to inhibit activity fronts but this could be reversed by splanchnic denervation (Bueno *et al*, 1978; Weisbrodt, 1987).

The migrating motility complex- Summary

In summary, it appears that motilin is important for initiating phase III activity in the stomach and duodenum. Activity fronts originating from the jejunum and ileum may be less dependent on motilin activity. There is strong evidence to suggest that motilin activity is mediated by intrinsic cholinergic neural pathways. 5-HT₃ receptors are also involved. Intrinsic neural pathways also need to be intact for synchronous aboral transmission of the MMC. Lastly, afferent information from extrinsic nerves may significantly modify MMC activity.

3.12 Converting from the MMC to the Fed Pattern

Code and Marlett (1975) found that the instillation of water and/or nutrients into the stomach of dogs resulted in a transient disruption in the MMC. The MMC was replaced with action potentials which resembled phase II MMC activity where approximately half the pacesetter potentials are followed by submaximal contraction of the antral muscle. However, unlike phase II MMC activity, this activity was of longer duration and the intensity did not increase with time. Also, unlike the MMC, the instillation of water and/or nutrients induced a pattern that is not cyclical but continues as long as ingesta is in the stomach. This is characteristic of "fed" action potential activity.

An infusion of 400 mls of saline, however, induced a fed motility pattern for only 10 to 60 minutes and it affected only one MMC cycle (Code and Marlett, 1975). The next MMC complex always started at the expected time in the duodenum, never in the stomach, and then proceeded distally in the normal sequence. Conversely, the instillation of 400 mls of milk disrupted MMC activity for much longer periods. MMC activity reappeared in the oral jejunum 2.5 to 4.0 hours after instillation. When the stomachs of dogs were distended with a balloon filled with 400 mls of air, Code and Marlett (1975) found that MMCs in the stomach and duodenum were replaced with a fed-like motility pattern. However, MMCs were variably disrupted in the remainder of the small bowel. Sometimes activity fronts which appeared in the jejunum were not propagated to the terminal ileum. From these early experiments, Code and Marlett (1975) concluded that the presence of non-nutrient and nutrient solutions in the stomach and gastric distention influenced the type and duration of gastrointestinal motility patterns.

In dogs, DeWever *et al* (1978) determined that there was a linear relationship between the volume of food ingested and the duration of the fed motility pattern. They determined that isocaloric quantities of carbohydrate, protein and lipids induced fed motility patterns of varying lengths with lipids being the most potent stimulator of fed motility and carbohydrates being the least potent. From these findings, it would be logical to assume that when a mixed nutrient meal is fed, the duration of the fed pattern would represent a summation of the effects of individual nutrient components. However, they found that feeding 30 kcal/kg of protein and 30 kcal/kg of lipid reduced the duration of the fed motility pattern to approximately half that observed when 30 kcal/kg of lipid was fed alone. These workers concluded that the physical and chemical composition of the meal is the most important factor in determining the duration of the fed motility pattern. The rate of gastric emptying was considered to play only a minor role because very small volumes of lipid had considerable effects on the duration of the fed state. However, the rate of gastric emptying was not assessed in this study.

Although Dewever *et al* (1978) concluded that the physical and chemical properties of a meal are important in maintaining the fed motility pattern, there is evidence that gastric distention may also play a vital role. A meal of polycarbophil (a synthetic, hydrophilic, bulk-forming laxative with no nutritive value) induced antroduodenal motor responses in dogs similar to those induced by a canned dog food meal (Russell and Bass, 1985b). Moreover, the polycarbophil meal and the canned food meal delayed the reappearance of the activity front of the MMC for comparable periods.

Is there a minimum gastric volume which must be obtained before a fed motility pattern is established? Weisbrodt *et al* (1976) partly addressed this issue by feeding canned dog food at different rates and recording gastrointestinal electrical activity. MMCs were not disrupted by feeding canned dog food at 6 g/kg; two out of three dogs converted to a fed pattern when 12 g/kg was fed; and all three dogs converted to a fed pattern when 24 g/kg was fed. However, DeWever *et al* (1978) found that a mere 1.2 g/kg of medium chain triglyceride oil successfully induced a fed motility pattern. Therefore, the volume of food required to disrupt the MMC is probably dependent on the types and quantity of nutrients that food contains.

3.13 Control of the Fed Pattern

Hormonal control

Since the ingestion of food can induce changes in serum concentrations of many hormones and the exogenous administration of a number of hormones to fasting animals or humans can produce changes in gastrointestinal motility patterns (Anderson *et al*, 1977; Mukhopadhyay *et al*, 1977; Wingate *et al*, 1979; Thor *et al*, 1982), it is reasonable to hypothesise that hormones may be responsible for the conversion from a fasting to a fed motility pattern. Intraluminal protein is a potent releaser of the hormone gastrin, particularly when the amino acids phenylalanine and tryptophan are present (Taylor *et al*, 1982). In dogs, the infusion of physiological doses of gastrin disrupted the MMC and induced a fed-like motility pattern (Marik and Code, 1975; Weisbrodt *et al*, 1978). Bueno and Ruckebusch (1976) postulated a role for insulin in converting motility from a fasting to a fed pattern in dogs when they demonstrated that exogenous administration of insulin induced a fed-like motility pattern. Insulin, like gastrin, is released by feeding. Furthermore, the disruption of the MMC in response to feeding was shorter in diabetic dogs than healthy dogs.

Several experiments have cast doubt on the physiological role of hormones in inducing the fed motility pattern, despite the plethora of indirect evidence indicating otherwise. Wingate *et al* (1978) closely examined the changes in motility induced by CCK-8 and gastrin in the fasting dog. They found that although MMC activity was inhibited, the motility patterns induced by these hormones are in fact dissimilar to the fed pattern. Russell *et al* (1982) found that dogs with autotransplanted antral pouches had chronically elevated serum gastrin concentrations and yet they still exhibited normal MMC activity. Two conclusions can be drawn from this study; either gastrin was not necessary for the abolition of MMC activity or the gastrointestinal tract adapted to hypergastrinaemia. Eeckhout *et al* (1978) established that although feeding increased serum concentrations of gastrin and insulin, MMCs reappeared before hormone concentrations returned to baseline concentrations. More importantly, they also determined that MCT oil and arachis oil inhibited MMCs without altering insulin and gastrin serum concentrations. Later, however, Eeckhout *et al* (1984) found that the infusion of an oil/bile/pancreatic enzyme mixture into the ileum inhibited intestinal MMCs and released neurotensin. Hence, lipids may modulate gut motility through other hormones or neurotransmitters. Sarr and Kelly (1981) determined that feeding dogs with extrinsically denervated Thiry-Vella loops did not inhibit MMC activity within the loops. It can be concluded from this study that either extrinsically denervated loops have a decreased sensitivity to hormones or endogenously released hormones are insufficient on their own to disrupt MMC activity.

Neural control

The role of the sympathetic nervous system on converting the fasting motility pattern to a fed motility pattern in response to feeding has not been appraised. Assessing this role may be difficult since severing the splanchnic nerves disturbed MMC activity even prior to feeding (see above).

It appears that vagotomy only has a minor effect on changing motility from a fasting to a fed pattern. In vagotomised animals, a greater volume of food is required to disrupt the MMC and the duration of disruption of the MMC is shorter than that of the vagally intact animal (Marik and Code, 1975; Ruckebusch and Bueno, 1977; Bueno and Ruckebusch, 1976). However, these findings should be interpreted with caution. Vagal denervation affects the rate of gastric emptying and hence the rate of exposure of the intestinal tract to nutrients. This could also alter the duration of the fed motility pattern. Nevertheless, when dogs with intrinsically denervated and extrinsically innervated Thiry-Vela loops are fed, MMCs are interrupted in the loop (Pearce and Wingate, 1979; Weisbrodt *et al*, 1976). This finding suggested that either hormones or the extrinsic nerves mediated the change in motility. The situation became somewhat clearer when the Thiry-Vela loops were also extrinsically denervated. In this situation MMCs are not interrupted in response to feeding. Considered together, these results suggest that the extrinsic nerves do have a role in mediating the change in motility, at least in the small bowel.

When Thiry-Vela loops are exposed to intraluminal glucose, MMCs are disrupted within the loops but not within the rest of the small intestine (Eeckhout and DeWever, 1979). This suggested that either the intrinsic nerves or paracrine influences were involved in disrupting the MMC in the small intestine.

Control of the fed pattern- Summary

The myoelectric pattern of the fed state resembles phase II of the MMC. Approximately half the pacesetter potentials are followed by action potentials and contraction of the antrum is submaximal. The duration of the fed pattern is governed by the type and quantity of nutrients exposed to the stomach and small intestine. Saline and gastric distension can convert motility from a fasting to a postprandial pattern, albeit for a short period. Similarly, fibre, with no nutritive value can induce fed-like motility. The fed pattern is probably regulated by a combination of hormonal and neural influences although currently these are poorly understood.

Chapter Four

Techniques for Assessing the Rate of Gastric Emptying

4.1 General Introduction

The properties of the "ideal" gastric emptying technique were discussed in Chapter One and it was emphasised that no single method possesses all these properties. This chapter reviews the techniques which have been used to determine the rate of gastric emptying in animals and humans. Following the review of each technique, a comparison is made with the hypothetical "ideal" technique and the relevance of each technique to private veterinary practice is also discussed. Table 4.2, at the end of this chapter, represents a summary of the findings on each of these techniques.

4.2 Positive contrast radiography

Review

A positive contrast radiography study involves taking abdominal radiographs at predetermined intervals prior to, and after, the administration of a radiodense contrast agent. The radiodense contrast agent is usually barium sulphate suspension either given alone or mixed with food.

Barium sulphate is available either as a fine, micropulverised salt which can easily be made into a colloidal suspension or as a pre-prepared stabilised, colloidal suspension (Barber and Mahaffey, 1986). Barium sulphate is not absorbed by the gastrointestinal tract and it is not soluble in water, hence it is not affected by gastrointestinal secretions. The recommendations regarding the volume and concentration of barium sulphate suspension to use in positive contrast studies are highly variable and include 20 to 100 mls of 100% w/v barium sulphate depending on the weight of the dog (Hertage and Dennis, 1989); 11 to 15 ml/kg (no concentration given) (Owens, 1982); 2 to 5 ml/kg (no concentration given) (Kealy, 1979); and 8-12 ml/kg for small to medium sized dogs and 5-7 ml/kg for large dogs (O'Brien, 1978).

Food should be withheld for 12 to 24 hours prior to a positive contrast radiographic study to ensure the stomach is empty and this should be confirmed by plain survey radiographs. The suspension can be administered either into the mouth by syringe or via a stomach tube. Radiographs are taken immediately after administration, at 15-30 minutes, 60 minutes, and at 2, 3 and 6 hours. Ventrodorsal, dorsoventral and right and left lateral recumbent radiographs are recommended by Hertage and Dennis (1989).

Emptying of barium sulphate suspension from the stomach should begin within 15 minutes of administration, however, in nervous animals the onset of emptying may be delayed for up to 45 minutes. Gastric emptying is considered abnormal if it has not started within 45 minutes. The stomach should be completely empty within four hours but in most dogs, gastric emptying is complete within 1-2 hours (Hertage and Dennis, 1989).

The disadvantages of barium sulphate as a contrast medium is that it can cause pneumonia if it is inhaled or it can cause granulomatous peritonitis if it enters the peritoneal cavity through a perforated viscus (Hertage and Dennis, 1989). If gastrointestinal perforation is suspected or if the patient needs surgery immediately after the positive contrast study, then water soluble iodide preparations should be used instead as these agents are absorbed from the peritoneal cavity (Hertage and Dennis, 1989).

Iodide containing preparations are all derivatives of triiodinated benzoic acid. The routine use of iodide preparations is not indicated because they have a bitter taste which makes oral administration difficult. Some iodide preparations (meglumine diatrizoate and sodium iothalamate) are also hypertonic and draw fluid from the vascular compartment into the intestinal lumen by osmosis which can cause both hypovolaemia and dilution of the contrast medium with consequent poor definition of the bowel (Hertage and Dennis, 1989). Recently, several water soluble iodide containing contrast media with low tonicity have been developed. These include iohexol, iopamidol, ioxaglate and metrizamide. Using iohexol at 10 mg/kg, Agut *et al* (1993) determined that in healthy Beagles, gastric emptying was completed within 30-60 minutes at concentrations of 525 and 700 mgI/kg and was completed within 90-120 minutes at a concentration 875 mgI/kg. At all concentrations, gastric emptying started immediately.

Recently in dogs, Miyabayashi and Morgan (1984) determined the gastric emptying rate of a dry kibbled diet coated with a 60% w/v barium sulphate suspension administered at 5 ml/kg bodyweight. The dogs were given the food at 8 g/kg bodyweight either as intact kibble or ground to a particle size with a diameter of less than 1 mm. They showed that when the kibbled diet was fed intact, the barium sulphate suspension separated from the food and both components left the stomach separately. Separation did not occur when the food was ground. In 50% of the dogs, gastric emptying began within 15 minutes of ingestion of the ground kibble/barium sulphate diet. Complete gastric emptying times ranged between 5 and 9 hours with a mean emptying time of 7 hours.

Burns and Fox (1986) repeated the methods of Miyabayashi and Morgan and utilised the same materials in an attempt to duplicate their data and increase on the number of animals studied. Their data were significantly different from those obtained by Miyabayashi and Morgan and consequently, the data from the two studies could not be combined. Burns and Fox concluded that "due to the wide range of normal values observed, accurate evaluation of clinical patients suspected of gastric abnormalities could be difficult unless there is gross abnormality in function". However, both studies demonstrated that there is little day to day variation in the same dog. Therefore, this technique appears to be a useful method to assess the effect a procedure or a drug may have on the completion of gastric emptying once a baseline value has been established.

Assessment

Positive contrast radiography studies have now been superseded by other techniques in human medicine. However, they are still widely used in veterinary medicine because they can be performed in veterinary clinics using standard radiography equipment and special skills are not required to interpret the radiographs. Moreover, positive contrast radiography studies are inexpensive compared with other methods of assessing the rate of gastric emptying.

However, there are significant problems with positive contrast radiography for assessing the rate of gastric emptying. Firstly, these studies provide very little quantitative information about gastric emptying. The initiation and termination of gastric emptying can be established but other values, such as the time taken to empty 50% of the stomach's content, cannot be determined. Secondly, only the gastric emptying of liquids (barium sulphate suspension) or suspended solids (food ground to a very small particle size and suspended in barium sulphate) can be assessed using this technique. Thirdly, there is considerable variation between dogs in the time it takes to complete gastric emptying and consequently, there is a wide range of reference values cited in the literature. This results in a poor positive and negative predictive value of the test. Additionally, there does not appear to be one standard barium sulphate suspension study used by all veterinarians. Variations in the methods employed make it difficult to apply "normal" values. Lastly, this technique can result in significant radiation exposure to the patient and hospital personnel.

In summary, positive contrast radiography studies provide, at best, a semi-quantitative method of assessing gastric emptying of liquids. Gastric emptying must be grossly abnormal before an abnormality can be detected using this technique. Nevertheless, the ease of performing positive contrast studies assures their continued use in veterinary medicine.

4.3 Plain Radiography

Review

In 1992, Arnbjerg determined the gastric transit times of three different types of food in dogs and cats from the interpretation of plain, right lateral recumbent abdominal radiographs. Dogs and cats were fasted for 24 hours and then radiographed. If the stomach contained a small volume of air, or if there was no ingesta visible between the stomach walls, then the stomach was considered empty and the subject was fed. The diameter of the stomach was determined immediately after eating. The initiation of gastric emptying was deemed to occur when the gastric diameter decreased for the first time. Gastric emptying was complete when the stomach appeared empty on radiographs.

In dogs, gastric emptying of 500 mls of dry dog food began 8 to 10 hours after eating (mean, 8.9 hours). The stomach was empty 14 to 16 hours after eating (mean, 15 hours). Gastric emptying of 500 mls of canned dog food began four to five hours (mean, 4.5 hours) after eating and was complete seven to eight hours (mean, 7.4 hours) after eating. Gastric emptying of 500 mls of fresh fish began 30 minutes after eating and was complete four to six hours (mean, 4.7 hours) after eating.

Assessment

Although the proximate analyses of the diets used in the study were stated, neither the product brand names nor the consistency of the diets were mentioned. The absence of this information makes it difficult to use the results of this study for clinical investigation. It should be noted however, that the intention of the study was to determine the duration of fasting required for complete gastric emptying rather than to develop a clinical method for assessing gastric emptying.

In general, the advantages and disadvantages of this technique are the same for those of a positive contrast radiography study. However, this technique may provide information about the gastric emptying rate of solid ingesta as well as liquid ingesta. Also, since a contrast agent is not required for this technique, plain radiography studies are cheaper and easier to perform.

4.4 Scintigraphy

Review

The scintillation camera

Scintigraphy requires the use of a scintillation camera which detects and measures the intensity of high energy radiation. In medicine, scintillation cameras specifically detect gamma radiation and are more commonly called gamma cameras. A component of the camera, called a phosphor, produces flashes of light when exposed to radiation. A photomultiplier then converts the light into pulses of electrical current. The electrical circuitry of the gamma camera then transforms the current into a two-dimensional image, termed a *scintigram*.

Scintigrams are commonly stored on computer tape and subsequently analysed by a computer connected to the scintillation camera. The amount of radiation in each scintigram is quantitated by summing the *pixel* counts. A pixel, or *picture element*, is represented by a dot on the scintigram. The greater the concentration of pixels, the brighter the image.

Recently, a simple, hand held, scintillation probe (Renatron Probe) was used to assess gastric emptying in healthy humans (Holt *et al*, 1990). Results obtained from the scintillation probe correlated well with those obtained from a gamma camera. To date, no work has been conducted on the use of scintillation probes in veterinary medicine but they may provide a less expensive alternative to gamma cameras for assessing gastric emptying in veterinary practice.

Test Meal Preparation

Although a scintigram has poorer image resolution than a radiograph, the strength of scintigraphy lies in its ability to directly assess the gastric emptying of food labelled with a radioisotope. Conversely, radiography techniques require the use of non-physiological contrast media to indirectly assess the gastric emptying of ingesta.

Chromium-51 (^{51}Cr) was the first radioisotope used in gastric emptying studies. However, this radioisotope could only be imaged and not counted quantitatively (Griffith *et al*, 1966) which led to the use of iodine-131 (^{131}I) (Malmud and Long, 1980). Unfortunately, although ^{131}I could be counted, it could also be cleaved from its marker (human serum albumin) in the duodenum, absorbed into the portal circulation and re-secreted in the gastric juice. This resulted in an underestimation of the rate of gastric emptying. Moreover, ^{131}I is a beta-emitter and had to be administered in low doses for radiation safety reasons (Malmud and Long, 1980). Today, the radioisotopes used include technetium-99m ($^{99\text{m}}\text{Tc}$), indium-111 (^{111}In) and indium-113m ($^{113\text{m}}\text{In}$). They fulfill the characteristics of the ideal radioisotope and are summarised in Table 4.1 below.

Table 4.1 Characteristics of the ideal radioisotope.

High count rates
Low radiation emission
Relatively short half-life
Suitable imaging characteristics
Gamma emitter with no associated beta emissions

Table modified from Scarpignato (1990), with permission.

Radioisotopes must be bound to a marker which can then be incorporated into a test meal. This process is termed *radiolabelling*. Radiolabelled markers can then be used to assess gastric emptying of solids (solid phase scintigraphy), gastric emptying of liquids (liquid phase scintigraphy) or the gastric emptying of both solids and liquids simultaneously (biphasic scintigraphy). Regardless of the radiolabelled marker used, they all must possess certain fundamental properties.

The radioisotope must remain bound to the marker as it passes through the gastrointestinal tract. If the radioisotope separates from the marker to which it is bound then it may leave the stomach in a different phase of gastric emptying, and hence, the scintigram may falsely depict the true gastric emptying rate of the marker. The capacity of the radioisotope to remain bound to the marker can be assessed *in vitro* by incubating the radiolabelled marker in gastric juice at 37°C and determining the quantity of radioisotope that has been eluted from its marker (Sagar *et al*, 1983).

The radiolabelled marker should not be adsorbed onto mucosa nor should it be absorbed through the gastric mucosa as this would alter the emptying rate of the marker. Marker absorption can be assessed by determining blood, urine and tissue concentrations of radioisotope (Malagelada *et al*, 1980b). Absorption and adsorption have also been assessed by determining radioactivity of the carcass, stomach, intestine and gastrointestinal content of rats following the ingestion of a radiolabelled marker (Sagar *et al*, 1983).

From a practical standpoint, the radiolabelled marker needs to be inexpensive, easily made and it must be homogeneously distributed within the test meal (Scarpignato, 1990).

Many different radioactive markers have been used to label food in gastric emptying studies. Currently, ^{99m}Tc -, ^{111}In -, or ^{113m}In -diethylenetriaminepentaacetic acid (DTPA) are the most common liquid phase markers (van den Brom and Happe, 1986). Markers of solid phase emptying are more numerous. Some radioisotopes can be attached to normal dietary constituents such as ^{131}I -labelled alpha cellulose (Malagelada *et al*, 1980b), ^{99m}Tc -labelled chicken liver (Meyer *et al*, 1976) and ^{99m}Tc -sulphur colloid labelled egg whites (Kroop *et al*, 1979). Other radioisotopes are labelled to carrier molecules such as ^{99m}Tc -labelled triethylenetetramine-polystyrene resin (Theodorakis, 1980). The majority of the radiolabelled markers are made *in vitro*, however, ^{99m}Tc -labelled chicken liver is made *in vivo* by injecting live chickens with ^{99m}Tc . The radioisotope is taken up by the Kupffer cells of the liver thus providing a stable intracellular label to the liver parenchyma (Meyer *et al*, 1976).

When biphasic scintigraphy is required, the solid and liquid phases are labelled with radioisotopes that have photopeaks which do not overlap. Commonly, ^{99m}Tc and ^{111}In are used together for biphasic scintigraphy. ^{99m}Tc has a photopeak at 130-155 keV whereas ^{111}In has photopeaks at 164-180 keV and 222-272 keV (Zeissman *et al*, 1992). The gamma camera will then be set to record the appropriate photopeaks for technetium-99m and indium-111.

Scintigraphy studies in human medicine

There is no standard method for performing scintigraphy studies. Each clinical or research institution appears to have a different test meal, recording technique and criteria for interpretation. Therefore, this section will attempt to outline the important similarities and differences in scintigraphy techniques.

A solid meal (eg, an omelette sandwich) or a liquid meal (eg, orange juice) containing the radiolabelled marker is given after an overnight fast. In many instances, both solid and liquid phase emptying may be assessed simultaneously by biphasic scintigraphy.

After ingesting the meal, patients are required to sit semi-upright at a 60° angle (Zeissman *et al*, 1992), or upright at a 90° angle (Holt *et al*, 1990). The gamma camera is positioned over the patient's stomach, often with the aid of an oscilloscope (Holt *et al*, 1990). *Acquisition* (the term for taking a scintigram) in the posterior view overestimates the rate of gastric emptying because as the meal moves from the posterior gastric fundus to the anterior gastric antrum and away from the gamma camera, a decrease in radiation energy is detected due to tissue absorption and scatter of photons. Conversely, acquisition in the anterior view underestimates the rate of gastric emptying. The loss of radiation energy due to absorption and the scatter of photons as the meal moves away from the gamma camera is termed *attenuation*.

The most common method of correcting for attenuation is to acquire both anterior and posterior views and then calculate the geometric mean for each imaging time point. This is usually performed by intermittent acquisition, typically one minute anterior and posterior images every 20 to 30 minutes for a total of 90 to 120 minutes. However, intermittent acquisition limits the number of data points on the gastric emptying curve. To illustrate this point, a two hour study with acquisitions every 20 minutes provides only seven data points. Dual headed gamma cameras allow for continuous anterior and posterior acquisition, however, most nuclear medicine laboratories do not have this facility.

Very recently, Zeissman *et al* (1992) at the Georgetown University Hospital demonstrated that continuous acquisition in the left anterior oblique (LAO) view with a single headed gamma camera provides very similar results to the geometric mean technique. In their 90 minute scintigraphy studies they could obtain gastric emptying curves with 60 data points by means of continuous acquisition.

Once acquisition is complete, gastric emptying versus time curves are created with the aid of an on-line computer system. The stomach, or *region of interest*, is delineated on each scintigram and the radioactivity in the stomach is determined by summing the pixel counts. The pixel count within the region of interest of the first scintigram has 100% activity and all subsequent scintigrams have activities which are expressed as a percentage of the pixel count of the first scintigram. Another means of deriving 100% activity is by placing the test meal in front of the gamma camera immediately prior to ingestion (Holt *et al*, 1990).

Due to the short half-life of the radioisotopes used in scintigraphy (^{99m}Tc has a half-life of 6.04 hours) emptying-time curves must be decay-corrected. Corrections may also be made for background radiation (Sagar *et al*, 1983) and in the case of biphasic scintigraphy, for scatter radiation. When two radioisotopes are used, counts from the first radioisotope may scatter into the recording window of the second radioisotope and vice versa. If biphasic scintigraphy utilises ^{99m}Tc and ^{111}In radioisotopes, scatter radiation is minimal and scatter corrections are unnecessary (Zeissman *et al*, 1992).

The time taken to reach 50% emptying, the percent emptying of liquids at 15 minutes, the percent emptying of solids at 90 minutes, the slope of the emptying-time curves and the shape of the emptying-time curves are examples of parameters which have been measured to assess whether a patient has a normal gastric emptying pattern. In one study, data from healthy individuals were used to derive 95% confidence intervals for all points on the emptying-time curves (Galil *et al*, 1993).

Scintigraphy studies in canine medicine

The earliest report of the use of scintigraphy as a clinical tool for the assessment of gastric emptying in veterinary medicine was provided by Theodorakis in 1980. He established that ^{99m}Tc -labelled triethylenetetramine-polystyrene resin was a suitable marker for use in the dog. A test meal of approximately 100 μCi ^{99m}Tc -labelled triethylenetetramine-polystyrene resin mixed with 225 grams of canned dog food was fed to healthy Beagles. After ingestion of the meal, the dogs were immobilised standing in a "restrainer". Images were obtained by continuous dorsal acquisition for 60 minutes. Computer generated gastric emptying-time curves were established for each dog. A mono-exponential pattern of gastric emptying was recorded in each dog. The gastric emptying half-life was then determined from these curves.

Six years later, van den Brom and Happe (1986) provided a mathematical model and reference values for the gastric emptying of a ^{99m}Tc -labelled semi-solid meal in mixed breed dogs. The semi-solid meal consisted of a mixture of 250 ml of water, 30 g of cornstarch, 20 g of glucose and 2 eggs. Ventral images were acquired intermittently for one minute over a two hour period. Restraint was aided by sedation with droperidol and dehydrobenzoperidol. Their model, which described the shape of the emptying-time curve, allowed parameters such as the initial half time, the real half time and the 95% emptying time to be calculated. This model was then used to show that there was no significant difference between the gastric emptying of a semi-solid meal in dogs which had surgery following an episode of gastric dilation-volvulus and healthy dogs (van Sluijs and van den Brom, 1988).

In 1989, Hornof *et al* published a technique for investigating solid-phase emptying in dogs. Their test meal consisted of one cup of a dry kibble diet (unspecified brand) moistened with 5 ml of saline containing 111 mBq of ^{99m}Tc Disofenin. A 2 oz jar of beef baby food was added for flavour. Dry food was chosen instead of canned food because there is less variation in the caloric density between brands of dry food. ^{99m}Tc Disofenin was regarded as a good marker because it was easy to prepare and it was neither absorbed through nor adsorbed onto the gastrointestinal mucosa. However, these workers did not demonstrate that ^{99m}Tc Disofenin remains well bound to the food when exposed to gastric juice and until this is determined the use of this cannot be recommended.

Pilot studies indicated that although smaller meals emptied more quickly than larger meals, thus requiring less imaging time. However, the data from small meals tended to be "noisy" with wide fluctuations in count rates early in the study and consequently larger meals were favoured. All dogs were fasted for at least 18 hours prior to the study. Dogs were offered the test meal and water for 15 minutes. After 15 minutes, the remaining meal and water were removed from the cage and the dogs were immediately positioned over a gamma camera. Thirty second images were acquired while dogs were restrained in left lateral recumbency (LLR), right lateral recumbency (RLR) and ventral recumbency. Acquisition was repeated every 30 minutes and the study was terminated at four hours.

Studies were not carried out in dorsal recumbency because pilot work had demonstrated considerable difficulty in restraining some dogs in this position. Also, the initial pixel counts were low in this view which indicated significant acquisition attenuation.

Decay-corrected emptying-time curves were established for each view and also for the geometric mean of the LLR and RLR restraint positions. Restraint in LLR resulted in scintigrams with the greatest initial pixel counts which suggested that this view resulted in the least attenuation acquisition. Although the ventral view produced the least deviation around the mean gastric emptying-time curves, restraint in this view was considerably more difficult than in either the LLR or the RLR views. Consequently, left lateral recumbency was considered to be the view of choice for scintigraphy in the dog and the emptying half-life and the 95% confidence interval for the linear slopes determined from the left lateral views were calculated.

Assessment

Compared with radiography studies, scintigraphy allows for quantitative assessment of intermediate rates of gastric emptying. The more points that can be established for the gastric emptying versus time curve, the more precisely gastric emptying can be defined. Therefore, parameters such as the shape and slope of the gastric emptying-time curve or the time taken to empty 50% or 95% of the ingesta can be determined.

Scintigraphy has the physiological advantage of being able to assess the gastric emptying of everyday foodstuffs because radiolabelled markers can be attached to food whereas radiography rely on the use of non-physiological markers to assess gastric emptying. The gastric emptying of solids and liquids can be assessed either separately or simultaneously by biphasic scintigraphy. Scintigraphy also results in less total body irradiation per procedure than radiography (Scarpignato, 1990).

The expense of the equipment required for scintigraphy, the problems associated with obtaining radioisotopes and the technical difficulties associated with manufacturing the radiolabelled markers, limits the availability of scintigraphy to referral institutions. Also, personnel with special technical training are required to perform the procedure.

In summary, scintigraphy, particularly with continuous acquisition, provides the clinician with a detailed, "physiological" assessment of gastric emptying in a patient. Consequently, scintigraphy is considered the "gold standard" technique for assessing gastric emptying in human and veterinary medicine. However, due to the disadvantages outlined in the preceding paragraph, scintigraphy will not replace radiography in general veterinary practice.

4.5 Real-time Ultrasound

Review

Ultrasonography can be used to determine gastric volume by imaging parallel, 1 cm cross-sectional slices of the stomach and adding the volumes (Bateman and Whittingham, 1982). Holt *et al* (1980) showed that there was good agreement between the change in gastric volume per unit time (as assessed by ultrasonography) and the rate of gastric emptying as assessed by scintigraphy. However, this approach was time consuming, required a computer for the calculation of volumes and was potentially inaccurate as gas accumulation in the fundus could artifactually increase gastric volumes. Furthermore, total gastric imaging could only be used to assess the emptying of liquid meals.

In an attempt to overcome these limitations, Bolondi *et al* (1985) measured changes in antral volume only to assess the rate of gastric emptying. The advantages of this technique were that the antral volume was unaffected by gas accumulation in the fundus and the gastric emptying of solids could also be assessed. The antral volume was calculated based on the assumption that every antral section was elliptical and that the change in cross-sectional area from the proximal to the distal antrum occurred in a linear fashion (Bolondi *et al*, 1985). Antral volume (expressed in millilitres) was calculated from the following formula

$$0.065h(2ab + 2ef + 4cd + cb + ad + ed + cf)$$

where h is the longitudinal length, in cm, of the antrum measured by an epigastric transverse scan; a , c and e are the craniocaudal diameters, in cm, measured in axial scans at the level of the angulus, at the mid-point of the antrum and at the prepyloric level respectively; b , d and f are the anteroposterior diameters, in cm, measured in axial scans at the level of the angulus, at the mid-point of the antrum and at the prepyloric level respectively.

All measurements were taken from the luminal side of the antral wall. From these measurements antral volume versus time curves were established.

However, the important issue of whether antral volume was proportional to the entire gastric volume remained unaddressed until 1993 when Ricci *et al* determined in ten healthy humans the antral volume by real-time ultrasound immediately after the oral ingestion of successive amounts of 100 ml 5% glucose solution up to a volume of 600 ml. These authors also determined antral volume after random, nasogastric administration of 100, 200, 300, 400 and 500 ml of 5% glucose solution. From these studies they showed that there was a linear trend between the amount of liquid administered and the antral volume. Moreover, they showed that ultrasound measurements were highly reproducible and that changes in posture did not affect the results.

Although calculating antral volume is less painstaking than calculating the entire gastric volume, it is still a laborious task. This prompted Marzio *et al* (1989) to evaluate the gastric emptying of liquids by measuring the anteroposterior and laterolateral measurements of a single section of the stomach at the zone of transition between the body and the antrum. This measurement correlated well with liquid phase scintigraphy (Marzio *et al*, 1989).

Currently, there are no reports of the use of real time ultrasound to assess gastric emptying in any animal species.

Assessment

When antral ultrasonography was compared to scintigraphy, there was a close correlation between the two techniques in the assessment of the gastric emptying of a solid meal (Corinaldesi *et al*, 1987) and a liquid meal (Bolondi *et al*, 1986). However, when biphasic scintigraphy was compared with antral ultrasonography, the ultrasound-obtained 50% emptying time is significantly different from the scintigraphically-obtained solid phase 50% gastric emptying time but it is similar to the liquid phase 50% emptying time (Dapoigny *et al*, 1988). This suggested that antral ultrasonography correlated to the liquid phase of emptying better than the solid phase.

The major advantage of real-time ultrasonography is that there is no exposure to radiation. Consequently, repeated ultrasound studies can be performed in the same patient. This is of particular benefit to patients who require serial gastric emptying studies to assess the effect that a drug or a procedure may have on gastric emptying.

Compared with the gamma camera which is required for scintigraphy, ultrasound equipment is found in most well-equipped human and veterinary hospitals throughout the world. Furthermore, the preparation of test meals for real-time ultrasound is simple compared with the procedures required to prepare the radiolabelled test meals required for scintigraphy.

A major disadvantage with real-time ultrasonography is that accuracy appears to be highly operator-dependent and exact measurements require a skilled ultrasonographer (Scarpignato, 1990).

In summary, the availability of ultrasound and its safety makes it an attractive alternative to scintigraphy in human medicine but it remains to be seen whether this technique can be modified for veterinary use. Although antral ultrasonography appears to measure the gastric emptying of liquids accurately, it is inferior to scintigraphy for the assessment of the gastric emptying of solids.

4.6 Applied Potential Tomography

Review

Applied potential tomography (APT) is a unique technique for assessing gastric emptying of liquid and semisolid meals in humans (Avill *et al*, 1987). In 1991, Mangnall *et al* also used APT to assess gastric emptying of a solid meal in humans. To date, the technique has not been used in veterinary medicine.

Applied potential tomography relies on the fact that different substances vary in their ability to conduct electricity. The ability of materials to conduct electricity can be compared by their *resistivity* constants. Copper wire, a good conductor, has a low resistivity constant, whereas wool, a good insulator, has a high resistivity constant. When a meal of low resistivity is fed to a patient, the resistivity measured in the gastric region falls but as the meal empties from the stomach the resistivity returns to fasting values.

The equipment^a for APT measurement is designed to detect changes in resistivity in the gastric region after a meal is ingested. It consists of a data-collection unit, a video display unit, a computer and a printer. Sixteen electrodes are placed on the skin surface in a circular array around the trunk at the level of the costal margin and leads are connected to the data collection unit. A current of 1 mA at 50 kHz is passed between two adjacent electrodes, known as the drive electrodes, and the potential difference is recorded at the remaining pairs of electrodes. Each pair of electrodes act as both drive and recording electrodes. From the data collected, a cross-sectional image of the abdomen in the plane of the electrodes, called a *tomographic image*, is created.

Applied potential tomography is conducted after an overnight fast. Four hundred milligrams of cimetidine, an H₂ receptor antagonist, is administered orally one hour prior to the study and 800 mg is administered orally at the commencement of the study. This step is taken to prevent changes in resistivity due to the secretion of gastric acid (Avill *et al*, 1987).

Applied potential tomography images are compiled over 30 seconds at two minute intervals. Imaging starts ten minutes before and finishes four hours after ingestion of the test meal. Subjects are seated during the study. The gastric region of interest is delineated by viewing an integrated image of the first ten APT images recorded after ingestion of the test meal. Once the region of interest has been determined, the resistivity within this region is calculated by the computer for each of the APT images. The percentage of the meal remaining in the stomach at any given time is calculated by expressing the resistivity at each time point as a percentage of the resistivity obtained when the meal was first ingested.

Assessment

Applied potential tomography has been validated by comparing gastric emptying times recorded by this technique and those measured by scintigraphy (Avill *et al*, 1987). However, APT was found to have a number of important shortcomings.

^aIBEES, Lodge Moor Hospital, Sheffield, England.

Firstly, there was only good correlation between APT and scintigraphy if gastric acid secretion had been inhibited. Although the inhibition of gastric acid secretion does not affect the gastric emptying of liquids and some solid meals (Houghton and Read, 1987), it did affect the gastric emptying of the beefburger meal used in the APT study of Mangnall *et al* (1991). It remains to be seen whether the inhibition of gastric acid secretion adversely affects the results obtained by APT when it is used in patients with diseases that alter gastric emptying.

Secondly, the curve obtained by APT consisted of oscillating peaks and troughs whereas the gastric emptying versus time curve obtained by scintigraphy was generally a smooth curve. To establish the slope of the APT graph and the 50% emptying time, a line of best fit must be made through the data points. Mangnall *et al* (1991) hypothesised that this variability was due to the movement of the test meal in and out of the plane of the electrodes.

Thirdly, there are some limitations as to the test meal which can be fed to a patient. The meal must be of sufficiently low resistivity so as to provide an adequate difference between pre- and postprandial conductance.

Despite the problems outlined above, APT has a number of advantages over other established techniques. It is simple to perform, non-invasive and does not involve exposure to radiation. The equipment is less expensive (Scarpignato, 1990) than the equipment required for scintigraphy. Applied potential tomography can be used to assess the gastric emptying of liquids, solids and semisolids, but it cannot be used to assess the gastric emptying of solids and liquids simultaneously.

Currently, this technique has not been used in veterinary medicine. Although this procedure is cheaper to perform than scintigraphy, it still utilises special equipment not normally found in veterinary practices. Therefore, even if the technique could be adapted to veterinary use, it would probably be restricted to universities and referral practices.

4.7 Biomagnetic Techniques

Review

In 1989, Ewe *et al* followed the transit of a 6 mm diameter metal sphere through the gastrointestinal tract of human subjects with a specially designed metal detector. The metal detector^b worked by emitting pulses of electromagnetic waves which caused eddy currents in the metal marker. The eddy currents in turn generated electromagnetic waves which influenced the primary electromagnetic field of the detector. This allowed the position of the metal particle to be determined.

In 1992, Basile *et al* developed a more sophisticated method of determining the location of a 2 mm diameter magnetic marker. In this technique, the three-dimensional location of the magnetised marker was determined by analysing isofield maps which were detected by a biomagnetic superconducting instrument. Although these workers determined only oroanal, small bowel and colonic total and segmental transit times, the technology could also be applied to determine gastric emptying times.

Currently, there are no publications on the assessment of gastrointestinal transit using biomagnetic techniques in animals.

Assessment

The biomagnetic techniques outlined above are only suitable for assessing the gastric emptying of large, indigestible, "non-physiological" objects. As such, these techniques assess the onset of the migrating motility complex and do not assess gastric emptying associated with the fed motility pattern. Moreover, the technique can provide information on the gastric emptying of only one object and therefore provides qualitative data only.

^bAS Metal Detector System Version EAS II, Kühlwetterstr. 28, Düsseldorf, Germany.

Determining the location of the marker in biomagnetic techniques relies on comparing the position of the marker with fixed anatomical structures. However, there is considerable person to person variation in the position of intra-abdominal organs which could make exact localisation of the marker difficult (Ewe *et al*, 1989). The technique outlined by Ewe *et al* (1989) has to be modified when obese people are being studied because the 6 mm diameter marker can only be detected at a distance of less than 12 cm away from the abdominal surface. Obesity did not pose a problem when the more sophisticated recording equipment used by Basile *et al* (1992) was employed.

In summary, biomagnetic techniques are better suited for determining intestinal transit times rather than gastric emptying times. The currently accepted technique for determining intestinal transit employs the use of radiopaque markers. Although both the radiopaque marker and biomagnetic techniques are non-invasive, the biomagnetic technique offers the advantage of not exposing the patient to radiation. Conversely, radiography with radiopaque markers provides more quantitative information since the passage of more than one markers can be traced. Furthermore, biomagnetic techniques require the use of specialised equipment which is not standard in most hospitals.

4.8 Magnetic Resonance Imaging

Review

Very recently, Schwizer *et al* (1992) have published a technique for assessing the gastric emptying rate of liquids in humans by magnetic resonance imaging (MRI). Like gastric ultrasound and applied potential tomography, MRI is a tomographic technique.

Twenty, consecutive, 10 mm thick transaxial slices covering the whole upper abdomen, including the lower chest, are recorded before and immediately after the administration of a liquid test meal. The liquid test meal contains 500 ml of a 10% glucose solution which has been mixed with an MRI marker, 400 $\mu\text{mol/l}$ gadolinium tetrazacyclododecane tetraacetic acid (Gd-DOTA). The 20 transaxial slices are then combined and displayed as a three-dimensional image with the aid of a computer. The pixel count can then be determined within the gastric region of interest for each sequence. The acquisition time for each sequence of 20 slices was 6-9 minutes. Sequences were taken every 15 minutes for 120 minutes. Gastric emptying, as assessed by MRI, correlated very well with scintigraphy and a dye dilution technique (Schwizer *et al*, 1992).

There have been no published reports of the use of MRI in assessing gastric emptying in animals.

Assessment

Unlike scintigraphy, MRI is a radiation-free technique. However, MRI is not without potential hazard because free gadolinium is toxic (Haley, 1965). When gadolinium is bound to DOTA however, there is very little gastrointestinal absorption. *In vitro* studies have shown that Gd-DOTA is a stable compound when incubated with human gastric juice at 37°C (Schwizer *et al*, 1992).

Anatomical structures surrounding the stomach can be clearly identified by MRI which helps to identify the gastric region of interest. Consequently, Gd-DOTA, which may be present in loops of bowel overlying the stomach, can be easily detected by MRI and can be excluded from gastric emptying calculations. Scintigraphy does not offer the same degree of resolution as MRI and radiolabelled markers which are present in overlying loops of bowel may be erroneously considered to be in the stomach.

Magnetic resonance imaging can also provide data on gastric secretion and motility concurrently with the determination of the rate of gastric emptying. Gastric secretion can be calculated because it is possible to measure gastric emptying and gastric volume simultaneously. Gastric wall movements can be assessed using the new generation gradient-echo MR imagers because they have fast acquisition times. The additional information provided by these determinations may allow clinicians to formulate better treatment regimens for their patients.

Magnetic resonance imaging has two major limitations. Firstly, the gastric emptying of solids cannot be determined by MRI because there is no solid phase MRI marker. Magnetite (Fe_3O_4) has recently been proposed as a possible solid phase marker but there have been no publications on the use of this marker for MRI gastric emptying studies. Secondly, only very well equipped human and veterinary hospitals will have MRI facilities because of the expense of the equipment. It is reasonable to assume that there is a high demand for MR imaging due to the limited number of MRI facilities. Combining this with the fact that two hours is required for an MRI gastric emptying study, it is unlikely that hospitals would favour the routine use of this technique.

4.9 Radiotelemetry

Review

A radiotelemeter is a device which measures a distant event and transmits the data via radio waves to a recording device. The Heidelberg capsule[°], a 7 mm x 20 mm radiotelemetry device, detects changes in gastrointestinal pH and transmits the data by radio waves to a receiver. The Heidelberg capsule was developed because it is less invasive than intubation techniques for determining gastrointestinal pH (Andres and Bingham, 1970; Connell and Waters, 1964).

[°]Telefunken Instrumentation, Heidelberg International Division, Norcross, Ga. USA.

Rather than use the Heidelberg capsule for determining changes in pH, Mojaverain *et al* (1985) used it to mark the onset of the MMC in humans. Passage of the capsule into the duodenum is characterised by a sudden increase in the pH detected by the Heidelberg capsule. Youngberg *et al* (1985) have assessed gastric pH changes and the gastric residence time of the Heidelberg capsule in the fasting and fed state in four healthy Beagles. Radiotelemetry has also been used to provide information on the gastric residence time of pharmacological products (Prescott, 1974; Levine 1970).

Assessment

The advantages of radiotelemetry are that it is non-invasive, it utilises inexpensive equipment and it is simple to perform. However, because of the large size of the Heidelberg capsule, radiotelemetry can only assess the onset of the MMC and it does not provide clinical information on the gastric emptying of food or liquids (Mojaverain *et al*, 1985). Moreover, this technique, provides information on the gastric emptying of only one object and therefore yields only qualitative data.

4.10 Indirect Methods

Review

In man and animals, some drugs are absorbed through the small intestine with negligible amounts being absorbed through the stomach. Therefore, the quantity of a drug (or its by-product) in the circulatory system is proportional to the rate of gastric emptying and if the drug can be measured from blood samples, then drug concentrations may serve as an indirect measurement of gastric emptying.

By using paracetamol solution as a marker, Heading *et al* (1973) used this principle to assess the gastric emptying of liquids. Similarly, paracetamol tablets have been used as a marker to assess the gastric emptying of solids (Sheehan *et al*, 1990). Recently, Asada *et al* (1990) have also used sulfamethizole as a marker for the gastric emptying of solids. These methods have been validated either by assessing the correlation between plasma drug concentrations and the quantity of drug emptied from the stomach or by comparison with scintigraphy.

Gastric emptying is not assessed directly by these methods but parameters such as the peak plasma concentration, the time taken to reach peak plasma concentration and the area under the plasma concentration versus time curve are used as indices of the emptying rate (Scarpignato, 1990).

Assessment

The indirect methods outlined above are simple and cheap to perform but they do require blood samples to be taken at intervals and are therefore more invasive than some of the other techniques discussed.

Indirect methods also assume that the rate of absorption, metabolism and excretion and the volume of distribution of the drug markers is the same in all subjects. Clearly, certain disease states may alter the pharmacokinetics of the drug marker which may influence the parameters measured above.

In summary, indirect methods do not provide the clinician with a direct measure of the gastric emptying rate. Due to the differences in pharmacokinetics between individuals with different disease states, intersubject comparisons may be inaccurate. However, the simplicity of the procedure makes it an attractive means of making intrasubject comparisons, especially when high precision is not required (Scarpignato, 1990).

4.11 Carbon-labelled Octanoic Acid Breath Test

Review

In 1993, Ghooos *et al* described an indirect test for assessing the gastric emptying of solids by a breath test. When ^{13}C or ^{14}C -labelled octanoic acid, which has been incorporated into a test meal, enters the duodenum it is rapidly absorbed across the gut mucosa, transported to the liver and preferentially oxidised to $^*\text{CO}_2$. The $^*\text{CO}_2$ is then excreted in the breath. The main parameter determining $^*\text{CO}_2$ appearance in breath is the rate of delivery of food from the stomach to the intestine.

The test meal used by Ghooos *et al* (1993) comprised of scrambled eggs labelled with ^{13}C or ^{14}C -octanoic acid, two slices of white bread, 5 g of margarine followed immediately by 150 ml of water. *In vitro* validation studies showed that there was excellent retention of the octanoic acid marker in the egg when it was incubated with gastric juice at 37°C . After ingestion of the test meal, breath samples were taken every 10 minutes for the first hour and then every 15 minutes for a further 3 hours. When ^{14}C -octanoic acid was used as the label, $^{14}\text{CO}_2$ was collected into 2 mmol/l hyamine hydroxide and measured by beta scintillation counting. When ^{13}C -octanoic acid was used as the label, $^{13}\text{CO}_2$ was measured by either an isotope ratio mass spectrophotometer^d or by an on-line gas chromatographic purification-IRMS^e.

^dmodel 250, Finnigan MAT, Bremen, Germany.

^eABCA, Europa Scientific, Crewe, England.

Ghoos *et al* (1993) validated the carbon-labelled octanoic acid breath test by labelling the scrambled egg with a second marker, ^{99m}Tc -albumin colloid, and simultaneously performing breath test measurements and scintigraphy in normal subjects and dyspeptic patients. In both groups studied, there was an excellent correlation between the breath test and scintigraphy over all parameters measured from the gastric emptying curves. Preliminary studies have also indicated that the carbon-labelled octanoic acid breath test is sensitive enough to detect pharmacological influences on gastric motor activity (Hiele *et al*, 1991).

Assessment

The carbon-labelled octanoic acid breath test has a number of major advantages compared with scintigraphy. Since the carbon-labelled octanoic acid breath test does not require the use of a gamma camera, it is significantly cheaper than scintigraphy. Breath samples can be collected away from the analytical unit and consequently, the carbon-labelled octanoic acid breath test can be conducted away from the hospital if necessary. When ^{14}C -octanoic acid is used, it is estimated that radiation exposure is 50 to 100 times less than that of scintigraphy. Radiation is totally avoided when ^{13}C -octanoic acid is used. Therefore, this technique provides an attractive alternative to scintigraphy in children and fertile women and when multiple studies are required.

However, at this stage, liquid phase and biphasic gastric emptying studies cannot be performed using breath test methodology. Also, since the carbon-labelled octanoic acid breathe test is an indirect method of assessing gastric emptying it has the same potential flaws as the other indirect methods covered in the preceding section. Further work also needs to be done to assess the effect different diseases have on the pharmacokinetics of carbon-labelled octanoic acid as these may potentially alter the accuracy of the technique.

In summary, this technique probably represents the best alternative to scintigraphy considered to date. It is simpler, cheaper and safer to perform than scintigraphy. However, the carbon-labelled octanoic acid breath test still needs to be evaluated over a wide range of disease states before its use will become widely accepted.

4.12 Intubation Methods

Review

Intubation methods involve the periodic sampling of ingesta from the stomach or duodenum through a gastrointestinal catheter. Gastric emptying studies using intubation methods were described more than 100 years ago by Ewald and Boas (1885). These workers administered a mixed solid-liquid test meal and completely aspirated the stomach contents 30 or 60 minutes later. Since this time, intubation methods have provided much of the current knowledge on gastric emptying in man and animals. Today, the gastric dye dilution technique is the most commonly performed intubation technique (Scarpignato, 1990).

This technique involves aspirating a sample of gastric content via a nasogastric tube and then adding to the gastric content an inert, non-absorbable marker of known concentration and volume. Suitable markers include phenol red or polyethylene glycol (PEG). The marker is then thoroughly mixed with the gastric content by repeatedly aspirating the chyme into a syringe and then returning it to the stomach. After thorough mixing has been achieved, a second gastric sample is taken. The time interval between taking the two samples should be less than one minute. The volume of gastric content can then be calculated by the following formula

$$[\alpha/(b-c)] - \delta$$

where α is the mmol of marker added to the stomach; b is the concentration of marker in sample two; c is the concentration of marker in sample one; δ is the volume of the marker added.

Marker concentration in the samples is determined by spectrophotometry. Increasing the concentration of marker substance added to each successive volume determination has been shown to improve the accuracy of gastric volume measurement (Hurwitz, 1981). It should be noted however that this method does not take into account ongoing gastric secretions which contribute to the gastric volume. However, corrections for gastric secretion can be made by determining the hydrogen ion concentration in both samples (Hunt, 1974).

Assessment

The gastric dye dilution technique is easy to perform, does not require the use of specialised equipment and does not expose the patient to radiation. However, due to the invasiveness of this procedure, the technique is of limited clinical use. Moreover, gastrointestinal catheterisation may influence gastric motility directly by its physical presence or indirectly by causing distress to the subject.

Although this technique accurately assesses the gastric emptying of liquid meals it cannot be used to assess gastric emptying of solid meals (Scarpignato, 1990). Another intubation method, the intraduodenal dye dilution technique (Go *et al*, 1970), can assess the gastric emptying of both liquids and solids but it is cumbersome to perform and it is not widely used in clinical investigations (Scarpignato, 1990).

Table 4.2. Summary of the advantages and disadvantages of the techniques used to determine the rate of gastric emptying.

Technique	Hazards	Expense ^a	Expertise ^a	Merit ^b	Invasive?	Quantitative?	Solid ^c	Liquid ^c
Radiography								
- Positive Contrast	Radiation	2	2	Poor	No	No	x	✓
- Plain	Radiation	1	1	Poor	No	No	✓	x
Scintigraphy^d	Radiation	8	8	Excellent	No	Yes	✓	✓
Real-time Ultrasound	None	6	10	Very good	No	Yes	?	✓
Applied Potential Tomography	None	7	7	Very good	No	Yes	✓	✓
Biomagnetic Methods								
- General	None	7	7	Poor	No	No	✓	x
- MRI	Toxin	10	8	Excellent	No	Yes	x	✓
Radiotelemetry	None	5	2	Poor	No	No	✓	x
Indirect Methods								
- General	None	4	3	Poor	Yes	No	✓	✓
- Octanoic Acid Breath Test	None	4	3	Very good	No	No	✓	x
Intubation Methods	None	3	5	Excellent	Yes	Yes	x	✓

^aNumbers represent a scale from 1 to 10 where 10 is the most expensive technique, or the technique requiring the most technical expertise, and 1 is the least expensive technique, or the technique requiring the least technical expertise.

^bRefers to the ability of a technique to truly assess the gastric emptying of liquids or digestible solids.

^cTicks imply that the technique assesses solid phase or liquid phase gastric emptying.

^dScintigraphy is the only technique which can assess gastric emptying of solids and liquids simultaneously (biphasic scintigraphy).

Chapter Five

Materials and Methods

Phase One- Radiopaque Marker Development and Selection of the Test Meal

5.1 Objectives

The first objective was to develop two types of radiopaque marker. The first marker type was designed to pass into the duodenum at the same rate as food and the second marker type was designed to remain in the stomach until all food had entered the duodenum and then leave at the onset of the migrating motility complex (MMC). The markers had to be made from an inert, non-toxic material which was sufficiently radiopaque to be visible on plain radiographs. The markers also had to be affordable for veterinarians in private practice.

The second objective was to select an appropriate diet which could be fed with the radiopaque markers. The diet needed to contain very little radiopaque material so as not to obscure the view of the markers in radiographs; be available in many countries in order for the technique to be applicable worldwide; and be made to stringent specifications with minimal batch to batch variation in its nutritional composition. Ideally, the diet should be moist or semi-moist to facilitate the mixing of the markers and it also should be low in fat, bland and highly palatable if it is to be suitable for feeding to dogs with gastrointestinal disease.

5.2 Background

The rate of gastric emptying of non-digestible solids in dogs is greatly affected by their size and density, whereas the shape and surface energy of the solid has a minimal effect (Meyer *et al*, 1985) . The rate of gastric emptying of non-digestible plastic spheres becomes progressively slower as particle diameter increases from 0.015 mm to 5.0 mm. There is no significant difference between the emptying rates of radiolabelled liver and spheres with a diameter of either 1.6 mm or 2.4 mm and a density of 1 g/ml. Spheres less than 1.6 mm in diameter and a density of 1 g/ml empty more rapidly than the liver. Spheres with a diameter of either 3.2 mm or 5.0 mm and a density of 1 g/ml empty more slowly than liver. Density plays a major role in determining the gastric emptying rate of spheres. At a density of 2 g/ml, spheres empty more slowly than radiolabelled liver. Given the results of this study, we considered that a radiopaque marker of approximately 1.6 to 2.4 mm in diameter with a density of approximately 1 g/ml should empty from the stomach at a rate similar to that of digestible solids.

The work of Russel and Bass (1985b) gave further support for this hypothesis. They showed that polycarbophil, a synthetic, hydrophilic, acrylate polymer, stimulated fed state motility patterns and that it empties from the canine stomach independently of the MMC. The hydrated polycarbophil particle had a diameter of 1-3 mm. Although the density of the hydrated polycarbophil was not mentioned in the study, it would be reasonable to assume that it is approximately 1 gm/ml given its high water content.

In contrast to the small indigestible solids used in the studies described above, larger, 7 mm diameter spheres, with a density of approximately 1 g/ml, pass into the duodenum late in phase II and early in phase III of the MMC (Mroz and Kelly, 1977).

Indirect evidence to corroborate Mroz and Kelly's findings can be obtained by determining if feeding has an effect on the gastric emptying rate of indigestible solids. The gastric emptying rate of indigestible solids which empty during the onset of the MMC will be delayed until the stomach is empty. Youngberg *et al* (1985) showed that feeding dogs delayed the emptying of a capsule which was 7 mm in diameter and 20 mm long. Smith and Feldman (1986) showed in humans, that ingestion of meals of increasing size progressively delayed the emptying of 2 and 10 mm lengths of polyvinyl tubing which had been filled with barium sulphate. This finding indicated that not only the 10 mm tubing but also the 2 mm tubing emptied with the onset of the MMC which conflicted with the findings in the study of Meyer *et al* (1985). However, Smith and Feldman (1986) did not specify the density of their markers and therefore it is possible that the density of the 2 mm markers had a major influence on the emptying rate in their experiment. Moreover, a species difference could explain the conflicting results; Meyer *et al* (1985) used dogs as their subjects compared with humans in the study of Smith and Feldman (1986).

Soft particles empty significantly more quickly than hard particles of the same size and shape (Meyer *et al*, 1989b). The soft particles used in this study had a rubbery consistency but they could not be compressed by digital pressure. This meant that a difference in particle size was not the explanation for the difference in emptying rate. However, there were two variables which were not controlled in this study. The density of the hard particles was 1.7 g/ml whereas the density of the soft particles was 1.4 g/ml and the surface textures of the two particles were different. The soft particles had a rubbery texture whereas the hard particles were completely smooth. Therefore, it was hard to reach a definite conclusion about the importance of particle consistency to gastric emptying. To the author's knowledge there are no other studies which address the issue of particle consistency on gastric emptying.

Table 5.1. Summary of the physical properties of an indigestible solid and their relative importance to the rate of gastric emptying of indigestible solids.

Property	Relevance to gastric emptying
Size	Important
Shape	Not important
Density	Important
Surface Energy	Not important
Consistency	Questionable importance
Surface Texture	Unknown importance

From the studies outlined above, it was determined that a radiopaque solid of approximately 1.5 to 2.5 mm in diameter and a density of approximately 1 g/ml could be a suitable marker for the evaluation of the gastric emptying rate of food whereas a radiopaque object with a density ≥ 2 g/ml and/or a diameter of approximately 7 mm could be suitable for the assessment of the MMC. Although it is technically easy to design a marker to fulfill the latter criteria it is considerably more difficult to design a small marker of suitable density to leave proportionally with food but still be visible on radiographs. The first part of our study focused on addressing this problem.

5.3 Selection of the Material for the Manufacture of Markers

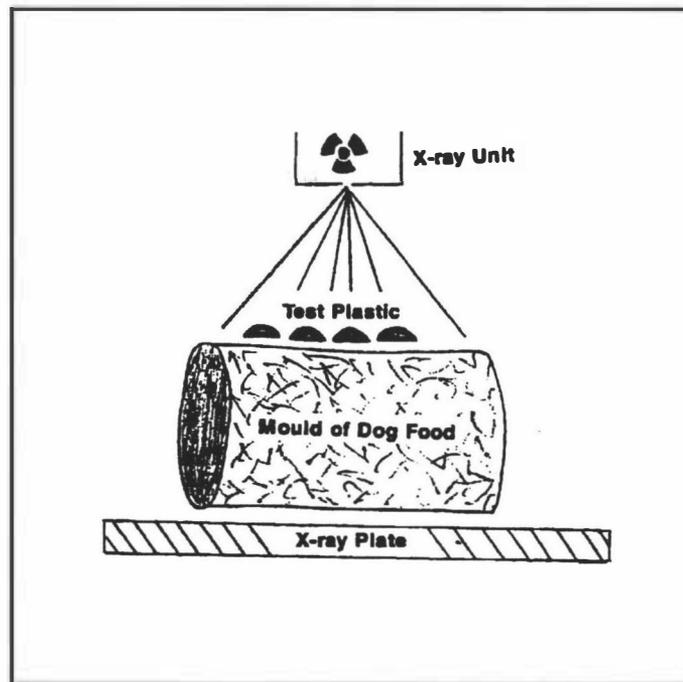
Initially, the gastric emptying of chromium coated 1 mm diameter steel bearing balls of approximately 6 g/ml was assessed. These balls were chosen because they were easily available, inexpensive, manufactured to stringent specifications and were not toxic. They were also visible on radiographs. A three year old, entire female, mixed breed dog was fed approximately 200 grams of canned pet food^a which had been thoroughly mixed with 10 balls. Radiographs were taken hourly after feeding. The balls separated from the food within the first hour and were visible in the ventral corpus. Emptying of the balls from the ventral corpus occurred when the stomach was free of food. Nine out of ten balls then emptied within an hour. These findings suggested that the high density of the balls resulted in their separation from the food and their expulsion from the stomach with the onset of the MMC. This study indicated that the marker had to be manufactured from a less dense radiopaque material.

Radiodensity of prospective materials was believed to be the most significant factor limiting their usefulness. Therefore, materials were assessed *in vitro* prior to their selection for use in this study. This was achieved by radiographing materials on a background of dog food^b. The dog food was removed from the can in an intact mould in order to keep its depth and density constant. The dog food mould was placed with its long axis on the radiographic plate and the sample of the material in question was then placed on top of the food (Figure 5.1). Radiographs were always exposed at 50 kilovolts and 8 milliamperere seconds using the x-ray equipment and film outlined in section 6.5. A direct comparison of the radiodensity of prospective materials could be made using this technique because the method was the same every time. It was assumed that if the test material was not sufficiently radiopaque to be seen by this method, then it was unlikely to be to be visible when mixed with food within the gastrointestinal tract of a dog.

^aChef, Best Friend Petfoods, Penrose, Auckland

^bWaltham's Canine Low Protein diet

Figure 5.1. Diagram representing how prospective marker material was assessed for radiopacity.



This method minimised the exposure of personnel and dogs to x-rays and it was easier than radiographing dogs. This technique was also used to assess the radiodensity of different food types and was employed to select the most appropriate test meal to be used in this study.

Initially, 2 mm x 2 mm squares of radiopaque plastic from radiopaque gastrointestinal feeding tubes was assessed for radiopacity using the method described above. However, this material was rejected as it was insufficiently radiodense.

A plastics manufacturer^c was then asked to supply a non-toxic, inert plastic which could be combined with radiodense compounds such as barium sulphate or ferric oxide. This step was taken because a material fitting the exact specifications required for the study could be obtained. The plastics manufacturer supplied high density polyethylene combined with either ferric oxide or barium sulphate powder, which had been extruded, pelletised and then moulded in small sheets 2 mm and 7 mm thick. A drill with a punch attachment was used to stamp out cylinders of plastic approximately 2 mm diameter x 2 mm deep and 7 mm diameter x 7 mm deep. Radiodensity of the plastic cylinders was then assessed using the *in vitro* method described above. It became apparent that the plastic cylinders containing ferric oxide were considerably less radiopaque than the plastic cylinders containing barium sulphate although the concentration of both substances was the same on a volume/volume basis. Further investigation of the ferric oxide-containing plastic was abandoned.

The concentration of barium sulphate in the plastic was increased until it was determined that the radiodensity of the plastic was sufficient to be clearly visible in the gastrointestinal tract of dogs. The radiopacity of the final plastic sample was assessed for suitability by combining both cylinder sizes in dog food and feeding it to a dog. The cylinders were clearly visible in the gastrointestinal tract of the dog. The density of the plastic was 1.37 g/ml. Now that an appropriate material had been selected, the next objective was to define the ideal shape and size of markers.

^cStallion Plastics Ltd, 73 Railway Rd, Palmerston North

5.4 Selection of the Shape of the Markers

Meyer *et al* (1985) had previously determined that non-digestible solids of similar dimensions, but of differing shapes, had comparable gastric emptying rates. However, we considered that marker shape was potentially important. Since the radiodensity of an object is proportional to its volume, if the volume of the markers could be maximised then they would be easier to see radiographically. Consequently, a spherical marker was selected because it has the greatest volume per surface area. Also, since spherical markers do not have any sharp edges we considered that they would be less likely to be retained in the gastric mucosa or rugal folds.

5.5 Selection of the Size of the Markers

As discussed above, the gastric emptying rate of indigestible solids is significantly influenced by their size and density. The concentration of barium sulphate required to make the plastic radiopaque predetermined the density of the markers used in this study. Therefore, the only remaining critical factor which could be altered was the size of the marker.

Based on the study of Meyer *et al* (1985), it was assumed that the gastric emptying of a marker between 1.6 and 2.4 mm in diameter with an approximate density of 1 g/ml would parallel the gastric emptying of food. However, we chose a slightly smaller, 1.5 mm diameter marker to compensate for the greater density of the markers used in our study.

As discussed above, Mroz and Kelly (1977) showed that 7.0 mm diameter spheres with a density of 1 g/ml left the stomach only during the MMC. However, 5.0 mm diameter markers were chosen for this study because of concerns that gastrointestinal obstructions could result if 7.0 mm diameter markers were used to assess gastric emptying in very small dogs. Financial constraints did not allow the manufacture of both 5.0 mm diameter and 7.0 mm diameter markers. Nevertheless, there is indirect evidence to suggest that the smaller, 5.0 mm markers leave at the onset of the MMC. Meyer *et al* (1985) showed that the gastric emptying of 5.0 mm markers, with a density of 1 gm/ml, began only after 60% of a meal had left the stomach and only 20% of the 5.0 mm markers had left after 90% of a meal had left the stomach.

5.6 Selection of the Test Diet

A number of important factors were considered when choosing the most appropriate food for the gastric emptying studies. The food needed to be available in many countries in order for the technique to be applicable worldwide. The food needed to be made to stringent specifications with minimal batch to batch variation in its nutritional composition. Batch to batch variation in nutritional composition could evoke different responses from the intestinal brake thereby altering the rate of gastric emptying of the markers. Ideally, the diet should be moist or semi-moist so the markers could be mixed with the food. Otherwise dry food would have to be moistened to facilitate the mixing of markers. Since this technique would normally be used in dogs which are vomiting, have diarrhoea or are anorectic, consideration also needed to be given to their dietary requirements. The most suitable diet is one that is bland, palatable and is based on a protein source that the dog is fed infrequently.

Canned Canine Select Protein^{®d} and Canine Low Calorie^{®d} and Prescription Diet^{®e} canned canine/feline d/d and canine i/d fulfilled the criteria outlined above and were considered good candidates for further assessment.

In order to make interpretation of radiographs easier, it was decided to choose the diet with the least amount of radiodense material. The radiodensity of each diet was compared by the *in vitro* technique described in section 5.3. Prescription Diet[®] canine/feline d/d was virtually free of radiopaque particulate matter and was subsequently chosen for use in this study. The other diets contained significant quantities of radiodense material.

5.7 Quantity of the Test Diet

The next consideration was to determine the quantity of test meal which was to be fed to each dog. De Wever *et al* (1978) demonstrated in dogs, that for a given type of food, the time it takes to complete gastric emptying is directly proportional to the quantity of food fed (expressed as kcal/kg bodyweight). Therefore, in order for this study to be of practical clinical value, it was determined that a small test meal would reduce the time taken to complete the study. However, when dogs are fed canned food, the meal size is important in changing the fasting motility pattern to a fed motility pattern (Weisbrodt *et al*, 1976). MMCs were not disrupted by feeding canned dog food at a rate of 6 g/kg; two out of three dogs converted to a fed pattern when 12 g/kg was fed; and all dogs converted to a fed pattern when 24 g/kg was fed.

^dWaltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire, England.

^eHill's Division of Colgate-Palmolive Company, Topeka, Kansas, USA.

Considering the studies outlined above, one quarter of the calculated daily maintenance calorie requirement of canned Prescription Diet® canned canine/feline d/d was chosen. The formula $132x \text{ kg}^{0.75}$ was chosen to represent maintenance daily calorie requirements in healthy adult dogs (Lewis *et al*, 1987). This meant that the lightest dog in this study was fed 12.2 grams of dog food per kilogram bodyweight and the heaviest dog was fed 9.5 grams of dog food per kilogram bodyweight. Although the volume of food used in our study was less than the volume required to convert all dogs to a fed motility pattern in the study of Weisbrodt *et al* (1976), we considered it sufficient because canned Prescription Diet® canine/feline d/d is energy dense compared to standard canned foods. The ability of a food to convert gastrointestinal motility from a fasting to a fed pattern is also proportional to its energy density (De Wever *et al*, 1978).

5.8 Number of Markers Per Test Meal

Thirty 1.5 mm and ten 5.0 mm markers were mixed with each test meal. This was the maximum number of markers that could be given without the markers overlapping which could make counting difficult. If fewer markers were given, then precision in terms of calculating the rate of gastric emptying was lost.

Chapter Six

Materials and Methods

Phase Two- Gastric Emptying Studies in Healthy Dogs

6.1 Radiopaque markers

Thirty 1.5 mm (small) and ten 5.0 mm (large) diameter markers were used in this study. The physical properties of these markers are summarised in Table 6.1 and the rationale for selecting markers with these properties is discussed in Chapter Five.

Figure 6.1. Photograph showing the two sizes of radiopaque marker used in the study.

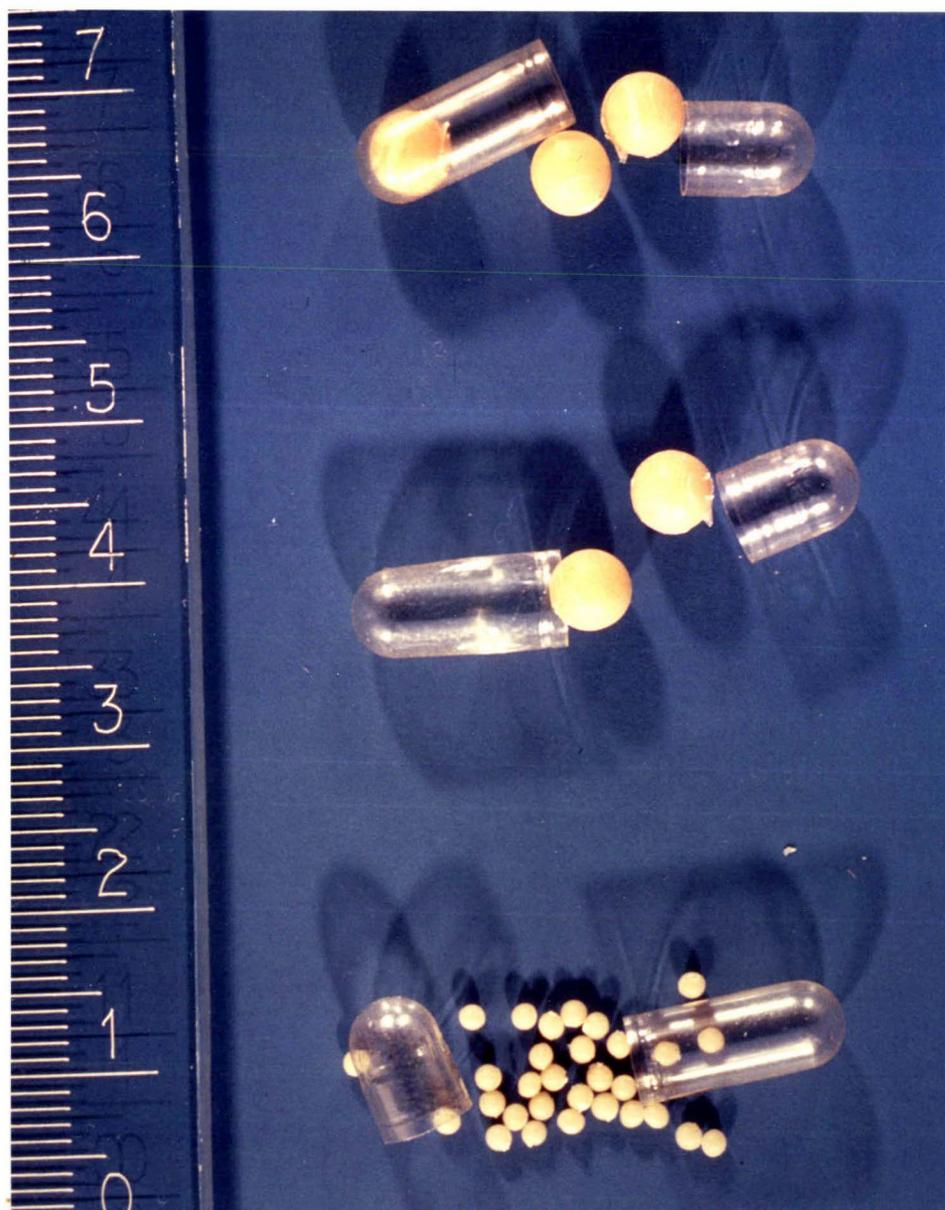


Table 6.1. Summary of the physical properties of the radiopaque markers used in this study.

Property	Description
Material	High density polyethylene mixed with barium sulphate
Size	1.5 and 5.0 mm diameter
Shape	Spheres
Density	1.37 g/ml
Surface energy	Not determined
Consistency	Hard
Surface texture	Smooth

6.2 The Test Meal

The test meal was canned Prescription Diet® d/d. The rationale for selecting this diet is discussed in the Chapter Five.

The formula, $33 \times \text{kg}^{0.75} / 1.402^a$, was used to calculate the weight, in grams, of canned d/d fed to each dog. This amount of food satisfies one-quarter of the daily maintenance energy requirements for an adult dog. The calculation was based on the bodyweight of the dogs on day one of the study.

^aThis formula is based on feeding the old Hill's canned Prescription Diet® canine/feline d/d. The denominator, which is the energy density of the food in kcal/g, became 1.372 when the new Hill's canned Prescription Diet® canine d/d was used.

The canned d/d was removed from the tin in an intact mould and the quantity required was weighed on a set of kitchen scales. The correct weight of moulded food was then halved, four holes were made in each piece and approximately equal numbers of markers were placed into each hole. The holes were then sealed with small wedges of food. This was performed to ensure an even distribution of markers throughout the food. If the markers were administered separately from the food, or with the food but in a bunch, there was the possibility that they may not mix thoroughly with the food once they were in the stomach. This could result in the markers leaving disproportionately from the food.

After each dog had eaten its meal, the cage floors were swept clean of any remaining crumbs of food or radiopaque markers which were dropped while eating. The left over food crumbs and markers were then fed to the dogs.

6.3 Prescription Diet® canine/feline d/d Formulation Change

Part way through the study, Hill's® Pet Products changed the formulation of the canned canine/feline d/d diet to a separate canned canine d/d diet and a canned feline d/d diet. Rather than use an obsolete dietary formulation for the entire study, 10 dogs were fed the old formulation and 10 dogs were fed the new canned canine d/d formulation. Since the study was already underway when the change in diet formulation was made, no attempt was made to randomly assign the two different formulae to the dogs. A summary of the differences in nutritional analyses between the old and new formulae is given in Table 6.2 below.

The most significant change was to the protein content of the diet. The canine d/d contained 38% less protein than the older canine/feline d/d. The reduction in the quantity of protein was offset primarily by an increase in the carbohydrate and fibre content of the diet. The diets were of very similar energy densities. The sources of protein, fibre and carbohydrate were identical in both diets.

Since the rate of gastric emptying is partly regulated by the energy density of food through the intestinal brake mechanism (McHugh and Moran, 1979) it was predicted that the change in diet would have little, if no, effect on the rate of gastric emptying of the markers.

Table 6.2. A comparison of the nutritional analyses of the old canned Prescription Diet® canine/feline d/d and the new canned Prescription Diet® canine d/d diets.

Analyte	Old		New		% Change ^b	
	Wet % ^a	DM % ^a	Wet %	DM %	Wet %	DM %
Protein	7.9	26.7	4.9	17.3	-38.0	-35.2
Fat	6.4	21.6	6.7	23.6	+4.7	+7.4
Carbohydrate	12.9	43.6	14.4	50.7	+11.6	+16.3
Fibre	1.0	3.4	1.2	4.2	+20.0	+23.5
Energy^c	1,402	-	1,372	-	-2.1	-

^aWet % and DM % refer to the percent weight of the analyte on a wet weight and dry matter weight basis respectively.

^b% change, is calculated according to the following formula, $[\text{new-old}]/\text{old} \times 100$.

^cenergy is expressed in kilocalories per kilogram.

6.4 Study Animals

Twenty crossbred dogs (7 entire males, 8 entire females and 5 speyed females) aged between 11 and 42 months were used in this study. The dogs were acquired from the Animal Health Service Centre, Massey University. The dogs weighed between 13.5 kg and 37.0 kg (20.6 ± 6.5 kg, mean \pm standard deviation). Each dog had no prior history of gastrointestinal disease and was healthy on physical examination. All the dogs had been treated for internal parasites with praziquantel^b and a pyrantel pamoate/oxantel pamoate combination^c within three months of the commencement of the study. The dogs had been inoculated with a combined canine parvovirus, canine adenovirus type II and canine distemper virus modified live vaccine^d within one year of the initiation of the study. A summary of the ages, sexes and weights of the 20 dogs used in this study are shown in Table 6.3. All the procedures performed on the dogs had been approved by the Massey University Animal Ethics Committee. The dogs were housed at the Massey University Companion Animal Hospital (MUCAH) for the 10 day duration of the study. Kennelling facilities included cages with a floor area of either 84 cm x 93 cm or runs with a floor area of 295 cm x 111 cm. During the study, all dogs were exercised in an outside courtyard for at least 10 minutes twice daily. The dogs were fed a maintenance ration of Friskies^e Go Dog[®], a kibbled dog food which has an energy content of 3,700 kcal/kg. Go Dog[®] meets the criteria set by the National Research Council of the United States of America for a nutritionally balanced and complete food for dogs. The proximate analysis of Go Dog[®] is shown in Table 6.4. Dogs were fed according to their maintenance metabolisable energy requirements which was calculated using the formula $132x \text{ kg}^{0.75}$ (Lewis *et al*, 1987). Dogs were given free access to water throughout the study.

^bDroncit[®], Bayer New Zealand Ltd.

^cCancare Plus[®], Ancare Distributors Ltd.

^dQuantum Canine 3 (DA₂ PARVO), Pitman-Moore NZ Ltd.

^eFriskies Pet Care, 1 Broadway, Newmarket, Auckland

Table 6.3. Summary of the sexes, ages and weights of the dogs used in the study and the type of test meal fed.

Dog	Sex ^b	Age ^c	Weights ^a			Diff ^d	Diet ^e
			Day 1	Day 6	Day 9		
A	M	11	18.0	18.5	18.0	0.0	Old
B	M	11	19.0	18.5	18.5	-0.5	Old
C	F	36	23.0	22.0	22.0	-1.0	Old
D	F	12	17.0	15.5	15.5	-1.5	Old
E	M	16	18.0	17.0	16.5	-1.5	Old
F	F	12	13.5	13.5	13.5	0.0	Old
G	F	12	13.5	14.0	13.5	0.0	Old
H	F	12	14.0	13.0	14.5	+0.5	Old
I	F	12	14.5	14.5	14.5	0.0	New
J	FS	14	17.0	16.0	15.5	-1.5	New
K	F	14	13.5	13.5	13.0	-0.5	New
L	F	36	29.5	29.0	29.0	-0.5	New
M	M	36	31.0	30.0	29.5	-1.5	New
N	FS	32	24.0	24.0	23.5	-0.5	New
O	FS	24	22.5	21.5	21.5	-1.0	New
P	M	16	18.5	18.5	18.5	0.0	New
Q	FS	32	25.0	24.5	24.5	-0.5	New
R	FS	24	37.0	36.5	35.5	-1.5	New
S	M	42	25.5	24.0	24.0	-1.5	Old
T	M	30	18.0	17.5	17.5	-0.5	Old

^aM, entire male; F, entire female; FS, spayed female.

^bages expressed in months.

^cweights expressed in kilograms.

^ddenotes the difference in bodyweight between the 9th day of the study and the first day of the study.

^eOld, Hill's canned Prescription Diet® canine/feline d/d diet; New, Hill's canned Prescription Diet® canine d/d diet.

Table 6.4. Typical Proximate Analysis of Friskies Go Dog®.

Analyte	% Weight Dry Matter
Crude Protein	21 (minimum)
Crude Fat	11 (minimum)
Crude Fibre	3 (maximum)

6.5 Experimental Method

Gastric emptying studies were performed on each dog on days 1, 6 and 9 of the study resulting in a total of 60 series of radiographs. On day one, each dog was radiographed within five hours of being admitted to the MUCAH. This step was taken to mimic the situation generally encountered in a veterinary practice, that is, the dogs undergo a radiographic procedure on the same day they are admitted to the clinic. On the days the dogs were not being radiographed (days 2-5, 7 and 8) they were taken to the radiography suite once daily and restrained on the radiography table in a manner identical to that required in the gastric emptying studies. This "training" procedure was undertaken to familiarise the dogs with the radiographic procedure and consequently, it was anticipated that the dogs would be less stressed when they were radiographed on days 6 and 9 of the study than on the first day.

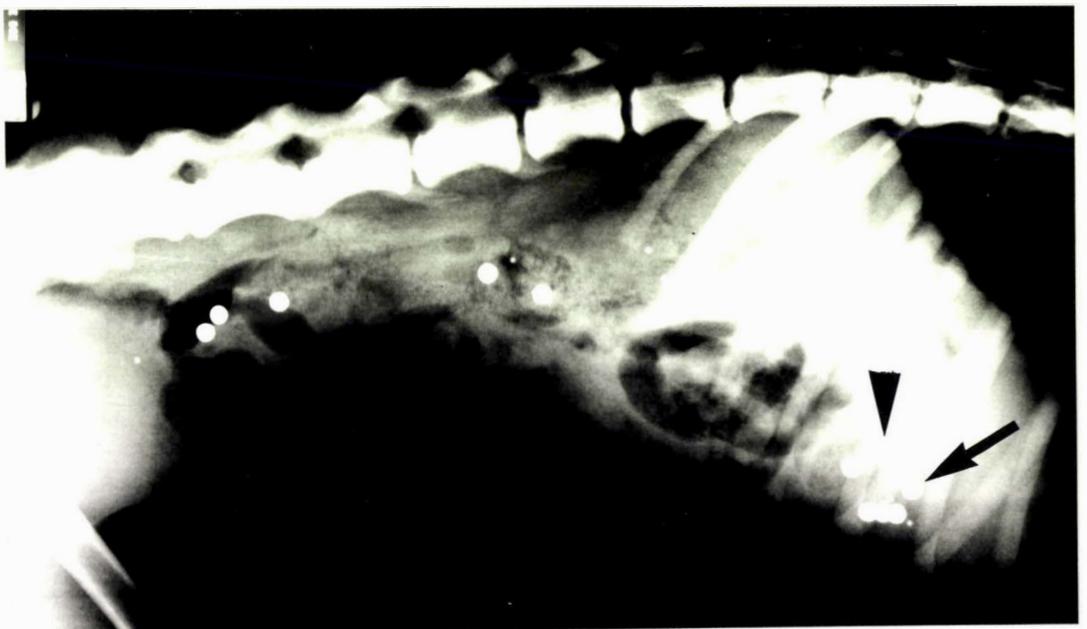
Dogs were radiographed on days 6 and 9 to assess the effect of training on the rate of gastric emptying (comparing the studies on day 1 with the studies of days 6 and 9) and to assess the repeatability of the procedure in trained dogs (comparing the studies on days 6 with the studies on day 9).

All the dogs were fasted for 24 hours prior to radiography to ensure that no food was in the stomach at the commencement of the gastric emptying studies. Since the ingestion of water does not affect the emptying of the solid components of a meal (Meyer *et al*, 1979), water intake was not restricted.

Dogs were manually restrained for radiography by two people. No chemical restraint was used in this study because sedatives may affect gastric motility and consequently the rate of gastric emptying. Ventrodorsal (VD) recumbent and left lateral (LL) recumbent radiographs (Figure 6.2) were taken immediately after feeding the test meal (designated as zero time) and hourly thereafter. Ventrodorsal restraint was aided by placing a foam cradle under the dogs thoracic spine which prevented rotation. Ventrodorsal, rather than dorsoventral (DV), recumbency was preferred because it was easier to keep the mid-sagittal plane of the dog in line with the primary beam. Also, when dogs are restrained in VD and LL recumbency, gas accumulation in the antrum and pylorus provided negative contrast to these areas which made it easier to determine when the markers had left the stomach and had entered the proximal duodenum. Conversely, restraint in DV and RL recumbency results in gas accumulating in the cardia and fundus of the stomach (Owens, 1982).

On most occasions, radiography terminated when all the large and small markers appeared to have left the stomach. Occasionally, radiography terminated before all the small and large markers had left the stomach because of the demands on the equipment by other hospital staff or because the study had finished in three out of the four dogs and the remaining dog had taken longer than 15 hours to empty all its markers.

Figure 6.2. Photographs of the ventrodorsal radiograph and the left lateral radiograph of Dog E on day 1 at 5 hours. The large markers (arrow) and small markers (arrowhead) are clearly visible.



6.6 Radiographic Equipment

Radiographs were exposed using a Philips^f super 120 CD X-ray unit. However, when this unit was unavailable due to the clinical demands of the MUCAH, a Picker^g Explorer mobile X-ray unit was used.

Radiographs were developed using a Kodak^h RP X-OmatTM automatic processor, model M6B. Kodak^h T-MatTM G, 35 cm x 43 cm, diagnostic film was used in Kodak^h X-omaticTM cassettes which contained Kodak^h lanexTM fast screens.

Radiographs were taken using a Lysholmⁱ 100 cm focused grid. Accordingly, the surface of the radiography table was always 100 cm from the bottom of the collimator.

Radiographic factors were selected to give good abdominal detail and depended on the size of the dog and the x-ray unit used. Factors ranged between 58 kilovolts and 12.5 milliamperere seconds and 64 kilovolts and 20 milliamperere seconds.

All personnel were required to wear lead gloves and aprons when handling dogs for radiography. The gloves had a lead equivalent exceeding 0.5 mm. The fronts of the aprons had a lead equivalent of 0.5 mm and the backs of the aprons had a lead equivalent of 0.3 mm. Thyroid shields were not compulsory. Personnel handling the dogs for radiography each wore a radiation dose meter attached to their clothing at the level of the shoulder.

^fPhilips NZ Ltd, PO Box 41021, Auckland, New Zealand

^gPicker Australia, PO Box 143, North Ryde, NSW 2113, Australia

^hKodak NZ Ltd, PO Box 2198, Auckland, New Zealand

ⁱElema-Schönander, Solna, Sweden

6.7 Radiographic Interpretation

Utilising the VD and the LL recumbent radiographic views obtained each hour, the radiologists allocated the markers to one of three groups: markers which were definitely in the stomach (DI); markers which were definitely in the intestinal tract (DO); and markers of indefinite location. The small markers were allocated separately from the large markers.

The radiologists were allowed to allocate markers after having viewed the radiographs as a series rather than after having viewed each hour's radiographs independently. It was believed that sequential analysis would increase the accuracy of radiographic interpretation because the radiologist could trace the passage of the markers. The percentage of markers that had emptied from the stomach were calculated using the formula

$$(DO/[DO + DI]) \times 100.$$

Percent gastric emptying versus time (GEvT) curves were generated for each gastric emptying study for both the 1.5 mm and 5.0 mm markers.

It was noted that during the initial part of most of the GEvT curves there was a period during which gastric emptying was very slow and <5% of the markers had left the stomach. This period was followed by a period of rapid gastric emptying. The time taken to reach the point of inflection on the GEvT curves that separated these phases, the lag phase, was measured for each curve.

The time taken to empty 25% (T_{25}), 50% (T_{50}), and 75% (T_{75}) of the markers was calculated. If the time to empty either 25%, 50% or 75% of the markers fell between two points on the GEvT curves then the greater time was taken to represent the T_{25} , T_{50} or T_{75} . For example, if 25% emptying occurred between 4 and 5 hours the T_{25} is 5 hours. In this way, inaccurate interpolation of data was avoided.

6.8 Observer Variation

In order for this technique to be considered suitable for veterinarians in private practice, it was considered necessary to show that there was minimal variation between veterinarians interpreting the radiographs. Although the author (FJA) interpreted all the radiographs, ten randomly selected series of radiographs were also interpreted by a specialist veterinary radiologist, Ian Robertson^j (IDR). Ian Robertson is a diplomat of the American College of Veterinary Radiologists and has completed three years postgraduate training.

A specialist veterinary radiologist was chosen as the second observer because it was considered more likely that he would correctly assess the location of the markers. Consequently, if the rate of agreement between the lay-radiologist (FJA) and the specialist-radiologist (IDR) was high, then this would indicate that the technique does not require specialist training in veterinary radiology and is therefore well suited for use in private practice.

The difference between the allocation of the markers made by both radiologists (IDR and FJA) was calculated using the formula

$$|\mathbf{DO}_{\text{IDR}} - \mathbf{DO}_{\text{FJA}}| + |\mathbf{DI}_{\text{IDR}} - \mathbf{DI}_{\text{FJA}}|$$

where \mathbf{DO}_{IDR} and \mathbf{DI}_{IDR} are the number of markers assessed by Ian Robertson to be definitely out of the stomach and definitely in the stomach respectively; \mathbf{DO}_{FJA} and \mathbf{DI}_{FJA} are the number of markers assessed by Frazer Allan to be definitely out of the stomach and definitely in the stomach respectively; and $||$ are absolute value bars.

^j Practising at the Chartwell Veterinary Clinic, PO Box 12-014, Hamilton, New Zealand.

To illustrate, consider the radiographs of dog E at 6 hours on day 1. Ian Robertson considered that 7 large balls were in the stomach ($DI_{IDR} = 7$), 2 large balls were in the small intestine ($DO_{IDR} = 2$) and there was 1 ball of unknown location whereas Frazer Allan deemed that 7 large balls were in the stomach ($DI_{FJA} = 7$) and 3 large balls were in the small intestine ($DO_{FJA} = 3$). In this example, the discordance between the radiologists was

$$|2-3| + |7-7| = \text{one marker.}$$

This calculation was done for every hour of radiographs assessed by both radiologists. The discordance data for the large markers is shown separately from the small markers. Discordance between the radiologists is summarised graphically for the small and large markers.

6.9 Statistical Methods

Statistical calculations were performed using The SAS System^k, version 6.08. Repeated measures analysis of variance (ANOVA) was used to assess the effect that sex, diet and the day of the study (ie. days 1, 6 and 9) had on the lag phase, T_{25s} , T_{50s} and T_{75s} . Linear regression analysis was used to compare the effect that dog age and weight had on the T_{50} . For all statistical analyses, a P value of <0.05 was considered significant.

Reference values for clinical purposes were calculated from the data obtained from day one of the study. Mean lag phases, T_{25s} , T_{50s} , T_{75s} and the mean GEvT curve with 95% confidence intervals are shown for the small and large markers.

^kSAS Institute Inc, Cary NC, USA

Chapter Seven

Results

7.1 Experimental protocol

All the dogs remained healthy over the ten day period of the study. One dog gained weight during the study, five dogs maintained the same weight and 14 dogs lost weight (see Table 6.3). Weight loss was never greater than 1.5 kg.

The test meal was eaten within 5 minutes of the food being offered in 17 of the 20 dogs. Dogs M, O and P had to be force fed the test meal on the first day of the study but on days 6 and 9, they consumed the test meals within 5 minutes.

More than 83% of the small markers were ingested (mean 95.2%, SD 6.3%) in 59 out of the 60 occasions the test meals were fed to the dogs. On day one of the study, dog P ingested only 60% of the small markers offered. The dogs always ingested at least 80% of the large markers offered (mean 97.2%, SD 5.5%).

All the dogs could be successfully restrained in VD and LL recumbency for radiography but struggling was a problem on day one of the study. Due to the struggling, approximately 20% of the radiographs needed to be repeated because of poor dog alignment or blurring. Struggling progressively reduced through day one and over the training period (days 2-5 inclusive). By day 6, the dogs were noticeably more relaxed when they were restrained on the radiography table and some dogs would voluntarily jump onto the table and lie down in anticipation of the procedure. At this stage, less than 5% of the radiographs had to be repeated.

Apart from dog B on day 9, all the radiographs were analysed and included in the results of this study. In this dog, the total number of large markers seen in the radiographs increased from 10 to 12 markers in the 7th hour and from 12 to 13 markers in the 17th hour. Consequently, the results obtained for the large markers were not included in any calculations.

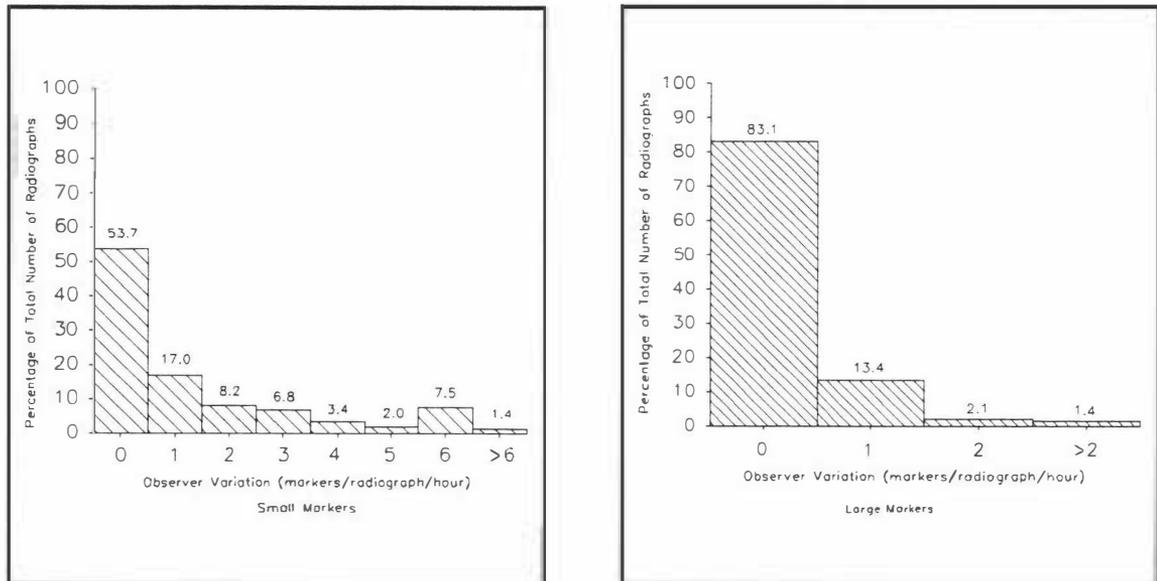
For the small markers, radiographs were taken until 100% emptying had occurred in 48 of the 60 studies; until >85% emptying had occurred in 11 of the 60 studies; and in one study until 60% emptying had occurred. For the large markers, radiographs were taken until 100% emptying had occurred in 50 of the 59 studies; until >70% emptying had occurred in six of the 59 studies; and in three series, until 60%, 50% and 10% emptying had occurred. The radiographic series in which only 10% of the large diameter markers had emptied was terminated after 22 hours.

The radiation dose meters worn by personnel handling the dogs for radiography always registered radiation levels less than 0.15 milliSieverts (mSv) per three months: 0.15 mSv representing the lowest detectable radiation level.

7.2 Observer variation and radiograph interpretation

Figure 7.1 summarises the differences in the radiologists' interpretation of the position of the markers in ten, randomly selected, series of radiographs. Interpretive differences between the radiologists occurred most often in the middle to later stages of a radiographic series for the small markers and towards the latter stages for the large markers.

Figure 7.1. Histograms showing the observer variation between two radiologists for the small and large markers.



Both radiologists were able to place all the large markers into the DO and DI categories for the total 142 hours of radiographs. For the small markers, FJA placed all the markers into the DO and DI categories for 140 of the total 147 hours of radiographs. For the remaining 7 hours, there were 4 hours where he placed one small marker into the indeterminate location category and 3 hours where he placed three small markers into the indeterminate location category. IDR placed all the small markers into the DO and DI categories for 132 hours. For the remaining 15 hours, there were 6 hours where he placed one small marker into the indeterminate location category; 4 hours where he placed two small markers into the indeterminate location category; 4 hours where he placed three small markers into the indeterminate location category; and 1 hour each where he placed four and seven markers into the indeterminate location category.

7.3 Effects of variables on gastric emptying parameters

Dog gender (Figure 7.2) and the change in diet formulation (Figure 7.3) had no significant effect on the T_{25} , T_{50} and T_{75} of the small and large markers.

Figure 7.2. Mean percent gastric emptying versus time curves for the male and female dogs. Error bar = standard error.

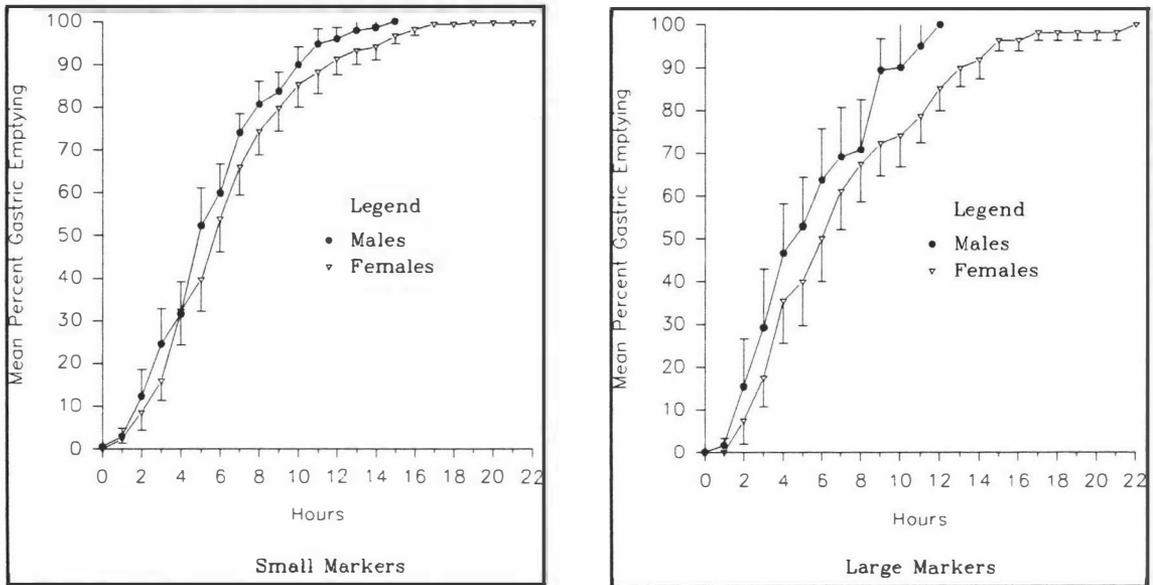
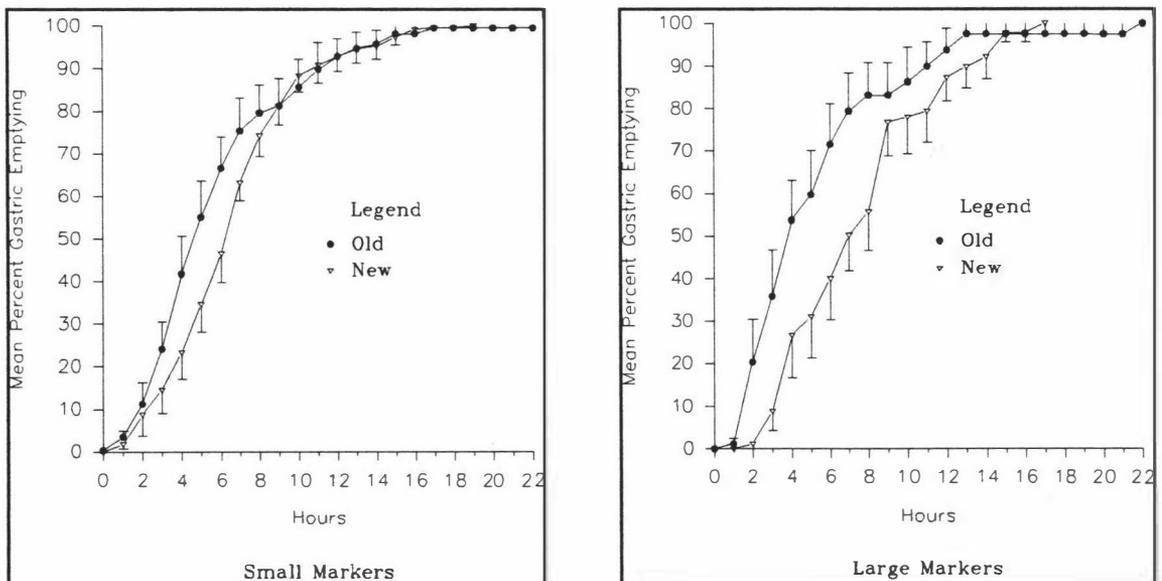
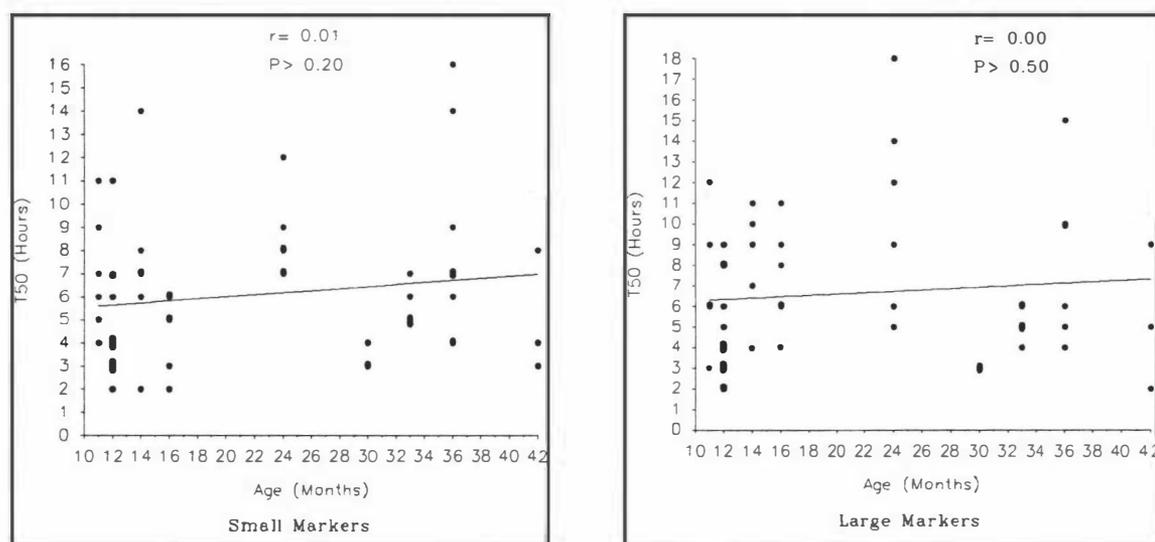


Figure 7.3. Mean percent gastric emptying versus time curves for the old and new diets. Error bar = standard error.



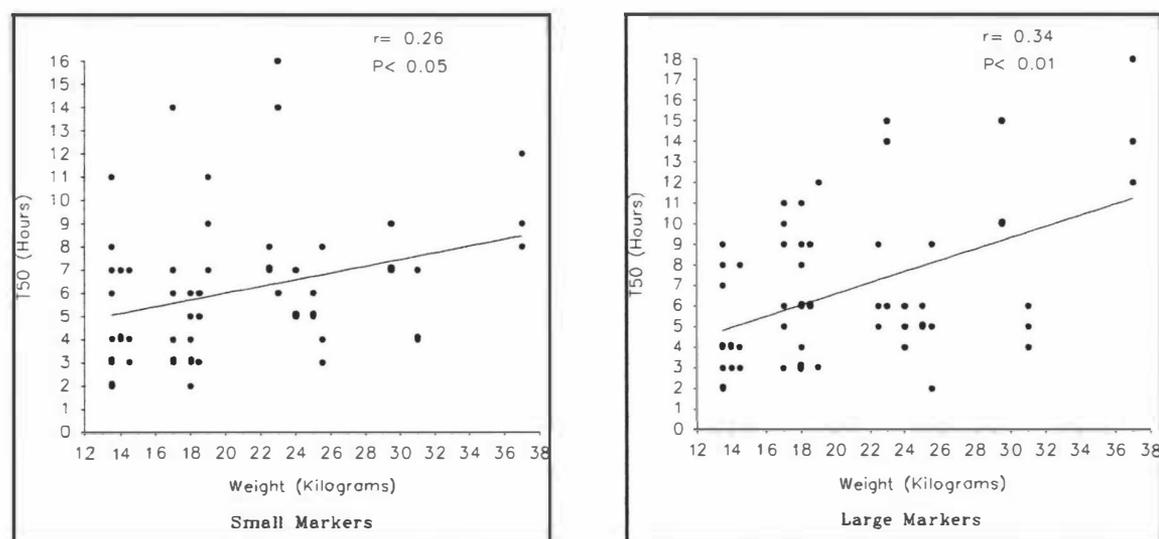
There was no correlation between dog age and T_{50} for the large and small markers (small markers, $r = 0.01$, $p > 0.20$; large markers $r = 0.00$, $p > 0.50$) (Figure 7.4).

Figure 7.4. Scattergram and line of best fit showing the effect of age on T_{50} for the small and large markers.



There was a weak but significant positive correlation between dog weight and T_{50} (small markers $r = 0.26$, $p < 0.05$; large markers $r = 0.34$, $p < 0.01$) (Figure 7.5).

Figure 7.5. Scattergram and line of best fit showing the effect of weight on T_{50} for the small and large markers.



There were no significant differences in the T_{25s} , T_{50s} and T_{75s} on days 1, 6 and 9 of the study (Table 7.1; Figure 7.6). Similarly, there were no significant differences in the T_{25s} , T_{50s} and T_{75s} between the large and small markers on days 1, 6 and 9 (Table 7.1).

Figure 7.6. Mean percent gastric emptying versus time curves for days 1, 6 and 9. Error bar = standard error.

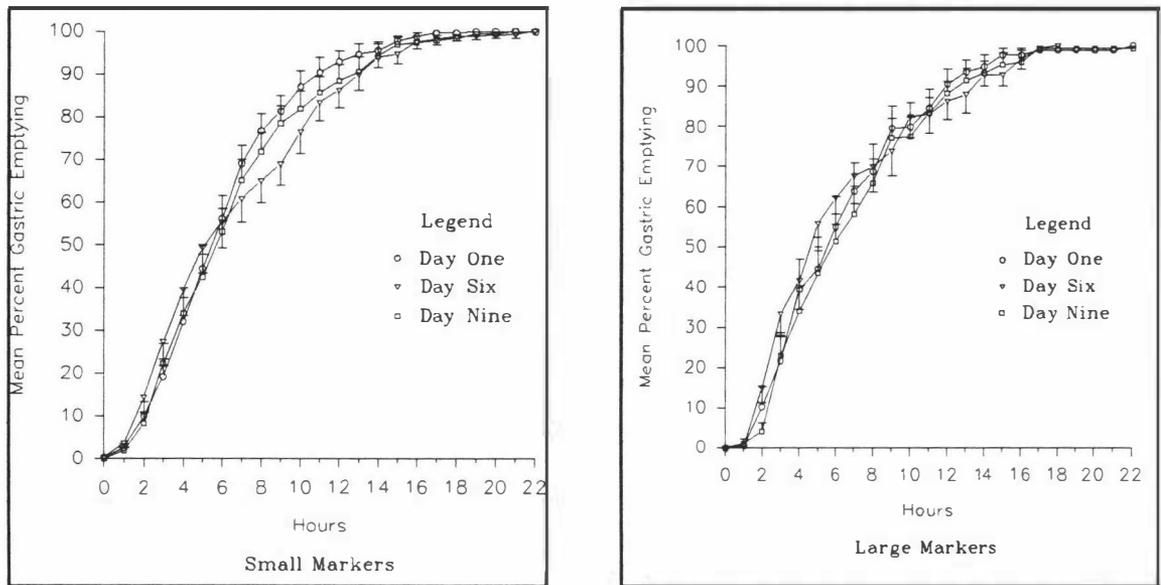


Table 7.1. Summary of the mean T_{25s} , T_{50s} and T_{75s} for the small and large markers on days 1, 6 and 9.

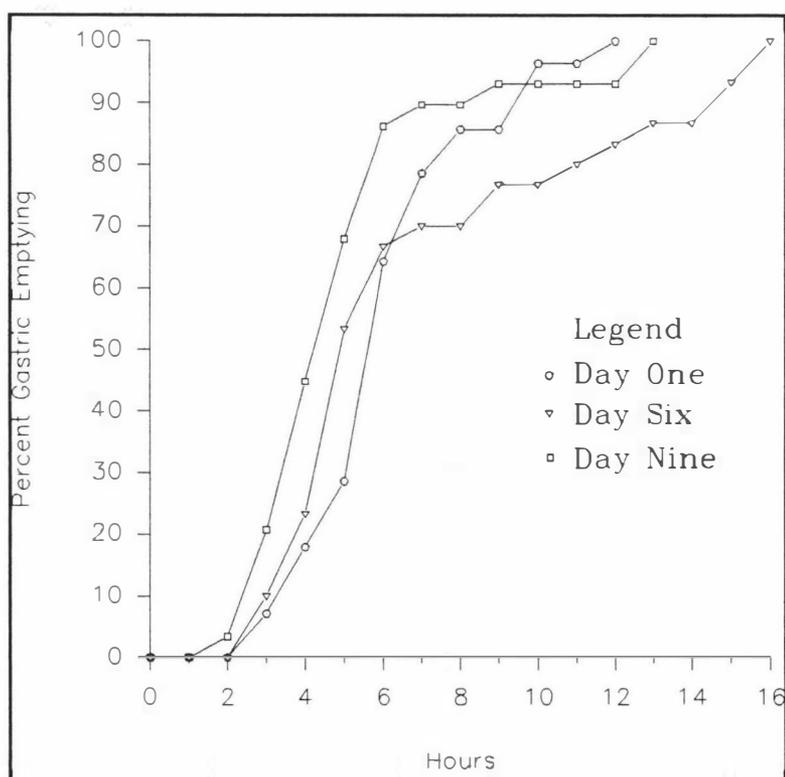
Marker Size	Day	$T_{25} \pm SD$	$T_{50} \pm SD$	$T_{75} \pm SD$
Small	One	4.85 ± 2.15	6.05 ± 2.99	8.32 ± 2.72
	Six	3.95 ± 1.77	6.25 ± 3.78	9.40 ± 3.58
	Nine	4.40 ± 1.59	6.35 ± 2.55	9.00 ± 3.30
Large	One	5.05 ± 2.63	7.11 ± 3.60	8.59 ± 4.02
	Six	5.05 ± 3.53	6.35 ± 3.90	8.28 ± 3.57
	Nine	5.37 ± 2.56	7.16 ± 2.34	8.53 ± 3.48

7.4 Individual GEvT curves and Lag Phases

Small markers

The majority of the individual GEvT curves for the small markers tended to have a sigmoid shape and there appeared to be very little intraindividual variation in the rate of gastric emptying of the small markers on days 1, 6 and 9 (Figure 7.7). Three phases of gastric emptying could generally be identified from these curves; an initial lag phase, an intermediate rapid phase and a terminal slow phase.

Figure 7.7. Percent gastric emptying versus time curves for the small markers in dog Q on days 1, 6 and 9.



The lag phase was a distinct, measurable period during which less than 5% of the small markers left the stomach (Table 7.2). There was no significant difference in the duration of the lag phase on days 1, 6 and 9. There was no significant difference between the duration of the lag phase for the small and large markers.

Table 7.2. Lag phases for the small and large markers on days 1, 6 and 9.

Marker Size	Day One	Day Six	Day Nine
Small	2.45 ± 2.04	1.80 ± 1.83	1.85 ± 1.31
Large	3.00 ± 2.37	2.35 ± 2.26	2.42 ± 1.50

The lag phase was followed by a linear, rapid phase of gastric emptying. Sometimes the linear, rapid phase continued until all the markers had emptied. More commonly however, the last 5-25 % of the markers emptied slowly from the stomach.

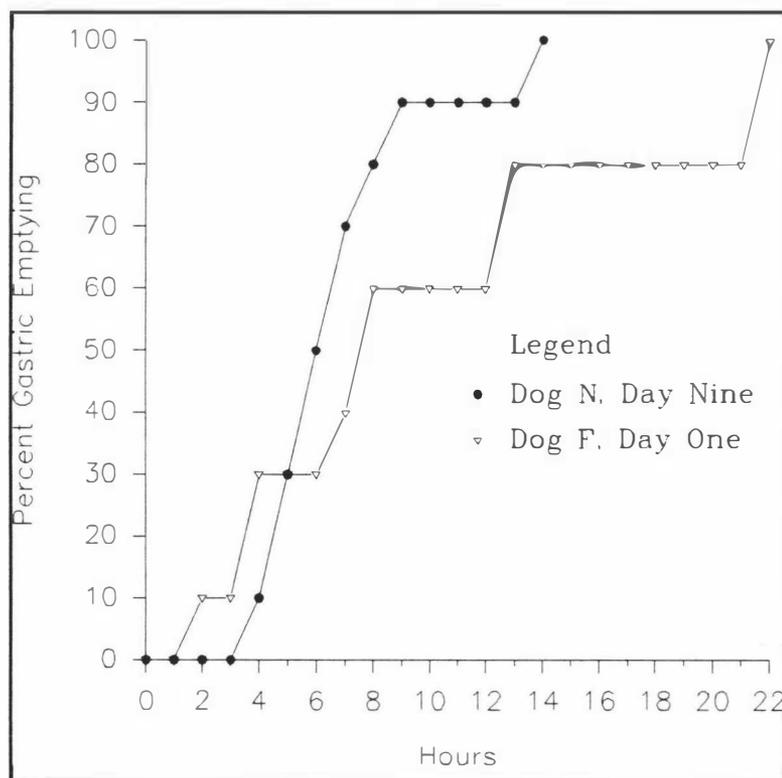
The terminal slow phase was often characterised by long periods where no markers left the stomach. Although the transition between the lag phase and the rapid phase was distinct, the transition between the rapid and slow phase was much less obvious and there was no attempt to quantify this phase.

Large markers

When individual large marker GEvT curves were examined, two curve shapes were commonly identified; a sigmoid curve and a "stepped" curve (Figure 7.8). There was more intraindividual variation in the rate of gastric emptying of the large markers compared with the small markers. The sigmoid curves were almost identical in shape to the GEvT curves for the small markers. The stepped curves were characterised by alternating phases where no markers leave followed by sudden gastric emptying.

Both the sigmoid shaped curves and the stepped shaped curves generally had an identifiable lag period (Table 7.2). The mean duration of the lag phase for the large markers was 2.59 ± 2.11 hours. There was no significant difference in the duration of the lag phase for the large markers on days 1, 6 and 9.

Figure 7.8. Percent gastric emptying versus time curves for the large markers in dog N on day 9 and dog F on day 1.

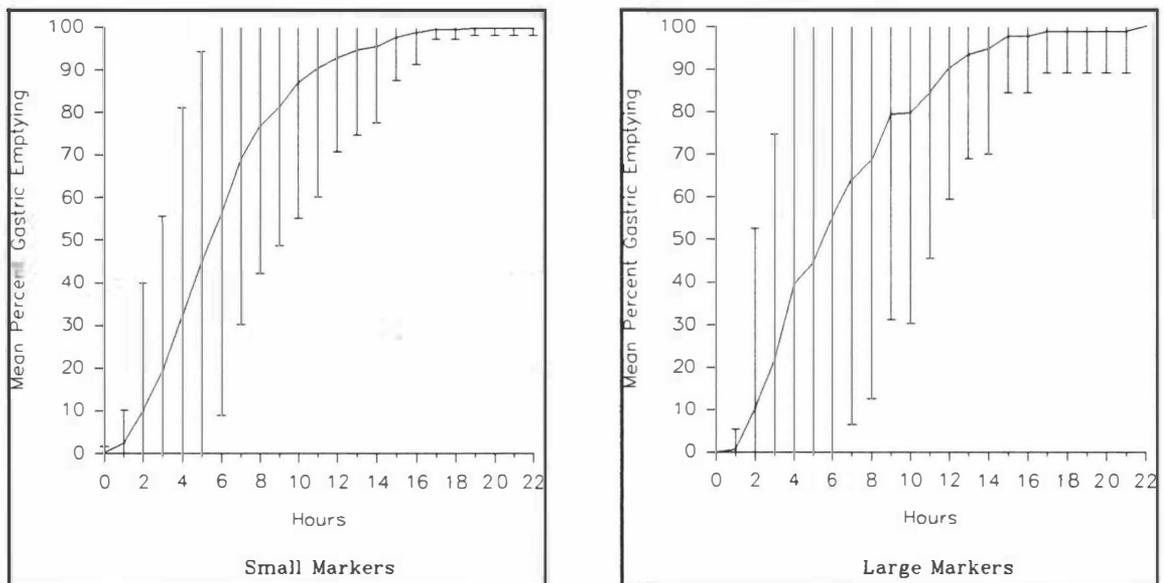


7.5. Clinical Data

One of the objectives of this study was to present the data in a form which could be used by veterinarians in private practice to assess whether the gastric emptying rate of a patient is "normal" or "abnormal".

Since most dogs in clinical practice are likely to be radiographed on the same day they are admitted to the clinic, the data obtained on day one of this study is the most appropriate data to use for establishing reference values. Figure 7.9 summarises the mean percent gastric emptying of the small and large markers on day one with 95% confidence intervals. It is convention to use 95% confidence intervals to represent the limits of "normality".

Figure 7.9. Mean percent gastric emptying versus time curves for the small markers and large markers on day one. Error bar = 95% confidence interval.



Chapter Eight

Discussion

8.1 Experimental protocol

Early in the study, most dogs did not eat their daily ration of the maintenance food, *Go Dog*. This accounted for the weight loss exhibited by 14 of the 20 dogs. Stress and/or low palatability of the food probably contributed to the initial poor appetites of these dogs.

Some markers always fell onto the floor while the dogs were eating their test meals. Although the dropped food scraps and markers were swept off the cage floor and fed back to the dogs, some markers were not recovered. This resulted in some dogs ingesting a smaller number of markers than the total number offered. However, since the percent gastric emptying of markers, rather than the absolute number of markers, was used in gastric emptying calculations, this loss of markers was of little consequence.

Sedation may have reduced the exposure of the dogs and handlers to x-irradiation and reduced wastage of film and chemicals because it may have calmed the dogs sufficiently to prevent poor dog alignment and movement blur of radiographs. However, sedatives were not used in this study because of their reported effects on gastric emptying (Zontine, 1973). Until the effects of sedation on gastric emptying has been assessed, the routine use of sedatives cannot be recommended.

In veterinary practices, staff may perform many radiopaque marker studies in their working life and consequently, they may be exposed to significant cumulative doses of radiation. The maximum level of radiation exposure allowed by the National Radiation Laboratory of New Zealand is 150 mSv per year for the eyes and 20 mSv per year for the trunk (J LeHeron, *pers comm*). In this study, the radiation exposure to personnel handling the dogs was well within acceptable limits, despite the large number of dogs studied within a short period of time. This was a satisfying finding given that one of the shortcomings of this technique is that it exposed personnel to x-irradiation. Radiation exposure was minimised by coning, wearing protective clothing and by using x-ray factor tables (New Zealand Department of Health, 1984).

Although the dogs received radiation doses many times higher than doses received by the handlers, the exposure of dogs to x-irradiation was not quantitated for two reasons. Firstly, this technique has been developed for use in veterinary practice and in this setting it is unlikely that dogs would be subjected to more than three radiopaque marker study in a lifetime. Secondly, dogs are much less likely than humans to develop radiation related illness because of their considerably shorter lifespan.

8.2 Observer variation and radiograph interpretation

An objective of this study was to assess the extent of observer variation between a specialist radiologist and a lay radiologist to help validate the use of this technique in veterinary practice. Most observer variation resulted from the presence of markers in five areas in which it was potentially difficult to determine whether the markers were within the stomach or intestine. The term "overlay regions" was coined for these areas. The overlay regions are described below and are illustrated in Figure 8.1.

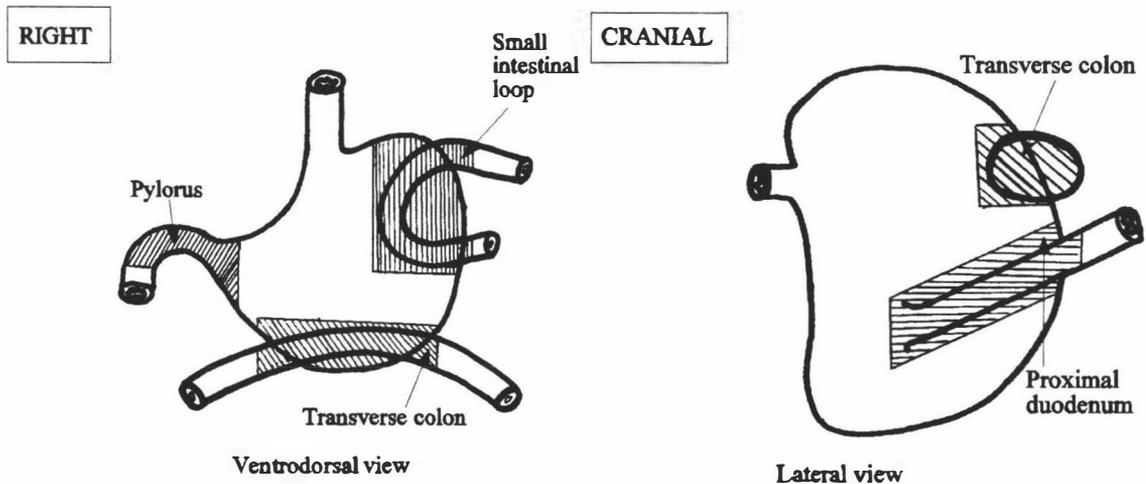
Region one- (VD view) The pyloric area. The close proximity of the distal antrum to the proximal duodenum made it difficult to be certain whether markers adjacent to the pylorus were in the stomach or duodenum.

Region two- (LLR view) The proximal duodenum overlays the stomach as it courses caudodorsally. Markers in the proximal duodenum may appear to be in the stomach. Often close examination of the VD view helped to resolve this dilemma because the duodenum courses caudally on the extreme right of the abdomen whereas the stomach is more centrally located in this view.

Region three- (VD view) A loop of small intestine may occasionally overlay the stomach, generally on the dog's left hand side. Markers in these intestinal loops may be mistakenly considered to be in the stomach.

Region four- (VD and LLR view) The transverse colon overlays the caudal body of the stomach. Difficulties can arise when there are markers in the stomach and in the colon at the same time. Markers in overlay region four proved to be the most troublesome because overlay occurred in both the VD and LLR views.

Figure 8.1. The four overlay regions where interpretive errors can be made.



LEGEND

-  Region One
-  Region Two
-  Region Three
-  Region Four

In general, there was little disagreement between both radiologists in the first few hours of a radiographic series. This could be attributed to two factors. Firstly, there was an initial delay in the gastric emptying of the markers (the lag phase) and it was not difficult to determine the location of the markers when they all appeared to be in the stomach. Secondly, markers in overlay regions one and two, which could cause observer variation in the initial stages of gastric emptying, appeared to be less troublesome than markers in overlay regions three and four.

The greatest observer variation occurred in the mid to later stages of a radiographic series when markers were in overlay region four. Markers were commonly found in this region and they could dwell there for many hours before passing on to more distal sites. Markers in region three also added to the disagreement in the mid to late periods although they were found less often in this region.

Having considered the potential difficulties arising from markers situated in the overlay regions, a number of potential improvements to the experimental protocol could be made.

- 1) Since the gastrointestinal tract is a relatively mobile structure, it is possible that markers in overlay regions could be separated more easily if RL and DV views were taken as well as the standard LL and VD views. In DV recumbency the stomach is situated more cranially in respect to the transverse colon than in VD recumbency (IDR, *pers com*) which may have made it easier to distinguish markers in region four. Furthermore, in the RL and DV views, gas accumulation in the fundus and cardia may provide negative contrast which may make it easier to determine the location of some of the markers. An obvious disadvantage with taking more views is the added expense and the extra exposure to radiation. Consequently, the additional views could not be recommended routinely.
- 2) Exposing radiographs at full expiration effectively flattens the diaphragm and results in the stomach occupying a more cranial position. This may also prevent the transverse colon from overlying the caudal stomach and it may have helped determine the location of markers in overlay region four.
- 3) Allowing the dog to urinate prior to radiography may have resulted in the small intestine occupying a more caudal position than if radiography was achieved with a full bladder. This may aid the distinction of region three markers.
- 4) Air enemas have been used successfully in clinical cases to provide negative contrast within the transverse colon. This has helped determine the location of markers in overlay region four.

When the radiologists felt uncertain about the location of the markers they were not obliged to allocate them to the DO or DI groups and they were not considered in gastric emptying calculations (see section 6.6). Potentially, large numbers of markers of indeterminate location could account for a large difference between the calculated percent of markers emptied and the actual percent of markers emptied. Consequently, this group of markers could cause substantial errors in gastric emptying calculations as Table 8.1 below illustrates.

Table 8.1. An example of how a large number of markers of indeterminate location can cause substantial errors in gastric emptying calculations.

Markers of unknown location	No. in stomach	No. in intestine	No. in unknown location	Calculated % in stomach	Real % in stomach*
Large number	5	9	16	35.7%	50%
Small number	10	17	3	37.0%	40%

*This calculation assumes that approximately two-thirds of the markers of unknown location were in the stomach and one-third were in the intestine.

In this study, there were very few markers of indeterminate location. Both radiologists could allocate all the large markers to the DO and DI groups. Ian Robertson could allocate all the small markers to these groups in 90% of the radiographs examined whereas FJA could allocate 95%.

Both radiologists felt that studying the radiographs as a series meant that the passage of the markers could be traced and consequently both radiologists felt more confident about the exact position of the markers in the overlay regions than if the radiographs were viewed independently. Viewing the radiographs independently, rather than as a series, would probably have increased the observer variation and/or increased the number of markers of indeterminate location.

It was interesting to note that there was greater observer variation with the small markers than the large markers (see Figure 7.1). This was probably due to two factors. Firstly, the larger number of small markers made it easier to miscount them, particularly when they were bunched together. Secondly, the smaller mass of the small markers meant that they had poorer abdominal contrast compared with the large markers. In the VD view it was particularly difficult to see the small markers when they overlaid the spine. Also, in an attempt to obtain the correct exposure for the cranial abdomen, the caudal abdomen was often overexposed. This meant that the small markers were often difficult to see and they could be missed if a bright light was not used.

In terms of this study, the level at which observer variation becomes unacceptable is a somewhat arbitrary value. However, it is highly improbable that observer variation of one ball for the large markers or 3 balls or less for the small markers^a would result in veterinarians drawing differing clinical opinions about the rate of gastric emptying in a dog. On this basis, the rate of agreement between both radiologists was excellent for the large markers; the radiologists disagreeing on the position of more than one large marker a mere 3.5% of the time. Observer variation was greater with the small markers but still remained low; the radiologists disagreeing on the position of more than three small markers 14.3% of the time.

In conclusion, the observer variation between the radiologist with specialist training (IDR) and the lay radiologist (FJA) was small and it can be concluded that this technique could be used confidently by other veterinarians who also do not have specialist radiology training. Furthermore, the high rate of agreement between both radiologists suggested that they were correctly locating the actual position of the markers. The contribution of markers of indeterminate location to the observer variation was minimal which could be attributed to the fact that the radiologists viewed the radiographs as a series rather than independently.

^aThis corresponds to approximately 10% of both the small and large markers.

8.3 Effects of Variables on Gastric Emptying Parameters

There is conflicting evidence on the effect of gender on the rate of gastric emptying in humans. Premenopausal women and postmenopausal women taking sex hormone replacement therapy have a slower emptying rate of solids than men whereas postmenopausal women, who are not on sex hormone replacement therapy, have a similar rate of gastric emptying of solids to men (Hutson *et al*, 1989). These findings suggested that oestrogen and/or progesterone inhibit gastric emptying. However, Madsen (1992) has recently shown that the gender of humans had no effect on the rate of gastric emptying of ^{99m}Tc -labelled cellulose fibre and 2-3 mm diameter ^{111}In -labelled plastic particles. No studies have been conducted to specifically examine the effect of gender on gastric emptying in dogs.

This study did not show a significant difference between males and females on the T_{25} , T_{50} and T_{75} . Dividing the group of female dogs into spayed and intact females was not done because there would have been too few dogs in each group for statistical comparison. A weight-match controlled study utilising larger numbers of dogs would be necessary to determine any effect of desexing on the rate of gastric emptying of the markers in dogs.

There was no significant effect of age on the T_{25} , T_{50} and T_{75} . However, all the dogs used in this study were young adults with ages ranging from 11 to 42 months. Extrapolation of these data to young, growing dogs (< 10 months of age) and to geriatric dogs (>8 years of age) may not be appropriate because the basal metabolic rate in dogs in these life stages is different to early adulthood and consequently, the rate of gastric emptying may also differ. The possible relationship between basal metabolic rate and the rate of gastric emptying is discussed below. Further studies need to be conducted to determine the rate of gastric emptying of the markers in very young and very old dogs.

The change in the dietary formulation from Prescription Diet® canned feline/canine d/d to canned canine d/d was an unwelcome variable introduced into this study. Fortunately, there was no significant effect of the change in diet on the T_{25} , T_{50} and T_{75} . The change in diet would only have been likely to influence the rate of gastric emptying of the markers if the energy density of the diets was significantly different (McHugh and Moran, 1979).

There was a weak but significant positive correlation between the T_{50} for both marker sizes and dog weight. Small dogs have a greater body surface area per unit of bodyweight than large dogs and consequently, they have a greater rate of heat loss and a higher basal metabolic rate (Lewis *et al*, 1987). In order to provide energy for a higher metabolic rate, smaller dogs need to eat more food per kilogram bodyweight and assimilate nutrients more rapidly than larger dogs (Lewis *et al*, 1987). Increasing the rate of gastric emptying is one of the mechanisms by which dogs may be able to achieve this.

In this study, the gastric emptying of the markers was assessed in dogs over a very small weight range (13.5 - 37.0 kg) but dog weights vary considerably depending on breed. An adult Chihuahua can weigh 1 kg whereas an adult St Bernard can weigh over 100 kg. Although the correlation between weight and T_{50} in this study was weak (small markers $r=0.34$, large markers $r=0.26$), if this correlation was extrapolated over the entire range of dog weights, T_{50} s would vary significantly at the weight extremes. Consequently, GEvT curves still need to be established for very small and very large breeds of dogs.

The exposure of dogs to acoustic stress slows the gastric emptying of solids during the first postprandial hour (Gué *et al*, 1989). However, in the present study there was no difference in the rate of gastric emptying by 2 hours. In the present study, the dogs were visibly stressed on the first day but they were relatively relaxed on days 6 and 9 (see section 7.1). Consequently, we expected that the gastric emptying of the markers may be slower on day 1 of the study compared to days 6 and 9. However, there was no significant difference in the T_{25s} , T_{50s} and T_{75s} on days 1, 6 and 9 of the study. The apparently transient nature of the effects of stress on gastric emptying documented by Gué *et al* (1989) may explain why a stress effect was not noted in this study. Furthermore, different types of stress have differing effects on the rate of gastric emptying. In humans, stress induced by immersing the hand in cold water delays gastric emptying (Thompson *et al*, 1984) whereas acoustic stress (dichotomous listening) does not (Cann *et al*, 1983). Although stress induced by restraint delayed gastric emptying in rats (Williams *et al*, 1987), it did not appear to affect gastric emptying in the dogs of this study.

8.4 Gastric Emptying of the Small Markers

The sigmoid shape of the individual GEvT curves for the small markers is very similar to the shape of the GEvT curves for digestible solids in dogs (Hinder and Kelly, 1977b). The most prominent feature of the individual GEvT curves was the presence of a lag phase, which had a mean duration of about 2 hours. The gastric emptying of food also has a lag phase which has been demonstrated clearly in scintigraphic studies (Zeissman *et al*, 1992; Collins *et al*, 1983; Collins *et al*, 1986).

The lag phase has been shown to represent the time it takes for most of the liquid component of a meal to empty and the time it takes for food to be triturated to particle sizes small enough to allow passage into the duodenum. Houghton *et al* (1988a) and Collins *et al* (1991) observed in humans that food remained in the fundus, where it was not exposed to antral contractions, until approximately 80% of the liquid component of the meal had emptied. Food then entered the antrum, trituration was initiated and solid phase emptying began. In dogs, Hinder and Kelly (1977b) showed that when liver was fed as cubes it took longer to initiate emptying than when the liver was fed in a homogenised form.

In this study, the small radiopaque markers were designed to be small enough to mimic the gastric emptying of triturated food. Therefore, the lag phase probably represented the time it took for the liquid component of the meal to leave the stomach plus the time it took for the chunks of food to be broken down so the markers could be presented to the pylorus.

The measurement of the lag phase may be clinically useful parameter. Humans suffering from morbid obesity (Horowitz *et al*, 1983) or diabetes mellitus (Zeissman *et al*, 1992; Loo *et al*, 1984) have a delayed lag phase. Furthermore, it has been shown that drugs such as metoclopramide improve gastric emptying in diabetic humans by shortening the lag phase (Loo *et al*, 1984). It would be interesting to determine whether a delay in the lag phase occurs in diseases which affect the gastrointestinal tract of dogs.

The rapid, linear phase of gastric emptying of the small markers is also commonly seen in the gastric emptying of solids (Houghton *et al*, 1988a; Collins *et al*, 1991; Hinder and Kelly, 1977b). Unlike the gastric emptying of solids, liquids tend to leave the stomach exponentially (percent volume remaining per unit time) (Houghton *et al*, 1988b; Collins *et al*, 1991). This is because the emptying of liquids is related to intragastric volume and fundic tone. The emptying of solids on the other hand is governed by mechanical factors such as antral, pyloric and duodenal contractility rather than changes in pressure. Accordingly, solids tend to leave the stomach in a linear fashion (constant volume per unit time).

The terminal part of the GEvT curves for digestible solids have a slower rate of gastric emptying than the intermediate part of the curve (Hinder and Kelly, 1977b). This pattern was also generally observed for the gastric emptying of the small markers in this study. However, there are no studies which have investigated the reason for this phenomenon. Possibly a critical volume of food is required to maintain antroduodenal contractility and below this volume contractility the rate of gastric emptying wane. Another possibility is that below a critical volume, the motility pattern may convert from the fed motility pattern to the quiescent phase I of the MMC. Food may remain in the stomach until the activity front (phase III) of the MMC clears the remaining food. Further studies need to be conducted to look at these possibilities.

Although the emptying patterns of the small markers were similar to the gastric emptying pattern of food observed by other workers, it cannot be concluded definitively from these observations that the small markers are leaving proportionally to food. In an attempt to address this issue, we measured the corporal diameter of the stomach in the LL recumbent view immediately after feeding and then hourly until the end of the study. We found that the mean T_{50} obtained from corporal measurement was approximately 6 hours which is similar to the T_{50} of the small markers. However, controlled studies are needed to confirm that the small markers do indeed leave the stomach proportionally to food.

8.5 Gastric Emptying of the Large Markers

It was anticipated that the large markers would remain in the stomach during the fed motility pattern and then leave suddenly during phase III of the MMC (see section 5.2). Consequently, the individual GEvT curves for the large markers were expected to have a long lag period followed by a rapid period of gastric emptying. However, the individual GEvT curves for the large markers either had a sigmoid shape, which was very similar to that of the small markers, or a stepped shape (Figure 7.8). Furthermore, the lag period for the large markers, and the mean T_{25} , T_{50} , and T_{75} was not significantly different from those of the small markers. These findings suggest that either the small markers were leaving during MMC activity or the large markers were leaving during the "fed" motility pattern.

A number of facts support the hypothesis the large markers were leaving during the fed motility pattern. Firstly, large markers could be seen in the duodenum while food was still present in the stomach. Secondly, the T_{50} obtained from corporal measurement (see section 8.4) was similar to the T_{50} of the large markers. Thirdly, if the small markers were emptying from the stomach during MMC activity, the small marker GEvT curves would be expected to have a "stepped" shape with steps occurring approximately every two hours and coinciding with phase III activity. Although individual curves with this shape were observed with emptying of the large markers, none of the small marker GEvT curves could be considered to have this shape.

If the large markers were indeed leaving the stomach during the fed pattern of gastric motility then the "stomach as a sieve" model must be redressed, at least with respect to the gastric emptying of indigestible solids. Given that food leaves the dog's stomach as particles less than 2 mm in diameter (Meyer *et al*, 1979), how can the stomach function as a sieve if 5 mm diameter markers apparently leave the stomach concurrently with 2 mm diameter food particles?

The answer may lie with the hydrodynamic model of Sirois *et al* (1990) (see section 3.10). This model suggests that three groups of variables influence the emptying of indigestible solids: the physical properties of the solid; the physical properties of the solution in which the solid is suspended; and stomach wall factors. Mathematically, the hydrodynamic model is defined by the gastric emptying coefficient (GEC),

$$\text{GEC} = (D_{py}/D_p) \cdot [g(\rho_f - \rho_p)D_p^2/\partial(v)]$$

where D_{py} is the pyloric diameter; D_p is the particle diameter; g is the gravitational constant, 9.8 m/s^2 ; ρ_f and ρ_p are the fluid density and particle density respectively; v is the average linear fluid velocity; and ∂ is the viscosity of the fluid.

The GEC is inversely related to the rate of gastric emptying of indigestible solids. In this study, the chyme is the fluid which suspends the markers. Chyme consists of partly digested food, any water which has been imbibed and gastric secretions.

By applying the hydrodynamic model to this study, if $\rho_f = \rho_p$ then $GEC = 0$, and consequently the gastric emptying of the markers is rapid and independent of marker size, D_p . In this study, ρ_p was 1.37 g/ml. Although ρ_f was not measured it would probably have been very close ρ_p since the density of water is 1 g/ml and chyme would probably be even denser. This factor alone may explain why marker size had no effect on gastric emptying rate. It may also explain why the gastric emptying of both sets of markers was rapid and appeared to occur proportionally with food.

However, the hydrodynamic model has been shown to apply only to indigestible solids >1 and <7 mm in diameter. Hinder and Kelly (1977b) have clearly shown that the gastric residence time of plastic spheres of 7 mm diameter is determined by the duration of the fed state. Conversely, particles less than 1 mm in diameter leave the stomach at the same rate, independent of particle size, particle density and fluid and stomach wall factors (Bechgaard *et al*, 1985; Davis *et al*, 1984).

In summary, it appears that the large markers emptied during the fed motility pattern rather than with the onset of the MMC as was intended. The hydrodynamic model provides a hypothetical reason for this occurrence but this needs to be confirmed by studying the gastric emptying of the large markers concurrently with electrogastrography. In order to detect the onset of the MMC, it would appear that the gastric emptying of a marker >7 mm diameter is required.

8.6 Clinical Implications

For clinical purposes, it is more appropriate to consider data derived from the GEvT curves for the small markers because they have smaller 95% confidence intervals than the data derived from the GevT curves for the large markers.

By utilising the mean GEvT curve for the small markers shown in Figure 7.9, veterinarians can compare the gastric emptying percent of a patient with this curve at any point in time. Consequently, veterinarians are not obliged to take radiographs hourly but they can take "spot" radiographs at intervals which suit their working schedule. Clinical experience with the radiopaque markers at Massey University suggests that taking two or three hours of "spot" radiographs is a satisfactory method of obtaining gastric emptying information in clinical cases (WG Guilford, *pers comm*). If gastric dumping is suspected then radiographs must be taken within the first five hours of the study. After five hours the 95% confidence interval of the mean GEvT curve incorporates 100% gastric emptying and the diagnosis will be missed. Conversely, if delayed gastric emptying is suspected, radiographs should be taken after five hours because prior to this point, the mean GEvT curve incorporates 0%.

Taking "spot" radiographs has a number of potential disadvantages. As the interval between radiographs increases, the veterinarian may be less able to trace the passage of markers through the intestinal tract. This may increase the number of markers of indeterminate location and consequently it may adversely affect the accuracy of the procedure (see discussion in section 8.2). Moreover, when spot radiographs are taken, the lag phase, T_{25} , T_{50} and T_{75} may be missed which reduces the information which can be gathered from the gastric emptying study. These disadvantages need to be weighed against the added expense, time and manpower required to take hourly radiographs in order to establish a complete GEvT curve.

An obvious feature of the mean GEvT curves is that the 95% confidence interval is wide. The wide 95% confidence intervals indicates considerable variation in the rate of gastric emptying. Given that there was very little variation in the gastric emptying rate of small markers within the same dog, this finding indicates that there is considerable dog to dog variation in the dog population and it is not an artifact of the technique. It remains to be seen whether the wide 95% confidence interval means that this technique is insensitive at detecting subtle disturbances of gastric emptying (see section 8.7).

A direct comparison between data on gastric emptying obtained using different techniques cannot be made because of differences in the quality and quantity of the test meals, variation in the populations of dogs and different experimental techniques. However, the mean \pm SD T_{50} of the small markers in this study (6.05 ± 2.99 hours) was similar to that obtained by Hornof *et al* (1989) who utilised scintigraphy to assess the gastric emptying of a meal containing 2 oz of baby food and one cup of dry kibble in dogs (4 ± 1 hours). This comparison provides further evidence that the gastric emptying of the markers was similar to the gastric emptying of food.

8.7 Validation of the technique

Any new diagnostic test must be validated before it can become an acceptable diagnostic procedure and this has yet to be done for this technique. In this case, validation would be a two step process. Firstly, it needs to be shown that the test indeed measures what it intends to measure, namely the gastric emptying of food. Secondly, it needs to be shown that the test is adequately sensitive and specific, ie. the test will detect abnormal gastric emptying when it is actually abnormal and it will show that gastric emptying is normal when it is actually normal. Both these steps require comparison with a "gold standard" technique. Most of the recently developed techniques for assessing gastric emptying are validated by comparison with scintigraphy (Avill *et al*, 1987; Holt *et al*, 1990; Mangnall *et al*, 1991).

To complete step one of the validation procedure, we are currently feeding the test meal and markers to dogs which are due to be killed at the conclusion of other studies. At different time intervals after eating, the dogs are humanely killed, radiographs are taken, the markers are counted and the wet and dry weights of food remaining in the stomach is measured. In this way, it can be determined if the percent gastric emptying of the markers at various times correlates with the percent gastric emptying of the test meal on a wet or dry matter basis.

Another method of validating gastric emptying of the markers is by comparing their rate of gastric emptying with the simultaneous gastric emptying of a radiolabelled component of the test meal in a group of healthy dogs. Given that the markers are indigestible, it may be more appropriate to correlate their rate of gastric emptying with the rate of gastric emptying of the fibre content of the test meal (cellulose) rather than the gastric emptying of a digestible component of the meal such as protein. Biphasic scintigraphy would allow the gastric emptying of digestible and indigestible solids to be assessed simultaneously with the radiopaque markers and this would provide an ideal comparison.

Once step one has been completed and if the markers have been shown to adequately reflect the gastric emptying of particulate digesta in healthy dogs, the same process can be carried out in dogs diagnosed with a range of gastrointestinal diseases. The sensitivity and specificity of the technique could be assessed by comparing the GEvT curves of the small markers with the GEvT curves of the radiolabelled marker.

8.8 Summary

This study was initiated because there were no technique suitable for use by veterinarians in private practice for assessing the gastric emptying of solids. A simple, inexpensive technique which utilises equipment commonly found in veterinary practices has been developed. This technique is easier to perform than the barium sulphate suspension studies which are currently used by veterinarians to assess the rate of gastric emptying. Dogs will not voluntarily ingest barium sulphate suspension and it must be administered by stomach tube, or alternatively, it needs to be force fed. Conversely, when the radiopaque markers are placed into the test meal, they are readily ingested by dogs.

Markers were made from a compound containing high density polyethylene and barium sulphate. It appears that both the small markers and the large markers leave the stomach during the fed motility pattern and validation studies are currently underway at the Department of Veterinary Clinical Sciences at Massey University in an attempt to show that the markers do leave proportionally with food and to show that the technique is sensitive enough to diagnose delayed or excessively rapid gastric emptying of solids in dogs.

A significant effect of the sex and age of the dogs and the day on which the study was performed was not detected in this study. However, the large variability in the gastric emptying rate between dogs in this study may have compromised the detection of subtle differences resulting from sex and age. Match controlled studies specifically designed to assess the effects of these variables on the gastric emptying of radiopaque markers would be required to assess their true significance.

There was a significant but weak positive correlation between dog weight and the T_{50} . The dogs used in this study were of medium weight and it is probably inappropriate to extrapolate the data obtained from these dogs to very small and very large dogs. Consequently, normal values need to be obtained for breeds of dogs at the weight extremes.

There was good concordance between a specialist radiologist, Ian Robertson, and a lay radiologist, Frazer Allan, in the interpretation of the radiographs. Two conclusions could be made from this finding. Firstly, specialist radiology training is not necessary to interpret the pattern of distribution of markers in abdominal radiographs and secondly, it suggests that both radiologists are accurately predicting the location of the markers.

The mean GEvT curve of the small markers on day one (with 95% confidence intervals) was considered to represent the most appropriate gastric emptying reference curve for clinical use. If delayed gastric emptying is suspected, taking two or three sets of radiographs at regular intervals from 6-16 hours after feeding and comparing the results with the reference curve is probably the most appropriate method of assessing gastric emptying in a patient. Conversely, if excessively rapid gastric emptying is suspected, taking two or three sets of radiographs at regular intervals from 0-5 hours after feeding and comparing the results with the reference curve is most appropriate.

Bibliography

- . Achem-Karam SR, Funakoshi A, Vinik AI, Owyang C. Plasma motilin concentration and interdigestive migrating motor complex in diabetic gastroparesis: Effect of metoclopramide. *Gastroenterology* 88, 492-9, 1985.
- . Aeberhard PG, Magnenat LD, Zimmermann WA. Nervous control of migratory myoelectric complex of the small bowel. *American Journal of Physiology* 238, G102-8, 1980.
- . Agut A, Sanchez-Valverde MA, Lasaosa JM, Murciano J, Molina F. Use of iohexol as a gastrointestinal contrast medium in the dog. *Veterinary Radiology and Ultrasound* 34, 171-7, 1993.
- . Allescher H-D, Daniel EE, Dent JE, Fox JET, Kostolanska F. Extrinsic and intrinsic control of pyloric sphincter pressure in the dog. *Journal of Physiology (London)* 401, 17-38, 1988.
- . Allescher H-D, Daniel EE, Dent J, Fox JET, Kostolanska F. Neural reflex of the canine pylorus to intraduodenal acid infusion. *Gastroenterology* 96, 18-28, 1989.
- . Allescher H-D, Tougas G, Vergara P, Lu S, Daniel EE. Nitric oxide as a putative nonadrenergic noncholinergic inhibitory transmitter in the canine pylorus in vivo. *American Journal of Physiology* 262, G695-702, 1992.
- . Anderson S, Rosell S, Hjelmquist U, Chang D, Folkers K. Inhibition of gastric and intestinal motor activity in dogs by (GI⁴) neurotensin. *Acta Physiologica Scandanavica* 100, 231-5, 1977.
- . Andres MR, Bingham JR. Tubeless gastric analysis with a radiotelemetering pill (Heidelberg capsule). *Journal of the Canadian Medical Association*. 102, 1087-9, 1970.
- . Andrews PLR, Grundy D, Scratcherd T. Reflex excitation of antral motility induced by gastric distension in the ferret. *Journal of Physiology (London)* 298, 79-84, 1980.
- . Anuras S, Cooke AR, Christensen J. An inhibitory innervation at the gastroduodenal junction. *Journal of Clinical Investigation* 54, 529-35, 1974.
- . Arnbjerg J. Gastric emptying time in the dog and cat. *Journal of the American Animal Hospital Association* 28, 77-81, 1992.
- . Asada T, Sako Y, Matsumoto M, Araki A, Fukushima Y, Murakami M, Yonekura Y, Kinishi J, Kita T. A new method for assessing gastric emptying: Sulfamethizole capsule method. *Gastroenterology* 98, A1, 1990.

- Aspiroz F, Malagelada J-R. Intestinal control of gastric tone. *American Journal of Physiology* 249, G501-9, 1985.
- Avill R, Mangnall YF, Bird NC, Brown BH, Barber DC, Seagar AD, Johnson AG, Read NW. Applied potential tomography: A new noninvasive technique for measuring gastric emptying. *Gastroenterology* 92, 1019-26, 1987.
- Balwinder S, Bedi MB, Kelly KA, Holley KE. Pathways of propagation of the canine gastric pacesetter potential. *Gastroenterology* 63, 288-96, 1972.
- Barber NL, Mahaffey RYB. The Stomach. In: Thrall DE (ed). *Textbook of Veterinary Diagnostic Radiology*. Pp 473-492. WB Saunders Company, Philadelphia, 1986.
- Basile M, Neri M, Carriero A, Casciardi S, Comani S, del Gratta C, di Donato L, di Luzio S, Macri MA, Pasquarelli A, Pizzella V, Romani GL. Measurement of segmental transit through the gut in man. A novel approach by the biomagnetic method. *Digestive Diseases and Sciences* 37, 1537-43, 1992.
- Bass P, Code CF, Lambert EH. Electric activity of the gastroduodenal junction. *American Journal of Physiology* 201, 587-92, 1961.
- Bateman DN, Whittingham TA. Measurement of gastric emptying by real-time ultrasound. *Gut* 23, 524-7, 1982.
- Bayguinov O, Sanders KM. Role of nitric oxide as an inhibitory neurotransmitter in the canine pyloric sphincter. *American Journal of Physiology* 264, G975-93, 1993.
- Beani L, Bianchi C, Crema A. Vagal non-adrenergic inhibition of guinea-pig stomach. *Journal of Physiology (London)* 217, 259-79, 1971.
- Bechgaard H, Christensen FN, Davis SS, Hardy JG, Taylor MJ, Whalley DR, Wilson CG. Gastrointestinal transit of pellet systems in ileostomy subjects and the effect of density. *Journal of Pharmacy and Pharmacology* 37, 718-21, 1985.
- Berger T. Studies on the gastric emptying mechanism. *Acta Chirurgica Scandinavica (Supplementum)*, 404, 1-51, 1969.
- Biancani P, CiCalzi LK, McCallum RW. Mechanism of histamine-induced excitation of the cat pylorus. *Journal of Clinical Investigation* 68, 582-8, 1981.
- Bitar KN, Makhoul GM. Relaxation of isolated gastric smooth muscle cells by vasoactive intestinal peptide. *Science* 216, 531-3, 1982.
- Bloom SR, Ralphs DN, Bessar GM. Effect of somatostatin on motilin levels and gastric emptying (abstract). *Gut* 16, 834, 1975.

- Boeckxstaens GE, Pelckmans PA, Bogers JJ, Bult H, De Man JG, Oosterbosch L. Release of nitric oxide upon stimulation of nonadrenergic noncholinergic nerves in the rat gastric fundus. *Journal of Pharmacology and Experimental Therapeutics* 256, 441-7, 1991.
- Bolondi L, Bortolotti M, Santi V, Calletti T, Gaiani S, Babo G. Measurement of gastric emptying time by real-time ultrasonography. *Gastroenterology* 99, 752-9, 1985.
- Bolondi L, Santi V, Bortolotti M, Li Bassi S, Turba E. Correlation between scintigraphic and ultrasonographic assessment of gastric emptying. *Gastroenterology* 90, 1349, 1986.
- Brown BP, Schulze-Delrieu K, Schrier JE, Abu-Yousef MM. The configuration of the human gastroduodenal junction in the separate emptying of liquids and solids. *Gastroenterology* 105, 433-40, 1993.
- Brown-Cartwright D, Smith HJ, Feldman M. Gastric emptying of an indigestible solid in patients with end-stage renal disease on continuous ambulatory peritoneal dialysis. *Gastroenterology* 95, 49-51, 1988.
- Bueno L, Ferre JP, Ruckebusch Y. Effects of anesthesia and surgical procedures on intestinal myoelectric activity in rats. *American Journal of Digestive Diseases* 23, 690-5, 1978.
- Bueno L, Ruckebusch Y. Insulin and jejunal electrical activity in dogs and sheep. *American Journal of Physiology* 230, 1538-44, 1976.
- Bult H, Boeckxstaens GE, Pelckmans RA, Jordaens FH, Van Maercke YM, Herman AG. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature (London)* 345, 346-7, 1990.
- Burns J, Fox SM. The use of barium meal to evaluate total gastric emptying time in the dog. *Veterinary Radiology* 27, 169-72, 1986.
- Campbell G. The inhibitory nerve fibres in the vagal supply in the guinea-pig stomach. *Journal of Physiology (London)* 185, 600-12, 1966.
- Cann PA, Read NW, Cammack J, Childs H, Holden S, Kashman R, Longmore J, Nix S, Simms N, Swallow K, Weller J. Psychological stress and passage of a standard meal through the stomach and small intestine in man. *Gut* 24, 236-40, 1983.
- Cannon WB. The movements of the stomach studied by means of Roentgen rays. *American Journal of Physiology* 1, 359-82, 1898.
- Cannon WB, Leib CW. The receptive relaxation of the stomach. *American Journal of Physiology* 29, 267-73, 1911.

- Carlson GM, Bedi BS, Code CF. Mechanism of propagation of intestinal interdigestive myoelectric complex. *American Journal of Physiology* 222, 1027-30, 1972.
- Carlson HC, Code CF, Nelson RA. Motor action of the canine gastroduodenal junction: A cineradiographic, pressure, and electric study. *American Journal of Digestive Diseases* 11, 155-72, 1966.
- Cataland S, Crockett SE, Brown JC, Mazzaferri EL. Gastric inhibitory polypeptide (GIP) stimulation by oral glucose in man. *Journal of Clinical Endocrinology and Metabolism* 39, 223-8, 1974.
- Chakder S, Rattan S. Release of nitric oxide by activation of nonadrenergic noncholinergic neurons of internal anal sphincter. *American Journal of Physiology* 264, G7-12, 1993.
- Chastain CB. Unusual manifestations of hypothyroidism in dogs. In: Kirk RW, Bonagura JD (eds). *Kirk's Current Veterinary Therapy XI (Small Animal Practice)*. Pp 330-4. WB Saunders, Philadelphia, 1992.
- Chaudhuri TK, Fink S. Gastric emptying in human disease states. *American Journal of Gastroenterology* 86, 533-8, 1991.
- Chung SA, Valdez DT, Diamant NE. Adrenergic blockade does not restore the canine gastric migrating motor complex during vagal blockade. *Gastroenterology* 103, 1491-7, 1992.
- Clarke RJ, Alexander-Williams J. The effect of preserving antral innervation and of a pyloroplasty in gastric emptying after vagotomy in man. *Gut* 14, 300-7, 1973.
- Code CF, Marlett JA. Canine tachygastria. *Mayo Clinic Proceedings* 49, 325-32, 1974.
- Code CF, Marlett JA. The interdigestive myoelectric complex of the stomach and small bowel of dogs. *Journal of Physiology (London)* 246, 289-309, 1975.
- Code CF, Schlegel JF. The motility correlates of the interdigestive myoelectric complex: A new motor pattern of the small bowel (abstract). *Rendiconti* 5, 151-2, 1973.
- Collins PJ, Horowitz M, Chatterton BE. Attenuation correction and lag period in gastric emptying (letter). *Journal of Nuclear Medicine* 27, 867-8, 1986.
- Collins PJ, Horowitz M, Cook DJ, Harding Pe, Shearman DJC. Gastric emptying in normal subjects- a reproducible technique using a single scintillation camera and computer system. *Gut* 24, 1117-25, 1983.

- Collins PJ, Houghton LA, Read NW, Horowitz M, Chatterton BE, Heddle R, Dent J. Role of the proximal and distal stomach in mixed solid and liquid meal emptying. *Gut* 32, 615-9, 1991.
- Collins SM, Lewis TD, Track N, Fox J, Daniel EE. Release of motilin. *Gastroenterology* 74, 1020, 1978.
- Colmer MR, Owen GM, Shields R. Patterns of gastric emptying after vagotomy and pyloroplasty. *British Medical Journal* ii, 448-50, 1973.
- Connell AM, Waters TE. Assessment of gastric function by pH telemetering capsules. *Lancet* ii, 227-30, 1964.
- Corinaldesi R, Bolondi L, Stanghellini BV, Barbara L. Comparison between radioisotopic and ultrasonographic techniques in the evaluation of gastric emptying of solids. In: Abstract book of the 11th International Symposium on Gastrointestinal Motility, p 171, Oxford, 1987.
- Cormack DH. The digestive system. In: *Introduction to Histology*. Pp 285-321. JB Lippincott, Philadelphia, 1984.
- Cortot A, Phillips SF, Malagelada JR. Gastric emptying of lipids after ingestion of an homogenized meal. *Gastroenterology* 76, 939-44, 1979.
- Cowles VE, Sarna SK. Relation between small intestinal motor activity and transit in secretory diarrhea. *American Journal of Physiology* 259, G420-9, 1990.
- Crider JO, Thomas JE. A study of gastric emptying with the pylorus open. *American Journal of Digestive Diseases* 4, 295-300, 1937.
- Cunningham KM, Daly J, Horowitz M, Read NW. Gastrointestinal adaptation to diets of differing fat composition in human volunteers. *Gut* 32, 483-6, 1991b.
- Cunningham KM, Horowitz M, Read NW. The effect of short-term dietary supplementation with glucose on gastric emptying in humans. *British Journal of Nutrition* 65, 15-19, 1991a.
- Dalziel HH, Thornbury KD, Ward SM, Sanders KM. Involvement of nitric oxide synthetic pathway in inhibitory junction potentials in canine proximal colon. *American Journal of Physiology* 260, G789-92, 1991.
- Dapoigny M, Bommelaer G, Bonnafous J, Kwiatowski F, Veyre A, Tournut R. Measure de l'evacuation gastrique par echographie. *Gastroenterologia* 2, 189-90, 1988.
- Davis SS, Hardy JG, Taylor MJ, Walley DR, Wilson CG. The effects of food on the gastrointestinal transit of pellets and an osmotic device (Osmet). *International Journal of Pharmaceutics* 21, 331-40, 1984.

- Debas HT, Farooq O, Grossman MI. Inhibition of gastric emptying is a physiological action of cholecystokinin. *Gastroenterology* 68, 1211-7, 1975.
- Delbro D, Lisander B, Anderson SA. Atropine-sensitive gastric smooth muscle excitation by mucosal nociceptive stimulation- The involvement of an axon reflex? *Acta Physiologica Scandinavica* 122, 621-7, 1984.
- Depoortere T, Peeters TL, Matthijs G, Cachet T, Hoogmartens J, Vantrappen G. Structure-activity relation of erythromycin-related macrolides in inducing contractions and in displacing bound motilin in rabbit duodenum. *Journal of Gastrointestinal Motility* 1, 150-9, 1989.
- Depoortere I, Peeters TL, Vantrappen G. The erythromycin derivative EM-523 is a potent motilin agonist in man and in rabbit. *Peptides* 11, 515-9, 1990.
- Devane SP, Ravelli AM, Bisset WM, Smith VV, Lake BD, Milla PJ. Gastric antral dysrhythmias in children with chronic idiopathic intestinal pseudoobstruction. *Gut* 33, 1477-81, 1992.
- De Wever I, Eeckhout C, Vantrappen G, Hellemans J. Disruptive effect of test meals on interdigestive motor complex in dogs. *American Journal of Physiology* 235, E661-5, 1978.
- Dozois RR, Kelly KA. Effect of a gastrin pentapeptide on canine gastric emptying of liquids. *American Journal of Physiology* 221, 113-7, 1971.
- Dubois A. Mechanical gastrointestinal obstruction and paralytic ileus. In: Kumar D, Gustavsson S (eds). *An illustrated guide to gastrointestinal motility*. Pp 365-82. John Wiley, Chichester, 1988.
- Dyce KM, Sack WO, Wensing CJG. The abdomen of the carnivores. In: *Textbook of Veterinary Anatomy*. Pp 411-28. WB Saunders, Philadelphia, 1987.
- Edelbroek M, Horowitz M, Fraser R, Wishart J, Morris H, Dent J, Akkermans L. Adaptive changes in the pyloric motor response to intraduodenal dextrose in normal subjects. *Gastroenterology* 103, 1754-61, 1992.
- Eeckhout C, Dewever I, Peeters T, Hellemans J, Vantrappen G. Role of gastrin and insulin in postprandial disruption of migration complex in dogs. *American Journal of Physiology* 235, E666-9, 1978.
- Eeckhout C, DeWever I, Vantrappen G. Intestinal motility after infusion of arachis oil into duodenum and ileum of dogs. *Digestive Diseases and Sciences* 29, 164-70, 1984.
- Eeckhout C, DeWever I, Vantrappen G, Hellemans J. Local disorganization of the interdigestive migrating motor complex (MMC) by perfusion of a Thiry-Vela loop. *Gastroenterology* 76, 1127, 1979.

- Ehrlein HJ, Heisenger E. Computer analysis of mechanical activity of gastroduodenal junction in unanaesthetized dogs. *Journal of Experimental Psychology* 67, 17-29, 1982.
- Ellenport CR. Canine digestive system. In: Getty R (ed). *Sisson and Grossman's The Anatomy of the Domestic Animals* (5th edition). Pp 1538-58. WB Saunders, Philadelphia, 1975.
- El Sharkawy TY, Szurszewski JH. Modulation of canine antral circular muscle by acetylcholine, noradrenaline and pentagastrin. *Journal of Physiology (London)* 279, 309-20, 1978.
- Evans HE, Christensen GC. Alimentary canal. In: Evans HE, Christensen GC (eds) *Miller's anatomy of the dog* (2nd edition). Pp 455-506. WB Saunders, Philadelphia, 1979.
- Ewald CA, Boas J. Beitrage zur Physiologie und Pathologie der Verdauung. *Virchows Archiv* 101, 325-75, 1885.
- Ewald CA, Boas J. *Archives of Pathology, Anatomy, Physiology and Clinical Medicine*. 104, 1886.
- Ewe K, Press AG, Dederer W. Gastrointestinal transit of undigestible solids measured by metal detector EAS II. *European Journal of Clinical Investigation* 19, 291-7, 1989.
- Fahrenkrug J, Galbo H, Holst JJ, Schaffalitzky de Muckadell OB. Influence of the autonomic nervous system on the release of vasoactive intestinal polypeptide from the porcine gastrointestinal tract. *Journal of Physiology (London)* 280, 405-22, 1978.
- Falko JM, Corckett SE, Cataland S, Mazzaferri EL. Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man. *Journal of Clinical Endocrinology and Metabolism* 41, 260-5, 1975.
- Feldman M, Smith HJ. Effect of cisapride on gastric emptying of indigestible solids in patients with gastroparesis diabeticorum. A comparison with metoclopramide and placebo. *Gastroenterology* 92, 171-4, 1987.
- Feldman M, Smith HJ, Simon TR. Gastric emptying of solid radiopaque markers: Studies in healthy subjects and diabetic patients. *Gastroenterology* 87, 895-902, 1984.
- Feng TP, Hou HC, Lim RKS. On the mechanism of the inhibition of gastric secretion by fat. *Chinese Journal of Physiology* 3, 371-8, 1929.
- Fisher RS, Lipshutz W, Cohen S. The hormonal regulation of pyloric sphincter function. *Journal of Clinical Investigation* 52, 1289-96, 1973.

- Fone DR, Horowitz M, Read NW, Dent J, Maddox A. The effect of terminal ileal triglyceride infusion on gastroduodenal motility and the intragastric distribution of a solid meal. *Gastroenterology* 98, 568-75, 1990.
- Fox S, Behar J. Pathogenesis of diabetic gastroparesis: A pharmacologic study. *Gastroenterology* 78, 757-63, 1980.
- Fraser R, Fone D, Horowitz M, Dent J. Cholecystokinin octapeptide stimulates phasic and tonic pyloric motility in healthy humans. *Gut* 34, 33-7, 1993.
- Furness JB, Costa M. The adrenergic innervation of the gastrointestinal tract. *Physiological, Biological, Chemical and Experimental Pharmacology*. 69, 1-51, 1974.
- Galil MA, Critchley M, Mackie CR. Isotope gastric emptying tests in clinical practice: expectation, outcome, and utility. *Gut* 34, 916-9, 1993.
- Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Measurement of gastric emptying rate of solids by means of carbon-labeled octanoic acid breath test. *Gastroenterology* 104, 1640-7, 1993.
- Glise H, Lindahl B-O, Abrahamsson H. Reflex adrenergic inhibition of gastric motility by nociceptive intestinal stimulation and peritoneal irritation in the cat. *Scandinavian Journal of Gastroenterology* 15, 673-81, 1980.
- Go VLW, Hoffinan AF, Summerskill WHJ. Simultaneous measurements of total pancreatic, biliary, and gastric outputs in man using a perfusion technique. *Gastroenterology* 58, 321-8, 1970.
- Gray G. Carbohydrate digestion and absorption. *Gastroenterology* 58, 96-107, 1970.
- Grider JR, Camble MB, Said SI, Makhoul GM. Vasoactive intestinal peptide as a neural mediator of gastric relaxation. *American Journal of Physiology* 248, G73-78, 1985.
- Grider JR, Murthy KS, Jin J-G, Makhoul GM. Stimulation of nitric oxide from muscle cells by VIP: prejunctional enhancement of VIP release. *American Journal of Physiology* 262, G774-8, 1992.
- Griffith GH, Owen GM, Kirkman S, Shields R. Measurement of rate of gastric emptying using chromium-51. *Lancet* i, 1244-5, 1966.
- Grivel ML, Ruckebusch Y. The propagation of segmental contractions along the small intestine. *Journal of Physiology (London)* 227, 611-25, 1972.
- Grundy D, Gharib-Naseri MK, Hutson D. Effect of immunisation against vasoactive intestinal polypeptide on gastric corpus tone and motility in the ferret. *Gut* 33, 1473-6, 1992.

- Gué M, Peeters T, Depoortere I, Vantrappen F, Bueno L. Stress-induced changes in gastric emptying, postprandial motility, and plasma gut hormone levels in dogs. *Gastroenterology* 97, 1101-7, 1989.
- Guilford WG. The enteric nervous system: function, dysfunction, and pharmacological manipulation. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 5, 46-56, 1990.
- Haba T, Sarna SK. Regulation of gastroduodenal emptying of solids by gastropyloroduodenal contractions. *American Journal of Physiology* 264, G261-71, 1993.
- Haley TJ. Pharmacology and toxicology of the rare earth elements. *Journal of Pharmaceutical Sciences* 54, 663-9, 1965.
- Hall KE, El-Sharkawy TY, Diamant NE. Vagal control of migrating motor complex in the dog. *American Journal of Physiology* 243, G276-84, 1982.
- Hausken T, Odegaard S, Matre K, Berstad A. Antroduodenal motility and movements of luminal contents studied by duplex sonography. *Gastroenterology* 102, 1583-90, 1992.
- Heading RC, Nimmo J, Prescott LF, Tothill P. The dependence of paracetamol absorption on the rate of gastric emptying. *British Journal of Pharmacology* 47, 415-21, 1973.
- Heddle R, Fone D, Dent J, Horowitz M. Stimulation of pyloric motility by intraduodenal dextrose in normal subjects. *Gut* 29, 1349-57, 1988.
- Hertage ME, Dennis R. Contrast media. In: Lee R (ed). *Manual of Radiography and Radiology in Small Animal Practice*. Pp 218-222. British Small Animal Veterinary Association, Cheltenham, 1989.
- Hiele Mi, Ghoo YF, Wensing C, Rutgeerts PJ, Vantrappen G. The effect of erythromycin and propantheline on gastric emptying rate as measured by the ¹⁴C octanoic acid breath test (abstract). *Gastroenterology* 100, A83, 1991.
- Hinder RA, Kelly KA. Human gastric pacesetter potential. *American Journal of Surgery* 133, 29-33, 1977a.
- Hinder RA, Kelly KA. Canine gastric emptying of solids and liquids. *American Journal of Physiology* 233, E335-40, 1977b.
- Holt S, Colliver J, Guram M, Neal C, Verhulst SJ, Taylor TV. Measurement of gastric emptying rate in humans. Simplified scanning method. *Digestive Diseases and Sciences* 35, 1345-51, 1990.
- Holt S, Ford MJ, Grant S, Heading RC. Abnormal gastric emptying in primary anorexia nervosa. *British Journal of Psychiatry* 139, 550-2, 1981.

- . Holt S, McDicken WN, Anderson T, Stewart IC, Heading RC. Dynamic imaging of the stomach by real time ultrasound- a method for the study of gastric motility. *Gut* 21, 597-601, 1980.
- . Hornof WJ, Koblik PD, Strombeck DR, Morgan JP, Hansen G. Scintigraphic evaluation of solid-phase gastric emptying in the dog. *Veterinary Radiology* 30, 242-8, 1989.
- . Horowitz M, Collins PJ, Cook DJ, Harding PE, Shearman DJC. Abnormalities of gastric emptying in obese patients. *International Journal of Obesity* 7, 415-21, 1983.
- . Horowitz M, Jones K, Edelbroek MAL, Smout AJPM, Read NW. The effect of posture on gastric emptying and intragastric distribution of oil and aqueous meal components and appetite. *Gastroenterology* 105, 382-90, 1993.
- . Horowitz M, McNeil JD, Maddern GJ, Collins PJ, Shearman DJC. Abnormalities of gastric and oesophageal emptying in polymyositis and dermatomyositis. *Gastroenterology* 90, 434-9, 1986.
- . Houghton LA, Read NW. A comparative study of the effect of cimetidine and ranitidine on the rate of gastric emptying of liquid and solid test meals in man. *Alimentary Pharmacology and Therapy* 1, 401-8, 1987.
- . Houghton LA, Read NW, Heddle R, Horowitz M, Collins PJ, Chatterton B, Dent J. Relationship of the motor activity of the antrum, pylorus, and duodenum to gastric emptying of a solid-liquid mixed meal. *Gastroenterology* 94, 1285-91, 1988a.
- . Houghton LA, Read NW, Heddle R, Maddern GJ, Downton J, Toouli T, Dent J. Motor activity of the gastric antrum, pylorus, and duodenum under fasted conditions and after a liquid meal. *Gastroenterology* 94, 1276-84, 1988b.
- . Hunt JN. Modification to the method of George for evaluation of gastric emptying. *Gut* 15, 812-3, 1974.
- . Hunt JN, Knox MT. The slowing of gastric emptying by nine acids. *Journal of Physiology (London)* 201, 161-79, 1969.
- . Hunt JN, Ramsbottom N. Effect of gastrin II on gastric emptying and secretion during a test meal. *British Medical Journal* 4, 386-7, 1967.
- . Hurwitz A. Measuring gastric volume by dye dilution. *Gut* 22, 85-93, 1981.
- . Hutson WR, Roehrkasse RL, Wald A. Influence of gender and menopause on gastric emptying and motility. *Gastroenterology* 96, 11-7, 1989.
- . Hyser CI, Mendell JR. Recent advances in Duchenne and Becker muscular dystrophy. *Neurologic Clinics* 6, 429-53, 1988.

- Iggo A. Tension receptors in the stomach and the urinary bladder. *Journal of Physiology (London)* 128, 593-607, 1955.
- Itoh Z, Nayaka M, Suzuki T, Arai H, Wakabayashi K. Erythromycin mimics exogenous motilin in gastrointestinal contractile activity in the dog. *American Journal of Physiology* 247, G688-94, 1984.
- Itoh Z, Takayanagi R, Takeuchi S, Isshiki s. Interdigestive motor activity of Heidenhain pouches in relation to main stomach in conscious dogs. *American Journal of Physiology* 234, E333-8, 1978b.
- Itoh Z, Takeuchi S, Aizawa I, Mori K, Taminato T, Seino Y, Imura H, Yanashara N. Changes in plasma motilin concentration and gastrointestinal contractile activity in conscious dogs. *Digestive Diseases and Sciences* 23, 929-35, 1978a.
- Kealy JK. The Abdomen. In: Kealy JK (ed) *Diagnostic radiology of the dog and cat*. Pp 9-145. WB Saunders Company, Philadelphia, 1979.
- Kelly KA. Gastric emptying of liquids and solids: roles of proximal and distal stomach. *American Journal of Physiology* 239, G71-6, 1980.
- Kelly KA, Code CF. Effect of transthoracic vagotomy on canine gastric electrical activity. *Gastroenterology* 57, 51-8, 1969.
- Kelly KA, Code CF. Canine gastric pacemaker. *American Journal of Physiology* 220, 112-8, 1971.
- Kihl B. Studies on the mechanisms by which fat inhibits gastric acid secretion in dog and man. Thesis. Pp 1-36. Department of Surgery, University of Gothenburg, Sweden, 1980.
- Kim CH, Azpiroz F, Malagelada J-R. Characteristics of spontaneous and drug-induced gastric dysrhythmias in a chronic canine model. *Gastroenterology* 90, 421-7, 1986.
- King PM, Adam RD, Pryde A, McDicken WN, Heading RC. Relationships of human antroduodenal motility and transpyloric fluid movement: Non-invasive observations with real-time ultrasound. *Gut* 25, 1384-91, 1984.
- Kleibeuker JH, Beekhuis H, Jansen JBMJ, Piers DA, Lamers CBHW. Cholecystokinin is a physiologic hormonal mediator of fat-induced inhibition of gastric emptying. *European Journal of Clinical Investigation* 18, 173-7, 1988.
- Kohn CW. Intestinal obstruction. In: Anderson NV (ed). *Veterinary gastroenterology (second edition)*. Pp 173-210. Lea and Febiger, Philadelphia, 1992.

- Kosaka T, Lim RKS. On the mechanism of the inhibition of gastric secretion by fat: the role of bile and cystokinin. *Chinese Journal of Physiology* 4, 213-20, 1930.
- Kroop HS, Long WB, Alavi A, Hansell JR. Effect of water and fat on gastric emptying of solid meals. *Gastroenterology* 77, 997-1000, 1979.
- Kumar D, Ritman EL, Malagelada J-R. Antral volume and propulsion-retropulsion in response to upper intestinal nutrients in a canine model. *American Journal of Physiology* 264, G1077-81, 1993.
- Lambert GA, Lang WJ, Friedman E, Meller E. Pharmacological and biochemical properties of isomeric yohimbine alkaloids. *European Journal of Pharmacology* 49, 39-48, 1978.
- Lee K, Chang TM, Chey W. Effect of rabbit anti-motilin serum on myoelectric activity and plasma motilin concentration in fasting dog. *American Journal of Physiology* 245, G547-53, 1983.
- Lee KY, Chey WY, Yagima H. Radioimmunoassay of motilin: Validation of studies on the relationship between plasma motilin and interdigestive myoelectric activity of the duodenum of dog. *American Journal of Digestive Diseases* 23, 789-95, 1978.
- Lefebvre RA, Sas S, Cauvin A. Relaxant effect of rat PHI, PHI-gly and PHV (1-42) in the rat gastric fundus. *Peptides* 12, 271-4, 1991.
- Leib MS, Wingfield WE, Twedt DC, Williams AR. Gastric emptying of liquids in the dog: Effect of volume. *American Journal of Veterinary Research* 47, 1019-21, 1986.
- Levine RR. Factors affecting gastrointestinal absorption of drugs. *American Journal of Digestive Diseases* 15, 171-88, 1970.
- Lewis LD, Morris ML, Hand MS. Nutrients. In: *Small Animal Clinical Nutrition III*. Pp 1-1 - 1-25. Mark Morris Associates, Kansas, 1987.
- Liddle R, Gertz B, Kanayama S, Beccaria L, Coker L, Turnbull T. Effects of a novel cholecystokinin (CCK) antagonist, MK-39, on gallbladder contraction and gastric emptying in humans. *Journal of Clinical Investigation* 84, 1220-5, 1989.
- Liddle RA, Morita ET, Conrad CK, Williams JA. Regulation of gastric emptying in humans by cholecystokinin. *Journal of Clinical Investigation* 77, 992-6, 1986.
- Lin HC, Doty JE, Reedy TJ, Meyer JH. Inhibition of gastric emptying by glucose depends on length of intestine exposed to the nutrient. *American Journal of Physiology* 256, G204-11, 1989.

- Lin HC, Kim BH, Elashoff JD, Doty JE, Gu Y-G, Meyer JH. Gastric emptying of solid food is most potently inhibited by carbohydrate in the canine distal ileum. *Gastroenterology* 102, 703-801, 1993.
- Lin HC, Moller NA, Wolinsky MM, Kim BH, Doty JE, Meyer JH. Sustained slowing effect of lentils on gastric emptying of solids in humans and dogs. *Gastroenterology* 102, 787-92, 1992.
- Lind JF, Duthie HI, Schlegel JF, Code CF. Motility of the gastric fundus. *American Journal of Physiology* 201, 197-202, 1961.
- Lloyd KCK, Maxwell V, Ohning G, Walsh JH. Intestinal fat does not inhibit gastric function through a hormonal somatostatin mechanism in dogs. *Gastroenterology* 103, 1221-8, 1992.
- Loo FD, Palmer DW, Soergel KH, Kalbfleisch JH, Wood SM. Gastric emptying in patients with diabetes mellitus. *Gastroenterology* 86, 485-94, 1984.
- Madsen JL. Effects of gender, age, and body mass index on gastrointestinal transit times. *Digestive Diseases and Sciences* 37, 1548-53, 1992.
- Malagelada J-R, Carter SE, Brown ML, Carlson GL. Radiolabeled fiber- A physiologic marker for gastric emptying and intestinal transit of solids. *Digestive Diseases and Sciences* 25, 81-7, 1980b.
- Malagelada J-R, Rees WDW, Mazzotta LJ, Go VLW. Gastric motor abnormalities in diabetic and postvagotomy gastroparesis: Effect of metoclopramide and bethanecol. *Gastroenterology* 78, 286-93, 1980a.
- Malmud LS, Long WB. Radionuclide studies of gastric emptying. In: Alavi A, Arger PH (eds). *Multiple Imaging Procedures*. 3. Abdomen. Pp 87-96. Grune and Stratton, New York, 1980.
- Mangnall YF, Kerrigan DD, Johnson AG, Read NW. Applied potential tomography: Noninvasive method for measuring gastric emptying of a solid test meal. *Digestive Diseases and Sciences* 36, 1680-4, 1991.
- Marik R, Code CF. Control of the interdigestive myoelectric activity in dogs by the vagus nerves and pentagastrin. *Gastroenterology* 69, 387-95, 1975.
- Marlett JA, Code CF. Effects of celiac and superior mesenteric ganglionectomy on interdigestive myoelectric complex in dogs. *American Journal of Physiology* 237, E432-6, 1979.
- Marzio I, Giacobbe A, Conoscitore P, Facciorusso D, Frusciante V, Modoni S. Evaluation of the use of ultrasonography in the study of liquid gastric emptying. *American Journal of Gastroenterology* 84, 496-500, 1989.

- McHugh PR, Moran TH. Calories and gastric emptying: A regulatory capacity with implications for feeding. *American Journal of Physiology* 236, R254-60, 1979.
- McSwiney BA. Innervation of the stomach. *Physiological Reviews* 11, 478-514, 1931.
- Melo JD-C, Summers RW, Thompson HH, Wingate DL, Yanda R. Effects of intestinal secretagogues and distension on small bowel myoelectric activity in fasted and fed conscious dogs. *Journal of Physiology (London)* 321, 483-94, 1981.
- Melone J, Mei N. Intestinal effects of the products lipid digestion on gastric electrical activity in the cat. Possible involvement of vagal intestinal receptors sensitive to lipids. *Gastroenterology* 100, 380-7, 1991.
- Meyer JH. Motility of the Stomach and Gastroduodenal Junction. In: Johnson LR (ed). *Physiology of the Gastrointestinal Tract* (2nd edition). Raven Press, New York, 1987.
- Meyer BM, Beglinger C, Jansen JBMJ, Rovati LC, Werth BA, Hilderbrand P. Role of cholecystinin in regulation of gastrointestinal motor functions. *Lancet* i, 12-5, 1989a.
- Meyer B, Beglinger C, Neumayer M, Stalder GA. Physical characteristics of indigestible solids affect emptying from the fasting human stomach. *Gut* 30, 1526-9, 1989b.
- Meyer JH, Dressman J, Fink A, Amidon G. Effect of size and density on canine gastric emptying of nondigestible solids. *Gastroenterology* 89, 805-13, 1985.
- Meyer JH, Elashoff J, Porter-Fink V, Dressman J, Amidon GL. Human postprandial gastric emptying of 1-3 millimeter spheres. *Gastroenterology* 94, 1315-25, 1988.
- Meyer JH, Gu Y, Elashoff T, Reedy J, Dressman JB, Amidon GL. Effects of viscosity and fluid outflow on postcibal gastric emptying of solids. *American Journal of Physiology* 250, G161-4, 1986.
- Meyer J, Jones R. Canine pancreatic responses to intestinally perfused fat and products of fat digestion. *American Journal of Physiology* 225, 1178-87, 1974.
- Meyer JH, MacGregor IL, Martin P, Cavalieri R. ^{99m}Tc tagged chicken liver: a new method for measuring the rate of gastric emptying of solid food. *American Journal of Digestive Diseases* 21, 296-304, 1976.
- Meyer JH, Thomson JB, Cohen MB, Shadchehr A, Mandiola SA. Sieving of solid food by the canine stomach and sieving after gastric surgery. *Gastroenterology* 76, 804-13, 1979.

- Miller J, Kauffman G, Elashoff J, Ohashi H, Carter D, Meyer JH. Search for resistances controlling canine gastric emptying of liquid meals. *American Journal of Physiology* 241, G403-15, 1981.
- Miolan JP, Roman C. Modification de l'electromyogramme gastrique du chien par stimulation des nerfs extrinsiques. *Journal de Physiologie (Paris)* 63, 561-6, 1971.
- Miolan JP, Roman C. Decharge unitaire des fibres vagales efferentes lors de la relaxation receptive de l'estomac du chien. *Journal de Physiologie (Paris)* 66, 693-704, 1974.
- Mir SS, Telford GL, Mason GR, Ormsbee HS. Noncholinergic, nonadrenergic inhibitory innervation of the canine pylorus. *Gastroenterology* 76, 1443-8, 1979.
- Miyabayashi T, Morgan JP. Gastric emptying in the normal dog. *Veterinary Radiology* 25, 187-91, 1984.
- Mizumoto A, Sano I, Matsunaga Y, Yamamoto, Itoh Z, Ohshima K. Mechanism of motilin-induced contractions in isolated perfused canine stomach. *Gastroenterology* 105, 425-32, 1993.
- Mogard MH, Maxwell V, Wong H, Reedy TJ, Sytnik B, Walsh JH. Somatostatin may not be a hormonal messenger of fat-induced inhibition of gastric functions. *Gastroenterology* 94, 405-8, 1988.
- Mojaverain P, Ferguson RK, Vlasses PH, Rocci ML, Oren A, Fix JA, Caldwell LJ, Gardner C. Estimation of gastric residence time of the Heidelberg capsule in humans: effect of varying food composition. *Gastroenterology* 89, 392-7, 1985.
- Morgan KG, Schmalz PF, Go VLW, Szurszewski JH. Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle. *American Journal of Physiology* 235, E324-9, 1978.
- Mroz CT, Kelly KA. The role of the extrinsic antral nerves in the regulation of gastric emptying. *Surgery, Gynecology and Obstetrics* 145, 369-77, 1977.
- Mukhopadhyay AK, Thor PJ, Copeland EM, Johnson LR, Weisbrodt NW. Effect of cholecystokinin on myoelectric activity of small bowel of the dog. *American Journal of Physiology* 232, E44-7, 1977.
- Nakazato Y, Saito K, Ohga A. Gastric motor and inhibitor response to stimulation of the sympathetic nerve in the dog. *Japanese Journal of Pharmacology* 11, 131-141, 1970.
- Nelsen TS, Kohatsu S. The stomach as a pump. *Rendiconti Romani di Gastroenterologia* 3, 65-70, 1971.

- New Zealand Department of Health. Code of safe practice for the use of X-rays in diagnosis (veterinary). National Radiation Laboratory, Christchurch, 1984.
- Nightingale JMD, Kamm MA, van der Sijp JRM, Morris GP, Walker ER, Mather SJ, Britton KE, Lennard-Jones JE. Disturbed gastric emptying in the short bowel syndrome. Evidence for a 'colonic brake'. *Gut* 34, 1171-6, 1993.
- O'Brien TR. Stomach. In: O'Brien TR (ed) *Radiographic diagnosis of abdominal disorders in the dog and cat*. Pp 204-278. WB Saunders Company, Philadelphia, 1978.
- Okike N, Kelly KA. Vagotomy impairs pentagastrin-induced relaxation of the canine gastric fundus. *American Journal of Physiology* 232, E504-9, 1977.
- Ormsbee HS, Mir SS. The role of the cholinergic nervous system in the gastrointestinal response to motilin in vivo. In: Duthie HL, Lancaster PA (eds). *Gastrointestinal Motility in Health and Disease*. Pp 113-22. MTP Press, Lancaster, 1978.
- Owens JM. The gastrointestinal system. In: Biery DN (ed). *Radiographic interpretation for the small animal clinician*. Pp 135-171. Ralston Purina, Saint Louis, 1982.
- Paintal AS. A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. *Journal of Physiology (London)* 126, 255-70, 1954.
- Pearce EA, Wingate DC. The role of the myenteric plexuses. *Gastroenterology* 76, 1215, 1979.
- Pearcy JF, van Liere EJ. Studies on the visceral nervous system. *American Journal of Physiology* 78, 64-73, 1926.
- Peeters TL, Matthijs G, Depoortere I, Cachet T, Hoogmartens J, Vantrappen G. Erythromycin is a motilin receptor agonist. *American Journal of Physiology* 257, G470-4, 1989.
- Poitras P. Motilin is a digestive hormone in the dog. *American Journal of Physiology* 87, 909-13, 1984.
- Poitras P, Steinbach J, Van Deventer G, Code C, Walsh J. Motilin independent ectopic fronts of the interdigestive myoelectric complex in dogs. *American Journal of Physiology* 239, G215-20, 1980.
- Pozzi M, Rivolta M, Gelosa M et al. Upper gastrointestinal involvement in diabetes mellitus: Study of esophagogastric function. *Acta Diabetologica Latina* 25, 333-41, 1988.

Prescott LF. Gastric emptying and drug absorption. *British Journal of Clinical Pharmacology* 1, 189-90, 1974.

Prove J, Ehrlein H-J. Motor function of gastric antrum and pylorus for evacuation of low and high viscosity meals in dogs. *Gut* 23, 150-6, 1982.

Read NW, McFarlane A, Kinsman I, Bates TE, Blackhall NW, Farrar GBJ, Hall JC, Moss G, Morris AP, O'Neill B, Welch I, Lee Y, Bloom SR. Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon. *Gastroenterology* 86, 274-80, 1984.

Reele SB, Ruwart MJ, Noah ML. Gastrointestinal propulsion measurement with radiopaque capsules during 16, 16- dimethyl PGE2 infusion. *Prostaglandins, Leukotrienes and Medicine* 9, 629-40, 1982.

Ricci R, Bontempo I, Corazziari E, La Bella A, Torsoli A. Real time ultrasound of the gastric antrum. *Gut* 34, 173-6, 1993.

Ruckebusch Y, Bardon T. The cyclic motor activity of the ovine gut: Its reset at a faster rhythm. *Experientia* 40, 819-20, 1984.

Ruckebusch Y, Bueno L. Migrating myoelectrical complex of the small intestine. *Gastroenterology* 73, 1309-14, 1977.

Russell J, Bass P. Canine gastric emptying of fiber meals: influence of meal viscosity and antroduodenal motility. *American Journal of Physiology* 249, G662-7, 1985a.

Russell J, Bass P. Canine gastric emptying of polycarbophil: An indigestible, particulate substance. *Gastroenterology* 89, 307-12, 1985b.

Russell J, Bass P, Shimizu M, Miyauchi A, Go VLW. Canine intestinal ulcer: Myoelectric components and the effect of chronic hypergastrinaemia. *Gastroenterology* 82, 746-52, 1982.

Sagar S, Grime JS, Little W, Patten M, Gulliford P, Critchley M, Bennett R, Shields R. Technetium-99m labelled bran: A new agent for measuring gastric emptying. *Clinical Radiology* 34, 275-8, 1983.

Sanders KM, Vogalis F. Organization of electrical activity in the canine pyloric canal. *Journal of Physiology (London)* 416, 49-66, 1989.

Sarna SK, Bowes KL, Daniel EE. Gastric pacemakers. *Gastroenterology* 70, 225-31, 1976.

Sarna S, Condon RE, Cowles V. Enteric mechanisms of initiation of migrating myoelectric complexes in dogs. *Gastroenterology* 84, 814-22, 1983b.

Sarna S, Condon RE, Cowles V. Morphine versus motilin in the initiation of migrating myoelectric complexes. *American Journal of Physiology* 245, G217-20, 1983a.

Sarna SK, Daniel EE. Vagal control of gastric electrical activity and motility. *Gastroenterology* 68, 301-8, 1975.

Sarna SK, Stoddard C, Belbeck LW, McWade D. Intrinsic nervous control of migrating myoelectric complexes. *American Journal of Physiology* 241, G16-23, 1981.

Sarr MG, Kelly KA. Myoelectric activity of the autotransplanted canine jejunioileum. *Gastroenterology* 81, 303-10, 1981.

Scarpignato C. Gastric emptying measurement in man. In: Scarpignato C, Bianchi Porro G (eds). *Clinical Investigation of Gastric Function*. Pp 198-246. Karger, Basel, 1990.

Scatcherd T, Grundy D. Nervous afferents from the upper gastrointestinal tract which influence gastrointestinal motility. In: M. Weinbeck (ed). *Motility of the Digestive Tract*. Raven Press, New York, 1982.

Schang JC, Devroede G. Beneficial effects of naloxone in a patient with intestinal pseudoobstruction. *American Journal of Gastroenterology*. 80, 407-11, 1985.

Schwizer W, Maecke H, Fried M. Measurement of gastric emptying by magnetic resonance imaging. *Gastroenterology* 103, 369-76, 1992.

Seal AM, Meloche RM, Liu YQE, Buchan AMJ, Brown JC. Effects of monoclonal antibodies to somatostatin on somatostatin-induced and intestinal fat-induced inhibition of gastric acid secretion in the rat. *Gastroenterology* 92, 1187-92, 1987.

Semba T, Hiraoka T. Motor response of the stomach and small intestine caused by stimulation of the peripheral end of the splanchnic nerve, thoracic sympathetic trunk and spinal roots. *Japanese Journal of Physiology* 7, 64-71, 1957.

Sheehan FG, Walsh TD, Go R, Beck G, VanLente R, Falk GW. The assessment of solid gastric emptying by acetaminophen absorption: Comparison with a standard radionuclide technique. *Gastroenterology* 98, A391, 1990.

Sirois PJ, Amidon GL, Meyer JH, Doty J, Dressman JB. Gastric emptying of nondigestible solids in dogs: a hydrodynamic model. *American Journal of Physiology* 258, G65-72, 1990.

Smith H, Feldman M. Influence of food and marker length on gastric emptying of indigestible radiopaque markers in healthy humans. *Gastroenterology* 91, 1452-5, 1986.

- Staadas JO, Aune S. Intra-gastric pressure/volume relationship before and after vagotomy. *Acta Chirurgica Scandinavica* 136, 611-5, 1970.
- Stark ME, Bauer AJ, Szurszewski JH. Effect of nitric oxide on circular muscle of the canine small intestine. *Journal of Physiology (London)* 444, 743-61, 1991.
- Stemper TJ, Cooke AR. Effect of a fixed pyloric opening on gastric emptying in the cat. *American Journal of Physiology* 230, 813-7, 1976.
- Stephen AM, Haddad AC, Phillips SF. Passage of carbohydrate into the colon. *Gastroenterology* 85, 589-95, 1983.
- Stoddard CJ, Smallwood RH, Duthie HI. Electrical arrhythmias in the human stomach. *Gut* 22, 705-12, 1981.
- Strombeck DR, Guilford WG. Chronic gastritis, gastric retention, gastric neoplasms, and gastric surgery. *Small Animal Gastroenterology (second edition)*. Pp 208-27. Stonegate, Davis, California, 1990.
- Strunz UT, Grossman MI. Effect of intra-gastric pressure on gastric emptying and secretion. *American Journal of Physiology* 234, E552-5, 1980.
- Strunz E, Wunsch E, Jaeger E, Demling L. Analysis of motor effects of 13-norleucine motilin on the rabbit, guinea pig, rat, and human alimentary tract. *Gastroenterology* 85, 1290-1300, 1975.
- Szurszewski JH. A migrating electric complex of the canine small intestine. *American Journal of Physiology* 217, 1757-63, 1969.
- Szurszewski JH. Electrical basis of gastrointestinal motility. In: Johnson LR (ed). *Physiology of the Gastrointestinal Tract (1st edition)*. Pp 1435-66. Raven Press, New York, 1981.
- Tack J, Janssens J, Vantrappen G, Peeters T, Annese V, Depoortere I, Muls E, Bouillon R. Effect of erythromycin on gastric motility in controls and in diabetic gastroparesis. *Gastroenterology* 103, 72-9, 1992.
- Tatsuta M, Iishi H, Okuda S. Gastric emptying in patients with fundal gastritis and gastric cancer. *Gut* 31, 767-9, 1990.
- Taylor I, Byrne W, Christie D, Ament M, Walsh J. Effect of individual L-amino acids on gastric acid secretion and serum gastrin and pancreatic polypeptide release in humans. *Gastroenterology* 83, 273-8, 1982.
- Telander RL, Morgan KG, Kruehen DL, Schmalz PF, Kelly KA, Szurszewski JH. Human gastric atony with tachygastria and gastric retention. *Gastroenterology* 75, 497-501, 1978.

- Theodorakis MC. External scintigraphy in measuring rate of gastric emptying in beagles. *American Journal of Physiology* 239, G39-43, 1980.
- Thomas PA, Kelly KA, Go VLW. Does motilin regulate interdigestive gastric motility? *Digestive Diseases and Sciences* 24, 577-82, 1979.
- Thompson DG, Richelson E, Malagelada JR. Perturbation of upper gastrointestinal function by cold stress. *Gut* 24, 277-83, 1984.
- Thor K, Rosell S, Rokaeus A, Kager L. (Gln⁴)-Neurotensin changes the motility pattern of the duodenum and proximal jejunum from a fasting-type to a fed-type. *Gastroenterology* 83, 569-74, 1982.
- Tomomasa T, Kuroume T, Arai H, Wakabayashi K, Itoh Z. Erythromycin induces migrating motor complex in human gastrointestinal tract. *Digestive Diseases and Sciences* 31, 157-61, 1986.
- Touqas G, Anvari M, Richards D, Dent J, Somers S, Stevesnson GW. Relationship of pyloric motility to transpyloric flow in healthy subjects (abstract). *Gastroenterology* 70, 550-5, 1987.
- Valenzuela JE. Effect of intestinal hormones and peptides on intragastric pressure in dogs. *Gastroenterology* 71, 766-9, 1976.
- van den Brom WE, Happe RP. Gastric emptying of a radionuclide-labeled test meal in healthy dogs: A new mathematical analysis and reference values. *American Journal of Veterinary Research* 47, 2170-4, 1986.
- van Sluijs FJ, van den Brom WE. Gastric emptying of a radionuclide-labeled test meal after surgical correction of gastric dilatation-volvulus in dogs. *American Journal of Veterinary Research* 50, 433-5, 1988.
- Vantrappen G, Janssens J, Peeters T, Bloom S, Christofides N, Hellemans J. Motilin and the interdigestive migrating motor complex in man. *Digestive Diseases and Sciences* 24, 497-500, 1979.
- Vogalis R, Sanders KM. Excitatory and inhibitory neural regulation of canine pyloric smooth muscle. *American Journal of Physiology* 259, G125-33, 1991.
- Weber J Jr., Kohatsu S. Pacemaker localization and electrical conduction patterns in the canine stomach. *Gastroenterology* 59, 717-26, 1970.
- Weisbrodt NW. Motility of the small intestine. In: Johnson LR (ed). *Physiology of the Gastrointestinal Tract* (2nd edition). Pp 631-63. Raven Press, New York, 1987.
- Weisbrodt NW, Badial-Aceves F, Copeland EM, Dudrick SJ, Castro GA. Small-intestinal transit during total parenteral nutrition in the rat. *American Journal of Digestive Diseases* 23, 363-9, 1978.

Weisbrodt NW, Copeland EM, Moore EP, Kearley RW, Johnson LR. Effect of vagotomy on electrical activity of the small intestine in the dog. *American Journal of Physiology* 228, 650-4, 1975.

Weisbrodt NW, Copeland EM, Thor PJ, Mukhopadhyay AK, Johnson LR. Nervous and humoral factors which influence the fasted and fed patterns of intestinal myoelectric activity. In: Vantrappen G (ed). *Proceedings of the Fifth International Symposium on Gastrointestinal Motility*. Pp 82-7. Typoff Press, Herentals, Belgium, 1976.

Weisbrodt NW, Wiley JN, Overholt BF, Bass P. A relation between gastroduodenal muscle contractions and gastric emptying. *Gut* 10, 543-8, 1969.

Wilbur BG, Kelly KA. Effect of proximal gastric, complete gastric, and truncal vagotomy on canine gastric electric activity, motility, and emptying. *Annals of Surgery* 178, 295-302, 1973.

Wilbur BG, Kelly KA. Gastrin pentapeptide decreases gastrin transmural pressure. *Gastroenterology* 67, 766-9, 1976.

Wilbur BG, Kelly KA, Code CF. Effect of gastric fundectomy on canine gastric electrical and motor activity. *American Journal of Physiology* 226, 1445-9, 1974.

Willard MD, Mullaney T, Karasek S, Yamini B. Diarrhea associated with myenteric ganglionitis in a dog. *Journal of the American Veterinary Medical Association* 193, 346-8, 1988.

Williams NS, Miller J, Elashoff J, Meyer JH. Canine resistances to gastric emptying of liquids after ulcer surgery. *Digestive Diseases and Sciences* 31, 273-80, 1986.

Williams CL, Peterson JM, Villar G, Burks TF. Corticotrophin-releasing factor directly mediates colonic responses to stress. *American Journal of Physiology* 253, G582-6, 1987.

Wingate DL, Pearce EA, Hutton M, Dand A, Thompson HH, Wunsch E. Quantitative comparison of the effects of cholecystokinin, secretin, and pentagastrin on gastrointestinal myoelectric activity in the conscious fasted dog. *Gut* 19, 593-601, 1978.

Wingate DL, Pearce E, Ling A, Boucher B, Thompson H, Hutton M. Quantitative effect of oral feeding on gastrointestinal myoelectric activity in the conscious dog. *Digestive Diseases and Sciences* 24, 417-23, 1979.

Wise LA, Lappin MR. A syndrome resembling feline dysautonomia (Key-Gaskell syndrome) in a dog. *Journal of the American Veterinary Medical Association* 198, 2103-6, 1991.

Wright RA, Krinsky S, Fleeman C, Trujillo J, Teague E. Gastric emptying and obesity. *Gastroenterology* 84, 747-51, 1983.

You CH, Lee KY, Chey WY, Menguy R. Electrogastrographic study of patients with unexplained nausea bloating and vomiting. *Gastroenterology* 79, 311-4, 1980.

Youle MS, Read NW. Effect of painless rectal distention on gastrointestinal transit of solid meal. *Digestive Diseases and Sciences* 29, 902-6, 1984.

Youmans WB, Meek WJ. Reflex and humoral inhibition in unanaesthetised dogs during rectal stimulation. *American Journal of Physiology* 120, 750-7, 1937.

Youngberg CA, Wlodyga J, Schmaltz S, Dressman JB. Radiotelemetric determination of gastrointestinal pH in four healthy Beagles. *American Journal of Veterinary Research* 46, 1516-21, 1985.

Zeissman HA, Fahey FH, Collen MJ. Biphasic solid and liquid gastric emptying in normal controls and diabetics using continuous acquisition in LAO view. *Digestive Diseases and Sciences* 37, 744-50, 1992.

Zontine WJ. Effect of chemical restraint drugs on the passage of barium sulfate through the stomach and duodenum of dogs. *Journal of the American Veterinary Medical Association* 162, 878, 1973.