

Motor Defects in Gastro- Oesophageal Reflux Disease

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*To Merryyn, Scott and Tom
For their love and dedication*

Declaration

The work reported within this thesis has been submitted to the University of Adelaide for the degree of Doctor of Philosophy. The studies described herein are original work performed by the author. None of the material contained in this thesis has been submitted for the award of any other degree or diploma in any university or other tertiary institution and the results of the original studies have not been previously published except as scientific papers. Material that has been previously published or written by another person has been acknowledged with due reference in the text.

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Thesis Summary

The work contained in this thesis investigates the nature of disordered oesophageal motor function that may be of importance in the pathogenesis of gastro-oesophageal reflux disease. Two major areas of dysfunction were explored; the mechanisms of gastro-oesophageal reflux and the motor function of the oesophageal body responsible for clearance of refluxate from the oesophagus. An additional study was performed in patients with non-obstructive dysphagia that arose from the studies in patients with reflux disease.

Secondary peristalsis is believed to be an important mechanism of oesophageal acid clearance. However, there are remarkably little authoritative data in the literature about this phenomenon. A detailed investigation of secondary peristalsis was therefore undertaken in normal subjects as a prelude to a study in patients with gastro-oesophageal reflux disease. This revealed that oesophageal distension with boluses of air or water was a reliable and reproducible means of triggering secondary peristalsis. The rate of triggering secondary peristalsis was proportional to the distension volume but not influenced by the site of oesophageal distension. Balloon distension of the oesophagus, on the other hand, produced a manometric response more like that seen with acute oesophageal obstruction and was not considered a good stimulus for examining secondary peristalsis. Triggering of secondary peristalsis was significantly inhibited by atropine

Using the methods validated in the normal subjects, it was found that patients with reflux disease had a major defect in triggering of secondary peristalsis in response to oesophageal distension with boluses of air or water. In the majority of cases, oesophageal distension with air or water failed to trigger any response in the reflux patients and it is postulated that this defect may well contribute to delayed oesophageal acid clearance in these patients. In contrast, the pattern of response to balloon distension was similar to that in the normal subjects. This difference is probably due to

differences in the nature of the distension induced by the balloon and the air or water boluses.

The use of oesophageal distension to assess secondary peristalsis also enabled the investigation of the lower oesophageal sphincter responses to distension. This study showed that in the normal subjects, the frequency of sphincter relaxation increased with distension volume and was not altered by the site of distension. The manometric characteristics of the relaxation were similar to that of primary peristalsis although the latency of onset of the relaxation from the triggering stimulus was longer for oesophageal distension than for swallowing. In the patients with reflux disease, there was a higher distension threshold required to trigger sphincter relaxation with a higher incidence of incomplete relaxation in response to air and water distension compared to the normal subjects. The rate and characteristics of sphincter relaxation associated with primary peristalsis and balloon distension, however, were similar to that in the normal subjects. The finding of reduced or incomplete sphincter relaxation in response to oesophageal distension is additional evidence to support the hypothesis that there is a disorder of oesophageal sensory function in patients with reflux disease.

Since the development of the sleeve sensor for continuous monitoring of lower oesophageal sphincter pressure, a considerable amount of information has accumulated about the mechanisms underlying gastro-oesophageal reflux. This information, however, is almost entirely limited to resting recumbent subjects. In order to examine the mechanisms of reflux during physical activity, a novel ambulatory perfused manometric system was developed that permitted accurate and continuous measurements of lower oesophageal sphincter pressure to be made in ambulant subjects. Using this system, the first detailed study of lower oesophageal sphincter function during reflux under ambulant conditions was performed in normal subjects. This study showed that the majority of reflux episodes occurred in the 3 hour post-prandial period and that virtually no reflux occurred during standardised exercise. Transient lower oesophageal sphincter relaxation was the most common mechanism of

reflux, irrespective of activity or body position, while swallow-related lower oesophageal sphincter relaxations and absent lower oesophageal sphincter pressure were less common mechanisms. Contrary to general belief, straining occurred at the time of reflux in only a minority of episodes. Acid clearance was largely by primary peristalsis when upright and by secondary peristalsis when recumbent. This study presents important information about the mechanisms of reflux in ambulant normal subjects and was a prelude to a subsequent study in ambulant patients with gastro-oesophageal reflux disease which was started during the writing of this thesis.

Hiatus hernia has been implicated in the pathogenesis of reflux disease for many years but the predisposing factors underlying the association are unclear. The effect of a hiatus hernia on the pressure profile across the gastro-oesophageal junction during straining was investigated in patients with large (≥ 5 cm) hiatus hernias. These studies confirmed that gastro-oesophageal reflux was more common during increased intra-abdominal pressure in patients with a hiatus hernia. This defect could be minimised, however, by contraction of the diaphragmatic crura which acts as an anti-reflux barrier by lumen occlusive compression of the herniated stomach. This study confirms the important physiological contribution of the diaphragm to the competence of the gastro-oesophageal junction in humans. Further studies are now needed to evaluate interaction of lower oesophageal sphincter and diaphragm to look for specific defects in diaphragmatic function in patients with gastro-oesophageal reflux disease.

Studies of secondary peristalsis and lower oesophageal sphincter function during oesophageal distension were also performed in patients with non-obstructive dysphagia. These were prompted by the simple and reliable method of triggering secondary peristalsis developed in normal subjects and the ready adaptability of this technique to clinical manometry. Defective oesophageal motor function is thought to be responsible for the symptoms in patients with non-obstructive dysphagia. In patients with reflux disease there is a defect in the triggering of secondary peristalsis and it was postulated that this defect may also be a cause of symptoms in patients with non-

obstructive dysphagia. The results of this study revealed a marked defect in the triggering of secondary peristalsis in response to oesophageal distension with boluses of air and water and an increased distension threshold required to trigger lower oesophageal sphincter relaxation. It was postulated that this defect may be a contributing factor to the symptoms in patients with non-obstructive dysphagia.

The studies described in this thesis have prompted a number of additional studies to evaluate the implications of the findings presented. The relevance of defective secondary peristalsis to delayed oesophageal acid clearance needs to be evaluated to more clearly delineate the clinical significance of this disorder as well as the effect of the prokinetic agent, cisapride, on the ability to trigger secondary peristalsis in patients with reflux disease. The issue of defective afferent innervation in patients with reflux disease is being addressed in animal studies designed to examine afferent oesophageal pathways and areas of possible neural dysfunction. Finally, a study evaluating the mechanisms of reflux in ambulant patients with reflux disease has been performed using the specially developed ambulatory manometric system described in this thesis.

Chapter 1

Oesophageal Body - Overview of Anatomy and Neural Controls

- 1.1 Gross Anatomy
- 1.2 Microscopic Anatomy
- 1.3 Innervation
 - 1.3.1 Extrinsic Nerve Supply
 - 1.3.2 Intrinsic Nerve Supply



1.1 Gross Anatomy

The oesophagus is a muscular tube connecting the pharynx to the stomach. It is approximately 25cm in length, beginning at the lower border of the cricoid cartilage at the level of the sixth cervical vertebra and ending at its junction with the stomach, slightly to the left of midline, at the level of the vertebral bodies of T10 or T11 [311].

The oesophagus is commonly divided anatomically into the cervical, thoracic and abdominal segments. Alternatively it may also be divided into the upper, middle and lower thirds, based on rough histological criteria with the upper segment being composed of skeletal muscle, the lower segment composed of smooth muscle and the middle segment corresponding to the transition zone between these two muscle types (see description of microscopic oesophageal anatomy below).

The cervical oesophagus is approximately 5cm in length and extends from the cricoid cartilage and cricopharyngeal muscle to the thoracic inlet at the level of the sternoclavicular joints. This corresponds to the vertebral bodies of C6 to T1. Anteriorly are located the larynx and trachea and anterolaterally, the oesophagus is adjacent to the carotid sheath, the inferior thyroid artery, the lobe of the thyroid gland and the recurrent laryngeal nerve. The thoracic duct is adjacent to the oesophagus on the left side up to the level of C7 before it descends to enter at the junction of the left subclavian and internal jugular veins. Posteriorly the cervical oesophagus is related to the alar fascia, the prevertebral fascia, the longissimus cervicis muscle and the vertebrae.

The thoracic oesophagus extends from the vertebral bodies of T1 to approximately T10 or T11. Anterior to the oesophagus lie the trachea and aortic arch, right pulmonary artery, left main bronchus, pericardium and left atrium, anterior vagal trunk and the oesophageal hiatus. The posterior aspect of the thoracic oesophagus is adjacent to the vertebral column and paravertebral muscles, the left thoracic duct (T7-T4), the right pleural sac, the azygos and hemiazygos veins and the aorta. The lateral relations are

often referred to as the “venous” or right side and “arterial” or left side. The mediastinal pleura, azygos vein, right main bronchus, root of the right lung, right vagus nerve and the oesophageal plexus are adjacent to the right or venous side of the oesophagus; the aortic arch, left subclavian artery, left recurrent laryngeal nerve, left vagus nerve, thoracic duct (T4-C7) and the descending thoracic aorta are on the left or arterial side.

The abdominal oesophagus is 0.5 to 2.5cm in length and lies at the level of the vertebral bodies of T11 or T12. The left lobe of liver, the left vagal trunk and the oesophageal plexus lie anterior to the abdominal oesophagus. The diaphragmatic crura and the aorta are posterior, the caudate lobe of the liver on the right and the fundus of the stomach on the left. The abdominal oesophagus passes through the oesophageal or diaphragmatic hiatus, an elliptical opening in the muscular part of the diaphragm. The margins of the hiatus are formed by the diaphragmatic crura and the median arcuate ligament. The crura arise from the anterior surface of the vertebral column and the fibres pass upwards and forwards to give rise to the arms of the muscle that form the hiatal ring. Anteriorly, the fibres insert into the transverse ligament of the central tendon of the diaphragm.

The arterial blood supply of the oesophagus arises from the superior and inferior thyroid arteries, bronchial arteries, left gastric artery and the splenic artery [214]. In addition, in two thirds of the patients examined in one study, there were also branches from the descending aorta [214]. A dense complex of submucosal vessels distributes blood from the main trunks to the oesophageal wall. The venous drainage of the oesophagus is split into three portions. The upper third drains into the superior vena cava; the middle third into the azygous system; and the lower one third into the portal vein via the gastric veins. All three systems, however, are interconnected and allow blood to be diverted via collateral flow if the veins at any level become obstructed.

The lymphatics of the oesophagus form a highly interconnected system: the upper one third of the oesophagus drains to the cervical nodes; the middle third, to the

mediastinal nodes; and the lower one third, to the coeliac and gastric lymph nodes. The widely interconnected lymphatic system draining the oesophagus explains why tumours of the oesophagus rarely remain localised.

1.2 Microscopic Anatomy

The oesophagus is lined by non-keratinising stratified squamous epithelium which is continuous with the lining of the oropharynx. The epithelium consists of a basal layer of two to three cells thickness. These cells contain dark nuclei. Covering the basal layer are a series of layers of squamous cells, heaped on one another. The nuclei of these cells become increasingly pyknotic in the superficial layers and eventually disappear before the luminal layer is reached. The squamous cells are filled predominantly with glycogen. There are also small lipid droplets and some phospholipid in the superficial squamous cells. The basal lamina of the epithelium separates it from the lamina propria which consists of loose connective tissue, lymph nodes, lymphocytes and plasma cells. The lamina propria extends into the epithelium in ridge like structures referred to as dermal pegs. These dermal pegs normally do not extend more than half way across the squamous epithelium but can be elongated in conditions such as reflux oesophagitis. The dermal pegs often contain capillaries.

The limited secretory and absorptive capacity of the oesophagus is reflected in the less extensive vascular and lymphatic system of the lamina propria compared to the rest of the intestine. The muscularis mucosae is the deepest layer of the mucosa and is composed predominantly of longitudinally arranged smooth muscle fibres. Beneath the mucosa lies the submucosa and the muscularis externa followed by an inner circular muscle layer and finally the outer longitudinal muscle layer. The submucosa contains an arteriolar plexus, elastic tissue, nerve cell bodies of Meissner's plexus and submucosal glands. The role of these glands remains largely unexplored but a recent study suggests that they contribute to oesophageal bicarbonate secretion [229].

The two major muscle layers of the oesophageal body consist of an inner circular layer and outer longitudinal layer. At the cranial end, the circular layer originates from the pharyngo-oesophageal sphincter and the longitudinal fibres from the posterior portion of the cricoid cartilage. In the upper oesophagus, both layers are skeletal muscle while the distal half of the oesophagus is entirely smooth muscle. There is a transition zone in the second quarter of the oesophagus where the circular and longitudinal muscle layers contain both skeletal and smooth muscle fibres. Between the two external muscle layers is the intermuscular nerve plexus named after Auerbach. Interconnections between the different plexuses form a local nerve network within the oesophagus that is responsible for the integration of the central inputs to the oesophagus and also for some of the more autonomous oesophageal muscular responses.

1.3: Oesophageal Innervation

1.3.1 Extrinsic Nerve Supply

The extrinsic control for oesophageal motor function resides in a brainstem “swallow centre”. This functional entity resides in two intimately connected regions in the medulla and pons. The brainstem “swallow centre” includes an afferent receptive system, an efferent system of motor nuclei and a complex organising or intermediary system of interneurons [97].

Afferent Innervation: Oesophageal afferent innervation remains poorly defined with little data available from humans. The afferent supply from the oesophagus has, however, been the recent focus of attention in animal studies and relatively well defined pathways are now described through which oesophageal sensory inputs may mediate reflex control of oesophageal motility and function.

The afferent innervation of the striated muscle portion of the oesophagus is both spinal and vagal [60] and arises from muscle spindles in the striated muscle [8] which are similar to spindles found in other striated muscles. A recent study with fast blue injections into the oesophagus of a cat has shown projection of the striated muscle spinal afferents to the dorsal root ganglia of C1 through to T8 [60]. These projections were predominantly localised to the upper cervical segments. The vagal afferents from the striated muscle region travel to the spinal tract of the trigeminal nerve and to the fasciculus and nucleus solitarius while some enter the commissural nucleus of Cajal [48]. The vagal afferent cell bodies are predominantly found in the rostral portion of the nodose ganglion [60].

The smooth muscle portion of the oesophagus is also supplied by vagal and spinal afferent innervation. The oesophageal spinal afferents arise from the C5 to L2 dorsal root ganglia [60] and travel along sympathetic pathways to the oesophagus. The central distribution of these afferents, however, has not been examined but a recent study of gastric afferents suggests that the fibres terminate in layers I and IIa of the dorsal horn with a few fibres ascending to the level of the dorsal column nuclei [74]. The projection of the subsequent spinal afferent interneurons is not known as they do not appear to make direct contact with preganglionic neurones [74].

The vagal afferents from the smooth muscle oesophagus travel through the superior laryngeal nerve, recurrent laryngeal nerve or the oesophageal branches of the vagus and project to the nucleus of the solitary tract in the medulla. The cell bodies of the vagal afferents are located diffusely throughout the nodose ganglion [60]. A prominent projection from the nucleus of the solitary tract to ipsilateral oesophageal motor neurones is probably important for connection of afferent and efferent pathways [74].

It has been reported that the vagal sensory pathways are important for the monitoring and modulation of oesophageal motor activity whilst the sympathetic afferent system is thought to be concerned with cognitive sensation and nociception [97]. This hypothesis is perhaps a little simplistic and not entirely in agreement with existing

literature. In an earlier study it has been shown in various animal species, that mechanoreceptor stimulation triggers afferent fibres that not only travel via vagal afferent fibres but also by sympathetic pathways [47]. In addition, in studies examining oesophageal thermoreceptors, recordings were made from vagal afferent units confirming that at least some cognitive sensory or nociceptive pathways travel via the vagus [125]. There is also considerable overlap in the central distribution of the vagal and sympathetic oesophageal sensory fibres which tends to suggest that there may be considerable overlap in function [60].

The sensory receptors associated with the various oesophageal afferents remain poorly defined but there are probably separate receptors that respond to mechanical, thermal and chemical stimuli [47]. It is controversial whether these sensory modalities have sensors with a specific anatomy or location within the oesophageal wall. Clerc and Mei have distinguished three functional types of oesophageal mechanoreceptor which were located in the mucosal, muscular and serosal layers of the oesophagus [53]. The mucosal receptor is rapidly adapting and discharges both at the onset and the offset of the distending stimulus while the serosal receptor, also rapidly adapting, discharges repetitively during the distension stimulus. The muscle receptors are slowly adapting and also respond during distension. The serosal receptors have a higher stimulus threshold than the muscle receptors and are probably only triggered under abnormal circumstances such as with vomiting or during painful stimuli. The mucosal receptors are innervated predominantly by vagal fibres while the serosal receptors trigger mostly spinal fibres. The muscular receptors are innervated by both vagal and sympathetic fibres.

The anatomical correlates of the various mechanoreceptors still needs to be conclusively identified. It is possible that the nerve cell bodies themselves are the mechanoreceptors, discharging upon deformation because of some localised functional sensitivity to deformation [47]. This hypothesis arose from a study of the anatomy of the myenteric plexus of the smooth muscled segment of the opossum oesophagus

[51]. It was observed that many ganglia in that region (10-50%, depending on the level) are pedunculated and not situated within the main nerve fibre bundle of the plexus, but outside these bundles and connected by short stalks. Within these parafascicular ganglia, there is often a paucity of neurophil (the mass of nerve fibres that usually surrounds the nerve cell bodies in the ganglion); this sparsity suggested that these cells are deficient in synaptic input and thus the notion arose that the cell bodies in such ganglia could be mechanoreceptors. This hypothesis is supported by the finding that some cells in the myenteric plexus of the small intestine of the rodent, discharge on deformation [350].

A second possibility, based on the extensive work of Rodrigo et al, is that there are structurally distinct mechanoreceptors in the smooth muscle oesophagus [288, 289]. In these studies, Rodrigo has suggested that specialised neural structures within myenteric ganglia, which he termed intraganglionic laminar nerve endings or IGLE's, function as sensory receptors. These structures, as acknowledged by Rodrigo, were first described by Lawrentjew [289]. The endings are highly variable in size and shape and are found in dogs, cats and monkeys. Subsequent elegant studies in the cat, showed that if the central portion of the nodose ganglion was destroyed, there was selective degeneration of the IGLE's consistent with the hypothesis that these structures are terminal specialisations of sensory fibres projecting to the oesophagus from the nodose ganglion. Selective section of the motor pathways to the oesophagus, on the other hand, did not result in degeneration of the IGLE's.

The thermoreceptors and the chemoreceptors of the oesophagus have received less attention and are poorly defined. El Ouazzani and Mei have described three types of thermoreceptor; warm, cold and mixed [125]. These receptors are not responsive to mechanical or chemical stimulation and appear to modulate oesophageal contractility and respiratory frequency in response to the temperature of an oesophageal bolus. During perfusion of the oesophagus with cold solutions in intact animals, there is a significant increase in the oesophageal contraction induced by electrical stimulation of

the superior laryngeal nerve and little effect on respiratory frequency. For warm solutions, there is an opposite effect for oesophageal contractility and a there is significant increase in the frequency of respiration. In vagotomised animals, the effect of temperature on oesophageal contractility is reversed and the respiratory changes are abolished. Oesophageal chemoreceptors have received the least attention in humans despite being the subject of many clinical studies into the oesophageal origin of chest pain [33, 205, 285, 313]. There are no clear data on how the chemical environment of the oesophageal lumen leads to the stimulation of nociceptive pathways and whether these travel along vagal or sympathetic pathways.

The sensory structures that mediate chemical or thermal changes are unknown but it has been suggested that the intra-epithelial nerve endings described by Rodrigo et al [290] may be responsible [47]. These endings are similar to the IGLE's described above, but rather than being confined to the myenteric plexus, extend to the base of the squamous epithelium. The intraepithelial nerve endings form a plexus of non-myelinated varicose fibres just beneath the basal epithelial layer. Some fibres from the plexus also penetrate the basal layer and terminate at various levels within the squamous epithelium. These fibres have a diverse morphology and possibly may be linked to the different sensory functions of thermosensitivity, chemosensitivity or various types of oesophageal pain. It has also been postulated that the superficial nerve endings respond to deformation of the oesophageal lumen during the passage of a bolus and thus may contribute to the modulation of the oesophageal motor response.

Efferent Innervation: Efferent nerves to the oesophagus are found in the cerebrospinal tract and the parasympathetic and sympathetic systems. The cerebrospinal innervation arises from the nucleus ambiguus and travels through the vagus to the ascending limb of the recurrent laryngeal nerve which supplies the skeletal muscle of the upper oesophagus. It is a direct supply from the motor neuron to the striated muscle cells. A small proportion of the striated muscle efferents have also been shown to arise from the dorsal motor vagal nucleus [59]. Smooth muscle efferents,

however, arise predominantly from the dorsal motor vagal nucleus and travel through the vagus nerves to the oesophagus [59]. Additional smooth muscle efferents arise from the nucleus retroambiguus and from the rostral nucleus ambiguus adjacent to the striated muscle efferents [59]. The right and left vagus nerves break up into the oesophageal vagal plexus which has inputs to the ganglia of the myenteric plexus. The smooth muscle of the distal oesophagus is supplied from this plexus via intramural neurones [293].

The sympathetic supply originates in the cervical ganglia, coeliac ganglion and the ganglia of the paravertebral chains. Sympathetic fibres reach the oesophagus by joining the vagus as it descends in the upper chest, through perivascular nerve pathways and from splanchnic nerves via the coeliac ganglia. The sympathetic fibres to the oesophageal body originate in preganglionic cells in spinal segments T5 and T6. The role of the sympathetic system in oesophageal motor function in humans remains to be defined. In the cat, the sympathetic system has been said to be unimportant as extensive sympathectomy has little effect on oesophageal motility [41]. In this study, however, 8 of the 12 animals subjected to extensive sympathectomy died within 24 hours due to severe hypotension leaving only 4 for subsequent examination. It is possible that the sympathectomy in the 4 remaining animals was incomplete compared to those animals that died and thus no difference in oesophageal function was detected after surgery. Nonetheless, sympathetic fibres synapse with the myenteric plexus ganglia in both the smooth and striated muscle segments of the oesophagus and so may well modulate efferent signals to the muscle (see section 1.3.2) [293].

Coordinating Region: The portion of the central nervous system that programs and controls the entire swallowing sequence is probably located in the solitary tract nucleus and the neighbouring reticular substance [97]. The dorsal area of this nucleus is involved in initiation of swallowing and organisation of the swallow sequence whilst the ventral area serves as a connecting pathway to the various motor neuron pools involved in the swallow sequence. The latter also includes integration of the

swallowing sequence with activity in the other medullary centres, such as the respiratory centre.

1.3.2 Intrinsic Nervous System

The intrinsic nervous system consists of two major components: Meissner's plexus in the submucosa and Auerbach's myenteric plexus in the connective tissue between the circular and longitudinal muscle layers. The myenteric plexus between the muscle layers is much more extensive than the submucosal plexus which is thin and contains few ganglion cells. The two components of the intrinsic nervous system are thought to act as relay neurons from the vagus to the smooth muscle cells. The greatest density of intrinsic ganglion cells, therefore, occurs in the smooth muscle oesophagus. The roles of the intramural ganglia in the striated muscle segment are unlikely to include direct relay of efferent signals to the muscle as the efferent fibres in this segment end directly at the neuromuscular junction with the striated muscle cells, rather than on the cells of the myenteric plexus [293]. This is similar to skeletal muscle elsewhere. The intramural ganglia in this part of the oesophageal body may supply innervation to oesophageal glands or have a sensory role with indirect effects on motor function [149].

The interstitial cells of Cajal are another important cell type within the myenteric plexus. These cells are neither nerve or muscle cells, but are found between them, forming tight gap junction contact with both [77]. It is proposed that these cells act as signal transducers or modulators of the nerve-smooth muscle interaction and as regulators of muscle function. If this were true, then dysfunction of the interstitial cells of Cajal is a potential mechanism for pathogenesis of oesophageal motor disorders. This intriguing possibility is an area of increasing research activity.

Sympathetic nerves are also present in the myenteric plexus of the striated and smooth muscle oesophagus. The sympathetic fibres have little effect on the motor function in the striated oesophagus but in the smooth muscle segment these nerves make both

axosomatic and axoaxonic contact with nerves of the myenteric plexus and occasionally with the smooth muscle cells themselves [97]. In the striated oesophagus, infusion of a beta-1 agonist resulted in small but significant reduction in peristaltic velocity but did not alter contraction amplitude [219]. Subsequent infusion of a beta-2 agonist and beta antagonists had no effect on motor function in the striated oesophagus. In the smooth muscle oesophagus, infusion of beta-1 and beta-2 agonists and antagonists had a significant influence on oesophageal motility [219]. Beta-adrenergic effects are inhibitory through both β_1 and β_2 receptors; the latter on the muscle and the former possibly at the prejunctional level of the myenteric plexus to decrease acetylcholine release [219]. Alpha-adrenergic effects are excitatory, acting on muscle (α_1) or on neurons (α_2), the latter increasing acetylcholine release and decreasing release of the NANC inhibitory transmitter [145]. This organisation suggests that the sympathetic nerves predominantly act in the smooth muscle oesophagus and serve mainly to modulate the activity of other neurons and the release of their respective neurotransmitters.

In the smooth muscle oesophagus, the function of the various morphological components within the myenteric plexus remains unclear. The two major types of effector neuron within the system include one that is capable of mediating cholinergic excitation of both the longitudinal and circular layers of the smooth muscle via M3 receptors, while the other is capable of mediating non-adrenergic, non-cholinergic (NANC) inhibition of the inner circular smooth muscle layer [69, 96, 98, 102, 112, 142, 143, 150, 293]. The neurotransmitter released by the latter neuron is likely to be nitric oxide or a related nitroso-compound [246, 332], although purine nucleotides and peptide transmitters, such as vasoactive intestinal polypeptide or calcitonin gene related peptide, have been among other substances proposed [96, 150, 152, 153, 274, 293]. The importance of these other transmitters, however, has been doubted in other studies [76]. Both the cholinergic and NANC systems are excited by cholinergic input from preganglionic vagal fibres and intramural interneurons. The cholinergic excitation of

the excitatory neuron is nicotinic, whereas that of the NANC neuron can be either nicotinic or muscarinic (M1 receptors) [97].

Chapter 2

Anti-Reflux Barrier - Overview of Anatomy and Neural Controls

- 2.1 Gross Anatomy
- 2.2 Microscopic Anatomy
- 2.3 Innervation of the Lower Oesophageal Sphincter
 - 2.3.1 Extrinsic Nerve Supply
 - 2.3.2 Intrinsic Nerve Supply
- 2.4 Innervation of the Costal and Crural Diaphragm

2.1 Gross Anatomy

The anti-reflux barrier is composed of the lower oesophageal sphincter, the crural diaphragm and the phreno-oesophageal ligament [238]. The combined action of these structures protects against retrograde movement of stomach contents from the higher pressure zone within the abdominal cavity to the oesophageal body in the lower pressure thoracic cavity.

The lower oesophageal sphincter can be readily identified by intraluminal manometry as a 2-4cm high pressure zone at the gastro-oesophageal junction but unlike other sphincters in the gastro-intestinal tract, macroscopic anatomical examination fails to detect any muscle specialisation or thickening in this region.

The human diaphragm consists of the costal and crural segments which not only have different actions but also different segmental innervations and embryological origins. It is for these reasons that the two segments are often regarded as separate muscles [3, 122, 220]. The embryological origin of the crural diaphragm is from the oesophageal mesentery while the costal diaphragm arises from the pleuroperitoneal membrane [238]. Anatomically, the costal diaphragm arises from the costal margin and inserts into the central tendon while the crural diaphragm stretches from the spinal column and the medial and lateral arcuate ligaments to the central tendon. These two muscle components combine with the central tendon to form one continuous sheet. Despite their structural unity, the muscle components remain more or less independent in their control and action. Functionally, contraction of the costal diaphragm in the dog causes caudad displacement of the anterior diaphragm and expansion of the volume of the pleural space whereas contraction of the crural diaphragm causes a caudad displacement of the posterior part of the diaphragm but no displacement of the rib cage [220]. The crural diaphragm is widely considered an integral part of the anti-reflux barrier [238]. It is the major component of the diaphragmatic hiatus, an elliptical orifice about 2.5cm in length, through which the oesophagus passes into the abdomen.

Manometrically, the high pressure zone associated with the crural diaphragm measures approximately 1-2cm in axial length and surrounds the proximal half of the lower oesophageal sphincter in normal volunteers [157].

The phreno-oesophageal ligament is the third component of the “anti-reflux barrier”; it is composed of loose areolar tissue and subperitoneal fascia derived from the undersurface of the diaphragm. It has two leaves; one extending cranially to its oesophageal attachment, 2 to 3 cm above the squamo-columnar junction and the second at the squamo-columnar junction itself [31]. Its role appears to be to anchor the distal oesophagus to the diaphragm. This ligamentous attachment of the oesophagus to the diaphragm allows 2-3 cm longitudinal movement of the distal oesophagus during peristalsis [101, 113, 188].

2.2 Microscopic Anatomy

Specialised histological techniques reveal regional specialisation with asymmetrical muscle thickening in the region of the human lower oesophageal sphincter. This increase in muscle thickness is due to an increase in the mass of the inner circular muscle coat with the muscle bundles forming horseshoe-like clasps rather than muscle rings as seen in other sphincters of the gut [213]. These clasps retain an overall transverse direction but are open at the anterior and posterior aspects of the oesophagus. The area of muscle thickening tapers toward both the stomach and oesophagus and measures approximately 3.1cm in length adjacent to the greater curvature of the stomach and 2.3cm on the lesser curvature side of the stomach [213]. It is angled obliquely upwards from the lesser to the greater curvatures of the stomach and is thickest at the greater curvature and successively thinner at the lesser curvature, posterior wall and anterior wall. The area of muscle specialisation correlates with the manometric high pressure zone that separates the stomach from the oesophagus.

The muscle bundles of the outer longitudinal layer in the region of the lower oesophageal sphincter run parallel with the long axis of the oesophagus and continue longitudinally along both gastric curvatures. At the anterior and posterior gastric wall, the fibres turn upwards toward the fundus and run at right angles to their initial direction. At their termination the fibres interlace either with the underlying inner muscle layer of the stomach or with the gastric serosa. The thickness of the outer longitudinal layer does not alter in the region of the lower oesophageal sphincter [213].

In addition to the histological features noted at the level of the lower oesophageal sphincter, ultrastructural studies of the opossum sphincter muscle show an alteration of the smooth muscle cell structure and arrangement. The circular muscle fibres of the sphincter have irregular protuberances from their surfaces whereas the circular muscle fibres of the rest of the oesophageal body have smooth surfaces [303]. This may, however, simply reflect the contracted nature of the muscle in this area. Furthermore, sphincter muscles cells also have a greater volume of mitochondria to overall cell volume, with the mitochondria located more centrally than in muscle cells from the oesophageal body [50]. Lower oesophageal sphincter muscle cells also contain more endoplasmic reticulum than oesophageal body muscle cells.

2.3 Innervation of the Lower Oesophageal Sphincter

2.3.1 Extrinsic Nerve Supply

The extrinsic innervation of the lower oesophageal sphincter is similar to the smooth muscle of the oesophageal body with both vagal and spinal inputs [231]. The vagus nerve contains both an excitatory cholinergic input that is blocked by atropine and inhibitory fibres that mediate sphincter relaxation. The spinal or sympathetic nerves act via adrenergic alpha-receptors. The role of the sympathetic innervation of the lower oesophageal sphincter in humans remains to be defined but it probably contributes to brief duration reflex augmentation of sphincter pressure.

Efferent Innervation: The lower oesophageal efferents have a similar central distribution to those of the oesophageal body (Chapter 1) [59]. The only significant difference between the oesophageal body and the lower oesophageal sphincter is that the rostral distribution of the efferent cell bodies in the dorsal motor vagal nucleus is shifted more caudally [59]. The efferent parasympathetic neurons arise from the dorsal motor nucleus of the vagus, enter the oesophagus at the level of the mid-thorax and travel intramurally to synapse with ganglia in the myenteric plexus [58, 72].

The efferent sympathetic innervation is derived from cell bodies in the intermediolateral columns of the spinal cord from the vertebral segments of T6-T10, and travel to the oesophagus via thoracic sympathetic nerves, greater splanchnic nerves and perivascular pathways [22, 231]. There are also some fibres that travel with the vagus. The sympathetic fibres innervate both intramural ganglia and smooth muscle directly [66]. Extensive sympathetic denervation of the lower oesophageal sphincter in the dog, did not influence the animals well-being or sphincter function [154].

Afferent Innervation: The afferent innervation of the lower oesophageal sphincter is similar to that described for the oesophageal body (Chapter 1) with both vagal and spinal fibres [22, 60]. The only exception is that lower oesophageal sphincter spinal afferents do not project to the cervical dorsal root ganglia but predominantly travel to the vertebral segments of T1-L3 [60].

2.3.2 Intrinsic Nerve Supply

Histochemical studies of the neural elements within the lower oesophageal sphincter have identified cholinergic and adrenergic nerves [18, 182] but it is not known if these neural elements are similar to those seen in the oesophageal body [149]. The sphincter is sensitive to a number of neurotransmitters such as nitric oxide, histamine, serotonin and dopamine which act through non-adrenergic and non-cholinergic pathways. It is also very sensitive to substance-P and vasoactive intestinal polypeptide which suggests that paracrine peptidergic transmitters may also have a role in sphincter function. The

role of these various neurotransmitters is discussed in chapter 4 where lower oesophageal sphincter function is reviewed in more detail.

2.4 Innervation of the Costal and Crural Diaphragm

The costal and crural diaphragm are innervated by both the right and left phrenic nerve branches. Functionally, however, the two components of the diaphragm have been shown to act independently during different phases of the respiratory cycle [122]. Sonomicrometry studies in anaesthetised animals have shown that in early inspiration (within the first 100 ms), there is shortening of the crural diaphragm while the costal diaphragm remains virtually inactive. During the subsequent 200 ms, the costal diaphragm muscle fibres begin to shorten. During this period, however, the crural muscle shortening is still much greater than costal muscle shortening. There are also differences in the peak EMG activity of the two components of the diaphragm during respiration, with a greater EMG signal from the crural diaphragm than the costal diaphragm, particularly during CO₂ re-breathing [122]. The most likely explanation to account for the different onsets of contraction of the two components of the diaphragm is that these have separate central neural controls which determine the initiation and degree of costal and crural diaphragm shortening. Alternatively, the differential shortening of the costal and crural diaphragm may be determined by mainly peripheral mechanical factors. If this were so then despite both segments being stimulated simultaneously, the crural diaphragm could be seen to contract first because of a lower mechanical impedance than the costal diaphragm. There is no direct evidence to refute this possibility, but the evidence discussed below is more supportive of the view that there are independent controls of costal and crural diaphragm contraction.

Experiments in cats [3], dogs [243] and sheep [331] have suggested that the costal and crural diaphragm are innervated independently. In these studies, oesophageal balloon distension and swallowing resulted in relaxation of the lower oesophageal sphincter

and inhibition of the respiratory-induced crural EMG activity with no effect on the EMG activity of the costal diaphragm. The differential innervation of the crural and costal diaphragm has also been observed during vomiting and eructation [243]. In these studies, the expulsion phase during vomiting was accompanied by a strong, simultaneous and sustained spike burst from the outer hiatal fibres, dome of the diaphragm and the rectus abdominis muscle with little or no activity seen in the inner hiatal fibres. During other movements resulting in increased intra-abdominal pressure, such as defecation, this effect was not seen with increased EMG activity seen in all areas of the diaphragm and the rectus abdominis muscle [243].

The inhibition of the crural diaphragm has not only been observed during swallowing, oesophageal distension and vomiting or belching but also during transient lower oesophageal sphincter relaxations (Chapter 4) [222, 233]. It was shown that the degree of crural diaphragmatic inhibition correlated with the magnitude of lower oesophageal sphincter relaxation [233]. The clinical implication of transient lower oesophageal sphincter relaxations are discussed further in chapter 5 of this thesis.

The inhibition of crural activity by oesophageal balloon distension and swallowing in the cat is triggered by mechanoreceptors in the upper oesophagus and mediated by the vagus nerve [3]. In contrast to these animal studies, Mittal et al found no effect of oesophageal balloon distension on crural diaphragm EMG recordings or intra-luminal pressure measurements during voluntary or involuntary diaphragmatic contraction in humans [241]. It is likely that this difference arises because of species differences and suggests that oesophageal afferents triggered by intra-oesophageal balloon distension do not influence the crural inhibitory reflex in humans.

The significance of the differential activation of the two segments of the diaphragm requires further investigation. A number of studies have postulated that the crural diaphragmatic contraction early in inspiration assists in maintaining competence of the gastro-oesophageal junction during this phase of the respiratory cycle [3, 35, 100, 122, 206, 236, 343]. In addition, selective relaxation or contraction of the crural

diaphragm appears important for facilitation of flow across the anti-reflux barrier during swallowing, oesophageal distension and vomiting or belching. This selective relaxation may also be an important pathophysiological mechanism in the development of gastro-oesophageal reflux disease. Some aspects of diaphragmatic function in humans and its relevance to gastro-oesophageal reflux disease have been addressed in work in this thesis.

Chapter 3

Normal Oesophageal Body Motor Function

3.1 Primary Peristalsis

3.1.1 Definition

3.1.2 Function

3.1.3 Manometric and Mechanical Characteristics

3.1.4 Control of Oesophageal Peristalsis

3.1.5 Extrinsic Control Mechanisms

3.1.6 Intrinsic Control Mechanisms

3.2 Secondary Peristalsis

3.2.1 Definition

3.2.2 Function

3.2.3 Stimuli for Secondary Peristalsis

3.2.4 Manometric and Mechanical Characteristics

3.2.5 Control Mechanisms

3.1 Primary Peristalsis

3.1.1 Definition

Primary peristalsis is defined as a propagated oesophageal motor response triggered by swallowing [226]. The initiation of this muscular activity is under voluntary control but once triggered, peristalsis is completed involuntarily [89]. Successful completion of the primary peristaltic sequence is not dependent on the presence of a bolus although complete peristalsis is more likely if a bolus is present [168, 185].

3.1.2 Function

Primary peristalsis transports a swallowed bolus into the stomach and so clears the oesophagus. During the oropharyngeal phase of swallowing, the bolus is moved voluntarily into the pharynx, and the pharyngeal contractions are initiated. A rapidly aborally progressing pharyngeal contraction transfers the bolus through the relaxed upper oesophageal sphincter and into the oesophagus. As the upper oesophageal sphincter closes, a progressive circular contraction begins in the upper oesophagus and proceeds distally along the oesophageal body to propel the bolus through the relaxed lower oesophageal sphincter.

3.1.3 Manometric and Mechanical Features

Oesophageal peristalsis begins at the upper oesophageal sphincter and progresses caudally at a velocity of 2 - 5 cm/sec, taking approximately 6 seconds to reach the stomach [149, 178]. The mechanism of primary peristalsis and bolus transport involves both the circular and longitudinal layers. The circular layer is responsible for the aborally progressive lumen occlusion of peristalsis while the longitudinal layer facilitates the passage of the bolus by shortening during peristalsis and “engulfing” the oncoming bolus. This shortening may reduce the oesophageal length by as much as 10% on swallowing, causing considerable transient upward movement of the lower oesophageal sphincter. Longitudinal muscle contraction and oesophageal shortening

may also increase the density of the circular smooth muscle fibres behind the bolus prior to their contraction [325].

The oesophageal body striated muscle, like most other non-postural striated muscles in the body, is quiescent at rest. Similarly, the oesophageal smooth muscle has no rhythmic or tonic contractions at rest [48, 294]. This is true for the circular and longitudinal smooth muscle layers, both of which are electromyographically silent between peristaltic sequences [5, 48, 293]. The oesophageal smooth muscle is unusual in this regard, as smooth muscle elsewhere in the gastrointestinal system has well documented rhythmic contractions such as those associated with the migratory motor complex.

In contrast to the electrophysiological studies of oesophageal body muscle, manometric tracings from the oesophagus reveal phasic alterations in resting pressure. These phasic alterations are not due to phasic contractions of the oesophageal musculature but arise from extrinsic pressure swings due to respiration or from variable direct compression of the oesophagus by the heart or the aorta [150].

When primary peristalsis is triggered, the oesophageal motor response consists initially of a period of inhibition followed by the peristaltic contraction [150, 308]. The initial inhibition, however, is not usually evident manometrically because there is little basal oesophageal tone, but becomes obvious if repeated swallows are taken within a short time interval when the second swallow inhibits the contraction evoked by the first swallow [9]. The interval between swallows that has been shown to inhibit oesophageal peristalsis is less than 15 seconds [9]. Swallows taken more frequently than every 15 seconds result in an increased incidence of failed or absent peristalsis which becomes more pronounced as the interval between swallows decreases. With “continuous” swallowing, that is every 2-3 seconds, there is complete inhibition of the oesophageal body until the last swallow has been taken. A few seconds after the last swallow, there is then a peristaltic contraction that is often of higher amplitude than

normal. The swallow frequency during normal drinking is high enough to cause sustained inhibition until after the last swallow is taken [150].

Following the wave of oesophageal inhibition associated with the onset of the primary peristaltic sequence, there is a small, short duration, negative deflection in oesophageal pressure. This pressure change is thought to be due to a brief inspiration that immediately precedes the peristaltic sequence. A small positive pressure wave is then seen in the proximal oesophagus which represents the pressure generated within the bolus as it is propelled into the upper oesophagus by the pharyngeal musculature [62]. This small positive pressure wave is referred to as "intra-bolus" pressure and, if seen, may be a discrete peak in the upper oesophagus or appear to progress down the entire length of the oesophageal body. An intra-bolus pressure wave is not usually seen with dry swallows but may be present if an air bolus is swallowed. Oesophageal outlet obstruction may be associated with increased intra-bolus pressure.

The positive pressure wave that follows the above events is the main peristaltic wave produced by the lumen occluding contraction of the oesophageal musculature. The characteristics of this wave vary with the segment of oesophagus in which it is measured [96, 150]. As the peristaltic contraction passes through the oesophagus, the amplitude of the wave is highest in the distal 5cm and lowest approximately 4 to 6cm below the upper oesophageal sphincter, corresponding to the 2 to 3cm transition zone from striated to smooth muscle. Normal contraction amplitudes have been reported by a number of different investigators using a variety of different manometric techniques. Contraction amplitudes are usually considered normal if greater than 10mmHg in the proximal oesophagus and approximately 30mmHg in the distal oesophagus [194]. In some parts of the oesophagus, contractions may exceed 150mmHg in healthy subjects but a mean contraction amplitude of greater than 150mmHg in the distal oesophagus is usually considered abnormal [323]. The duration of the oesophageal contraction at any one level within the oesophagus is normally less than 7 seconds and it takes 6 to 8 seconds for a peristaltic front to proceed through the oesophagus with an average

velocity of 3 to 4 cm/second. Proximally, the velocity is 3.0 to 3.5 cm/second; this increases in the lower third to 5.0 cm/second and then slows to 2.0 cm/second in the very last 2 to 3cm of the oesophageal body.

Several factors may influence the amplitude, duration and propagation velocity of the peristaltic pressure wave. Within the same individual and with the same technique, peristaltic amplitude remains reasonably constant when examined serially [247]. In the upright position, the amplitude of oesophageal contraction is less, with an increased velocity in the upper oesophagus and a decrease in the mid and lower oesophagus compared to the supine position [200]. Also, whether a bolus is present has an important impact on the duration and amplitude of the peristaltic pressure wave. Compared to a “dry” swallow, water swallowing results in increased duration and amplitude of the peristaltic pressure wave, along with a decreased velocity of propagation [107]. Other factors also alter peristalsis; intra-abdominal pressure slowing peristaltic velocity [108], while warm boluses augment, and cold boluses inhibit peristaltic contractions [81, 125, 348].

After the main peristaltic contraction wave there is a refractory period during which it is difficult to evoke an oesophageal response to swallowing; this is referred to as the oesophageal refractory period. The refractory period of the oesophagus is approximately 5-6 seconds. Swallowing at a rate of 8-12 per minute (ie. 1 swallow/ 7.5- 5 seconds) results in successful peristalsis with most swallows while at a swallow rate of 15 per minute (ie. 1 swallow/ 4 seconds), a significant proportion of peristaltic sequences fail to occur. A swallow interval of less than 4 seconds invariably results in complete inhibition of the oesophageal body until the last swallow is taken [141].

3.1.4 Control of Oesophageal Peristalsis

The physiology of the oesophageal peristaltic contraction is complex as different mechanisms are involved in generating peristalsis in the striated and smooth muscle segments [89] as well as in the longitudinal and circular muscle layers [324, 325].

It is generally believed that oesophageal peristalsis relies on a combination of extrinsic and intrinsic neurogenic pathways as well as specific properties of the oesophageal musculature. The action of these various mechanisms results in oesophageal peristalsis. The physiology of the striated muscle segment of the oesophagus is comparable to that of other striated muscles in the body and is controlled by excitatory nerves [293]. In the smooth muscle oesophagus, however, there are at least four potential mechanisms for the stimulation and modulation of peristalsis which reside at different levels of control: (1) signals from the central nervous system that result in the sequential firing of efferent motor fibres during both primary and secondary peristalsis [137, 294]; (2) an intramural neural mechanism that can be excited to produce peristalsis by vagal stimulation or intraluminal balloon distension - the "on" contraction [69, 102, 112, 143, 330]; (3) another intramural neural mechanism that can be excited to produce peristalsis that begins after the termination of vagal stimulation or balloon distension - the "off" response [69, 102, 112, 143, 330]; and (4) a myogenic mechanism for propagation of a contraction, suggesting that muscle properties also contribute to the modulation of peristalsis [17, 298].

The interaction and relative contribution of each of the above four mechanisms in the generation of the final peristaltic response needs to be fully defined. There are two hypotheses that have been suggested. The first hypothesis proposes that the central nervous system stimulates and coordinates oesophageal peristalsis by triggering the cholinergic excitatory neurons as the final common pathway to the oesophageal muscle. In this proposed system, the intramural neural mechanisms are viewed as modulators of the peristaltic response via local and CNS reflexes as well as providing

a local mechanism for the oesophageal inhibition responsible for the oesophageal refractory period.

The second hypothesis proposes that the CNS swallowing control mechanisms trigger peripheral intramural neural mechanisms, which are then primarily responsible for coordinating the peristaltic contraction. The central mechanisms are, however, able to modulate the peripheral neural pathways through temporal and frequency differences in neural discharge. This hypothesis has been developed from data in animal species, such as the opossum and the cat, where intramural mechanisms are capable of producing neurally mediated peristalsis in isolation [102, 112, 137, 143, 152, 294, 330]. The “truth” is probably more complex and is likely to involve a combination of the two above hypotheses.

In the following sections the extrinsic and intrinsic control mechanisms of oesophageal peristalsis will be reviewed separately and in more detail. This separation is somewhat artificial but will hopefully result in a clearer review of the existing literature. In addition, many of the physiological control mechanisms for primary and secondary peristalsis are shared and the reader is reminded that the ultimate peristaltic response, either primary or secondary, will utilise various common pathways.

3.1.5 Extrinsic Control Mechanisms

Striated Muscle Oesophagus: The striated muscle has bilateral cortical innervation [292] and is supplied by excitatory vagal fibres. As with any other striated muscle in the body, the striated muscle component of the oesophagus has no intrinsic capacity to trigger contractions and is entirely dependent on excitatory nerve activity from lower motor neurons [293]. This view is supported by electrical stimulation of the distal end of the cut vagus which results in simultaneous contraction of the oesophageal striated musculature [150, 293]. Transection of the striated oesophagus does not interfere with the peristaltic sequence, which implies that intramural pathways are not required to complete a primary peristaltic sequence in this region [184].

Peristalsis in the striated muscle oesophagus is controlled by sequential central firing of the vagal fibres [5, 96, 150, 293] and is abolished by bilateral cervical vagotomy above the pharyngo-oesophageal branches [150]. Unilateral nerve section has no effect because of extensive bilateral central innervation [292]. Experiments devised by Roman [292] in which the central portion of a sectioned vagus on the left side was used to reinnervate the sternocleidomastoid and trapezius muscles revealed that swallowing triggered a sequential contraction of the reinnervated muscle fibres, and that this coincided with peristaltic contractions simultaneously measured by manometry in the oesophagus.

Smooth Muscle Oesophagus: The triggering of primary peristalsis in the smooth muscle segment of the oesophagus is also dependent on central activation with transmission of excitatory neural impulses through vagal pathways. Disruption of the vagal supply by bilateral cervical vagotomy or vagal cooling abolishes primary peristalsis in the smooth muscle oesophagus [268, 281, 293, 296, 330]. Transection with re-anastomosis of the smooth muscle oesophagus, however, results in deterioration of peristalsis due to interruption of intramural fibres but not in disappearance of the centrally activated peristaltic contraction. These transection studies suggest that sequential activation by central vagal efferents extends along the entire length of the oesophagus [184]. The circular layer of the smooth muscle segment of the oesophagus, however, differs from the circular layer of the striated segment in that the progressive nature of the peristaltic contraction does not solely arise from a programmed sequence of activation of the central swallowing control mechanisms. Brief duration, mass stimulation of the distal stump of the cut vagus results in a propagated, not simultaneous, contraction of the smooth muscle circular layer [150, 324] suggesting that there is an intrinsic mechanism capable of generating a peristaltic sequence in this region. Pharmacological studies suggest that this intrinsic mechanism is triggered by inhibitory non-adrenergic, non-cholinergic vagal pathways [61, 118].

Gidda and Goyal identified two types of efferent vagal fibres using single-fibre recording techniques after stimulation of the proximal end of the superior laryngeal nerve in the opossum [137]. One fibre type had a low activation threshold and a short latency (<1 second) while the second type had a longer latency and higher activation threshold. The activation of the short latency fibres correlated temporally with the onset of activation of the inhibitory non-adrenergic, non-cholinergic vagal pathways. It was therefore postulated that these short latency fibres were responsible for triggering the intrinsic mechanism of oesophageal peristalsis. Activation of the second, longer latency fibres correlated with the latencies of peristaltic contractions and were considered the likely mediators of central sequential oesophageal excitation and contraction.

The longitudinal layer of the smooth muscle segment of the oesophagus is similar to the striated segment of the oesophagus; peristalsis being achieved by sequential firing of cholinergic vagal fibres [102, 325]. In a study by Sugarbaker et al, the onset of the longitudinal muscle contraction occurred in an aboral sequence with swallowing but with stimulation of the distal end of the cut vagus there is simultaneous contraction of the longitudinal layer [325]. This contrasts to the circular smooth muscle layer which contracts in a sequential fashion after stimulation of the distal end of the cut vagus. This difference between circular and longitudinal smooth muscle layers is partly because the longitudinal muscle only receives excitatory innervation, whereas the circular muscle receives dual inhibitory and excitatory innervation. The longitudinal muscle layer cells also lack the intrinsic myogenic properties that are able to generate peristalsis in vitro (see intrinsic control mechanisms below).

In summary, the extrinsic control mechanisms are responsible for the initiation and propagation of peristalsis in the striated muscle layers and in the longitudinal layer of the smooth muscle segment of the oesophagus. In the circular smooth muscle layer, the central mechanisms may initiate and coordinate peristalsis but are not essential for peristaltic success.

3.1.6 Intrinsic Control Mechanisms

As presented above, Roman and Tieffenbach suggested that sequential activation of the vagal preganglionic fibres caused sequential activation of the postganglionic cholinergic excitatory neurons that were responsible for peristalsis in the smooth muscle portion of the oesophagus [294]. This concept, however, fails to explain several other well founded observations of peristalsis in the oesophageal smooth muscle.

These observations are that;

- 1) electrical stimulation of the distal end of the cut vagus nerve can result in peristalsis in oesophageal smooth muscle [102, 140].
- 2) there is a contraction latency gradient that mimics peristalsis with transmural stimulation of intramural nerves in circular muscle strips from different oesophageal levels [69, 70].
- 3) there is an initial period of neural inhibition preceding the oesophageal contractions induced by vagal efferent stimulation [141].
- 4) there is active membrane hyperpolarisation that occurs prior to depolarisation and spike burst activity in response to swallows or vagal efferent stimulation [273, 324].

These phenomenon are accounted for by intrinsic neural mechanisms in the oesophageal smooth muscle wall.

Since the days of Bayliss and Starling, there has been evidence of an intrinsic mechanism capable of generating peristalsis within the intestine [19]. In their original experiments, only the small intestine of the dog was studied, but in further studies in the early 1920's and 1930's, this hypothesis was also extended to the oesophagus. Ingelfinger, in his review of oesophageal motility in 1958 [178], summarised these data by saying; "the orderly propagation of intestinal peristalsis depends on an intact myenteric plexus....this is also true for the esophagus...".

Data to account for this intrinsic phenomenon were first reported in an elegant study by Weisbrodt and Christensen in the 1972 [341]. In this study, it was shown that short-train electrical stimulation, applied directly to isolated smooth muscle strips, triggered a contraction a short time after the stimulus had finished. This was called the “off response” and is a rebound response to antecedent neurogenic inhibition produced by the pulse train stimulation. The latency of this contractile response increased progressively in muscle strips that originated from more distal sections of the oesophagus. This gradient in latency in sequential muscle strips simulates a peristaltic sequence although it was acknowledged that the latencies for occurrence of the “off response” were shorter than the latency of primary peristalsis at corresponding levels. It was also reported that the response was not inhibited by cholinergic or adrenergic antagonists and that it was therefore mediated by non-cholinergic, non-adrenergic inhibitory nerves. Based on these studies, the hypothesis was developed that swallow-induced stimulation of the inhibitory nerves to the smooth muscle oesophagus was the mechanism responsible for oesophageal peristalsis. It was suggested that after a swallow, the responsible inhibitory nerves to the entire smooth muscle segment are excited simultaneously, with consequent circular muscle inhibition. Since all of the oesophageal muscle except the lower oesophageal sphincter is already normally fully relaxed, neural inhibition would only be evident at the lower oesophageal sphincter. Upon recovery from this inhibition, the muscle at all levels demonstrates a sequential rebound contraction, which because of the latency gradient appears as a peristaltic contraction down the oesophagus. This hypothesis has subsequently been supported in vivo with primary peristalsis triggering initial oesophageal relaxation simultaneously along the length of the oesophagus, followed by a contraction with a progressively increasing latency of onset in the distal oesophagus [308].

The cellular basis for the latency gradient in the distal oesophagus is not well understood and it is not clear whether the rebound is due to the action of muscle cell membrane ion channels or to a separate excitatory nerve supply. This latter possibility seems unlikely as pharmacological studies in the opossum have shown that the

rebound or “off response” is not affected by atropine, hexamethonium, methysergide, propranolol, phenoxybenzamine, tolazoline, nicotine, curare or succinylcholine [48]. In vivo, the initial inhibitory wave triggered by swallowing results in membrane hyperpolarisation of the smooth muscle cells of the circular layer [98, 273, 324]. The contraction that subsequently follows this hyperpolarisation only occurs when the membrane depolarises and overshoots the resting membrane potential [273]. This change in membrane electrical potential difference is presumably mediated by the muscle cell membrane ion channels.

A number of recent studies have suggested that nitric oxide may be the neurotransmitter that mediates the non-adrenergic, non-cholinergic inhibitory influence seen in the “off response” and in normal oesophageal peristalsis [61, 246, 355]. Antagonism of nitric oxide synthesis reduces the amplitude and duration of both electrical field stimulation (EFS) induced hyperpolarisation and depolarisation with an associated reduction in the contraction latency gradient in the distal oesophagus [61, 118]. The effects of the antagonist were overcome by the competitive agonist, L-arginine or by addition of nitric oxide to the organ bath. Tetrodotoxin blocked the EFS-induced sequence of membrane hyperpolarisation and depolarisation but had no effect on the hyperpolarisation induced by the exogenous addition of nitric oxide. This suggests that nitric oxide is the neurotransmitter released by the non-adrenergic, non-cholinergic inhibitory pathways and that its site of action is directly on smooth muscle cells.

Nitric oxide may be responsible for the initial neural-induced smooth muscle inhibition, and thus membrane hyperpolarisation, but this does not explain the progressive latency of onset of muscle cell depolarisation and contraction, which is greatest in the distal oesophagus [138]. This finding may be explained by either differences in the passive rebound of the membrane potential, some active intrinsic property of the muscle or a combination of these two factors. The latter is suggested by the demonstration of a decreasing gradient of intracellular potassium concentration

along the distal oesophagus which has been shown to be due to a gradient in the permeability of the muscle to potassium. This may also account for the greater difference in amplitude of hyperpolarisation seen electrophysiologically in the proximal oesophagus [48]. Other factors that may contribute to the latency of the response include central neural delays, conduction velocities of efferents, synaptic delays at the myenteric ganglia, and electromechanical delay during excitation-contraction coupling.

In addition to the “off response”, an intra-stimulus response is also seen in isolated smooth muscle strips with long-train electrical stimulation [69, 70, 102, 112, 139, 143]. This was termed the ‘A’ wave or “on response”. The “on response” is sensitive to tetrodotoxin and atropine, suggesting it is mediated by excitatory cholinergic nerves. This intra-stimulus response has also been demonstrated in vivo [149].

The interaction of the non-adrenergic, non-cholinergic inhibitory innervation and the excitatory cholinergic innervation of the smooth muscle of the oesophagus combine to generate the oesophageal peristaltic sequence. Recent studies in the opossum have shown that the cholinergic “on response” mechanism is more prominent proximally and decreases distally [69, 143], whereas the non-adrenergic, non-cholinergic “off response” is more prominent distally [69, 143]. This produces contractions that are atropine-sensitive proximally but atropine-resistant distally. In the opossum, a combination of the above mechanisms has been shown to contribute to peristalsis with a transition from the “on” mechanism to the “off” mechanism as the contraction proceeds distally. Furthermore, different frequencies of vagal stimulation in this species, can alter and shift the mechanism from one to the other and therefore change either the propagation velocity or even direction of peristalsis [69]. The peripheral gradient of cholinergic innervation also explains why anticholinergic drugs increase contraction latency in the proximal oesophagus and produce a net decrease in the contraction latency gradient along the distal oesophageal body.

Although peripheral intramural mechanisms are capable of producing peristalsis, the characteristics of this response do not entirely mimic primary peristalsis. For example,

swallow-induced peristalsis is significantly slower than peristalsis induced by vagal or transmural stimulation and its amplitude and duration are also substantially different [138]. It is therefore necessary to have central excitatory cholinergic inputs to regulate peristalsis in the smooth muscle segment of the oesophagus. The combination of these finely tuned mechanisms in the intact animal results in normal peristalsis which sweeps distally along the oesophagus without any discernible break. The central swallowing control mechanisms set the timing of this process so that correct progression is possible and the anatomical overlap of muscle fibre type and the intrinsic neural control mechanisms ensure that the transition between different control mechanisms is uniform.

In the section dealing with extrinsic control mechanisms it was shown that the peristaltic nature of longitudinal muscle contraction was determined by the central nervous system with efferent neural signals passing via the vagus nerve. With swallowing, the latency of longitudinal muscle activation is shorter than for the circular muscle so that longitudinal muscle contraction precedes circular muscle contraction at all levels within the oesophagus. The reason that the longitudinal muscle contraction does not simply outrun the circular muscle peristaltic sequence is because of the greater duration of contraction of the longitudinal muscle. This duration increases progressively along the oesophagus. This suggests that there are distinct intrinsic neural or myogenic control mechanisms which modulate the contraction of the longitudinal smooth muscle separately from the circular muscle contraction [324, 325].

3.2 Secondary Peristalsis

3.2.1 Definition

Secondary peristalsis, first described by Meltzer [226], is an important auxiliary physiological mechanism to empty the oesophagus [89, 149]. Most commonly, secondary peristalsis is defined as the oesophageal body response stimulated by oesophageal distension which is not associated with swallowing or any other form of oropharyngeal contraction [68, 129, 149, 305]. This definition has been adopted in this thesis. Under normal conditions, secondary peristalsis is triggered by oesophageal distension induced by reflux of food or fluid from the stomach or by incomplete clearance of a bolus after primary peristalsis.

3.2.2 Function

Secondary peristalsis frequently occurs after spontaneous gastro-oesophageal reflux in normal subjects [90, 109], suggesting a significant role in oesophageal volume clearance after physiological reflux. Secondary peristalsis has been found to occur less frequently after reflux in patients with reflux oesophagitis compared with normal subjects implying that this is a clinically relevant dysfunction in these patients [109]. The contribution of secondary peristalsis to oesophageal acid clearance in reflux disease is unknown. In one recent study, it was argued that because secondary peristalsis failed to restore oesophageal pH to above pH 4 after reflux, it was not of physiological importance [38]. In a more detailed study of acid clearance, however, it has been shown that secondary peristalsis acts primarily by clearing the bulk of the refluxate volume from the oesophagus [162]. This occurs without a change in oesophageal pH, which does not rise until primary peristalsis delivers bicarbonate rich saliva into the oesophagus. As secondary peristalsis, when it occurs, is the initial oesophageal motor event after reflux [38], it would not by itself be expected to restore oesophageal pH, but to complement and accelerate the effects of the primary peristalsis that follows.

3.2.3 Stimuli for Secondary Peristalsis

Secondary peristalsis has been studied using intra-oesophageal balloon distension [68, 129, 269] and infusion of boluses of air or water [68, 115, 227] but there is no consensus on the most appropriate or reliable method of triggering or evaluating secondary peristalsis.

The pattern of the secondary peristaltic response is determined by the site and characteristics of the distending stimulus [178]. Injection of boluses of air or water into the oesophageal lumen trigger a peristaltic response that begins in the upper striated segment and progresses aborally down the entire length of the oesophagus [68, 115, 227]. Balloon distension produces a different pattern of response with a synchronous contraction above the balloon during distension and a propagated contraction wave below the balloon after deflation [68, 129, 269, 349]. When balloon distension is prolonged, the manometric pattern resembles acute oesophageal obstruction at the level of the balloon with high amplitude repetitive contractions above the balloon during inflation and motor quiescence below the balloon [349]. From a physiological point of view, the air and water boluses are probably more appropriate for examining secondary peristalsis than balloon distension. An anchored balloon cannot be displaced by the induced motor response and thus is a stimulus that poorly models the normal situation of oesophageal bolus transport. The sudden deflation of the balloon may also influence the response as this removes the sensory stimulus to the oesophageal wall that may contribute to the successful completion of the secondary peristaltic sequence [168, 338].

The difference in the manometric response induced by air or water boluses compared with that induced by a balloon presumably results from the different nature of the distension resulting from these stimuli. Air or water distension results in a diffuse distension of the oesophagus, there being virtually immediate distribution of the bolus along the length of the oesophagus [194]. In addition, the air and water boluses are mobile and can be cleared by the induced oesophageal response. The presence of a

moveable bolus in this situation is likely to increase the rate of successful peristalsis in much the same way as the presence of a bolus during swallowing increases the rate of successful primary peristalsis [107, 168]. The motor response associated with balloon distension is also more likely to be peristaltic if a fixed balloon is released and allowed to move with the peristaltic contraction [349].

The effects of site and volume of oesophageal distension on the triggering of secondary peristalsis have not been examined in any detail. The studies that have attempted to examine this issue are limited by unreliable and outdated recording methods making evaluation of the data difficult. Furthermore, the different stimuli and volumes used in each study make it virtually impossible to compare the findings amongst studies. Despite these limitations, it has been suggested that the site of oesophageal distension is relevant in the evaluation of secondary peristalsis. In the cervical oesophagus, secondary peristalsis is triggered less commonly by balloon distension than in the distal oesophagus, while the reverse is true for distension with a liquid bolus [178]. The volume of distension is also an important determinant of the response. In a study using balloon distension, secondary peristaltic response rates were greater as balloon diameter increased and this effect was seen for both brief and prolonged balloon distension [30, 269]. Data for diffuse oesophageal distension using different volumes of air and water are more limited but in a study by Creamer and Schlegel, a 50ml bolus was more likely to trigger a response than a 5ml bolus [68]. The rate of oesophageal infusion with a liquid bolus also appears to be a significant variable for stimulation of secondary peristalsis. Slow oesophageal distension (7ml/minute) [328] is less likely to trigger a response than rapid distension (50ml/3.3seconds) [68]. Other physical properties of the bolus such as pH [64, 328], temperature or salt concentration do not influence patterns of stimulation of secondary peristalsis [178].

3.2.4 Manometric and Mechanical Characteristics

The manometric characteristics of secondary peristalsis are variable and depend on the oesophageal preparation used and the method of oesophageal distension employed. Data have been published from human studies [68, 115, 129, 305, 349] and various in vitro or in vivo animal models [46, 49, 185, 226, 269], using either anchored [46, 49, 68, 129, 269, 305, 349] or unrestrained balloons [349] as well as mobile boluses such as air and water [68, 115, 185, 226]. Unfortunately, many early studies suffer from significant technical limitations as they used non-perfused manometric techniques, slow chart recording speeds and limited sampling points. Also, most studies failed to distinguish effectively between primary and secondary peristalsis by monitoring of swallowing [49, 68, 115, 129, 185, 226, 305, 349]. These limitations make it impossible to confidently derive measures such as amplitude and propagation velocity from these studies. Consequently the findings of these studies are reported qualitatively with no attempt being made to derive detailed manometric values.

In studies where values for secondary peristaltic amplitude and velocity are quoted, the velocity has been reported to vary from 3 cm/second [129] to 4-8 cm/second [349]. The velocity depends on the region of the oesophagus examined and the stimulus used, with peristaltic velocities in the mid oesophagus recorded up to as fast as 16cm/second [266] or as slow as 0.2 cm/second when a balloon is being pushed down the oesophagus [349]. The amplitude of the response has received even less attention but in one well performed study [266], the amplitude of the pressure wave above an oesophageal balloon was greater than that seen in the same area with primary peristalsis, while below the balloon, the amplitude was less than primary peristalsis. The actual data for the pressure wave amplitude, however, were not provided.

There remain many unanswered questions regarding the optimal stimulus and site or volume of distension required for the evaluation of secondary peristalsis. The

manometric characteristics of the response also need to be defined with reliable and accurate recording methods. A detailed evaluation of secondary peristalsis in both normal subjects and two patient groups, using modern manometric techniques, comprises a large part of this thesis with the aim of addressing these questions in detail.

3.2.5 Control Mechanisms

The pattern of the motor response induced by oesophageal balloon distension is similar to the response induced by balloon distension in the intestine and consistent with that predicted by "The Law of the Intestine" as described by Bayliss and Starling [19]. The neural and myogenic mechanisms that control secondary oesophageal body peristalsis, however, have received little attention in humans. Animal studies have suggested that many of the mechanisms that control secondary peristalsis are shared with primary peristalsis [305]. The evidence for this partly depends on the fact that primary and secondary peristalsis are manometrically similar and that disruption of intramural pathways does not necessarily alter the characteristics of the secondary peristaltic response. Direct comparisons between human and animal studies, however, are difficult because of species differences in oesophageal physiology and muscle cell type and the different methods of oesophageal distension used in the various studies.

In the opossum, distension of an anchored balloon triggers distal neural inhibition with hyperpolarisation of the smooth muscle cell membrane. This phase is then followed by repolarisation of the cell membrane and then a progressive wave of cell depolarisation and muscle contraction, with an increasing latency of onset along the oesophagus [267]. This results in oesophageal peristalsis. The pathway for this effect in the distal oesophagus involves intramural non-cholinergic fibres as the responses persists after bilateral cervical vagotomy, and electromyographic activity is minimally affected by atropine [267, 269].

In the proximal oesophagus of the opossum, a simultaneous contraction is seen during distension of an anchored distal balloon. This response is abolished by either bilateral cervical vagotomy or atropine with loss of the initial hyperpolarisation phase in the electromyographic sequence [264]. The effect of atropine, however, was noted to be greater in the proximal than the distal oesophagus. This is consistent with the differing contribution of cholinergic innervation in the proximal versus the distal oesophagus of the opossum as described earlier (section 3.1.6) [69, 143]. Currently there is no satisfactory explanation as to why the response above the balloon is synchronous rather than peristaltic.

The above mechanisms indicate that secondary peristalsis in the opossum requires both a central vagally mediated cholinergic pathway and an intra-mural non-adrenergic, non-cholinergic pathway. The interaction of these two, depending on the afferent information from the bolus, will determine the response. This is similar to the postulated mechanism of primary peristalsis in humans with a gradual transition from cholinergic innervation in the proximal oesophagus to non-adrenergic, non-cholinergic neural innervation distally.

In the only detailed study in humans which compares primary and secondary peristaltic contractions, it was suggested that the neural pathways for primary and secondary pathways were different as contractions distal to an oesophageal balloon are of lower amplitude, less atropine sensitive, and more often non-peristaltic when compared to the swallow-induced contractions at the same level [266]. From these data, it was concluded that the two motor events were controlled by different neural mechanisms. The validity of this conclusion may be questioned, as the oesophageal response to distension very much depends on how it is triggered. As suggested earlier in this chapter, fixed balloon distension, as used in this human study, is a poor model for an oesophageal bolus as it induces a motor response significantly different to that associated with swallowing and to spontaneously occurring secondary peristalsis. The site of distension may also be relevant as distension of the striated muscle segment

probably mediates the peristaltic response by central mechanisms and hence mimics primary peristalsis. Conversely, the response to distension in the smooth muscle segment may be different because it is mediated largely by intrinsic nerves. This argument is supported by animal studies as balloon distension in the proximal oesophagus of the cat results in peristalsis which is identical to that induced by swallowing and appears to be exclusively mediated by central mechanisms [30]. By comparison, the aboral distension-induced responses in the opossum smooth muscle oesophagus occur independently of central innervation and differ from swallow-induced peristalsis in much the same way as in the above human study [269]. To accurately compare primary and secondary peristalsis, the physical characteristics and volume of the distending stimulus must be similar, and this probably means the use of an air or water bolus. These data do not exist in either animals or humans.

The effect of atropine on the distension-induced responses above and below an oesophageal balloon in humans reveal that the oral contractions are extremely atropine sensitive whereas contractions induced at the same location with a balloon located proximally are minimally affected by atropine [266]. This observation is perhaps not surprising as the motor response evoked by balloon distension is quite different above and below the point of distension. These differences in atropine sensitivity are similar to the pattern of the response seen in the opossum where the oral atropine-sensitive contractions are abolished by vagotomy whereas the aboral atropine-resistant contractions persist despite vagotomy [264]. The similarity of the response pattern suggests that distension of the human smooth muscle oesophagus evokes proximal excitation that is mediated by cholinergic neurons, whereas the distal response is mediated by intrinsic and largely non-cholinergic neural mechanisms.

By comparison with primary peristalsis, secondary peristalsis is much less pre-programmed and stereotyped, being modulated by the characteristics and position of the bolus. For large boluses or those with a relatively high resistance to passage through the oesophagus, such as boluses with high viscosity, the propagation velocity

of the secondary peristaltic response is slow, as if the oesophageal muscle is working in "low gear" [114]. For smaller or more mobile boluses, however, the response progresses more rapidly [349]. In addition, the progression of peristalsis can be halted altogether if the bolus is held up; only to restart when the bolus moves aboral again. It has been proposed that this pattern of function results from activation of the intramural interneurons in the oesophagus. For example, a proximal contraction might reactivate intramural inhibitory neurons just as the distal site is recovering from the initial hyperpolarisation, thus causing either a suppression of the impending depolarisation or a further hyperpolarisation-depolarisation sequence. This mechanism is a physiological method of fine tuning the timing and strength of the contractions distally depending on the size of the bolus and its rate of transport through the more proximal areas. This capability means that the oesophageal response to distension is infinitely variable and depends on the nature of the distension present.

The control mechanisms of air or water bolus induced secondary peristalsis, have received virtually no attention. The utilisation of purely intra-mural pathways to explain the motor events induced by air and water distension, however, is unlikely as the motor response triggered by oesophageal distension in the distal oesophagus can trigger peristalsis in the proximal oesophagus after it has been transected and excluded from the distal segment [184]. In addition, the secondary peristaltic response can begin in the striated oesophagus and, as described previously (section 3.1.6), this segment lacks the intrinsic capacity to generate peristalsis that is seen in the smooth muscle oesophagus. It is likely, therefore, that afferent pathways are diffusely stimulated throughout the oesophagus and a central pathway is then triggered to assist in coordinating the response [305]. The existence of the sensory limb of this postulated reflex arc is supported by the fact that stimuli, such as balloon distension, are often uncomfortable or even painful. Furthermore, balloon distension with a volume that is below the cognitive sensory threshold, produces characteristic cerebral evoked potentials proving that information is received directly from oesophageal afferents [44, 95, 314].

The efferent pathways involved in secondary peristalsis have not been examined in detail but, in anecdotal evidence from an experiment in a dog, transection and re-anastomosis of the cervical oesophagus did not alter cricopharyngeal relaxation or proximal oesophageal peristalsis induced by balloon distension in the distal oesophagus [305]. It is likely, therefore, that the excitatory muscarinic pathways involved in the generation of primary peristalsis are also utilised for secondary peristalsis. This is further supported by the manometric similarities reported between primary and secondary peristalsis in some studies [30].

Secondary peristalsis is a complex motor event that is controlled by both local and central pathways. The oesophagus is able to distinguish the nature of the distending stimulus and modify its response accordingly. The physiology, neural controls and manometric characteristics of this process, however, are still to be fully established.

Chapter 4

Lower Oesophageal Sphincter Motor Function

- 4.1 Function
- 4.2 Manometric Characteristics
 - 4.2.1 Basal Tone
 - 4.2.2 Relaxation
 - 4.2.3 Reflex After-Contraction
- 4.3 Control
 - 4.3.1 Basal Tone
 - 4.3.2 Relaxation
 - 4.3.3 Reflex After-Contraction
- 4.4 Other Factors that Influence Sphincter Pressure
 - 4.4.1 Meals
 - 4.4.2 Migratory Motor Complex
 - 4.4.3 Physical Activity
 - 4.4.4 Sleep

4.1 Function

The existence of a sphincter at the gastro-oesophageal junction has been the subject of controversy for over a century [78]. The first successful recordings of lower oesophageal sphincter responses during deglutition were obtained by Burget and Zeller in 1936 in a series of experiments in the dog [42]. It was not until 20 years later that Fyke et al. demonstrated a high pressure zone between the oesophagus and the stomach in humans [136]. In these experiments it was also demonstrated that lower oesophageal sphincter pressure decreased in response to increases in intra-oesophageal pressure. A similar high pressure zone has been found in dogs [300], cats [167], monkeys [349], and ruminants [321]. The relationship between swallowing and the changes in intra-sphincteric pressure in these animals is similar to that observed in human subjects. The major function of the lower oesophageal sphincter is to control antegrade and retrograde flow across the gastro-oesophageal junction.

Many of the early studies which examined the function of the lower oesophageal sphincter were limited by sub optimal recording techniques and the lack of understanding of the pitfalls of sphincter pressure measurement. The subsequent development of the low compliance pneumo-hydraulic perfusion systems [7] overcame some of the problems and, with increasing awareness of the appearances of various manometric artefacts, there was an improvement in the fidelity of the measurement systems and the quality of manometric recordings. Difficulties have also been encountered in the choice of the most appropriate recording sensor for sphincter pressure measurements. Side-holes have been used most widely for the measurement of lower oesophageal sphincter pressure but movement of a stationed side-hole relative to the sphincter has been shown to be a major technical limitation. Such movement makes recording of maximal sphincter pressure difficult, and can result in recorded pressure changes suggestive of sphincter relaxation when in fact none occurred. The pull-through techniques have been used with side-hole manometry to assess basal sphincter pressure, but these give only brief glimpses of basal pressure and do not

allow reliable correlation of sphincter pressure with associated oesophageal or gastric pressure events. A major advance came with the development of the “sleeve sensor” which allowed continuous measurement of lower oesophageal sphincter pressure without continual catheter manipulation [88]. Although the sleeve catheter has some limitations in pressure rise rate, it is still the best means of long term sphincter pressure measurement. Further discussion of these manometric techniques and their use in the studies described in this thesis are provided in chapter 6.

This chapter examines various aspects of lower oesophageal sphincter function with first a detailed review of the literature concerning the manometric characteristics of basal sphincter tone, relaxation and the reflex sphincter contraction following relaxation. Following this are discussed the various intrinsic and extrinsic controls that govern sphincter function. In the final section of this chapter, the effect of various external influences such as meals, the migratory motor complex, sleep and physical activity are discussed, as these are relevant background to the evaluation of sphincter function performed in subsequent chapters.

4.2 Manometric Characteristics

4.2.1 Basal Tone

The lower oesophageal sphincter generates a zone of high pressure at rest. The axial sphincter pressure profile, assessed by withdrawal of a side hole through the sphincter, shows a bell-shaped configuration with the higher pressure area in the distal segment of the sphincter. There is also radial pressure asymmetry with slightly higher pressures exerted on the left side in the distal half of the sphincter [342, 343]. The reason for the asymmetry is not clear but probably relates in part to passive external compression by surrounding anatomical structures such as the diaphragmatic crura. The sphincter segment is also angled as it enters the stomach and thus is exposed to uneven extrinsic compression by the diaphragmatic hiatus. It is also possible that

asymmetrical buttressing of the lower oesophageal sphincter by gastric sling fibres is important as patients with hiatus hernia still show radial asymmetry despite displacement of diaphragmatic hiatal support [186].

Basal sphincter pressure varies in association with intra-thoracic and intra-abdominal pressure changes associated respiration. The crural diaphragm also contributes to respiration related pressure changes, as in the cat, respiratory fluctuations are reduced when the crural diaphragm is inactive [35, 343]. The net effect of these influences is a negative pressure deflection in the oesophagus and upper part of the sphincter during inspiration, while in the lower half of the sphincter and the stomach, inspiration results in a positive pressure deflection. The transition zone between these two pressure influences is termed the respiratory reversal point or pressure inversion point. The location of the respiratory inversion point is sometimes used as a guide to the location of the lower oesophageal sphincter but this may be unreliable in subjects with hiatus hernias. In addition to the respiratory related changes, the lower oesophageal sphincter of the opossum and the human can show rhythmic pressure changes that occur at a rate of 3-4 per minute [150]. Lower oesophageal sphincter pressure is also influenced by other factors such as the slow phasic contractions associated with the gastric migratory motor complex [92, 169, 171], by the sleep cycle [257, 258] and by meals [90].

Measurement of basal lower oesophageal sphincter pressure figures prominently in the literature and particularly in many of the early studies which investigated the pathogenesis of gastro-oesophageal reflux disease [56, 155, 271, 347]. It was thought that absent or low basal sphincter pressure was the major pathogenic factor in this condition and thus basal pressure was the subject of intense study. More recently, absent basal pressure has been shown to be a mechanism in only a minority of reflux events, with transient lower oesophageal sphincter relaxation now being recognised as the major factor predisposing to gastro-oesophageal reflux [90, 91, 93, 109, 134, 170, 173-175, 180, 193, 218, 223, 235, 240, 352]. Despite this evidence, the measurement of basal lower oesophageal sphincter pressure is still the primary focus

in many studies that address questions relevant to sphincter function in reflux disease. The values for basal sphincter pressure vary widely within the literature, depending on the population being studied and whether allowance is made for other factors such as the posture of the subject during recording and whether the measurements are made during fasting or post-prandially. In addition, different recording methods can make a significant difference to the basal sphincter pressure recorded [342] and thus make comparisons between studies difficult.

Studies with the sleeve sensor have shown that basal lower oesophageal sphincter pressure varies widely between and within subjects. Basal pressure of anywhere between 5-60 mmHg are common in normal subjects depending on the recording conditions [90]. It is difficult to validate a single value for lower oesophageal sphincter pressure due to the various pressure fluctuations associated with respiration, stage of interdigestive motor cycle and sleep status. Because definition of normal may vary in different studies, each method needs appropriate validation before adequate conclusions may be drawn.

4.2.2 Relaxation

The uninterrupted progression of a bolus from the oesophagus to the stomach is dependent upon the integrated function of oesophageal peristalsis and lower oesophageal sphincter relaxation. When relaxation of the sphincter is impaired, oesophageal transport is impaired. In patients with achalasia, the impaired relaxation of the lower oesophageal sphincter during swallowing is the major determinant of the clinical features of this disease. Lower oesophageal sphincter relaxation can be triggered by swallowing and oesophageal or gastric distension.

Swallow-induced relaxation: Lower oesophageal sphincter relaxation associated with swallowing begins with the onset of deglutition and lasts up to 6-10 seconds. When so triggered, lower oesophageal sphincter pressure falls within 1.5 to 2.5 seconds and remains low as the peristaltic wave traverses the oesophageal body [96].

Lower oesophageal sphincter relaxation occurs with virtually all swallows, including those swallows that may result in failed or synchronous oesophageal peristaltic responses. Complete primary peristalsis is normally associated with relaxation to within 2 or 3 mmHg of intra-gastric pressure.

Transient lower oesophageal sphincter relaxation: A “transient lower oesophageal sphincter relaxation” (tlosr) is another distinctive pattern of lower oesophageal sphincter relaxation [90]. By definition, a tlosr is not associated with swallowing although one controversial study has suggested that many tlosr’s may be triggered by “sub-threshold” swallows [235]. This notion has been refuted by other investigators in a subsequent study [193]. Transient lower oesophageal sphincter relaxation is the major mechanism which allows gastro-oesophageal reflux of gas [190, 223] or fluid [90] from the stomach and is triggered by a number of factors such as gastric distension [93, 131, 134, 170, 173, 174, 352] and duodenal fat receptors [180]. The site and nature of the stimulus required to induce this relaxation are complex but the abrupt onset and brief duration of these relaxations suggests that they are neurally mediated [172]. A detailed description of the triggering and controlling mechanisms of tlosr’s is presented in section 5.1.1. Studies of the manometric characteristics of tlosr’s have shown that in the majority of these events, sphincter relaxation is complete (<4mmHg residual pressure) and lasts between 7-40 seconds [235] with a mean duration of 14.9 seconds [93].

Oesophageal distension-induced sphincter relaxation: Sphincter relaxation may also be produced by oesophageal distension, with or without associated secondary peristalsis. This response was first described by Fyke et al [136] and subsequently examined in more detail in studies with balloon and water bolus oesophageal distension [68, 129]. More recently, animal studies have examined the neural pathways of this response and the characteristics of the distending stimulus required to induce lower oesophageal sphincter relaxation [265, 272]. In the human, there is less information and the value of many of the early studies are limited by what

are now known to be invalid or sub-optimal recording methods [68, 129]. In addition, existing studies are confined to examination of the sphincter response during balloon distension with no data on more physiological stimuli such as oesophageal distension by air or water boluses or during gastro-oesophageal reflux. Little or no information is available about the manometric characteristics of the sphincter response to oesophageal distension.

4.2.3 Reflex After-Contraction

Following swallow-induced sphincter relaxation there is a 7-10 second after-contraction which occurs in peristaltic sequence with the oesophageal contraction. The lower part of the lower oesophageal sphincter does not show after-contractions and in this region the sphincter pressure simply returns to the resting level [149]. There are no studies in the literature which examine the after-contraction following sphincter relaxation in any detail.

Short-lived increases in lower oesophageal sphincter pressure also occur secondary to increases in intra-abdominal pressure. Considerable controversy exists as to whether this rise in sphincter pressure is due to reflex contraction or merely from passive transmission of the increased intra-abdominal pressure [36, 106, 111, 164, 183, 199, 208, 211, 215, 252]. This issue is discussed in more detail in section 5.3.

4.3 Control

4.3.1 Basal Tone

The level of basal lower oesophageal sphincter pressure *in vivo* reflects the cumulative effect of the intrinsic smooth muscle sphincter and the superimposed extrinsic compression that adds to the pressure generated by the sphincter itself. It has been suggested that it may be more appropriate to refer to the pressure barrier at the lower oesophageal sphincter as the “anti-reflux barrier” [238] and the measured cumulative

pressure as the oesophago-gastric junction pressure [232] in order to recognise the contribution of extrinsic sphincter compression. This terminology has merit in that it acknowledges that the intraluminal pressure measured in the region of the lower oesophageal sphincter is the net result of more than the smooth muscle sphincter. For the purposes of this thesis, however, the components will be referred to by current convention with each component presented separately in an effort to clarify the respective roles of the intrinsic smooth muscle sphincter and extrinsic factors, namely the diaphragm.

Extrinsic control of basal lower oesophageal sphincter tone: The extrinsic factors that influence basal lower oesophageal sphincter pressure may be divided into extrinsic neural influences, humoral agents and circulating hormones based on studies using electrical nerve or tissue stimulation and various pharmacological manipulations in anaesthetised animals or isolated organs. In humans, the extrinsic controls of sphincter tone have been examined by pharmacological manipulation of various neural pathways using a limited variety of neurotransmitter agonists and antagonists. The results of these different studies are variable depending on the method and species studied as well as the obvious limitation of attempting to study the isolated effects of neural and humeral pathways in a complex integrated system that ultimately controls sphincter function in vivo.

There are significant species differences in the neural control of basal lower oesophageal sphincter pressure. In humans, dogs and cats there are both excitatory and inhibitory vagal fibres that influence basal sphincter pressure [59, 103, 154, 223, 272] while in the opossum, the vagus predominantly mediates sphincter inhibition [147, 151, 268, 275, 296]. In dogs and cats, excitatory vagal fibres contribute to the maintenance basal sphincter pressure as vagal cooling, vagotomy or cholinergic antagonists all cause a significant reduction in basal pressure [59, 154, 223, 272]. Vagal inhibitory fibres in these species, trigger sphincter relaxation and are discussed in more detail in section 4.3.2. In the anaesthetised opossum, there is no significant

alteration in basal sphincter pressure after vagotomy, vagal cooling or cholinergic antagonists [147, 151, 268, 275, 296]. In humans, sphincter pressure is virtually abolished by cholinergic blockade suggesting that basal sphincter pressure is maintained by an excitatory cholinergic input [103]. It is likely that this effect is mediated by vagal fibres but the effect of bilateral cervical vagotomy is not known. Previous studies which have attempted to address the role of the vagus in humans have examined the effect of high abdominal vagotomy. This approach is inappropriate as vagal efferents to the sphincter enter the oesophagus at the mid thoracic level and travel intramurally in the distal oesophagus. It is therefore not surprisingly that high abdominal vagotomy does not influence lower oesophageal sphincter pressure [73, 252, 326].

There is also evidence that sympathetic nerve activity can influence lower oesophageal sphincter tone in the cat [22, 130, 145]. Stimulation of splanchnic sympathetic efferents in the cat causes sphincter contraction that is sensitive to atropine, hexamethonium, phentolamine and tetrodotoxin. Cervical sympathectomy or splanchnicectomy in this species, however, does not significantly alter basal lower oesophageal sphincter pressure so the normal physiological role of these mechanisms is probably unimportant [22, 130]. In a detailed study of the sympathetic supply to the lower oesophageal sphincter of the cat, it has been shown that sympathetic stimulation excites intrinsic excitatory cholinergic neurons and inhibits vagal inhibitory fibres to the sphincter [145]. This dual effect is achieved chiefly through an action at the level of myenteric neurons and, to a lesser degree, through a direct action on muscle cells. In both cases alpha-adrenergic receptors are involved. The effect of the sympathetic nervous system on lower oesophageal sphincter pressure in humans remains poorly understood. Studies with adrenergic agonists and antagonists are limited but in one, propranolol increased basal lower oesophageal sphincter pressure [150]. The effect of these agents are transient and no published study has yet supported a major role of tonic adrenergic activity in control of basal lower oesophageal sphincter tone.

The effects of other neurotransmitters on lower oesophageal sphincter pressure have been studied with various agonists and antagonists but interpretation of the data are often difficult because of species differences and the method of study. The receptors and transmitters which play a physiologically important role can be recognised by investigating the effects of elimination, inactivation, or antagonism of the receptors or the various neurotransmitters. If such a manoeuvre produces an abnormality in function, then physiological importance can be assumed. If no effect is seen, then the pathway is either unimportant or there is compensation by other mechanisms that disguise an important effect of the mechanism being examined. If an effect is seen then replacement of the missing substance at the appropriate site in the appropriate concentration should restore physiological function but this is not always possible.

Agonist studies are helpful in determining the distribution and action of various neurotransmitters and their receptors but do not clarify which of these are normally active under basal conditions. Antagonist studies are more helpful under basal conditions, but the use of these agents is often limited to animal studies because of the antagonist's often widespread action and undesirable systemic side effects. The difference in agonist and antagonist studies is illustrated by the effect of cholinergic agonists and antagonists on lower oesophageal sphincter function. In humans and most animals, muscarinic agonists cause lower oesophageal sphincter contraction [279, 291]. The administration of muscarinic antagonists such as atropine, however, does not change basal lower oesophageal sphincter pressure in the opossum, cat or monkey but substantially decreases basal pressure in the human and dog [22, 103, 150, 293]. In no species does atropine completely abolish basal lower oesophageal sphincter pressure. This indicates that there are excitatory inputs to the lower oesophageal sphincter in all the species studied but only in some species are these necessary for the maintenance of basal sphincter tone.

Other biological agents have been suggested as contributing to the tonic contraction of the lower oesophageal sphincter. These include serotonin, histamine, dopamine,

substance P, enkephalin, bombesin, prostaglandins and vasoactive intestinal polypeptide. Dopamine decreases lower oesophageal sphincter pressure whereas histamine, serotonin and opioids have complex effects because of different receptor subtypes and activities [150]. The selective antagonism of these agents, however, does not abolish basal lower oesophageal sphincter pressure and thus it is unlikely that they are primarily responsible for the generation of basal tone. The other agents have been shown to cause lower oesophageal sphincter contraction but their physiological role is unclear.

Interest in the effects of circulating hormones on basal lower oesophageal sphincter pressure arises from the variations in sphincter pressure associated with feeding and during the day and night. It was postulated that these variations were due to alterations in hormone levels, particularly gastrin. Evidence for the role of gastrin was initially based on the fact that gastrin resulted in lower oesophageal sphincter contraction [144] and that the changes in sphincter tone associated with changes in gastric pH were mediated by gastrin [43]. Numerous studies have since refuted this proposed physiological role of gastrin [150]. Other hormones such as secretin, cholecystokinin and glucagon have also been suggested as influencing lower oesophageal sphincter pressure but they do not appear to have any role in maintaining basal sphincter tone.

Intrinsic control of basal lower oesophageal sphincter tone: The intrinsic factors that influence basal sphincter tone include local myogenic and physiological properties of the sphincter muscle as well as intrinsic neural and electrophysiological factors. These physiological and mechanical features distinguish lower oesophageal sphincter muscle from the muscle in the distal oesophagus and proximal stomach [24, 26, 27]. The importance of the intrinsic controls for basal tone, in the cat at least, are revealed by extrinsic denervation of the sphincter by sectioning of the cervical vagus nerves, the spinal cord, the left splanchnic nerves and the left gastric artery, all of which did not affect basal sphincter pressure [22]. Administration of atropine in

humans virtually abolishes basal lower oesophageal sphincter pressure, which suggests that interruption of the extrinsic vagal cholinergic efferent supply would also reduce lower oesophageal sphincter pressure.

An important intrinsic capability of the lower oesophageal sphincter, like other smooth muscle sphincters, is the maintenance of tonic contraction for prolonged periods of time. The precise mechanism responsible for this characteristic is not clear. One proposed mechanism for the maintenance of basal sphincter tone in the lower oesophageal sphincter is the "latch phenomenon". This is an energy dependent phosphorylation reaction between actin and myosin which also requires calcium and its binding protein, calmodulin. Sphincteric phosphorylation rates decrease to 4% of maximum during sphincter relaxation, increase to 33% during tone development and then fall to 16% during maintenance of the tonic contraction [150]. These findings suggest that the myofibrils are able to temporarily "latch" in the contracted state and thereby save energy while maintaining basal tone. Calcium may be important in this process as levels of free intracellular calcium increase during tone development and subsequently returning to baseline levels during tone maintenance. Levels of free intracellular calcium are higher in sphincter muscle at rest than in the oesophageal muscle and calcium channel blocking drugs are able to reduce sphincter tone [150].

In addition to the calcium related effects on sphincter tone, electrophysiological studies in the opossum and the cat have shown continuous spike activity in sphincter muscle which increases in frequency with increased tone [10, 279, 280]. The contribution of the spike activity, however, is unclear as spiking is abolished by calcium antagonists which only partially reduce basal sphincter tone. The mechanism which maintains the residual, spike-independent tone is not known but it may be related to a difference in basal resting membrane potential of the smooth muscle cells [150], as the resting membrane potential is -40mV in the sphincter muscle and -50mV in the oesophageal muscle [75]. In the cat, there is a linear dependence between the degree of depolarisation of the sphincter muscle and the degree of tonic muscle tension [262].

4.3.2 Relaxation

The control of lower oesophageal sphincter relaxation is a complex process which integrates both central and local neural pathways, the combination of which determine the timing, extent and duration of sphincter relaxation. Lower oesophageal sphincter relaxation may be triggered by primary or secondary peristalsis or independently of swallowing or any peristaltic oesophageal activity such as occurs during transient lower oesophageal sphincter relaxation.

Sphincter relaxation is an active process associated with inhibition of the continuous spike activity and hyperpolarisation of the muscle cell membrane. These electrical phenomenon may be related to decreases in free intracellular calcium which leads to cessation of myosin phosphorylation or to inhibition the other unknown proteins involved in the latch phenomenon. Agents such as isoproterenol and prostaglandin E which increase intracellular c-AMP and sodium nitroprusside that increase c-GMP also cause lower oesophageal sphincter relaxation, presumably by activating protein kinase A and G respectively [150]. These kinases may lower free intracellular calcium and also inhibit myosin phosphorylation by inactivating the enzyme myosin light-chain kinase [150].

Neural control of sphincter relaxation: The swallow-induced sphincter relaxation is triggered by the deglutitive wave of oesophageal neural inhibition and is sustained if swallowing occurs in rapid succession. Restoration of the sphincter pressure only occurs after the last swallow. Animal studies show that sphincter relaxation associated with primary peristalsis is mediated by vagal efferent nerves as it is abolished by bilateral cervical vagotomy or vagal cooling [150].

Reflex lower oesophageal sphincter relaxation is also triggered by distension of either the skeletal or smooth muscle portions of the oesophagus and is usually associated with secondary peristalsis [68, 129, 268, 349]. The neural pathways involved in distension of the striated or smooth muscle segments, however, are different. Lower

oesophageal sphincter relaxation triggered by distension of the striated muscle is mediated centrally and is abolished by vagotomy while the response to smooth muscle distension is mediated by intramural nerves and remains after vagotomy [150]. The neural pathways that mediate distension-induced sphincter relaxation are also different to those that mediate vagal-induced sphincter relaxation [265]. In studies in the opossum, a combination of atropine, hexamethonium and 5-methoxydimethyltryptamine blocked ganglionic transmission in the vagal inhibitory pathway to the sphincter but did not affect sphincter relaxation induced by balloon distension. The vagus nerve, however, may exert a facilitatory influence on the lower oesophageal sphincter relaxation triggered by distension of the smooth muscle oesophagus [268, 282]. The depth and duration of sphincter relaxation is proportional to the distension volume and duration [269].

In addition to the triggering of lower oesophageal sphincter relaxation associated with swallowing, the sphincter is also able to relax during vomiting, retching or belching. The mechanisms which trigger lower oesophageal sphincter relaxation associated with these events are not well understood. Gastric distension in humans increases the frequency of transient sphincter relaxations [353] and in the dog, this response to gastric distension is significantly impaired after vagal cooling [223] or vagotomy [322]. It is possible therefore that the afferent nerves triggered by gastric distension induce sphincter relaxation by activating central vagal inhibitory efferent fibres. A second postulated mechanism is that lower oesophageal sphincter relaxations are triggered via intramural pathways within the gastric wall as electrical stimulation of the gastric intramural nerves can cause sphincter relaxation [150].

Neurotransmitters involved in sphincter relaxation: The lower oesophageal sphincter receives inhibitory influences from both the vagus and the intramural neural plexus; the latter involves a chain of pre- and postganglionic neurons. The vagal preganglionic neurons synapse on inhibitory neurons present in the intramural plexus of the lower oesophageal sphincter. The intramural pathways are less well defined but

probably involve sensory neurons that also synapse with the postganglionic inhibitory neurons.

The preganglionic vagal neurons release acetylcholine which combines with nicotinic and muscarinic receptors to activate the postganglionic inhibitory neurons [151]. This response in the opossum is significantly impaired by a combination of hexamethonium and atropine [151]. Rattan and Goyal subsequently reported that in most opossums a serotonergic receptor antagonist was also required to completely antagonise ganglionic transmission in this pathway [276]. Serotonin (5-HT) stimulates two types of nerves, which lead to an inhibitory and an excitatory response of the lower oesophageal sphincter respectively [152]. The neurally mediated inhibitory effects of 5-HT are antagonised by tetrodotoxin but not by adrenergic blockade, catecholamine depletion, or atropine, suggesting that non-cholinergic, non-adrenergic inhibitory neurons are involved in the effect of 5-HT on the lower oesophageal sphincter. A number of other neurotransmitters have also been shown to influence sphincter relaxation [152]. These include adrenergic agents via beta adrenergic receptors, dopamine, histamine (H1 & H2 receptors), prostaglandins (PGE1, PGE2, PGA2) and various other gastrointestinal polypeptides such as gastrin, cholecystokinin, secretin, glucagon, vasoactive intestinal polypeptide and gastric inhibitory polypeptide.

The synaptic transmitter used by the intramural neurons is unknown. It is different to the vagal inhibitory pathway to the lower oesophageal sphincter, as electrical stimulation of the oesophageal intramural pathways or oesophageal balloon distension will still trigger lower oesophageal sphincter relaxation in the presence of nicotinic, muscarinic and serotonergic antagonists [265]. The inhibitory neurotransmitter released by the postganglionic intramural neuron is not cholinergic or adrenergic in nature and has therefore been previously referred to as a nonadrenergic, noncholinergic neurotransmitter [151, 334]. There are also data to suggest that histamine, dopamine and prostaglandins are not involved [150]. Vasoactive intestinal polypeptide (VIP) was, until recently, considered the most likely candidate for the

non-adrenergic, non-cholinergic inhibitory transmitter to the lower oesophageal sphincter. This was based on four lines of evidence: (i) VIP immunoreactivity is present in abundance in myenteric neurons of both the opossum [278] and human [1, 4] lower oesophageal sphincter and is known to be released in association with nerve-mediated relaxation [25], (ii) patients with achalasia, a condition characterised by failure of lower oesophageal sphincter relaxation, have been reported to have a marked paucity of VIP immunoreactive neurons in the region of the lower oesophageal sphincter [1], (iii) VIP has a direct inhibitory effect on smooth muscle of the lower oesophageal sphincter that is resistant to tetrodotoxin [25, 277], and (iv) specific VIP antisera [21, 25, 153, 306] as well as putative VIP antagonists [263] have been reported to antagonise nerve-mediated lower oesophageal sphincter relaxation both in vivo and in vitro. There is not unanimity about the role for VIP as the non-adrenergic, non-cholinergic neurotransmitter to the lower oesophageal sphincter. Significant doubts are cast by the findings of Daniel et al that VIP does not cause the hyperpolarisation of the oesophageal circular muscle that is associated with activation of the inhibitory neural pathway [76]. Daniel's study has however been criticised on the basis that oesophageal muscle and not sphincter muscle was studied. Miller et al have also reported that VIP induces lower oesophageal sphincter relaxation in the cat by increasing intracellular c-AMP. By contrast, stimulation of intramural nerves resulted in increased production of c-GMP levels with no change in c-AMP [230].

Calcitonin gene related peptide is another possible inhibitory neurotransmitter to the lower oesophageal sphincter. This is also localised in the sphincter and has been shown to cause lower oesophageal sphincter relaxation [274]. The significance of this compound is yet to be defined.

More recently, it has been suggested that nitric oxide is the major mediator of the inhibitory nonadrenergic, noncholinergic transmission in the oesophagus [118, 356]. Nitric oxide fulfils a number of criteria as a putative inhibitory nonadrenergic, noncholinergic neurotransmitter in the oesophagus that VIP fails to satisfy. Its release

is triggered by vagal and intramural stimulation and electrically it results in membrane hyperpolarisation prior to depolarisation and contraction. In addition, nitric oxide increases c-GMP levels in opossum oesophageal body circular muscle [119] and it is assumed that this effect is similar in the lower oesophageal sphincter.

The major difficulty with the hypothesis that nitric oxide is the major inhibitory neurotransmitter to the oesophagus and lower oesophageal sphincter is that the net effect of nitric oxide release in the oesophageal body is to trigger contraction (via the "off response") and yet in the lower oesophageal sphincter, nitric oxide triggers prolonged relaxation. This problem has recently been addressed in elegant microelectrode studies by Conklin et al [61]. In these studies, smooth muscle strips from the opossum oesophagus and lower oesophageal sphincter were placed in an organ bath and the force of muscle contraction as well as transmembrane potential difference were measured in response to transmural electrical field stimulation. At rest, the transmembrane potential of the oesophageal body and lower oesophageal sphincter muscle were identical. With transmural electrical field stimulation membrane hyperpolarisation was seen in both muscle types. The important difference noted, however, was that oesophageal muscle tone was unaltered during membrane hyperpolarisation while in the sphincter muscle there was relaxation. The subsequent electrophysiological responses of the oesophageal and sphincter muscle strips were also different. In the oesophageal body muscle, there was initial hyperpolarisation followed by depolarisation whereas in the sphincter, only hyperpolarisation was observed. The duration of the hyperpolarisation was also significantly longer for the sphincteric muscle compared to the oesophageal muscle. These effects were all abolished by tetrodotoxin and the addition of L-NNA, a nitric oxide synthase blocker, to the preparation reduced the degree of hyperpolarisation but not its duration. To further test the role of nitric oxide in this process, exogenous nitric oxide was added to the preparation and this resulted in prompt hyperpolarisation of the sphincter muscle which gradually returned to normal. The nitric oxide induced hyperpolarisation was not followed by depolarisation. Tetrodotoxin blocked the hyperpolarisation of the

sphincter muscle induced by electrical field stimulation but not that produced by exogenous nitric oxide. From these studies it has been concluded that mediation by nitric oxide best fits the pattern of cellular events seen with non-adrenergic, non-cholinergic inhibitory signals to the oesophagus and lower oesophageal sphincter in the opossum.

The mechanism(s) for the different electrophysiological behaviours of the sphincter muscle and the oesophageal muscle is not clear. One possible mechanism, put forward by Ward et al. based on electrophysiological studies in the canine colon, is that the depolarisation following nerve-induced hyperpolarisation of colonic muscle is mediated by the release of an excitatory eicosanoid [340]. Such a mechanism may also be present in oesophageal muscle but not in the lower oesophageal sphincter. Alternatively, nitric oxide in the oesophageal body may either be co-released with, or stimulate the release of an excitatory neurotransmitter that is not present in the lower oesophageal sphincter. The converse of this could also be true; that is, nitric oxide and another inhibitory neurotransmitter such as vasoactive intestinal polypeptide or calcitonin gene-related polypeptide may be co-released in the sphincter and not in the oesophagus. There is evidence to support this from other studies as both peptides have been shown to cause lower oesophageal sphincter relaxation (as discussed above). In addition, studies by Costa et al. show co-localisation of nitric oxide synthase and vasoactive intestinal polypeptide immunoreactivity in enteric neurons of the guinea pig [65]. The exact role of nitric oxide and any associated neurotransmitters in lower oesophageal sphincter relaxation in humans need to be confirmed.

4.3.3 Reflex After-Contraction

The contraction of the lower oesophageal sphincter after-relaxation occurs in sequence with the peristaltic wave in the oesophageal body. The after-contraction is more prominent with deep sphincter relaxation and as muscle tone increases, the after-contraction is abolished [150]. The control of the after-contraction has not been adequately studied.

4.4 Other Factors that Influence Sphincter Pressure

4.4.1 Meals

There is considerable diurnal variation in basal lower oesophageal sphincter pressure and one of the major influences is food ingestion. Post-prandially there is a significant decrease in basal sphincter pressure [90] and a significant increase in the frequency of transient lower oesophageal sphincter relaxations [170, 270]. The majority of studies on the effect of various food substances, however, are limited by the sub optimal recording methods used to measure sphincter pressure changes. In addition, most of these studies only examine the effect of a specific food substance on basal sphincter pressure and ignore their potential effects on other aspects of sphincter function such as transient lower oesophageal sphincter relaxation.

The type of food ingested determines the predominant effect on the lower oesophageal sphincter. Despite the significant limitations discussed above, most studies have shown that basal lower oesophageal sphincter pressure is reduced by fat, chocolate, alcohol and carminatives and is increased by high protein meals. Studies in human volunteers with equi- caloric test meals have shown significant decreases in sphincter pressure after fat ingestion, and augmentation after protein-rich meals [249, 250]. This has suggested to some the possible role of a protein-rich diet in the management of patients with gastro-oesophageal reflux disease. The depressant effect of fat on the lower oesophageal sphincter also occurs if the fat is infused directly into the duodenum [180, 249]. Although the lowering of sphincter pressure by chocolate was originally considered to be due to the high fat content of most chocolate preparations, the use of defatted chocolate syrup has shown that chocolate itself decreases sphincter pressure [286]. The mechanism of action is most likely explained by the high content of methyl xanthines, and their inhibition of phosphodiesterase, resulting in accumulation of cyclic AMP [297]. This increase in c-AMP can induce sphincter relaxation [148].

Carminatives, including peppermint and spearmint, which are often included in after-dinner foods and liqueurs, have been shown to result in decreased lower oesophageal sphincter pressure and may provide another explanation for post-prandial heartburn.

There are conflicting data on the response of the lower oesophageal sphincter to coffee and caffeine [286]. Some studies have shown that coffee reduces sphincter pressure while others have noted an increase in pressure or simply no effect. Similar conflicting data are also available for caffeine. It is difficult to reconcile these different studies but it has been suggested that the conflicting data may be related to the type of coffee or raw green coffee bean used and the method of processing [337]. The major criticism of all these studies again remains the unreliable techniques used for the measurement of lower oesophageal sphincter pressure and the focusing of the effect of these various substances on basal sphincter pressure rather than other potential mechanisms of sphincter function that are more relevant in the pathogenesis of disease states such as gastro-oesophageal reflux disease.

4.4.2 Migratory Motor Complex

Lower oesophageal sphincter pressure fluctuates in response to the different phases of the migrating motor complex [92, 169, 171]. Lower oesophageal sphincter pressures are maximal during phase 3 of the gastric migratory motor complex activity and lowest during phase 1 [92]. During phase 2, particularly phase 2b, the sphincter pressure pattern has a “roller coaster” appearance, with the peaks of lower oesophageal sphincter contraction corresponding closely to the gastric body contractions. There is transient inhibition of the sphincter pressure oscillations associated with the MMC cycle during swallowing [92]. Similar fluctuations in lower oesophageal sphincter pressure are also noted in the opossum and these are abolished by atropine, anaesthesia or feeding [169]. In the opossum, the cyclical changes in lower oesophageal sphincter pressure during the migratory motor cycle are mediated by motilin [171].

4.4.3 Physical Activity

Prior to the studies presented in chapter 12 of this thesis, lower oesophageal sphincter pressure has not been recorded in subjects while walking or exercising and so it is not known if there is any significant fluctuation in sphincter pressure during these activities. It is possible that there is a significant effect as many patients with reflux disease complain that their symptoms are aggravated by physical activity. This is discussed in more detail in section 5.3.

4.4.4 Sleep

The effect of sleep on oesophageal motor function has been known for many years. In 1965, Lear et al. reported that swallowing frequency was greatest during eating and least during sleep, with other activities occupying an intermediate place [209]. Records during sleep invariably showed periods of 20 minutes or more during which deglutition was absent, swallowing only occurring when subjects were falling asleep or during arousal periods [212]. Measurements of lower oesophageal sphincter pressure were not possible until much later when techniques were developed to allow continuous recordings over prolonged periods [88]. It was then demonstrated that there is a clear rise in basal sphincter pressure during the night in both healthy subjects and patients with gastro-oesophageal reflux disease [90, 104]. Sphincter relaxations only occurred during arousal periods when the subject either swallowed or a transient lower oesophageal sphincter relaxation occurred [90, 104, 132].

Chapter 5

Mechanisms of Gastro-Oesophageal Reflux and Oesophageal Acid Clearance in Health and Disease

- 5.1 Role of the Lower Oesophageal Sphincter
 - 5.1.1 Defective Basal Lower Oesophageal Sphincter Pressure
 - 5.1.2 Lower Oesophageal Sphincter Relaxation
- 5.2 Effect of Hiatus Hernia and the Diaphragm
- 5.3 Effect of Exercise and Straining
- 5.4 Mechanisms of Oesophageal Acid Clearance
 - 5.4.1 Acid Clearance in Normal Subjects
 - 5.4.2 Acid Clearance in Patients with Reflux Disease

5.1 Role of the Lower Oesophageal Sphincter

The lower oesophageal sphincter is the major structure that prevents gastro-oesophageal reflux [178] and with the diaphragm constitutes what is commonly referred to as the anti-reflux barrier [238]. The role of these structures in the pathogenesis of gastro-oesophageal reflux will be examined in this chapter.

Traditionally, reflux was thought to occur as a result of a chronically weak lower oesophageal sphincter that was simply overcome by the gastro-thoracic pressure gradient [105]. This view, however, failed to explain the mechanism of reflux in a significant proportion of patients with gastro-oesophageal reflux disease who have normal basal lower oesophageal sphincter pressures [329]. This hypothesis was also unable to account for the episodes of gastro-oesophageal reflux seen in healthy subjects - so called physiological reflux.

In more recent studies, lower oesophageal sphincter pressure has been correlated with individual episodes of reflux by concurrent measurements of oesophageal motility and pH [88, 90, 93, 104, 240]. From these studies it was established that under resting conditions, the lower oesophageal sphincter pressure must be absent ($< 2\text{mmHg}$) for reflux to occur. The absence of sphincter pressure during the majority of these episodes, however, was not due to a chronically weak or absent sphincter pressure but to intermittent episodes of lower oesophageal sphincter relaxation. Basal sphincter pressures in the range of 5-10mmHg, although generally regarded as being abnormally low, were sufficient to prevent reflux [91].

In healthy volunteers and patients with mild reflux disease, transient lower oesophageal sphincter relaxation accounts almost exclusively for the absence of lower oesophageal sphincter pressure at the time of reflux. Transient lower oesophageal sphincter relaxation is also the mechanism allowing reflux in nearly two thirds of reflux episodes in patients with more severe reflux disease [104, 110], the remaining episodes in these patients occurring during periods of minimal or absent lower

oesophageal sphincter pressure. Lower oesophageal sphincter dysfunction in patients with gastro-oesophageal reflux disease thus is not primarily a question of defective smooth muscle tone. Rather it is a question of a control dysfunction which leads to an increased frequency of transient lower oesophageal sphincter relaxations. Secondly, there is also the question as to why a greater proportion of these transient lower oesophageal sphincter relaxations are associated with gastro-oesophageal reflux [172].

5.1.1 Defective Basal Lower Oesophageal Sphincter Pressure

Absent basal sphincter pressure is the mechanism associated with reflux in 18-23% of reflux episodes [93, 104] with the frequency of reflux due to this mechanism increasing proportional to the severity of the reflux disease [93]. In addition, in patients with severe reflux disease, lower oesophageal sphincter pressure is likely to be absent for short periods (10-15 minutes) after a meal and further predisposes these patients to gastro-oesophageal reflux [172]. The cause of these variations in basal lower oesophageal sphincter pressure is unknown but suggests a defect in lower oesophageal sphincter control rather than an intrinsic abnormality of the sphincter muscle.

In humans, the major component of lower oesophageal sphincter tone appears to depend on sustained cholinergic vagal input as atropine virtually abolishes lower oesophageal sphincter pressure [103, 283]. There is evidence that some patients (25-50%) with reflux disease have vagal nerve dysfunction on the basis of impaired gastric secretory responses to insulin-induced hypoglycaemia or abnormal cardiovascular reflexes [253]. From these data, it was extrapolated that there is also impairment of the vagal inputs to the lower oesophageal sphincter in some patients with reflux disease. These data, however, only provide indirect evidence of vagal dysfunction to the upper gastrointestinal tract and the validity of testing the integrity of the vagal inputs to the lower oesophageal sphincter with these indirect measures is uncertain.

In a few patients with reflux disease and complete absence of lower oesophageal sphincter pressure, there is evidence for sphincter muscle dysfunction. This type of abnormality is most commonly seen in patients with smooth muscle myopathies such as scleroderma [245]. There are also some patients who do not have any overt signs of smooth muscle dysfunction and who have abnormal responses to smooth muscle stimulants such as gastrin, suggesting decreased muscle contractile function [128]. The role of gastrin in lower oesophageal sphincter dysfunction, as discussed in section 4.3.1, remains confused. Initially, it was reported that the incompetent lower oesophageal sphincter in patients with reflux disease responded to pentagastrin, but that the response was diminished compared to that in normal subjects [55, 128]. Subsequently, Lipshutz et al showed a similar percentage change in lower oesophageal sphincter pressure with similar doses of pentagastrin in normals and in patients with an incompetent lower oesophageal sphincter and concluded that in reflux disease there was normal lower oesophageal sphincter responsiveness but decreased endogenous gastrin secretion [217]. Reconciliation of these studies was attempted by Siewert et al who suggested that each observation may be correct and that different groups were evaluated in each study [307]. In Siewert's study, it was suggested that normal lower oesophageal sphincter responsiveness to pentagastrin may be found in mild cases of reflux disease while in more severe cases, the response was impaired. The hypothesis was that lower oesophageal sphincter incompetence in reflux disease varied in different patient groups and perhaps at different stages of their illness. If this were the case, it would be tempting to suggest that the variability in sphincter response is due to inflammation of the sphincteric muscle secondary to oesophagitis. The failure of lower oesophageal sphincter pressure to improve after healing of the oesophagitis [117] and the rapid relapse of endoscopic oesophagitis after cessation of anti-reflux therapy [165] is evidence against this hypothesis.

5.1.2 Lower Oesophageal Sphincter Relaxation

Lower oesophageal sphincter relaxation is the usual mechanism which allows reflux in both normal subjects and patients with gastro-oesophageal reflux disease. Detailed analysis of sphincter function during reflux has shown that sphincter relaxation can be classified as either being swallow-induced or as sphincter relaxation independent of swallowing. The latter are usually referred to as transient lower oesophageal sphincter relaxations.

Swallow-induced lower oesophageal sphincter relaxation: Lower oesophageal sphincter relaxation is a physiological response to swallowing and is coordinated with oesophageal body motor activity. The sphincter relaxation triggered by swallowing is rarely associated with reflux [90, 93, 104], with this type of lower oesophageal sphincter relaxation accounting for only 5-10% of reflux episodes. It has been suggested that the infrequent association of reflux with swallow-induced sphincter relaxation is due to its brief duration; maximal sphincter relaxation usually lasting less than 5 seconds. In addition, even if reflux occurs, the advancing peristaltic wave triggered by swallowing limits the extent of the reflux to the distal oesophagus and the continuation of the peristaltic sequence promptly returns the refluxate to the stomach. In the majority of cases where significant reflux occurs during swallow-induced sphincter relaxation, the associated peristaltic wave is defective and fails to traverse the entire oesophageal body [90, 93]. In addition, the defective peristalsis also delays subsequent acid clearance and further exacerbates the problem.

Transient lower oesophageal sphincter relaxation: The majority of reflux episodes in both normal subjects and patients with gastro-oesophageal reflux disease occur during episodes of spontaneous sphincter relaxation [90, 104]. These relaxations can occur without any preceding pharyngeal or oesophageal motor activity or may immediately follow a peristaltic wave or synchronous contractions within the

oesophagus. The term transient lower oesophageal sphincter relaxation is used to differentiate this type of sphincter relaxation from that attributable to swallowing.

Reflux does not always accompany transient lower oesophageal sphincter relaxation. In healthy subjects about 35% of transient lower oesophageal sphincter relaxations are associated with reflux whereas in patients with gastro-oesophageal reflux disease the proportion is about 65% [104, 174, 240]. The factors that favour reflux during this type of sphincter relaxation are unclear. The duration of maximal sphincter relaxation is longer than that seen during swallow-induced sphincter relaxation [235] and this may increase the chance of spontaneous reflux. Sudden increases in intra-abdominal pressure during a transient lower oesophageal sphincter relaxation may also precipitate reflux. Other factors that may be relevant to the frequency of reflux during transient lower oesophageal sphincter relaxation include body position, intragastric volume or pressure, relaxation of the diaphragmatic hiatus and longitudinal shortening of the oesophagus [339].

Control of transient lower oesophageal sphincter relaxations: In view of the fact that transient lower oesophageal sphincter relaxation is the crucial event that allows gastro-oesophageal reflux, it is important to examine the factors that trigger and control this event. Transient lower oesophageal sphincter relaxations are a normal physiological event and it appears that disordered control contributes to pathological reflux.

Transient lower oesophageal sphincter relaxations are neurally mediated as they are abolished by bilateral cervical vagal blockade in dogs [223]. This centrally driven inhibition to the sphincter also affects the oesophageal body, as peristalsis is also abolished during transient lower oesophageal sphincter relaxations [93]. The final neurotransmitter in the vagal pathway generating transient lower oesophageal sphincter relaxations is likely to be similar to that responsible for swallow-induced sphincter relaxation since there is an absence of both swallow-induced and transient lower oesophageal sphincter relaxations in patients with achalasia [175]. This suggests that

the control mechanism or neurotransmitter involved in triggering a transient lower oesophageal sphincter relaxation is finally mediated through the same inhibitory non-adrenergic, non-cholinergic nerves that trigger swallow-induced sphincter relaxation.

The triggering of transient lower oesophageal sphincter relaxations is not well understood. Gastric distension is known to be a potent stimulus [173, 352] and physiologically this is an important mechanism that allows venting of gas from the stomach [353]. This mechanism may also partly explain the increase in transient lower oesophageal sphincter relaxations seen after meals [134, 174]. In addition to meal-induced mechanical stimulation, there is also evidence that the composition of the meal can influence the frequency of transient lower oesophageal sphincter relaxations. Duodenal fat infusion, in the absence of any gastric distension or stimulation, significantly increases the frequency of transient lower oesophageal sphincter relaxations compared to control infusions [180, 249-251].

The neural pathways that mediate transient lower oesophageal sphincter relaxations in association with gastric distension are thought to be triggered by mechanoreceptors in the gastric wall with the afferent signals being transmitted via the vagus nerve [172]. Such mechanoreceptors have been localised to the fundus and body of the ferret stomach and are sensitive to stretch [6]. Gastric partitioning studies in the dog, however, suggest that the cardia and lesser curve aspect of the body of the stomach are the most important regions in triggering transient lower oesophageal sphincter relaxations [131]. Distension of a fundal pouch that excludes the cardia causes a much smaller response while bands sewn around the gastric cardia to prevent distension, abolish belching during gastric insufflation.

The afferent information from gastric mechanoreceptors is transmitted via the vagus to the brain stem region of the nucleus solitarius [13]. The information is then sent via interneurons to the dorsal motor nucleus of the vagus [79, 127, 304] which has been shown in electrophysiological studies to produce transient lower oesophageal sphincter

relaxation. A vago-vagal pathway, therefore, exists that could mediate transient lower oesophageal sphincter relaxations in response to gastric distension.

Intramural pathways within the gastric wall are the other possible pathway for gastric distension to mediate transient lower oesophageal sphincter relaxation. It is unlikely that this pathway is of any major importance, if it exists at all, in view of the abolition of transient lower oesophageal sphincter relaxations with cervical [223] and high thoracic [322] vagotomy in animal studies.

The studies of Mittal and McCallum are at odds with the view that afferent information from the stomach and duodenum is the major trigger for transient lower oesophageal sphincter relaxations. These investigators suggested that incomplete swallows were a trigger of many (42-43%), but not all transient lower oesophageal sphincter relaxations [235, 240]. By definition, transient lower oesophageal sphincter relaxations are not preceded by any pharyngeal pressure wave attributable to swallowing, but Mittal and McCallum found that electromyographic activity in the sub-mental muscles preceded a significant proportion of transient lower oesophageal sphincter relaxations. This activity is similar to that seen prior to pharyngeal peristalsis but is less intense and not followed by any pharyngeal pressure wave consistent with a complete swallow. Pathways for this effect certainly exist in animals, as lower oesophageal sphincter relaxation without oesophageal peristalsis in the opossum can be triggered by mechanical stimulation of the pharynx or by electrical stimulation of the superior laryngeal nerve [268]. An incomplete swallow is the postulated mechanism triggering this type of transient lower oesophageal sphincter relaxation. This stimulus is presumed to be associated with a subthreshold vagal stimulus that fails to trigger oesophageal contraction but is capable of inducing sphincter relaxation [235]. A more recent study has re-examined the relationship between submental electromyographic activity and transient lower oesophageal sphincter relaxations and failed to find any correlation between the two, thus casting doubt on the importance of this postulated

mechanism [193]. The reasons for this disparity of findings between the two studies are unclear.

Posture and the subject's level of consciousness are also important in the control of transient lower oesophageal sphincter relaxations. Transient lower oesophageal sphincter relaxations do not occur during steady state sleep and are only seen during periods of arousal. It is during these episodes that reflux has been shown to occur [90]. In dogs, transient lower oesophageal sphincter relaxations are also abolished by general anaesthesia [67]. The mechanism by which the level of consciousness influences the triggering of transient lower oesophageal sphincter relaxations is not known.

In humans [352, 353] and animals [116, 218] there is a marked decrease in the frequency of transient lower oesophageal sphincter relaxations in the supine position. It was originally suggested that afferent receptors, responding to food or fluid in the fundus and cardia, influenced the triggering of the transient lower oesophageal sphincter relaxations to help prevent reflux while supine [116]. It has since been shown in dogs, that postural suppression of transient lower oesophageal sphincter relaxations still occurs when the animals are fasted and any gastric secretion is aspirated from the stomach [218]. In human studies, the triggering and postural suppression of transient lower oesophageal sphincter relaxations were found to be unaffected by vestibular signalling as head position had no effect on the frequency of transient lower oesophageal sphincter relaxations when supine or sitting [179]. The basis of postural suppression of transient lower oesophageal sphincter relaxations needs further study; this phenomenon perhaps being an important clue to the understanding of control dysfunction that leads to reflux disease. In some patients with gastro-oesophageal reflux disease, transient lower oesophageal sphincter relaxations are abnormally frequent in the supine position suggesting a possible loss of the postural suppression in these patients.

The defect in the control of transient lower oesophageal sphincter relaxations that leads to reflux disease is not known. Patients with gastro-oesophageal reflux disease have higher rates of transient lower oesophageal sphincter relaxations than normal subjects [93, 104, 174] and this results in an increased frequency of reflux episodes. One study has suggested that the rates of transient lower oesophageal sphincter relaxations are similar in normals and patients with reflux disease, but that transient lower oesophageal sphincter relaxations in the reflux patients are more often accompanied by reflux [240]. These differences between normal subjects and patients with gastro-oesophageal reflux disease, remain to be explained but may include abnormally intense afferent signals from the stomach or, as mentioned above, impaired postural control of transient lower oesophageal sphincter relaxations [352]. In addition, the altered anatomy associated with a hiatus hernia, an anatomical defect commonly seen in patients with gastro-oesophageal reflux disease, may influence the tension and responsiveness of mechanoreceptors within the herniated stomach and lower oesophageal sphincter. It is also possible that delayed gastric emptying found in some patients with gastro-oesophageal reflux disease [225] prolongs gastric distension and so prolongs triggering of transient lower oesophageal sphincter relaxations.

5.2 Effect of Hiatus Hernia and the Diaphragm

Additional factors also contribute to the competence of the gastro-oesophageal junction. These include the diaphragmatic crura and the position of the lower oesophageal sphincter within the abdominal cavity. Hiatus hernia exposes the sphincter to abnormal external pressures and removes diaphragmatic support; both of which may influence sphincteric function. The relative contributions of the diaphragm and a hiatus hernia to the pathogenesis of reflux disease is considered below.

Hiatus hernia and reflux: The relationship of a hiatus hernia to gastro-oesophageal reflux disease is disputed but the high incidence of hiatal hernias in

patients with reflux disease suggests a significant role [23, 260, 351]. Certainly, in much of the older literature, hiatus hernia and gastro-oesophageal reflux disease are diagnoses that have been used interchangeably and this has caused much confusion in the discussion of the contribution of a hiatus hernia to the pathogenesis of reflux disease.

Simplistically, it is likely that the disruption of the anatomy of the gastro-oesophageal junction, resulting in the formation of a hiatus hernia, impairs the competence of this region to gastro-oesophageal reflux. The movement of the lower oesophageal sphincter into the thoracic cavity displaces the buttressing effect of the diaphragm and the diaphragmatic crura. This defect is probably not particularly significant under resting conditions [35] but becomes more important during episodes of abdominal straining [242] where diaphragmatic contraction is thought to externally compress the sphincter and help prevent reflux.

The displacement of the lower oesophageal sphincter from an intra-abdominal pressure environment to that of the thoracic cavity exposes the sphincter to potentially large negative intra-thoracic pressures. This is particularly likely with large hiatal hernias [172]. As a result, sudden increases in intra-abdominal pressure, which normally compress the sphincter region and assist in sphincter closure, may “blow open” the lower oesophageal sphincter which is exposed to the thoracic pressure environment and result in reflux. This has prompted the recent considerable interest in the length of the intra-abdominal segment of the lower oesophageal sphincter and correlation of this measurement with the capacity of the sphincter to resist reflux [71, 82, 84-86, 126, 187]. The reduction in intra-abdominal sphincter length in patients with reflux disease and its restoration after anti-reflux surgery was postulated as a mechanism by which surgery restored “normal” sphincter function in these patients [71, 82, 84-86, 126, 187]. The measurement of sphincter length, however, depends on the factors that are responsible for the generation of basal tone and these are often unknown after surgery. It is therefore not surprising that the measurement of basal lower oesophageal

sphincter pressure and intra-abdominal length correlates poorly with the success of the surgical repair [11, 12, 83]. Recent studies of sphincter function after anti-reflux surgery have used manometric catheters with a ring sides holes which are slowly pulled through the sphincter region. The radial pressure data so obtained are then used to construct a three-dimensional image of the sphincter pressure profile around an axis which represents the gastric pressure baseline. This technique is reported to offer some promise in assessing the success of surgical repair [319]. The major difficulty with all these measurements is that they only reflect sphincter characteristics at the time of the evaluation. As mentioned earlier in this chapter, these glimpses of sphincter function correlate poorly with mechanisms of gastro-oesophageal reflux. A more valid assessment would be to examine the effect of different surgical "repairs" on mechanisms of sphincter function known to correlate with reflux events such as transient lower oesophageal sphincter relaxations.

The creation of a closed hernial pouch between the diaphragm and the lower oesophageal sphincter may also predispose patients with a hiatus hernia to reflux during episodes of abdominal straining. The pressure within the hernia pouch may exceed the lower oesophageal sphincter pressure and result in reflux of the hernia contents into the oesophagus [172]. Also it is possible that stretching of the gastric wall within the hernia during these episodes influences the tension of the mechanoreceptors within the hernia wall, resulting in increased triggering of transient lower oesophageal sphincter relaxations [172].

Diaphragm and reflux: The contribution of the diaphragm to lower oesophageal sphincter competence is complex and difficult to study in isolation in normal subjects. Traditionally, the phasic variation in lower oesophageal sphincter pressure was thought to be due to sphincter movement caused by respiration but it has since been established that the pressure swings recorded in the lower oesophageal sphincter are greater than the respiratory swings in intra-abdominal and intra-thoracic pressures [238]. An alternative explanation was provided by Dodds et al [101, 113] who

suggested that the exaggerated pressure oscillations were due to movement of the recording apparatus in and out of the lower oesophageal sphincter during respiration. This is undoubtedly an important contributor to the observed pressure variations but studies by Boyle et al. in the cat, showed that substantial respiratory oscillations in sphincteric pressure persisted despite the manometric catheter being anchored by pin to the lower oesophageal sphincter [35]. In this study, the amplitude of the pressure swing was directly proportional to the depth of inspiration and the oscillations were abolished by paralysis of the skeletal muscle. It was hypothesised that the phasic alteration in lower oesophageal sphincter pressure was not only influenced by intra-abdominal and intra-thoracic pressure differences but was also due to the effect of active contraction of the crural diaphragm. In a more recent study, sphincter pressures were recorded continuously with a sleeve sensor and correlated with the simultaneous measurement of diaphragmatic electromyography [236]. This revealed that the increased pressure recorded during inspiration was associated with increased crural diaphragmatic electromyographic activity and that the increase was proportional to the depth of inspiration and the force of the diaphragmatic contraction during forced respiration. This effect was even shown to persist during lower oesophageal sphincter relaxation induced by oesophageal distension [241].

It has been suggested that the diaphragm acts as an “auxiliary sphincter”, assisting in the maintenance of gastro-oesophageal competence [242]. During expiration, the intrinsic sphincter pressure is considered sufficient to maintain an effective anti-reflux barrier but during deep inspiration, when the gastro-thoracic pressure gradient is potentially increased, there may be decreased competence of the anti-reflux barrier and a tendency to gastro-oesophageal reflux [238]. This tendency is counteracted with an increase in oesophago-gastric junction pressure by contraction of the crural diaphragm. The importance of this phenomenon in the maintenance of gastro-oesophageal competence during the respiratory cycle depends on the level of basal lower oesophageal sphincter tone and is more important when sphincter pressure is defective.

For the prevention of reflux during the respiratory cycle, it is important for the oesophago-gastric junction pressure to increase before the increase in the gastro-thoracic pressure gradient. It is, therefore, necessary for the crural diaphragm to contract and compress the lower oesophageal sphincter prior to the contraction of the costal diaphragm and the subsequent development of a negative intra-thoracic pressure. The independent actions of these two components of the diaphragm have since been shown in experiments in dogs [122]. In these studies, the crural diaphragm contracted earlier than the costal diaphragm and increased the gastro-oesophageal junction pressure before the gastro-thoracic pressure gradient was altered. The independent control of the costal and crural diaphragm has also been confirmed by Boyle et al. in electromyographic studies in cats [34]. These studies showed that crural muscle activity could be inhibited without any effect on costal diaphragmatic activity. The control of diaphragmatic function is discussed in more detail in chapter 2 in the anatomy and innervation of the anti-reflux barrier.

The diaphragm, thus, plays a crucial role in maintaining competence across the gastro-oesophageal junction. The displacement of this important structure, as in a hiatus hernia, is likely to significantly impair sphincteric function and predispose to gastro-oesophageal reflux.

5.3 Effect of Exercise and Straining

Patients with gastro-oesophageal reflux disease commonly complain that straining or exercise exacerbate their symptoms. This suggests that these manoeuvres in some way alter the gastro-oesophageal pressure gradient, thereby reducing the competence of the gastro-oesophageal junction and predisposing to reflux. This may particularly be a problem if straining or physical activity coincides with periods of low or absent lower oesophageal sphincter pressure. Straining may also be a problem in patients with a hiatus hernia as diaphragmatic contraction may increase the likelihood of reflux rather

than helping to augment sphincter pressure. The effect of abdominal straining and physical exercise on the mechanisms of reflux are discussed below.

Increased intra-abdominal pressure: During resting conditions, the positive gastro-oesophageal pressure gradient is resisted by the lower oesophageal sphincter and its extrinsic supporting structures. Increases in intra-abdominal pressure, either sustained as in ascites or transient, as seen with coughing, increase the pressure gradient between the abdomen and thorax and potentially promote reflux. The steady-state increase in intra-abdominal pressure seen in obese patients [228] or in patients with ascites [327] appears to predispose these patients to gastro-oesophageal reflux [20, 156, 309]. In addition, an association has been reported between obesity and both patients with a hiatus hernia and patients with mild but not severe reflux oesophagitis [320]. The possible mechanisms responsible for this association have not been studied in detail but it has been shown that basal lower oesophageal sphincter pressure in asymptomatic obese subjects with reducible hiatus hernias is similar to that seen in normal lean volunteers [228]. The motor events associated with reflux in obese patients have yet to be examined but it would seem likely that obesity exacerbates the effect of transient increases in abdominal pressure on lower oesophageal sphincter competence. It may also increase the likelihood of reflux during transient or more prolonged periods of absent sphincter tone, particularly if associated with a hiatus hernia.

The effect of straining has not been formally examined in patients with gastro-oesophageal reflux disease but there are analyses of spontaneous abdominal straining in studies which examined the mechanisms of reflux in resting recumbent subjects. In one such study, reflux associated with straining was only seen during periods of low sphincter pressure (≤ 5 mmHg) [93]. Strain-induced reflux was more prevalent in patients with severe oesophagitis than in those without endoscopic evidence of oesophageal inflammation. The true significance of the straining, however, is difficult to assess, as strain-induced reflux accounts for only a minority of reflux episodes in

resting studies [93, 104] while in a clinical setting it anecdotally seems a far greater trigger of reflux symptoms.

Although it has been established in normal subjects that reflux at rest can only occur during periods of absent lower oesophageal sphincter pressure [91], sudden increases in intra-abdominal pressure may overcome low, but detectable, sphincter pressures. If the increased intra-abdominal pressure is associated with diaphragmatic contraction, however, then reflux does not occur because of the protective pinchcock effect of diaphragmatic contraction [234]. A study evaluating diaphragmatic function in patients with hiatus hernia during straining is presented in chapter 12 of this thesis.

Exercise and reflux: The roles of exercise and physical activity in gastro-oesophageal reflux disease have received limited attention. It has been shown that oesophageal acid exposure increases during running and rowing but the reasons for this have not been directly evaluated [52, 207, 357]. It has also been reported that significant exertional reflux may be the cause of non-cardiac chest pain in patients without other evidence of gastro-oesophageal reflux disease [87, 302] but this is not a universal finding [210].

The physical limitations of current manometric recording equipment has meant that studies which examine lower oesophageal sphincter function and the mechanisms of reflux have been confined to resting, recumbent subjects [90, 93, 104]. Whether the mechanisms of reflux are similar in ambulant subjects is unknown. A recent study has suggested that 63% of reflux episodes in ambulant subjects are associated with a “visible” sphincter relaxation when measured by a sphinctometer [14]. This method, however, does not simply measure sphincter pressure but is also influenced by sphincter length thus potentially shows a “visible relaxation” if there is a reduction in sphincter length without any change in actual sphincter pressure. In addition, the effect of exercise and physical activity were not specifically addressed in this study.

Detailed evaluation of the mechanisms of reflux and the effect of exercise in ambulant subjects is required to establish the relative contributions of lower oesophageal sphincter relaxation and sudden or sustained increases in intra-abdominal pressure. A study which evaluates these factors is presented in chapter 11.

5.4 Mechanisms of Oesophageal Acid Clearance

The duration that the oesophageal mucosa is exposed to a $\text{pH} < 4$ after a reflux event is referred to as the acid clearance time. This concept was first employed by Booth et al in developing the acid clearance test [32]. This test was proposed in order to develop a standardised clinical test to evaluate oesophageal acid clearance. During this test, a pH probe was inserted into the distal oesophagus and the time measured for restoration of normal oesophageal pH after the injection of a 15ml bolus of hydrochloric acid. The advantage of this test was that it removed variables such as the volume and pH of the endogenous refluxate and spontaneous patterns of swallowing which might otherwise make comparison of events difficult. It was not until 15 years later that the concept of oesophageal acid clearance was re-examined and the physiological mechanisms resulting in the restoration of oesophageal pH after gastro-oesophageal reflux were established [159-163].

5.4.1 Acid Clearance in Normal Subjects

The mechanisms of acid clearance in normal subjects were defined in an elegant series of experiments by Helm et al [159, 162, 163]. In one of these studies, Helm investigated normal acid clearance by simultaneous measurement of refluxate volume and oesophageal pH [162]. This showed that the bulk of the refluxate volume was cleared almost immediately after reflux by oesophageal peristalsis but that it took some time before the oesophageal pH returned to normal. The subsequent neutralisation of the residual acid "film" is achieved by the bicarbonate and non-bicarbonate buffering components in swallowed saliva [159, 162]. The restoration of oesophageal pH

occurs in a stepwise fashion with each increment in pH occurring with each swallow that brings more saliva into the oesophagus. If the saliva is aspirated from the mouth then acid neutralisation is severely delayed despite multiple primary peristaltic sequences. Acid clearance, therefore, cannot result from peristalsis alone as a progressive reduction in intra-oesophageal acid volume by itself would not change the pH of the acid remaining in the oesophagus [161]. Oesophageal acid clearance is a two-step process with emptying of the acid volume from the oesophagus and subsequent acid neutralisation. These are related but not equivalent events.

Acid volume emptying: Radiographic [194] and scintigraphic [244] studies have shown that oesophageal volume clearance is usually completed by the first or second peristaltic sequence after gastro-oesophageal reflux. The potential impact of impaired oesophageal peristalsis, however, is considerable as the neutralising capacity of saliva is limited if there is any significant residual acid volume in the oesophagus [159].

Acid neutralisation: Acid clearance time from the oesophagus is more dependant on the rate of saliva production than the swallow rate [32]. Acid neutralisation is severely delayed if the subject does not swallow any saliva but the acid clearance time is similar if the swallow rate is slow or rapid. A rapid swallow rate simply brings down smaller volumes of saliva with each swallow [196]. Acid clearance in normal subjects with normal peristaltic function can be shortened by increasing the production of saliva with oral lozenges or the administration of bethanechol. Conversely, aspiration of saliva or reduction of the production of saliva with atropine results in delayed oesophageal acid clearance. It is interesting to note that saliva production is virtually abolished during sleep and thus acid clearance during this time will be severely impaired [301].

In addition to swallowed saliva it has also been shown that oesophageal bicarbonate secretion can contribute to acid neutralisation [229]. In these studies in normal subjects, it was shown that the amount of oesophageal bicarbonate secretion over 5 minutes was similar to that reported for basal salivary secretion and capable of

neutralising amounts of acid similar to the residual present within the oesophageal lumen after an episode of reflux and bolus clearance by peristalsis.

5.4.2. Acid Clearance in Patients with Reflux Disease

Delayed oesophageal acid clearance in gastro-oesophageal reflux disease was first demonstrated by Booth et al in their original description of the acid clearance test [32]. Similarly, in 24 hour oesophageal pH recordings in 100 patients with reflux disease, mean acid clearance time was markedly prolonged compared to that in normal volunteers [85]. The population of reflux patients, however, is heterogeneous and not all patients have delayed acid clearance. Stanciu and Bennett showed that clearance times were only abnormal in 50% of patients when this was evaluated with the standard acid clearance test [318]. This is similar to the proportion of patients found to have delayed acid clearance in a study of spontaneous reflux events in patients with gastro-oesophageal reflux disease [109]. Similar heterogeneity within the reflux population has also been noted by Johnson in a review of data from a series of 24 hour oesophageal pH studies where patients with hiatus hernia tended to have the most prolonged recumbent acid clearance times [189]. In all of these studies, however, there are a number of uncontrolled variables such as the frequency of primary peristalsis, the flow rate of saliva and the occurrence of two or more reflux episodes within a short time interval that go undetected with conventional methods of pH tracing analysis [354].

Delayed oesophageal acid clearance in patients with gastro-oesophageal reflux disease can be attributed to defective oesophageal peristalsis or to an abnormality of saliva secretion. These two mechanisms will be discussed separately below.

Oesophageal volume clearance in gastro-oesophageal reflux disease:

Oesophageal volume clearance depends on intact oesophageal peristalsis [162, 244]. Patients with peristaltic abnormalities invariably have some delay of acid clearance. In reflux disease, motility disturbances associated with oesophagitis were first suggested

by Olsen and Schlegel when they reported an increased incidence of weak and failed peristaltic contractions in patients with increasing severity of oesophagitis [254]. This finding was also noted by Ahtaridis et al in a comparison of normal subjects, patients with mild reflux disease and patients with peptic strictures [2]. More recently, Kahrilas et al compared peristaltic function in normal volunteers and a series of patients with mild to severe reflux disease [191]. In this study it was shown that the prevalence of peristaltic dysfunction increases greatly with increasing severity of oesophagitis. Only 25% of patients with mild oesophagitis had abnormal peristalsis versus 50% of patients with severe oesophagitis.

In patients with gastro-oesophageal reflux disease, the most common patterns of peristaltic dysfunction that result in delayed oesophageal volume clearance are failure of the peristaltic contraction to traverse the entire oesophagus (failed peristalsis) or low amplitude peristaltic contractions [194]. Failed peristalsis results in very poor volume clearance with invariable loss of bolus propulsion whereas low amplitude peristalsis usually only clears part of the swallowed bolus because of partial bolus escape. The contraction amplitude required to clear a swallowed bolus varies according the oesophageal segment. In the proximal oesophagus, a contraction wave of amplitude of 10mm Hg or more is effective in clearing the bolus whereas in the distal oesophagus, contraction amplitudes of at least 30mm Hg are required to ensure bolus transit. These results are similar to those of Richter et al who reported that a single peristaltic sequence effectively cleared the oesophagus provided that the contraction amplitude exceeded 30mmHg [284].

Hiatus hernia is also relevant to the assessment of oesophageal volume and acid clearance [239]. In a study by Mittal et al, patients with hiatus hernia, with and without oesophagitis, had delayed acid clearance because of "re-reflux" from the hernia sac during swallowing. This "re-reflux" phenomenon was observed in 75% of the patients studied. The overall role of a hiatus hernia as an aetiological factor in the development

of prolonged acid clearance time is not fully known but as many patients with oesophagitis have hiatal hernias, this is potentially an important observation.

Salivary function in gastro-oesophageal reflux disease: Reduced or absent salivation can have as profound an effect as defective oesophageal peristalsis and thus is also of critical importance in the evaluation of acid clearance in reflux disease. Sonnenberg et al studied salivary function in 44 patients with reflux oesophagitis and 88 control subjects but failed to find any difference in basal secretion rate or buffering capacity of saliva between the two groups [316]. There was, however, a significant difference in the salivary secretory response to oesophageal acidification. In the young normal subjects there was a 2-3 fold increase in saliva secretion after oesophageal acidification whereas in the age-matched normals and the reflux patients this effect was lost. The significance of this finding is unclear as the latency for the increased saliva flow was 20-30 minutes which is too long to significantly contribute to the acid clearance of individual reflux episodes. The increased salivary flow in response to oesophageal acidification was also noted by Dutta et al who demonstrated a significant increase in salivary flow and bicarbonate secretion in normal subjects but only with acidic solutions of $\text{pH} \leq 1.8$ [121]. The mechanism for this response has not been studied in detail. It does not appear to be due to oesophageal mucosal receptors as the response only occurs in subjects who developed heartburn in association with the oesophageal acid infusion [158]. It is thus likely that the central nervous system triggers the increased salivary production under these conditions.

Some patients with gastro-oesophageal reflux disease are likely to have impairment of oesophageal acid clearance times on the basis of hyposalivation. These include cigarette smokers and people who drink alcohol [120, 192, 204]. The data regarding smoking, however, are conflicting, with one study failing to find a correlation between smoking and oesophagitis, acid contact time or frequency of reflux episodes [317] while another study demonstrated an increased frequency of reflux after smoking [195]. Nonetheless, cigarette smokers without oesophagitis have been shown to have

only 60% of the titratable salivary base secretion of non-smokers and acid clearance times 50% longer than those of non-smokers [192]. These effects were presumably long-lasting effects of cigarette smoking as they were present after the subjects refrained from smoking for at least 6 hours prior to the study. It has been postulated that cigarette smoking exerts an anticholinergic (nicotinic) effect similar to that seen in patients who take medication with anticholinergic effects. Similarly after the ingestion of a single large dose of alcohol, salivary flow rate is reduced and composition is altered [120].

A final consideration in the delayed oesophageal acid clearance seen in reflux disease is that some patients are more prone to reflux when supine at night [110, 258, 259]. If a reflux event should occur at this time then the combination of absent peristalsis and hyposalivation [301] drastically increases oesophageal acid clearance time. This is true for both healthy subjects and patients with reflux disease [259] but as normal subjects rarely reflux when supine, it is usually of no clinical importance. Nocturnal reflux is uncommon in the majority of patients with reflux disease but in some patients, nocturnal reflux may be a significant contributor to the 24 hour oesophageal pH score.

Chapter 6

Recording Methods

- 6.1 **Standard Oesophageal Manometry**
 - 6.1.1 Recording Devices
 - 6.1.2 Manometric Assemblies
 - 6.1.3 Standard Manometric Techniques
 - 6.1.4 Manometric Data Analysis
- 6.2 **Ambulatory Manometry and pH Measurement**
 - 6.2.1 Recording Devices
 - 6.2.2 Manometric and pH Assemblies
 - 6.2.3 Ambulatory Manometric Perfusion
 - 6.2.4 Manometric and pH Data Analysis

This chapter summarises the recording methods used in the studies presented in the thesis. Many of the recording methods are common to the various studies and they have been presented here to save unnecessary duplication in subsequent chapters. This chapter should be read as a guide to the various techniques used and is referenced in subsequent chapters where relevant. The subsequent chapters presenting original studies, however, may include details of minor variations in the recording methods relevant to that particular chapter. Details of the study protocol used for each study appear in the individual chapters and are only presented here to highlight the reasoning for using specific methods.

6.1 Standard Oesophageal Manometry

6.1.1 Recording Devices

All static manometric measurements were made in a purpose designed manometric laboratory. Specific attention had been given to electrical safety requirements and suppression of any possible external electrical noise or interference. The perfusion pump, pH and electrical meters used to measure trans-mucosal potential difference were all housed in a Faraday cage to prevent outside electromagnetic or radio interference.

All static measurements were made using a low-compliance pneumohydraulic capillary infusion system similar to that described by Arndorfer et al [7]. The perfusion pressure used in all static studies was 1bar or 100kPa. Degassed distilled water was used as the standard perfusate in all recording channels except when the channel was also used to measure trans-mucosal potential difference (TMPD). In this situation, the channels used for TMPD measurement were perfused with degassed sterile normal saline (0.9% NaCl). The measurement of TMPD is described in detail below. The perfusate passes from the pressure chamber through a filter to remove any particulate material before

entering a manifold connected to an array of fine bore capillary tubes. The flow rate allowed by a single capillary at a perfusion pressure of 100kPa is 0.6ml/minute. Flow rates of 0.3ml/minute were achieved by using two capillaries in series. After leaving the capillaries, the perfusate passed through an external pressure transducer (Deseret Medica Inc., Park Davis & Co., Sandy Utah, USA., Model 38-8000-1) and then into the manometric catheter. The pressure transducers were calibrated to atmospheric pressure as zero and to a pressure of 80mmHg. Calibrations were performed at the beginning of every study and the zero baseline checked at 30 minute intervals during the study for any baseline drift.

Pressures sensed by the external pressure transducers were recorded by a 12 channel polygraph recorder (Grass Instrument Co., Quincy, MA, USA. Model 7D). Recordings were made at a paper speed of 5mm/sec. The chart recorder was also used to record pH and TMPD measurements simultaneously with the pressure tracings by using a combination of pre-amplifiers designed for these various inputs.

Oesophageal pH was recorded 5cm above the proximal margin of the lower oesophageal sphincter by an antimony electrode (Synectics Medical, Sweden). The pH probe was fixed by wax tape to the outside of the manometric catheter for ease of insertion and to prevent displacement. The pH measurements from the pH meter were recorded by the chart recorder. Calibrations were made in pH1 and pH7 buffer solutions at the beginning and end of every study. Any drift in pH when measured in the buffer solutions at the end of the study was assumed to have occurred in a linear fashion over the duration of the study and an appropriate correction was then made when the tracing was analysed.

Transmucosal potential difference measurements were made using previously reported techniques [335], as illustrated in figure 6.1. The channels used to measure TMPD were perfused separately with normal saline (0.9% NaCl) via the perfusion pump. Two pressure transducer connections were modified so that a KCl-agar-filled polyethylene bridge could be inserted and contact made with the saline perfusate. A

similar KCl-agar-filled polyethylene bridge was connected to an intravenous cannula, flushed with saline, that was inserted subcutaneously into the volar aspect of the subjects forearm. The agar bridges were inserted in test tubes of saturated KCl solution containing calomel reference electrodes. These electrodes were connected to a low voltage, high impedance differential amplifier with a remote power source. The signal from the voltage meter was then fed simultaneously with the pressure measurements to the chart recorder.

6.1.2 Manometric Assemblies

The standard manometric assemblies used in all static studies were constructed of silicone rubber which was either extruded as multi-lumen tubing or made by bonding the individual silicone rubber tube elements with silicone rubber glue. The diameter of the catheters varied depending on the complexity of the construction and the number of recording channels but was usually less than or equal to 5mm.

The recording configuration used in the manometric catheters consisted of a combination of side holes and one or more sleeve sensors [88]. The side holes were used to measure pressures from regions such as the pharynx, oesophageal body, hiatus hernia and stomach while sleeve sensors were used to continuously monitor lower oesophageal sphincter and diaphragmatic pressure as required. This technique allowed the catheter to be fixed in one position for the duration of the study and avoided potential pressure artefacts because of inevitable catheter movement.

Assessment of Secondary Oesophageal Peristalsis and Lower Oesophageal Sphincter Responses to Oesophageal Distension: The manometric catheters used in these studies (described in chapters 7, 8, 9, & 10) shared many similar features and will be described together. Minor catheter modifications were made in the different studies and these are illustrated for clarity.

In the four studies mentioned above, oesophageal motility was recorded with a multi-lumen manometric assembly made from a 4.5mm diameter silicone rubber extrusion

that incorporated a 6cm sleeve sensor. Side holes, spaced 3cm apart, recorded pressures from 7 sites along the oesophageal body starting at 2cm above the proximal margin of the lower oesophageal sphincter. A side hole in the gastric fundus recorded gastric pressure and a side hole in the pharynx monitored swallowing. The sleeve sensor was used to continuously record lower oesophageal sphincter pressure. The catheter was fixed and maintained in position so that the mid portion of the sleeve sensor was located within the lower oesophageal sphincter. The oesophageal side holes and sleeve sensor were perfused with degassed distilled water at 0.6ml/minute, and the pharyngeal side hole at 0.3ml/minute.

In chapter 7, secondary oesophageal peristalsis was evaluated in response to oesophageal distension in a group of young healthy volunteers. The two manometric catheters used for this study are illustrated in figure 6.2.

One manometric assembly incorporated three silicone rubber balloons for distension of the upper, middle and lower oesophagus. During the study, the balloons were inflated for 5 seconds to diameters of 1, 2 and 3cm, corresponding to balloon volumes of 1ml, 4.5ml and 17ml respectively. The second assembly had three infusion ports, corresponding to the position of the balloons, for the rapid injection of boluses of air or water. The infusion ports and the centre of the balloons were located at 6.5, 12.5 and 18.5cm above the lower oesophageal sphincter. The recording configurations of the catheters were otherwise identical.

In chapters 8 and 9, the oesophageal motor responses were examined in response to oesophageal distension in patients with gastro-oesophageal reflux disease and non-obstructive dysphagia. For these studies, a single catheter was used that incorporated both an oesophageal balloon and an infusion port (Figure 6.3). The balloon was inflated to a diameter of 3cm and provided focal oesophageal distension 12.5cm above the lower oesophageal sphincter. The infusion port was located immediately above the balloon for the rapid injection of boluses of air or water.

In chapter 11, data are presented for the lower oesophageal response to oesophageal distension. These data were recorded by the sleeve sensors in the manometric catheters described above and were obtained with the same distension stimuli as those used to evaluate secondary oesophageal peristalsis.

Assessment of the Effect of Hiatus Hernia on Lower Oesophageal Sphincter Pressure and Gastro-oesophageal Reflux During Straining:

Studies described in chapter 12 used a 14 lumen manometric assembly that incorporated two, 6cm sleeve sensors and an intra-oesophageal balloon. The configuration of the assembly and its positioning across the gastro-oesophageal junction are shown in figure 6.4.

The distal sleeve recorded pressure at the diaphragmatic hiatus and was reverse perfused in order to maximise the recording fidelity [310]. The proximal sleeve recorded lower oesophageal sphincter pressure and was perfused in an antegrade direction. Side holes recorded pressures from the stomach, 3 sites in the oesophageal body (5cm intervals) and 4 sites within the hernial sac (1cm intervals). A side hole in the pharynx monitored swallowing. A 3cm long silicone rubber balloon, which fitted tightly around the catheter assembly when deflated, provided focal distension 6.5cm above the proximal margin of the lower oesophageal sphincter. The catheter was positioned so that the mid portion of the proximal sleeve sensor was located within the lower oesophageal sphincter. The intra-hernia and gastric side holes and the sleeve sensors were perfused at 0.6ml/minute, while the oesophageal and pharyngeal side holes were perfused at 0.3ml/minute. Oesophageal pH was recorded 5cm above the proximal margin of the lower oesophageal sphincter by an antimony electrode. The position of the manometric and pH assembly was monitored constantly and correct positioning of the proximal sleeve across the gastro-oesophageal junction confirmed during the study by measurement of the transmucosal potential difference that exists across the gastro-oesophageal junction [256, 335]. This was measured through the side holes at the proximal and distal margins of the proximal sleeve.

6.1.3 Standard Manometric Techniques

All static manometric studies were performed supine in the manometric laboratory. Prior to the study the subjects fasted for a minimum of 6 hours and were asked not to smoke for at least 12 hours. Subjects were intubated in the sitting position after the nasal mucosa had been anaesthetised with 2% Xylocaine spray. The catheter was passed pernasally for all studies. During intubation the subjects sipped small amounts of water to facilitate passage of the catheter through the pharynx and upper oesophageal sphincter. Once the catheter had been introduced, the subjects were placed in the supine position and the catheter manipulated into the correct position using standard manometric criteria. After successful placement of the catheter, the subjects were allowed a 10 minute adaptation period to become accustomed to the catheter prior to the commencement of any measurements.

On completion of the study the subjects were extubated and the perfusion baselines of the catheter and the transducer baselines checked in order to calculate the pressure offset between channels; this allowed the subsequent comparison of pressures recorded by different recording channels. The transducer baselines were also checked at half hourly intervals during the study and reset to zero if any drift was detected.

6.1.4 Manometric Data Analysis

Analysis of all manometric tracings was performed manually. Strict guidelines and definitions were used to ensure uniform and reproducible analysis of the data within studies and for comparison of data between studies. The following definitions were used in the analysis of oesophageal body contractions and lower oesophageal sphincter responses to oesophageal distension.

Analysis of Oesophageal Body Contractions: The contraction amplitude at each recording site and the latency of the wave onset between adjacent recording sites were measured for all oesophageal contractions analysed. Amplitude was measured from basal end expiratory intra-oesophageal pressure to the peak of the pressure wave.

The onset of the major upstroke of the pressure wave was used as the reference point for determination of the wave latency.

Primary peristalsis was classified as complete if a propagated pressure wave of ≥ 12 mm Hg in the proximal two oesophageal body channels and ≥ 25 mmHg in the distal five oesophageal channels, traversed all the recording sites [166, 194]. The minimum latency of wave onset between adjacent recording sites that defined peristaltic progression was set at 0.5 seconds, corresponding to a peristaltic velocity of 6 cm/second. Criteria for failed peristalsis were either failure of a pressure wave, ≥ 12 mm Hg in the proximal two oesophageal channels and ≥ 25 mmHg in the distal five channels, to traverse each of the oesophageal recording sites or synchronous pressure waves occurring at two or more recording sites. In addition, no response to distension was judged to have occurred if a pressure wave ≥ 10 mm Hg was seen in less than two recording sites. For complete peristaltic responses, mean amplitude and velocity were calculated for the five distal recording sites corresponding to the distal 12 cm of the oesophageal body.

Secondary peristalsis in response to air and water bolus injection was typically characterised by a propagated pressure wave that traversed the entire oesophagus, and was analysed in the same manner as primary peristalsis. Secondary peristalsis associated with balloon distension, however, was characterised by separate responses above and below the balloon and each component was analysed individually using the same criteria as for primary peristalsis.

Lower Oesophageal Sphincter Responses to Oesophageal Distension:

For studies presented in chapter 10, basal lower oesophageal sphincter pressure was calculated by taking a visual mean of the manometric tracing over a 15 second period before each oesophageal distension was performed. End-expiratory basal sphincter pressure was then referenced to intra-gastric as zero. Sphincter relaxation was said to have occurred if there was a visible relaxation of ≥ 3 mmHg in response to the stimulus. The latency of the sphincter response was measured as the time from the onset of the

stimulus to the time of the usually sudden onset of sphincter relaxation. Nadir lower oesophageal sphincter pressure was calculated as the lowest end expiratory sphincter pressure induced by the stimulus, referenced to intra-gastric. The duration of the response was measured, in seconds, from the time that sphincter relaxation reached 80% of maximal to the time where it subsequently rose above this point. In some cases, sphincter pressure failed to return to basal pressure until after the next complete primary peristaltic sequence. In these cases the duration of the sphincter response was said to be 20 seconds. The measurement parameters are shown graphically in figure 6.5.

6.2 Ambulatory Manometry and pH Measurement

6.2.1 Recording Devices

Pressure and pH measurements made during the ambulatory study described in chapter 11 were recorded by a portable datalogger (MMS Medical, Enschede, Holland) and transferred on completion of each study to a computer for subsequent display and analysis.

Prior to the commencement of each study, the permanent memory of the datalogger was erased and the unit re-programmed. The input configuration of the datalogger was factory installed and allowed for simultaneous measurements from 5 pressure transducers and one pH channel. The pressure and pH sample rate could be varied from 8Hz to 0.125Hz and this was determined at the time of re-programming. Due to datalogger memory limitations (1Mb), pressures for the ambulatory study were sampled at a rate of 4Hz while the pH channel was sampled at a rate of 0.25Hz. During data acquisition, a compression algorithm was used to store the data in the permanent memory. This enabled the collection of approximately 1.5Mb of data with only a built in memory capacity in the datalogger of 1Mb. Events during the study

such as meals, exercise periods and any time spent supine were also recorded by the datalogger by using one of five programmable event markers.

The datalogger was powered by two alkaline AA batteries and during pre-study bench testing these were shown to last more than 48 hours if the datalogger was programmed as described above. Data were stored in a permanent memory that was maintained even in the presence of battery failure. It was necessary on one occasion to change the batteries mid way through a study and this only resulted in loss of one minute of recording time. Data gathered prior to the exchange were preserved and the study was successfully completed with the new batteries.

On completion of a study, the data were downloaded to an IBM compatible computer (Compaq 386SX with 4Mb extended memory) and stored in a database as part of the software package provided by MMS Medical. Data was subsequently displayed and analysed on the computer screen as described in section 6.2.4.

6.2.2 Manometric and pH Assemblies

Ambulatory manometric and pH measurements were made concurrently. Pressures were recorded by intra-luminal solid state transducers from the lower oesophageal sphincter, stomach, oesophageal body (2 sites) and pharynx. Three separate manometric catheters were used in the study to allow accurate placement of the various transducers in the different recording regions. The position of the various catheters was determined from measurements made of the positions of the upper and lower oesophageal sphincters during a standard static manometric study performed before the insertion of the ambulatory catheters. A pH electrode was also attached to the bundle of manometric catheters so that acid reflux events could be detected. The recording configuration used for the study is illustrated in Figure 6.6.

Gastric and oesophageal pressures were recorded by an assembly that incorporated three intraluminal solid-state transducers, spaced at 10cm intervals (Gaeltec, Isle of Skye, Scotland). The assembly was positioned so that the lower oesophageal sphincter

was located mid-way between the distal two transducers. A separate transducer, 1mm in diameter, (Braun Medical B.V., Uden, The Netherlands) was positioned in the hypopharynx to monitor swallowing.

A sleeve sensor was used to monitor lower oesophageal sphincter pressure. The standard sleeve sensor used in static studies could not be used for the ambulatory study as it transmits pressure to an external pressure transducer. This arrangement is not suitable for ambulatory studies as the hydrostatic pressure difference between sleeve and external transducer will vary with the subjects body position and the height of the sleeve compared to the transducer. It was therefore necessary to manufacture a specially designed sleeve sensor with a built in solid-state transducer suitable for use in ambulant subjects. The device that was developed and manufactured for this purpose, by Professor John Dent, was a 6 cm long and 0.25 cm wide reverse-perfused sleeve sensor. The pressure sensed by the sleeve was registered by a miniature solid-state transducer (Braun Medical B.V., Uden, The Netherlands) embedded in the perfusion channel at the mid point of the base of the sleeve sensor (Figure 6.7).

The sleeve was reverse-perfused for two reasons. First, it allowed the embedded transducer to be positioned at the mid-point of the sleeve, thereby minimising differences in hydrostatic pressure between different body positions. Second, lower oesophageal sphincter pressure measurements during physical activity also includes a component of diaphragmatic squeeze of the gastro-oesophageal junction. The use of reverse perfusion has been shown to improve the response rate of the sleeve during diaphragmatic contraction [310].

The sleeve was perfused at 0.3ml/min in order to minimise the amount of perfusate delivered during the study. The use of a narrower sleeve bed compared with that of a standard sleeve assembly, allowed the use of this relatively low perfusion rate whilst maintaining an adequate rise rate. Bench testing of the sleeve at this perfusion rate gave a rise rate at the proximal end of the sleeve of 13.6 cmH₂O/sec and 136 cmH₂O/sec

with occlusion at the distal end of the sleeve. In a sphincter model, the sleeve was accurate to within 1 cmH₂O, at pressures ranging from 2 cmH₂O to 50 cmH₂O.

Oesophageal pH was monitored using a bipolar glass pH electrode (Ingold, AG Urdorf, Switzerland, model LOT 440), positioned 5cm above the LES. The electrode was calibrated in buffers of pH 1 and pH 7 before and after each study. The glass pH electrode was very stable over the 24 hour study and did not show any significant measurement drift during use.

The diameter of the four catheter bundle used in the ambulatory study was approximately 6mm and in all subjects was passed through one nostril without difficulty.

6.2.3 Ambulatory Manometric Perfusion

A single channel ambulatory perfusion system for the sleeve sensor was not commercially available and this had to be developed in order to perform the desired study. The performance characteristics required were the ability to perfuse a single channel with a constant flow rate of 0.3ml/min for a full 24 hours. The system had to be portable and therefore needed to be compact and light. Another important consideration was that the perfusate reservoir needed to be able to function both horizontally and vertically in order to accommodate the different body positions assumed by the subject during the study. In view of the problems associated with mechanical and syringe based perfusion devices, it was decided that the principals of a low compliance pneumo-hydraulic perfusion system needed to be adapted for the ambulatory system. The design that was finally used for the study is illustrated in Figure 6.8. The gas cylinder used had a volume of 0.75 litre and was pressurised from a large cylinder of medical air to approximately 250bar. This pressure was reduced by a first stage regulator to approximately 10bar which was the input pressure to the adjustable second stage regulator. The first and second stage regulators have been combined in the figure for the sake of clarity. A final pressure of 100KPa (1bar) was

delivered to the pressure chamber as the driving pressure. A safety valve built into the pressure chamber was set at 250KPa to ensure that the chamber would not be exposed to inadvertent high pressures.

The air in the pressure chamber and the degassed distilled water used for the perfusate were separated by a double membrane “plastic bag” that was impermeable to water and air. This allowed the perfusion system to be put into any position without the risk of air mixing with the perfusate. The impermeable bag was also required as standard polyethylene bags were found to be permeable to small amounts of air and these caused problems with bubble formation in the perfusate if the chamber was left pressurised for 24 hours. The perfusate was driven from the pressure chamber via a long perspex outlet pipe that had multiple perforations along its length. Initial bench testing with the system revealed that the bag collapsed down onto the outlet pipe and occluded flow if the pipe was too short. For this reason, a long pipe was manufactured and the multiple perforations were made to ensure that the perfusate outflow was not obstructed. Final outflow of the perfusate from the pressure chamber was controlled with a tap. Connected to the tap was a plastic capillary that limited flow to a rate of 0.3ml/minute when the perfusion pressure was maintained at 100KPa. The volume of the bag of perfusate was 300ml which, at a perfusion rate of 0.3ml/min, gave a perfusion time of 16.6 hours. The bag was therefore routinely filled with degassed distilled water after 12 hours of recording so that measurements could be made for the full 24 hours. The datalogger, perfusion system and associated hoses and tubing weighed approximately 5 kg, and were carried in a small back pack.

A photograph of the perfusion system, datalogger and various manometric catheters is shown in Figure 6.9. One of the major difficulties in using this system was tangling of the various hoses, catheters and wires. In order to simplify the system, a second manometric perfusion system was built and has been used in subsequent studies in ambulant subjects (not reported in this thesis). This second perfusion system is built along the same principles but is more robust and compact compared to the first

version. The second device also has a larger pressure chamber that accommodates a 500ml bag of perfusate which eliminates the need to fill the bag half way through the study. The second perfusion system is photographed in Figure 6.10.

6.2.4 Manometric and pH Data Analysis

One of the aims of the ambulatory study was to examine the effect of physical activity on the mechanisms of gastro-oesophageal reflux. It was therefore important to carefully document the subjects activity and posture during the entire study. This was done by means of a structured protocol of activity with specific details being recorded in a study diary and by using one of five event keys on the datalogger. These keys activated a marker that was recorded with the manometric and pH data and which gave a precise reference for the beginning and end of various activities and events during the study. The first task was to divide the recording time into upright and supine periods. As subjects were not allowed to lie down during the day, the only supine recording time was after they had gone to bed in the evening. While upright, the subjects moved quietly around the laboratory or sat reading or watching television, this activity being defined as sedentary. Ambulant and exercise periods were defined as the time during which subjects were out of the hospital and on the exercise bicycle or treadmill. The time periods when the subject was considered ambulant or exercising did not include movement around the motility laboratory.

The pH recording was analysed manually. Acid reflux episodes were identified and counted and were defined as either an abrupt drop in oesophageal pH below 4 for at least five seconds or, if oesophageal pH was already below 4, a further abrupt drop of at least 1 pH unit for at least five seconds. Slow downward drifts on pH over a period of several minutes to below pH4 were not included in the analysis of the duration of oesophageal acid exposure or scored as reflux episodes. The acid clearance time after gastro-oesophageal reflux was measured as the time taken for the oesophageal pH to rise above 4.

The onset of the abrupt drop in oesophageal pH associated with acid reflux episodes served as the reference time for the analysis of the motor events associated with reflux. For all reflux episodes, the following variables of motility were measured: (i) the lower oesophageal sphincter pressure at the time of reflux, (ii) minimum lower oesophageal sphincter pressure in the 5 seconds preceding reflux, (iii) basal lower oesophageal sphincter pressure during the 30 seconds prior to reflux, (iv) the pattern of lower oesophageal sphincter pressure for the 30 seconds before reflux, (v) the pattern of pharyngeal and oesophageal motility for the 30 seconds before the onset of reflux and (vi) the first oesophageal motor event following the onset of the reflux episode.

Basal end-expiratory lower oesophageal sphincter pressure, referenced to intragastric pressure, was determined at 15 minute intervals by taking a visual mean of the tracing over this period after exclusion of swallow-induced sphincter relaxations. During the formal exercise periods, however, basal lower oesophageal sphincter pressure was calculated over one minute intervals.

The lower oesophageal sphincter pressure recording was also analysed for the occurrence of lower oesophageal sphincter relaxations that occurred independently of swallowing. These transient lower oesophageal sphincter relaxations were defined as abrupt (>1 cmH₂O/sec) falls in lower oesophageal sphincter pressure of >5 cmH₂O to a level ≤ 3 cmH₂O above intragastric pressure for at least 5 seconds in which no swallowing was evident for 5 seconds before the onset of lower oesophageal sphincter relaxation until maximal relaxation had been achieved [170]. For the purposes of this analysis, acid reflux was judged to have accompanied a transient lower oesophageal sphincter relaxation if an abrupt fall in oesophageal pH of >1 pH unit occurred during the relaxation [170, 240]. The lower oesophageal sphincter pressure recording was initially analysed in a blinded fashion with the pH recording masked. The subsequent association of reflux and sphincter relaxation was then determined after the sphincter relaxation analysis had been completed. The presence or absence of straining in

association with transient lower oesophageal sphincter relaxations was also recorded. Strains were defined as sharp and brief positive elevations in gastric and oesophageal pressure, greater than two or more times the normal respiratory pressure excursions in gastric pressure. The onset of the major upstroke of the positive pressure wave was used as the reference point for determination of the onset of straining.

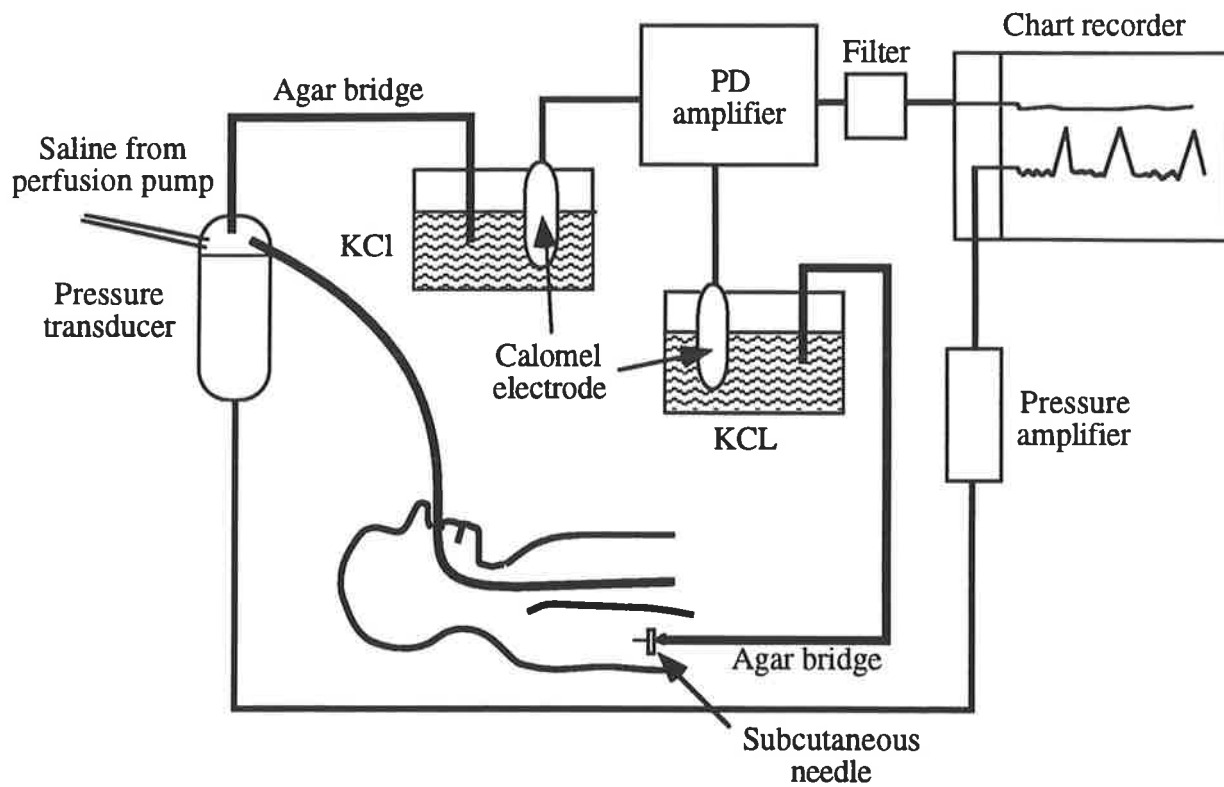


Figure 6.1 Schematic illustration of the method for obtaining simultaneous oesophageal pressure and transmucosal potential difference (TMPD) measurements.

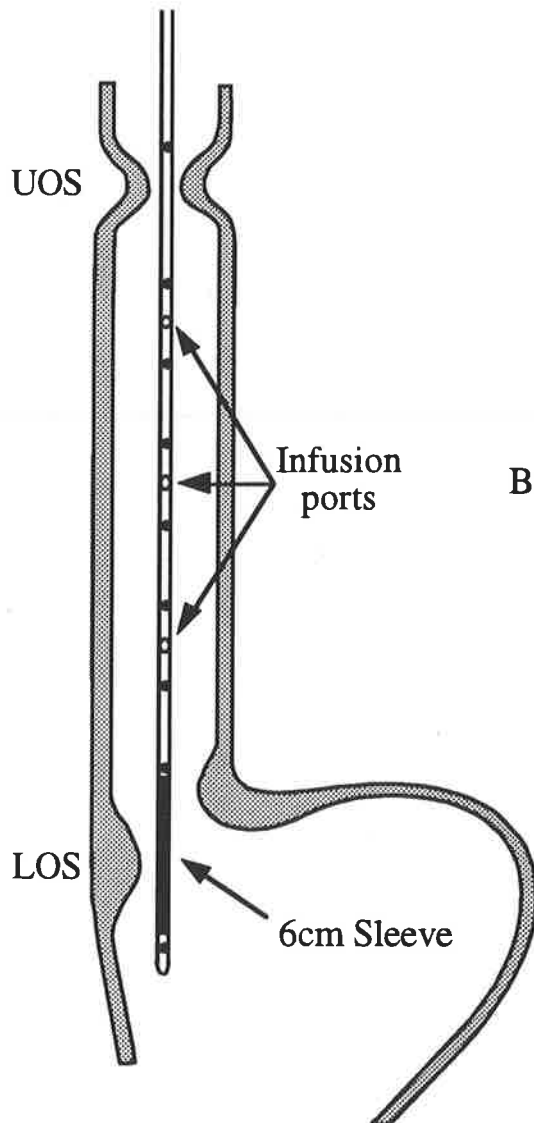
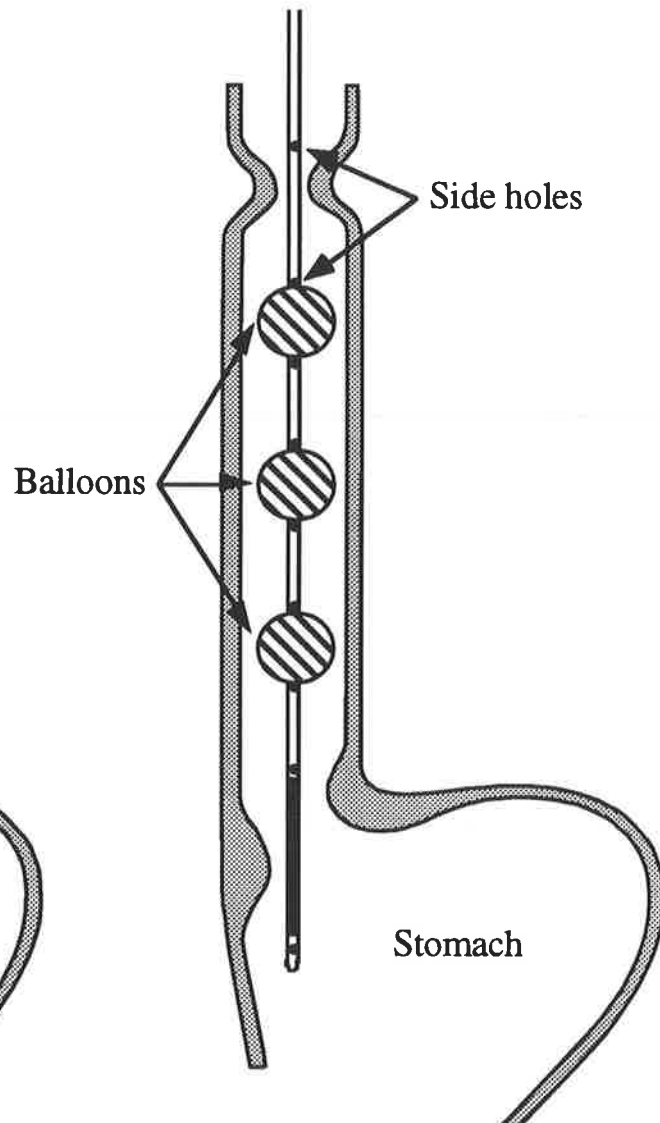
Infusion Catheter*Balloon Catheter*

Figure 6.2 Air and water infusion catheter and balloon catheter used in the evaluation of the oesophageal body and lower oesophageal sphincter motor responses to oesophageal distension in healthy young subjects.

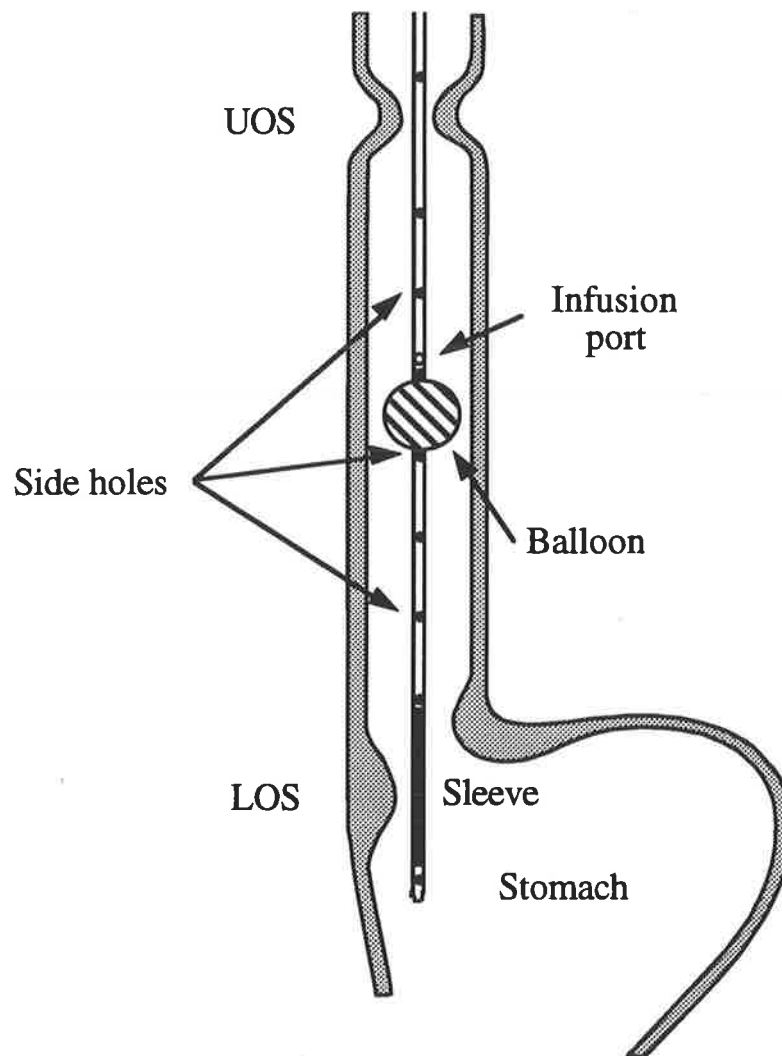


Figure 6.3 Manometric catheter used in the evaluation of the oesophageal body and lower oesophageal sphincter motor responses to oesophageal distension in patients with gastro-oesophageal reflux disease and non-obstructive dysphagia.

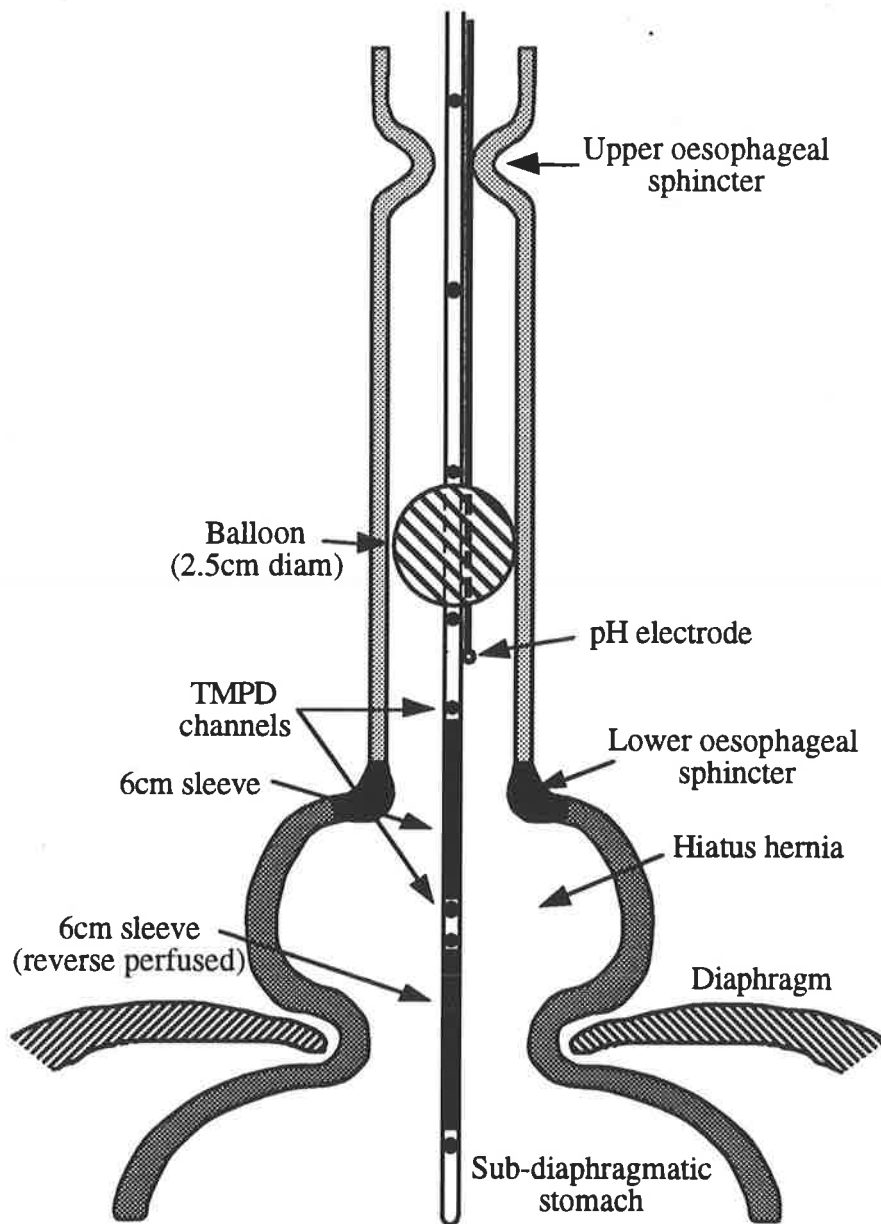


Figure 6.4 Manometric catheter used to evaluate the effect of changes in intra-abdominal pressure and diaphragmatic contraction on mechanisms of gastro-oesophageal reflux in patients with a hiatus hernia. In the normal volunteers, only the proximal sleeve was used to measure the combined lower oesophageal sphincter and diaphragmatic pressures. In these experiments, the distal sleeve and intra-hernia side holes were not perfused.

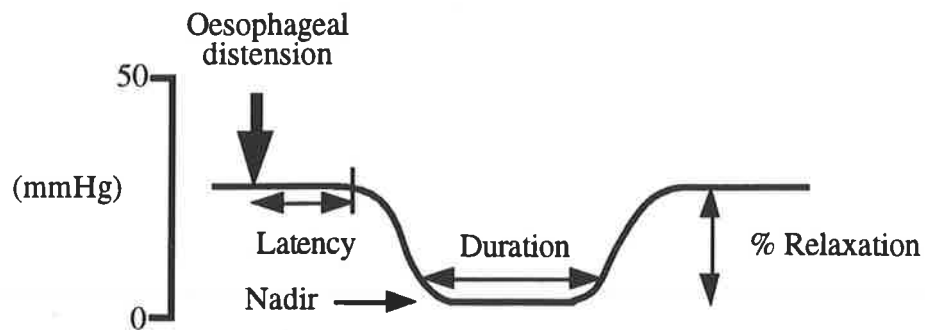


Figure 6.5 Schematic diagram of the measurement parameters used in evaluating lower oesophageal sphincter relaxation to oesophageal distension

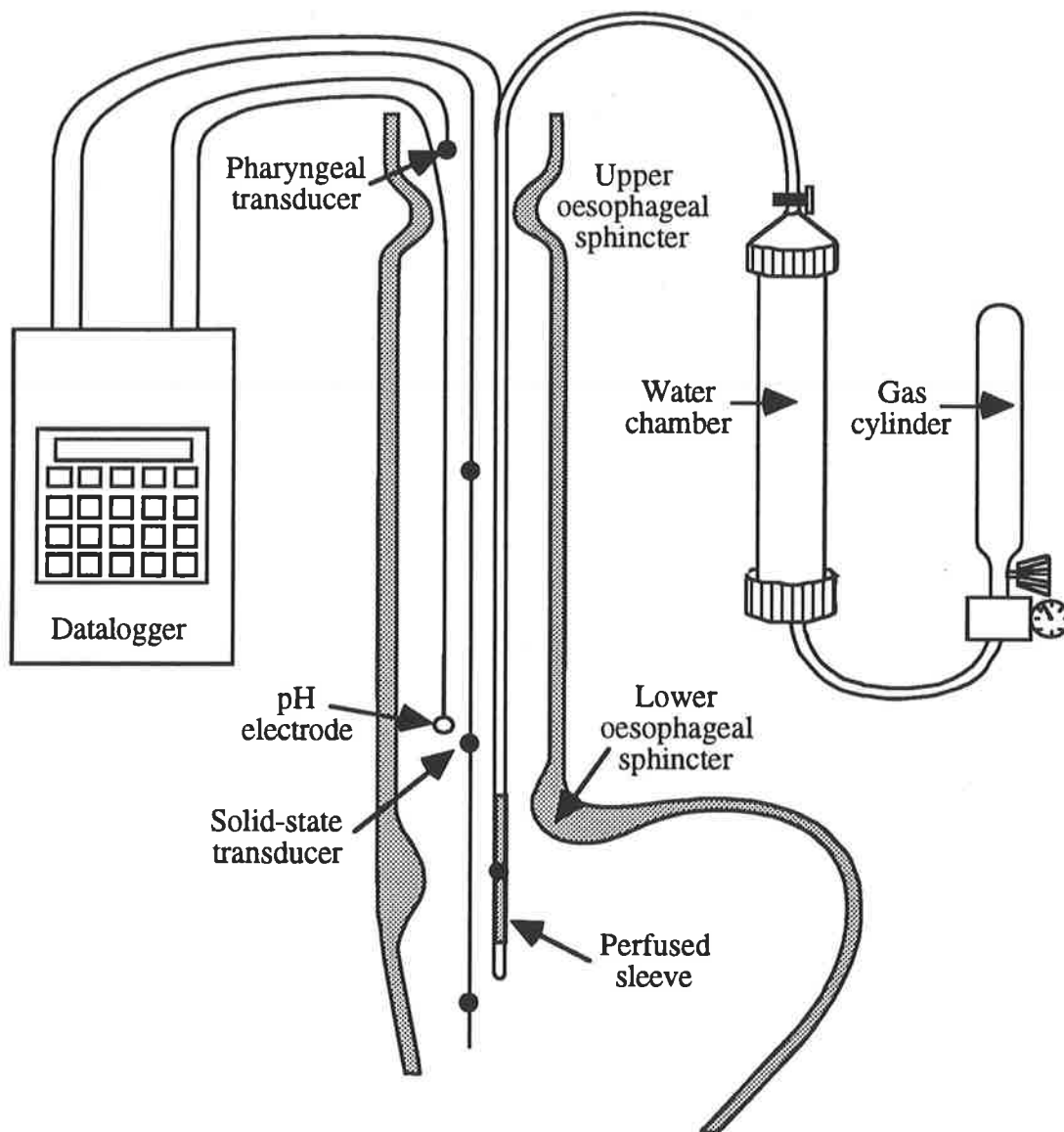


Figure 6.6 Schematic diagram of the recording configuration used in the ambulatory manometry study presented in chapter 11.

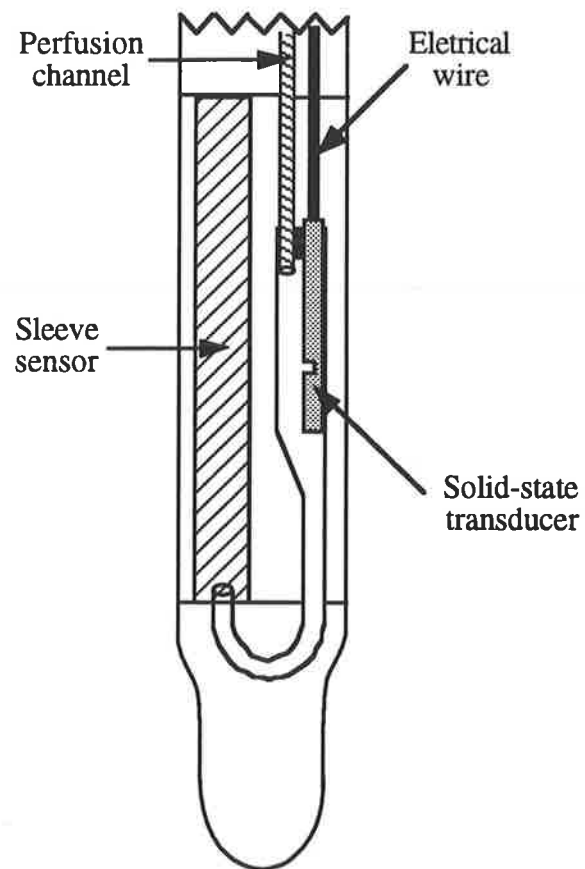


Figure 6.7 Expanded view of the reverse-perfused ambulatory sleeve sensor.

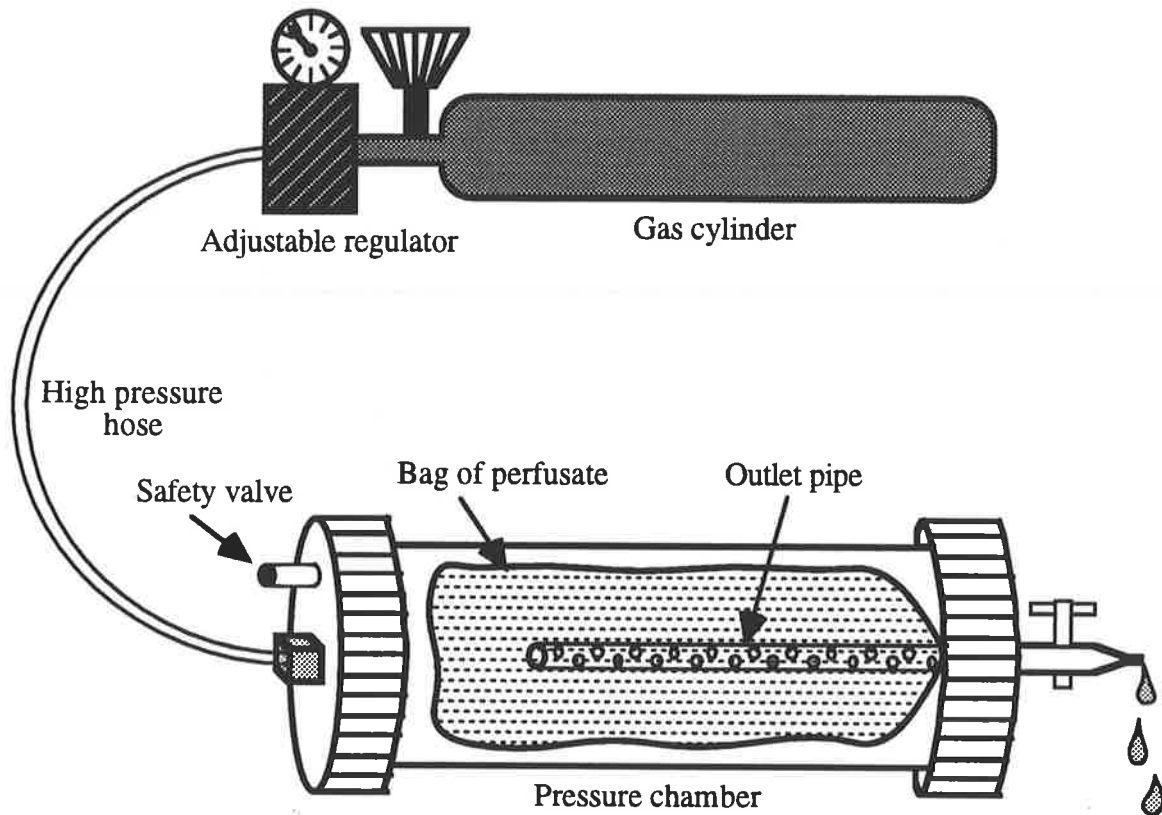


Figure 6.8 Schematic diagram of the ambulatory perfusion system



Figure 6.9 Photograph showing the ambulatory manometric system used in the studies presented in chapter 11. The photograph shows the datalogger (MMS Medical), the ambulatory perfusion system and the three manometric catheters. The connection of the sleeve catheter to the perfusion system via the plastic capillary is not shown.

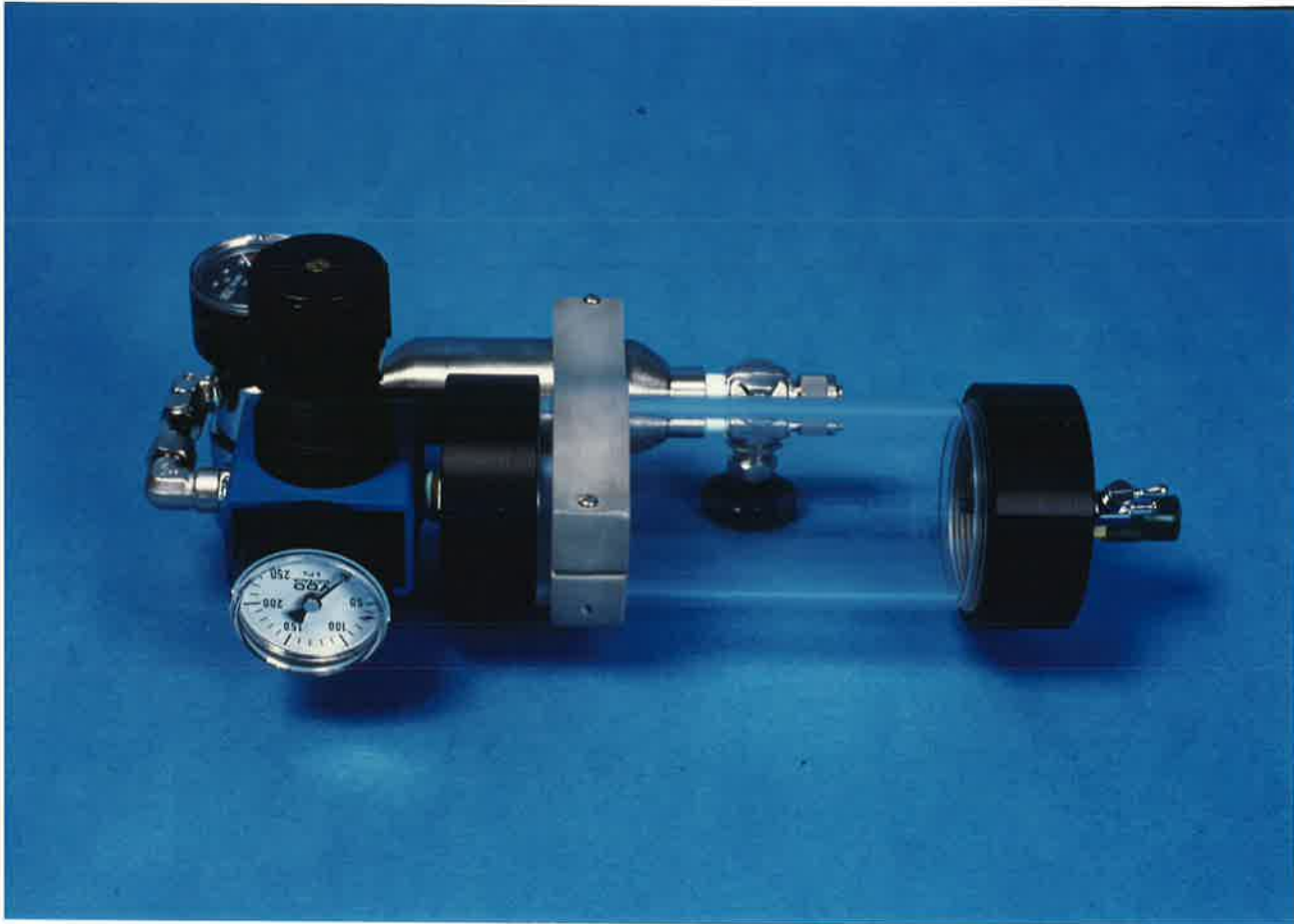


Figure 6.10 Photograph showing modified ambulatory perfusion system. This device is more compact than the earlier version as the air cylinder and pressure chamber have been clamped together eliminating the need for the long section of high pressure hose. The pressure chamber is also larger than in the first model and now accommodates sufficient perfusate for a full 24 hour study.

Chapter 7

Secondary Oesophageal Peristalsis in Normal Subjects

7.1 Introduction

7.2 Methods

7.2.1 Subjects

7.2.2 Recording Techniques

7.2.3 Protocol

7.2.4 Data and Statistical Analysis

7.3 Results

7.4 Discussion

7.1 Introduction

Secondary peristalsis occurs in response to oesophageal distension. Physiologically this occurs if food, fluid or air are retained in the oesophagus after a failed primary peristaltic wave or after reflux from the stomach. Functionally, it is of protective importance in maintaining an empty oesophagus by clearing the bulk of the volume of the refluxate after a reflux event [162]. This assists the normalisation of oesophageal pH by primary peristalsis and swallowed saliva [162] by preventing prolonged contact time between refluxed gastric acid and the oesophageal mucosa [64].

Considerable data are available in the literature about primary peristalsis with regard to its reproducibility, contraction characteristics, and abnormalities in various disease states [107, 287]. Secondary peristalsis, on the other hand, has received comparatively limited attention. Meltzer first defined secondary peristalsis in animal experiments in 1906 [226]. Since then, stimuli such as oesophageal balloon distension and air or water infusion have been studied but differences in distension volume, level of infusion and duration of distension make comparison of the results difficult [68, 115, 129, 227, 269, 305, 349]. In addition, early studies sampled relatively few points in the oesophagus and used manometric techniques with low recording fidelity that impair the validity of data on peristaltic amplitude and velocity. Recent studies have investigated oesophageal responses to prolonged balloon distension [344] and slow intra-oesophageal infusion of liquid [64, 328] but these did not examine the secondary peristaltic responses in detail. The most appropriate method of triggering secondary peristalsis and the normal ranges for peristaltic amplitude and velocity, therefore, remain to be adequately defined.

The aims of this study were to test the efficacy of different distending stimuli within the oesophagus in order to establish a reliable means of examining secondary peristalsis and to define the distension induced oesophageal motor responses in healthy

subjects as a prelude to the evaluation of patients with suspected oesophageal body motor dysfunction.

7.2 Methods

7.2.1 Subjects

Twenty five healthy subjects (11F : 15M) ranging in age from 19-40 years (median: 21 years) were recruited for the study. Subjects were free of any gastrointestinal symptoms, had no history of upper gastrointestinal surgery, and were not taking antacids on a regular basis. No subject took any medication known to alter oesophageal motor function. Each volunteer gave written informed consent and the study was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

7.2.2 Recording Techniques

The manometric recording techniques for this study have been described in detail in chapter 6 of this thesis. The specific details of the recording apparatus used in this study are described and illustrated in section 6.1.

In brief, oesophageal motility was recorded using perfused multi-lumen manometric catheters with side holes located at 7 sites along the oesophageal body. One catheter included three silicone rubber balloons for distension of the upper, middle and lower oesophagus while a second assembly incorporated three infusion ports, corresponding to the position of the balloons, for the rapid injection of boluses of air or water.

7.2.3 Protocol

Subjects were studied following an overnight fast. After positioning of the manometric assembly and a 10 minute adaptation period, each subject underwent assessment of both primary and secondary peristalsis. Primary peristalsis was tested with ten, 5ml water swallows and ten dry swallows. Each swallow was separated by a 30 second interval.

Secondary peristalsis was triggered by oesophageal distension using three stimuli. In 10 subjects the oesophagus was distended by balloons that were inflated for 5 seconds to diameters of 1, 2 and 3cm, corresponding to volumes of 1ml, 4.5ml and 17ml respectively. In a further 10 subjects, 2, 5 and 10ml boluses of water and 5, 10 and 20ml boluses of air were injected rapidly by hand into the oesophagus at levels corresponding to those of the balloons. One subject took part in both protocols. The rate of injection was determined by the volume and viscosity of the stimulus used. The 10ml water bolus was injected within 1.5 seconds while the injection of the boluses of air was more rapid with the 20ml bolus being injected within 0.5 seconds. Each stimulus was tested in triplicate and was separated by an interval of at least 15 seconds from any preceding primary peristaltic activity. An interval of 20 seconds was allowed after each test for any response to occur. During this time the subjects were instructed not to swallow. After 20 seconds, each stimulus was followed by a dry swallow to clear any residual air or water and reduce the desire to swallow. Subjects were then given intravenous atropine in a bolus dose of 15 μ g/kg followed by a continuous intravenous infusion at 4 μ g/kg/hour [103], and the stimuli retested in triplicate. The degree of cholinergic blockade was monitored at 15 minute intervals by assessment of pulse rate, pupillary dilatation and the subject's reporting of mouth dryness and visual blurring. Mouth dryness was self reported and scored on a scale of 0-10 with normal being rated as ten and very dry as zero.

The reproducibility of testing primary and secondary peristalsis was examined in an additional six subjects. Primary peristalsis was tested using water swallows and secondary peristalsis was triggered using rapid bolus injection of 10ml of air and water into the mid oesophagus. Each stimulus was repeated 10 times and the study performed twice, at least one week apart.

7.2.4 Data and Statistical Analysis

The oesophageal body contractions triggered in this study were analysed as described in section 6.1.4. The contraction amplitude at each recording site and the latency of the wave onset between adjacent recording sites were measured for both primary and secondary peristalsis. The primary and secondary peristaltic responses were then classified as complete or failed according to the previously stated criteria. No response was scored if a pressure wave ≥ 10 mm Hg was seen in less than two recording sites. For peristaltic responses, mean amplitude and velocity were calculated for the five distal recording sites corresponding to the distal 12cm of the oesophageal body.

For each stimulus, response rates were determined for each subject. Additionally, data for all tests in all subjects were combined. The differences in response rates between stimuli were analysed using a Chi-squared test, and peristaltic amplitude and velocity were compared using analysis of variance. Differences among sites for each stimulus were compared using log-linear modelling techniques [28]. Re-test reliability of primary and secondary peristalsis was examined using multivariate analysis for single-sample matched pairs [40]. Data in the text are expressed as mean $\pm$ standard error of the mean.

7.3 Results

Primary peristalsis

In all subjects, complete primary peristalsis occurred with at least 8 of the 10 water swallows. The mean success rate was 96%. Pressure wave amplitudes and propagation velocities are summarised in Table 7.1.

Secondary Peristalsis

a) Air and water boluses

Both air and water boluses produced complete secondary peristaltic responses that, regardless of the level of injection, started at the most proximal oesophageal recording site and traversed the entire length of the oesophageal body (Figure 7.1). For both air and water boluses, the response rate of complete peristalsis increased significantly as the injected volume increased ($p < 0.0001$, Figure 7.2) and were similar for air and water boluses of equal volume. In addition, when equal bolus volumes of air and water were compared, the level of injection did not influence the number of complete peristaltic responses ($p = 0.4$, Figure 7.3).

Mean pressure wave amplitude and propagation velocity are summarised in Table 7.1. The amplitudes of secondary peristalsis stimulated by air and water were less than that of primary peristalsis. Propagation velocity of secondary peristalsis stimulated by air was similar to that of primary peristalsis while secondary peristalsis stimulated by water was slower than primary peristalsis. The three volumes of air triggered pressure waves of similar amplitude as did the 5ml and 10ml water boluses. The 2ml water bolus, however, triggered only 2 peristaltic responses. Propagation velocities for all three volumes of air-stimulated secondary peristalsis were similar, while the propagation velocity with the 10ml bolus of water was less than that stimulated by the 5ml bolus ($p = 0.04$).

Table 7.1: Manometric characteristics of primary and secondary peristalsis

STIMULUS	AMPLITUDE (mmHg)	VELOCITY (cm/sec)
PRIMARY PERISTALSIS		
Water swallows	88.9 ± 2.1	2.9 ± 0.04
SECONDARY PERISTALSIS		
Air		
5ml	72.3 ± 3.9	2.8 ± 0.14
10ml	72.9 ± 2.6	2.9 ± 0.10
20ml	74.6 ± 2.4	3.1 ± 0.10
Mean (all vols)	73.6 ± 1.6*	2.9 ± 0.06
Water		
2ml (n=2)	n/a	n/a
5ml	59.4 ± 3.0	2.8 ± 0.14
10ml	66.7 ± 2.6	2.5 ± 0.07 ¶
Mean (all vols)	63.6 ± 2.0*	2.6 ± 0.07 †
Balloon		
1cm (n=1)	n/a	n/a
2cm	59.1 ± 8.6	2.5 ± 0.38
3cm	64.8 ± 5.4	2.9 ± 0.30
Mean (all diams)	64.0 ± 4.3*	2.7 ± 0.23

Data expressed as mean ± SE; * p=0.0001 compared with water swallow amplitude; ¶ p=0.04 compared to 5ml water responses; † p=0.01 compared to primary peristaltic velocity; n/a = not analysable

b) Balloon distension

Balloon distension produced a pattern of secondary peristalsis different from that of the air or water boluses (Figure 7.4). Characteristically, during distension there was a high amplitude synchronous contraction above the balloon while below there was motor quiescence. After distension, the synchronous contraction above the balloon subsided and a peristaltic contraction wave progressed distally from the level of the balloon. Peristalsis above the balloon was rare and occurred with only 2 of the 270 balloon distensions. Response rates for the different motor components are shown in

Figure 7.5. The ability to trigger secondary peristalsis below the balloon was volume dependent with a 3cm distension more likely to elicit a response than a 1cm distension ($p=0.004$). In addition, the middle balloon was more likely to stimulate a peristaltic response than the upper or lower balloons ($p=0.04$, Figure 7.6). The amplitude of secondary peristalsis elicited by balloon distension was less than that of primary peristalsis, while propagation velocity was similar (Table 7.1). Balloon volume did not influence the amplitude or propagation velocity of the peristaltic responses triggered by the 2cm and 3cm balloons ($p>0.05$). The manometric responses induced by the 1cm balloon could not be compared because of the limited number of responses.

Reproducibility

Intra-subject secondary peristaltic response rates did not differ significantly between the first and second test days (Table 7.2). Similarly, the pressure wave amplitude and velocity of secondary peristalsis was the same on the first and second test days.

Table 7.2: Reproducibility of secondary peristalsis

	TEST 1	TEST 2
Response Rate *		
Air	2.5 (1-6)	4.5 (0-7)
Water	1.0 (0-5)	3.5 (0-9)
Amplitude (mm Hg) †		
Air	76.6 ± 5.8	71.4 ± 4.7
Water	66.1 ± 3.6	66.0 ± 4.0
Velocity (cm/sec) †		
Air	3.1 ± 0.15	3.3 ± 0.11
Water	2.8 ± 0.10	3.0 ± 0.11

* Data expressed as median (interquartile range); † Data are expressed as mean ± SE

Atropine

All subjects demonstrated clinical features of muscarinic blockade after the loading dose and throughout the duration of the atropine infusion. Clinical assessment 15 minutes after the start of the atropine infusion revealed a significant increase in pulse rate (pre: 69 ± 3 beats/min, post: 108 ± 3 beats/min; $p=0.0001$), mouth dryness (pre: 8.5, post: 2; $p=0.0001$) and pupillary dilatation (pre: 4.0 ± 0.2 mm, post: 4.85 ± 0.3 mm; $p=0.02$).

Atropine significantly reduced the rate of complete peristaltic responses to all the stimuli (Figure 7.7). Primary peristalsis and the secondary peristaltic responses to 5ml air and water boluses were virtually abolished. The reduction in secondary peristalsis was associated with an increase in the frequency of failed and no-responses with the frequency of synchronous responses remaining unchanged. The effect of atropine on the distension induced responses below the balloon was similar to that on the responses to air and water distension (Figure 7.7). Above the balloon, atropine significantly reduced the frequency of synchronous responses to balloon distension at all sites with a decrease in mean response frequency from 47% to 18% ($p<0.01$).

Atropine significantly reduced the amplitude of secondary peristalsis to the air but not water boluses. The number of primary peristaltic responses and secondary peristaltic responses to balloon distension, however, was too small to permit statistical comparison (Table 7.3). Atropine reduced the amplitudes of the synchronous responses above the middle and lower balloon but had no effect on the amplitude of the synchronous responses above the upper balloon. Atropine had no effect on the propagation velocities for secondary peristalsis.

Table 7.3: Manometric characteristics of primary and secondary peristaltic pressure waves before and after atropine.

STIMULUS	AMPLITUDE		VELOCITY	
	(mmHg)		(cm/sec)	
	Before atropine	After atropine	Before atropine	After atropine
Primary Peristalsis				
Water swallows †	88.9±2.1	n/a	2.9±0.04	n/a
Secondary Peristalsis§				
Air	73.6±1.6	50.9±3.7*	2.9±0.06	3.2±0.51
Water	63.6±2.0	54.9±4.3	2.6±0.07	2.4±0.36
Balloon †	64.0±4.3	n/a	2.7±0.23	n/a

Data are expressed as mean ± SE; † Triggered only 2 responses after atropine; § All volumes combined; * $p=0.0002$ compared with pre-atropine; n/a = not analysable

7.4 Discussion

Although it has been almost 90 years since Meltzer's original description of secondary peristalsis [226], remarkably little data exist in the literature about the phenomenon. Debate persists about the nature of the oesophageal response and its similarity to primary peristalsis [266, 269]. In the present study, the oesophageal responses to distension were examined using high-fidelity manometry that gave accurate measurement and sensitive spatial resolution of pressures. The effect of site and volume on the response to three different stimuli were investigated. The findings establish that, with an appropriate stimulus, a secondary peristaltic response can be evoked that has similar manometric characteristics to those of primary peristalsis. The

response is reproducible and can be readily incorporated into diagnostic clinical manometric studies.

The results indicate that the oesophageal response to distension is variable and influenced by the type of stimulus. When the oesophagus is distended with air or water boluses, a peristaltic response that traverses the entire oesophagus is produced. In contrast, the response to balloon distension is usually characterised by synchronous contractions above the balloon; secondary peristalsis is uncommon and occurs predominantly below the distension point after deflation. This variability in the response to oesophageal distension explains, at least in part, the conflicting results of previous studies, and indicates that oesophageal responses to distension can only be considered in the context of a specified stimulus.

The variability of the response most likely arises from the physical characteristics of the stimulus. The balloon provides a focal stimulus that cannot be moved by any induced motor response. In contrast, air and water disperse along the oesophagus and are able to be moved ahead of any induced propagated wave. The moving bolus may also serve to reinforce the response in a manner similar to that of water swallows in primary peristalsis [107, 168, 185]. Previous studies suggest that stimulation of the striated muscle segment of the oesophagus may be an important factor in triggering secondary peristalsis through activation of a central reflex pathway [30, 266]. The air and water boluses invariably induced a small common cavity pressure rise throughout the oesophagus at the time of injection indicating that the striated muscle segment was distended whatever the site of injection. This effect was not seen with balloon distension which, in the case of the distal two balloons, would be confined to the smooth muscle segment. Interestingly, however, the balloon distension at the mid-oesophagus, was significantly better at stimulating peristalsis than when applied to the upper oesophagus. The reason for this difference is not clear but may relate to the transition from striated to smooth muscle at this level.

The manometric characteristics of complete secondary peristalsis were similar to those of primary peristalsis suggesting common neural mechanisms. In contrast to earlier findings [266, 269], peristaltic velocity with balloon distension was similar to that for air and water distension and for primary peristalsis. This discrepancy may be explained by differences in data analysis, with the analysis in this study being limited to the mean velocity of the peristaltic responses over the distal 12cm of the oesophagus rather than between adjacent recording sites. Calculation of regional differences were not performed as the time intervals between contractions at adjacent side holes spaced 3cm apart, recorded at a paper speed of 5mm/second, were beyond the limits of accurate resolution using manual analysis. Peristaltic velocity was not influenced by balloon diameter or the size of the air boluses but was slower for the largest water bolus. A similar effect has been observed with primary peristalsis [338] and suggests sensory feedback from the oesophageal wall to the intramural neural mechanisms underlying the peristaltic response.

Atropine reduced the frequency of secondary peristalsis triggered by all three stimuli. This effect appeared to be due largely to a reduction in pressure wave amplitude as the pattern of failure after atropine was usually characterised by focal low amplitude contractions rather than an alteration in peristaltic velocity or complete peristaltic failure. This response to atropine is consistent with an effect at the level of the smooth muscle [139]. Similar observations have been made in the cat [30]. The reduction in peristaltic response rate might also be explained by either increased oesophageal compliance induced by atropine with subsequent reduction in the number of oesophageal stretch receptors triggered or failure to detect weak non-lumen occluding peristaltic responses [224].

Atropine also decreased the amplitude of the synchronous responses above the balloon. This reduction however, was seen only with the middle and distal balloons and may reflect inclusion of responses from the proximal part of the smooth muscled portion of the oesophagus. It may also be due to a reduction in the intensity of the

stimulus resulting from the effects of atropine on the oesophageal smooth muscle at the site of distension. Consistent with an earlier report [266], atropine did not affect the amplitude of the synchronous responses induced in the striated segment of the oesophagus above the proximal balloon.

Secondary peristalsis is an important mechanism for the clearance of retained material or refluxate from the oesophagus [90] and defective secondary peristalsis might be a mechanism contributing to the pathogenesis of reflux disease or dysphagia. Measurement of secondary peristalsis could thus be useful in assessing these problems. It has been shown that secondary peristalsis can be easily and reliably tested by injecting air or water boluses through the manometric catheter. The reproducibility of the response is excellent with no significant variation in response rates or manometric characteristics between test days. Whilst balloons have been the traditional means of testing secondary peristalsis, the findings in this study suggest that they are an inferior stimulus and, from a practical point of view, they are susceptible to damage.

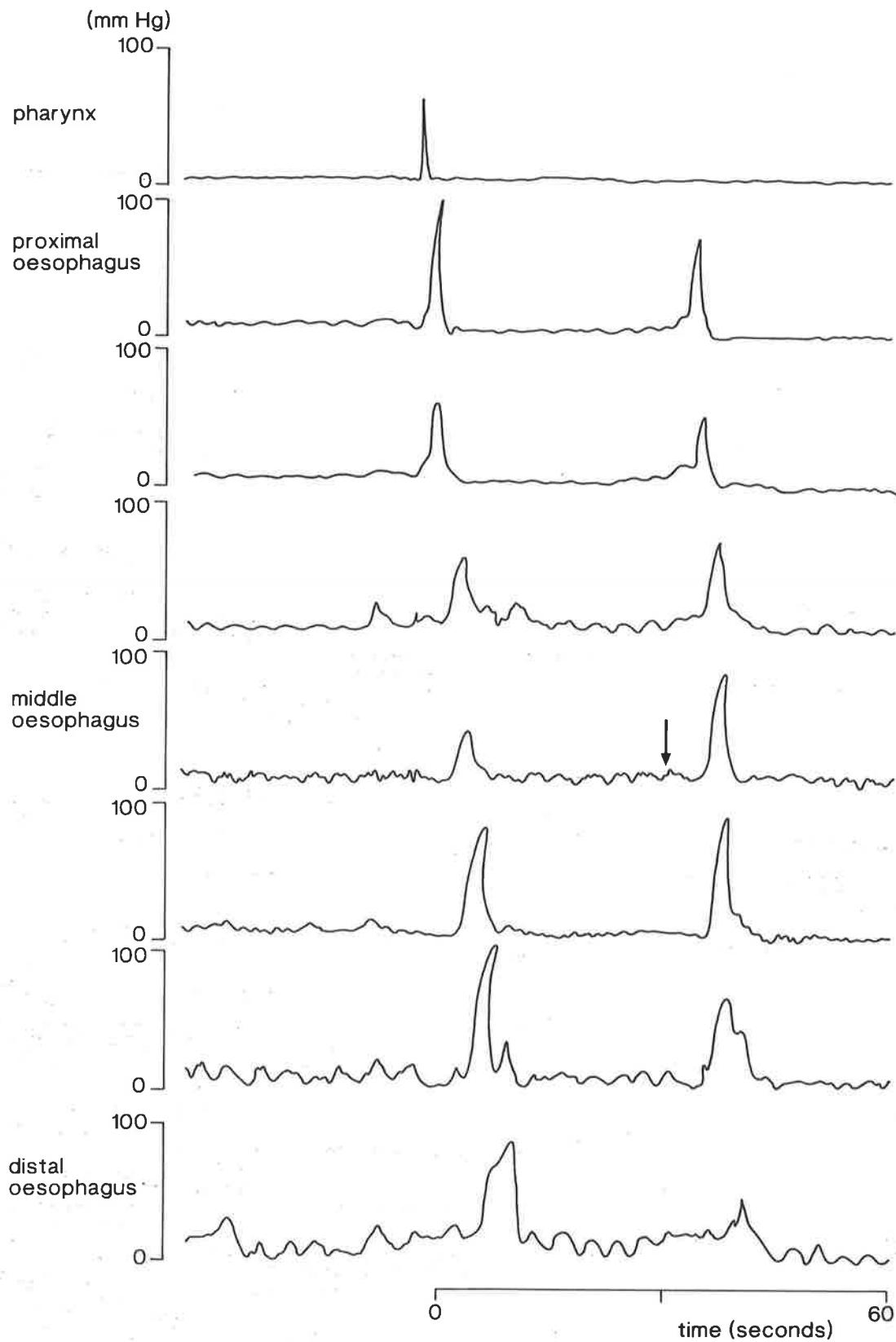


Figure 7.1: Pressure tracing showing primary peristalsis triggered by a water swallow and secondary peristalsis triggered by a 10ml air bolus injection. The position of the arrow indicates the onset and level of bolus injection

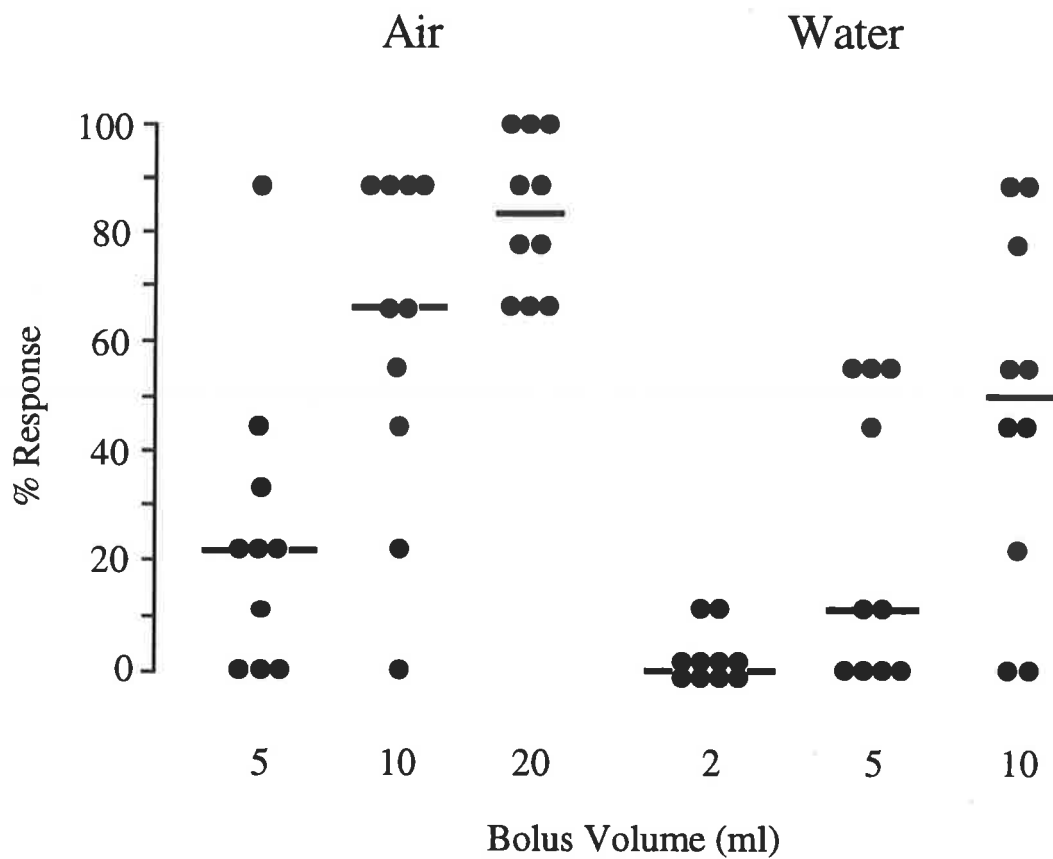


Figure 7.2: Effect of bolus volume on the secondary peristaltic responses to air and water. Data for the three oesophageal sites are combined with each volume tested nine times in each subject. Overall response rates for each individual subject are shown by the dots and median response rates are indicated by the bars. The frequency of secondary peristaltic responses increased significantly with bolus volume ($p < 0.0001$).

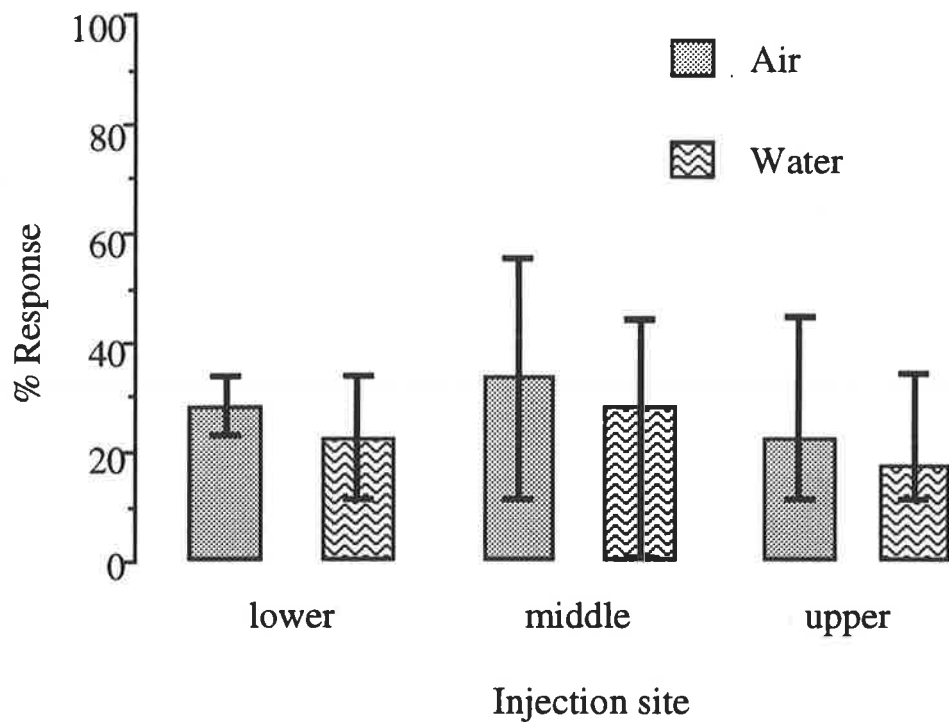


Figure 7.3: Effect of the site of injection on the proportion of secondary peristaltic responses to air and water boluses. Combined data for the 5 and 10ml boluses are shown. The columns represents the median frequency of secondary peristaltic responses for each stimulus at each site and the interval lines indicate the interquartile range. The 5 and 10ml volumes were tested in triplicate in 10 volunteers giving a total of 60 tests at each level. The frequency of responses for air and water boluses were similar at each site.

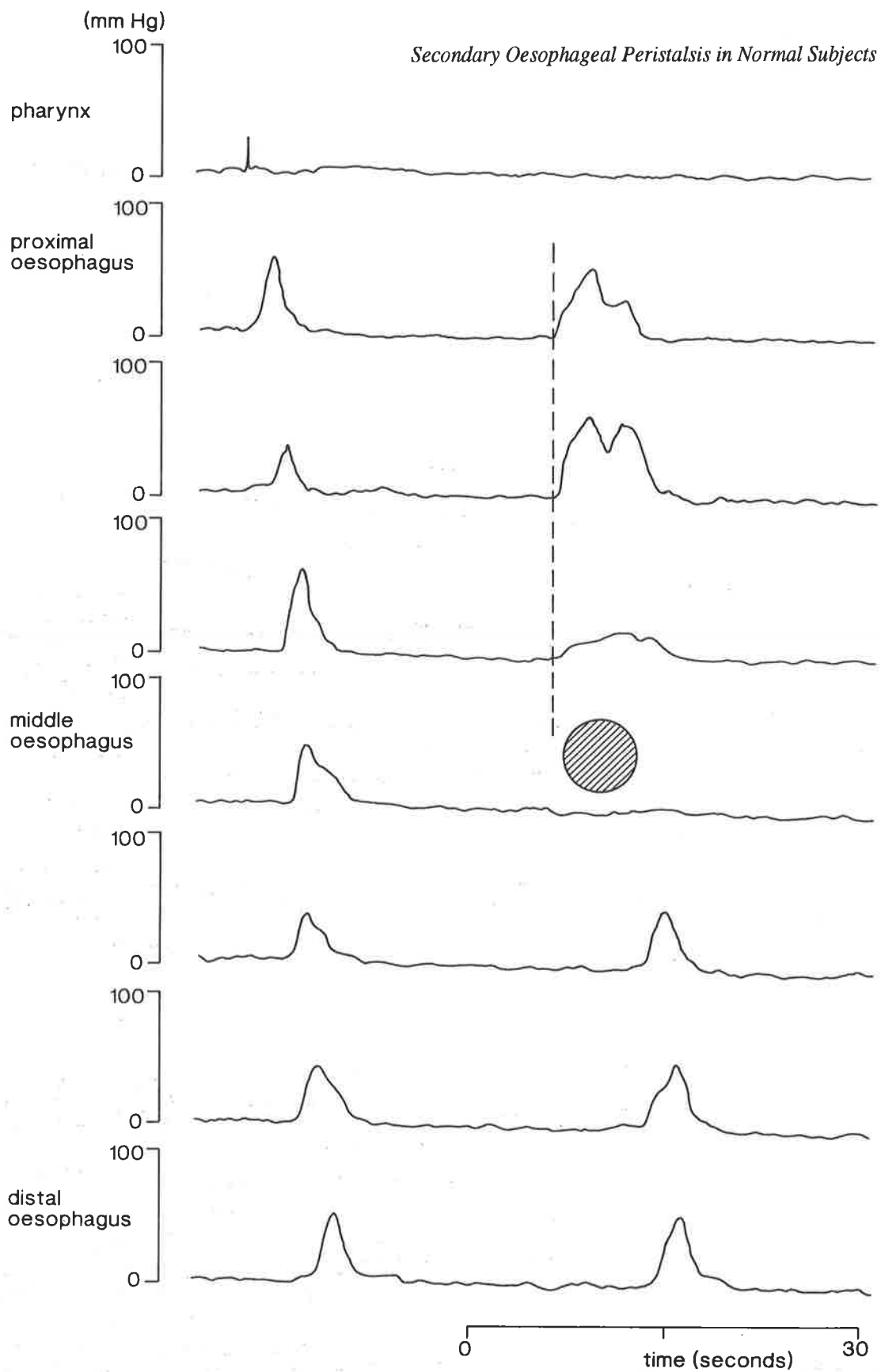


Figure 7.4: Pressure tracing showing primary peristalsis triggered by a water swallow and secondary peristalsis triggered by balloon distension. The position of the schematic balloon illustrated indicates the level and duration of balloon distension.

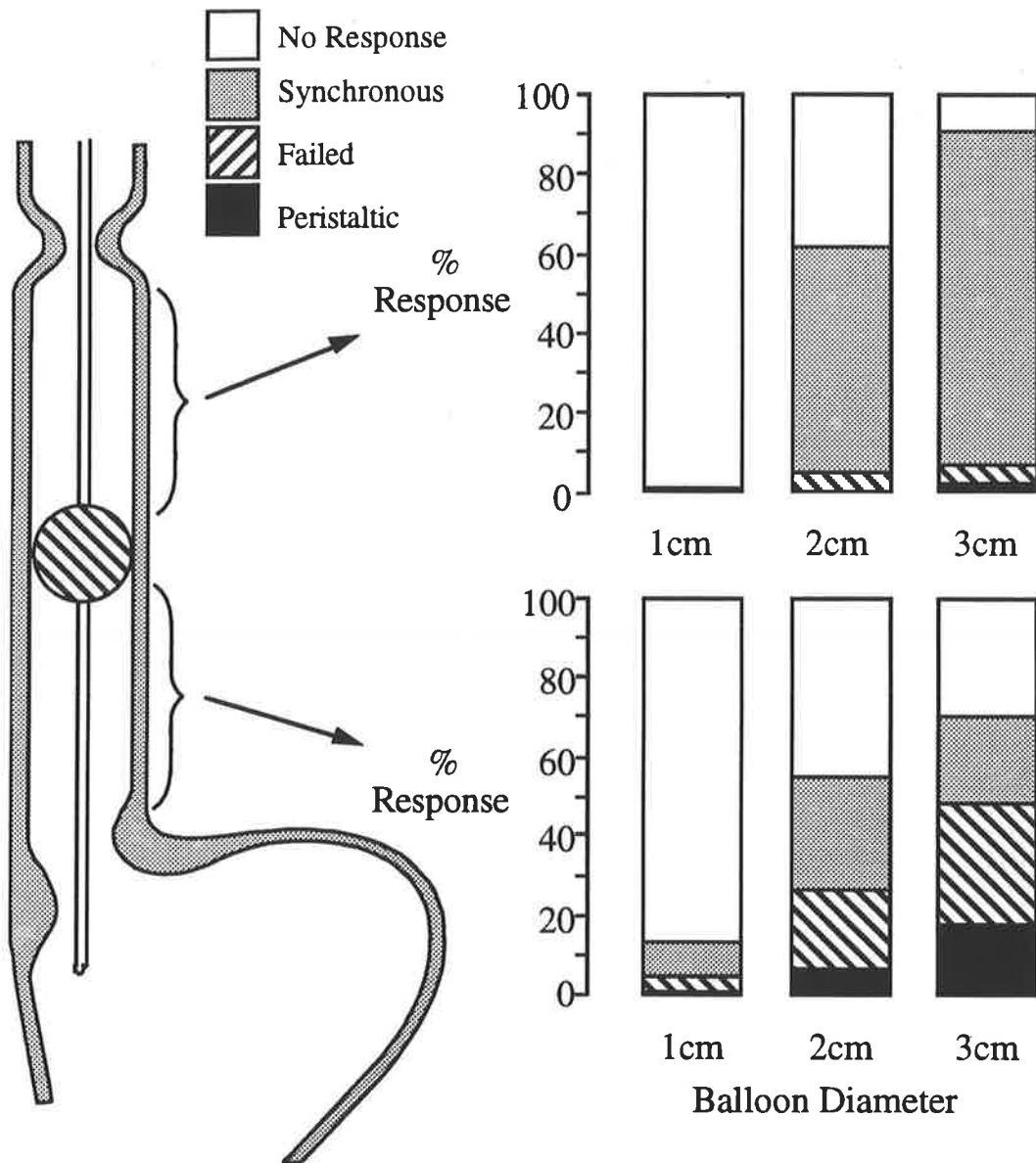


Figure 7.5: Manometric responses to oesophageal balloon distension. Responses have been characterised separately above and below the balloon for the three distension volumes. The responses above the balloon occurred during inflation and the responses below the balloon occurred after deflation. Data for the three sites have been combined and expressed as a percentage of the total number of tests. Each diameter was tested in triplicate at three levels in the oesophagus in 10 volunteers giving a total of 90 tests. The frequency of secondary peristaltic responses increased significantly with balloon volume ($p=0.004$).

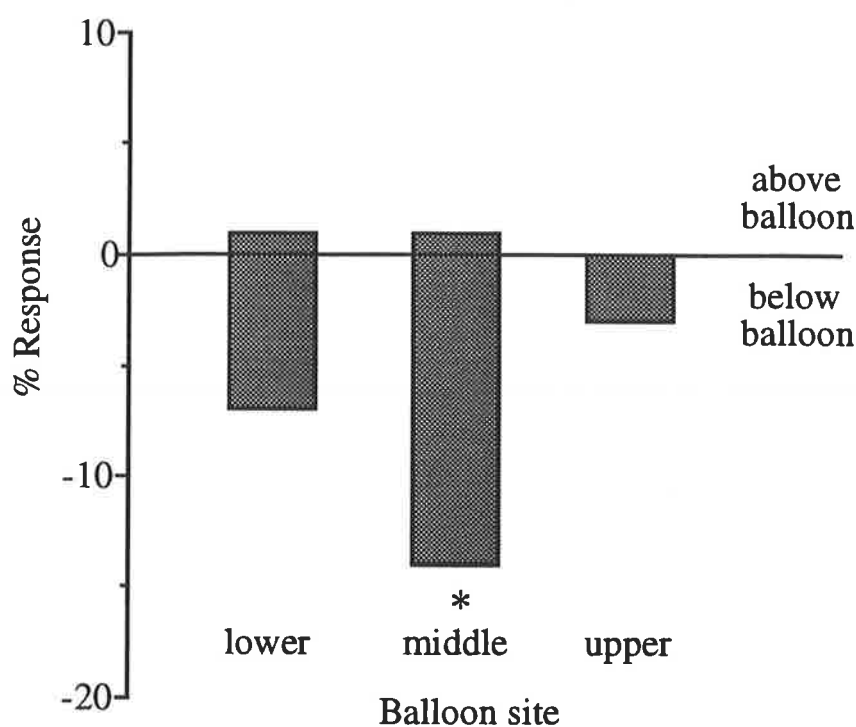


Figure 7.6: Effect of site of balloon distension on secondary peristaltic responses. Data for the three volumes have been combined. Each column represents the proportion of peristaltic responses, expressed as a percentage of the total number of tests. The three diameters were tested in triplicate in 10 volunteers giving a total of 90 tests at each level. The middle balloon was more likely to trigger secondary peristalsis than the upper or lower sites (* $p=0.04$).

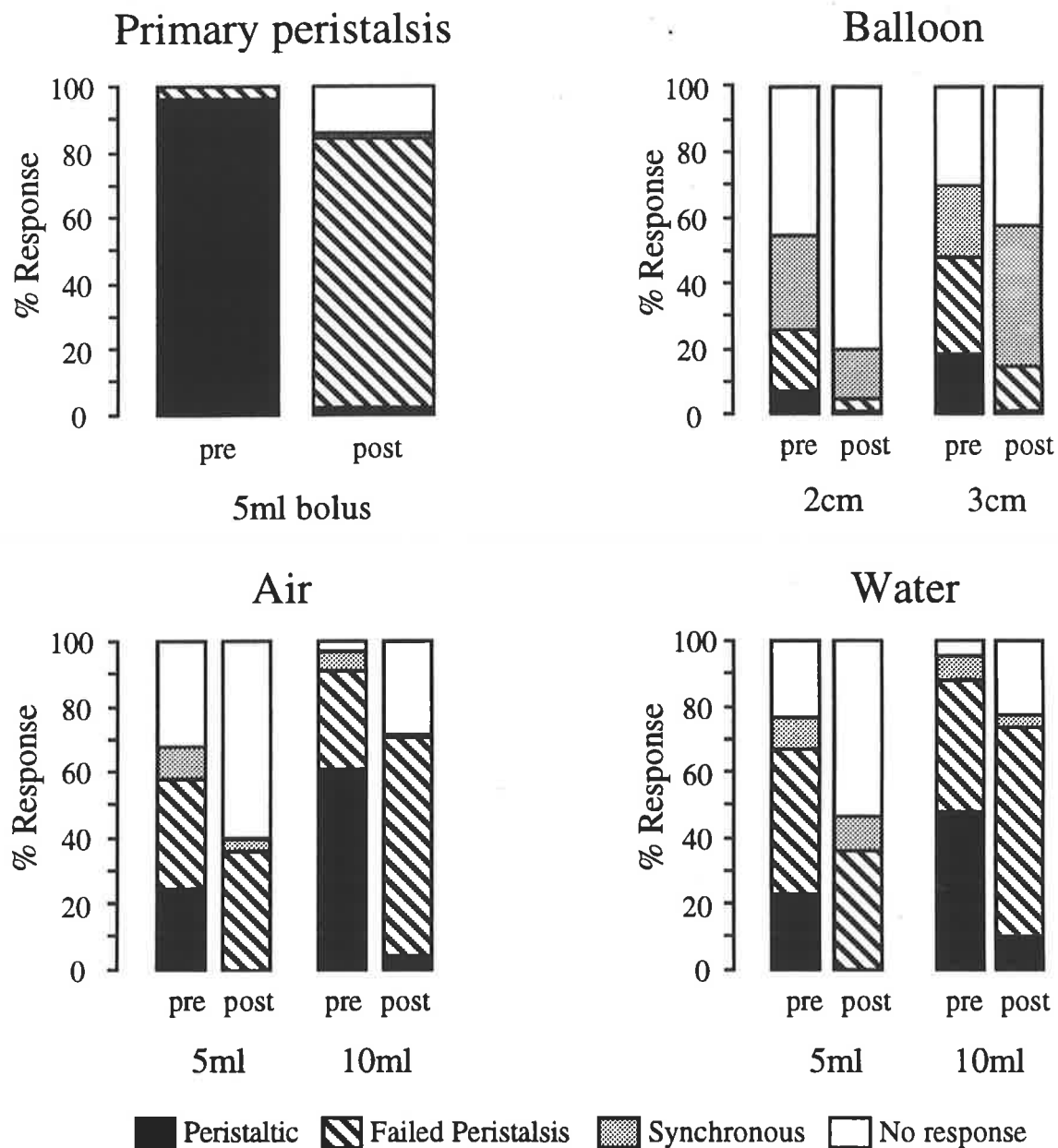


Figure 7.7: Effect of atropine on the pattern of primary peristalsis and secondary peristaltic responses to water, air and balloon distension. Data for the three sites of stimulation of secondary peristalsis have been combined and frequency of response is expressed as a percentage of the total number of tests. Atropine significantly decreased the frequency of primary and secondary peristaltic responses and increased the frequency of failed and no responses with little effect on the proportion of synchronous responses.

Chapter 8

Secondary Peristalsis in Patients with Gastro-Oesophageal Reflux Disease

8.1 Introduction

8.2 Methods

8.2.1 Subjects

8.2.2 Recording Techniques

8.2.3 Protocol

8.2.4 Data and Statistical Analysis

8.3 Results

8.4 Discussion

8.1 Introduction

Secondary peristalsis is triggered by oesophageal distension [68, 115, 129, 269, 305, 349] and contributes to oesophageal volume clearance after reflux [90, 162]. It is the initial oesophageal motor event after the majority of reflux episodes in normal subjects [90]. In patients with reflux disease, however, it has been reported to occur less often after reflux [110] suggesting that triggering of secondary peristalsis may be abnormal in such patients. A systematic study of secondary peristalsis in patients with reflux disease, however, has not been reported. Studies of the oesophageal motor responses to prolonged balloon distension have shown a higher threshold to distension and fewer contractions during the period of distension in patients with reflux disease, these findings being correlated with greater severity of oesophagitis and delayed oesophageal acid clearance [344]. Other studies however, have found the threshold to trigger a secondary peristaltic response to relatively slow intra-oesophageal instillation of water or acid in patients with reflux disease to be similar to that in normal subjects [63, 64]. In chapter 8 of this thesis, data were presented examining secondary peristalsis in normal subjects. Based on this work, the aims of the following study were to investigate in detail, the integrity and characteristics of secondary peristalsis in patients with gastro-oesophageal reflux disease and to examine the effect of different stimuli on the motor response.

8.2 Methods

8.2.1 Subjects

Twenty two patients with gastro-oesophageal reflux disease (F8:M14), ranging in age from 23-85 years (median 57yrs), were recruited for the study. All patients complained of reflux symptoms, such as heartburn and acid regurgitation and had either erosive oesophagitis at endoscopy (n=17) or excessive oesophageal acid exposure (pH<4 for >5% of total time) during 24 hour oesophageal ambulatory pH monitoring (n=5). Those with endoscopic evidence of oesophagitis were studied before treatment. Nine of the 22 patients complained of intermittent dysphagia but none of these had any structural obstructive lesion at endoscopy. Patients taking medication that may have affected oesophageal motility ceased these at least 48 hours prior to the study.

Twenty age-matched healthy subjects (F8 : M12) ranging in age from 23-76 years (median 50yrs) served as controls. Subjects were free of gastrointestinal symptoms, had no history of upper gastrointestinal surgery, were not taking antacids regularly or any medication known to alter oesophageal motor function.

All patients and volunteers gave written informed consent and the study was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

8.2.2 Recording Techniques

The manometric recording techniques for this study have been described in detail in chapter 6 of this thesis. The specific details of the recording apparatus are described and illustrated in section 6.1.

In brief, oesophageal motility was recorded using perfused multi-lumen manometric catheter with side holes located at 7 sites along the oesophageal body. The manometric assembly also incorporated a silicone rubber balloon for distension of the mid

oesophagus and, immediately above the balloon, an infusion port for the rapid injection of boluses of air or water.

8.2.3 Protocol

Subjects were studied following an overnight fast. The catheter was passed pernasally and the subjects studied supine. After a 10 minute adaptation period, both primary and secondary peristalsis were tested in each subject.

Primary peristalsis was tested with ten, 5ml water swallows. Each swallow was separated by an interval of 30 seconds. Secondary peristalsis was triggered by oesophageal distension with 10ml boluses of air and water and by inflating the intra-oesophageal balloon. Each stimulus was tested five times. Balloon distension was only performed in 16 of the 22 patients because of technical problems such as balloon rupture (2 subjects) or intolerance of balloon distension due to chest discomfort (4 subjects). The air and water boluses were rapidly injected through the infusion port by hand. The 10ml air bolus was injected within 0.5 second, while the 10ml water bolus was delivered within 1.5 seconds. The balloon was inflated with 17ml of air to a diameter of 3cm within 0.5 seconds and sustained for 5 seconds before being quickly deflated over 0.5 seconds. The distending stimuli were given between 15 to 20 seconds after any preceding primary peristaltic wave and there were no systematic differences in this interval among stimuli or between subject groups. An interval of 20 seconds was allowed after each stimulus for any response to occur. This interval was selected as it is comparable to the latency of the secondary peristaltic response after physiological reflux events [110]. During this time the subjects were instructed not to swallow. At the end of the 20 second period the subject was asked to perform a dry swallow that served to reduce the desire of the subject to swallow during the distension stimulus as well as to clear any residual air or water.

8.2.4 Data and Statistical Analysis

The oesophageal body contractions triggered in this study were analysed according to the methods described in section 7.1.4. The contraction amplitude at each recording site and the latency of the wave onset between adjacent recording sites were measured for both primary and secondary peristalsis. The primary and secondary peristaltic responses were then classified as complete or failed according to the previously stated criteria. No response was scored if a pressure wave ≥ 10 mm Hg was seen in less than two recording sites. For peristaltic responses, mean amplitude and velocity were calculated for the five distal recording sites corresponding to the distal 12 cm of the oesophageal body.

The frequencies of successful primary and secondary peristalsis were determined in each subject as a percentage of the number of tests for each stimulus. Differences in response rates among stimuli were analysed using log linear modelling techniques [28]. Data for peristaltic amplitude and velocity for all tests in all subjects were pooled for each stimulus and subjected to an analysis of variance [40]. The proportions of the various motor responses to distension for all tests in all subjects were also pooled for each stimulus and subjected to Chi-squared analysis. The relationship between secondary peristalsis and oesophageal acid clearance was assessed using Spearman's rank correlation. Group data for response rates are expressed as median values and those for peristaltic amplitude and velocity as mean $\pm$ standard error of the mean.

8.3 Results

Primary peristalsis

Nineteen of the normal subjects (95%) and 14 of the 22 reflux patients (64%) exhibited complete peristaltic responses with at least 8 of the 10 water swallows and were defined as having normal primary peristalsis (Figure 8.1). Whilst the median frequency of successful primary peristalsis in the reflux patients (90%) was the same as that in the normal subjects (90%), eight of the 22 reflux patients (36%) had an abnormal frequency of successful primary peristalsis with a median response rate in these subjects of only 5%. The median response rate in patients with endoscopic oesophagitis (85%) was not significantly different from those in patients without oesophagitis (95%). Of the nine patients with reflux disease who complained of dysphagia, five (56%) had normal primary peristalsis. Similarly, 9 of the 13 patients (69%) without dysphagia had normal primary peristalsis. All of the abnormal primary peristaltic responses were of the failed pattern. Contraction amplitudes and propagation velocities are summarised in Table 8.1. Primary peristaltic amplitude was similar in the two groups but peristaltic velocity was significantly slower in the reflux patients ($p=0.001$).

Table 8.1: Primary and secondary peristaltic amplitude and velocity

	NORMAL SUBJECTS	REFLUX PATIENTS
AMPLITUDE (mm Hg)		
Primary Peristalsis	80.6 ± 2.1	82.0 ± 1.9
Secondary Peristalsis		
Air injection	77.1 ± 3.5	75.9 ± 5.1
Water injection	73.2 ± 3.2	76.6 ± 5.4
Balloon distension †	68.1 ± 3.2	77.9 ± 8.5
VELOCITY (cm/s)		
Primary Peristalsis	2.8 ± 0.04	2.6 ± 0.05 *
Secondary Peristalsis		
Air injection	2.9 ± 0.08	2.7 ± 0.22
Water injection	2.6 ± 0.11	2.1 ± 0.16 §
Balloon distension †	2.7 ± 0.19	2.9 ± 0.26

Data expressed as mean ± standard error; † Secondary peristaltic responses induced by balloon distension occurred below the balloon after deflation; * p=0.001 compared to normals; § p=0.04 compared to normals

Secondary Peristalsis

a) Air and water boluses

Secondary oesophageal motor responses to air and water boluses were typically characterised by a propagated pressure wave that traversed the entire oesophagus (Figure 8.2). The frequencies of successful secondary peristalsis in response to oesophageal distension with air and water injections were significantly higher in the normal subjects than in the reflux patients (Figure 8.3) but were not significantly different in patients with or without oesophagitis. These differences were maintained when patients (and the one normal subject) with abnormal primary peristalsis were

excluded from the analysis. The median response rate with air injection was 50% in the normal subjects and 0% in the patients ($p=0.0005$). Similarly, the median success rate with water injections was 30% in the normal subjects and 0% in the reflux patients ($p=0.003$). Within each group, however, the frequency of secondary peristalsis in response to air did not differ from that in response to water. There was no correlation between the frequency of primary and secondary peristalsis with either stimulus and most patients with abnormal secondary peristalsis had normal primary peristalsis. In patients with normal primary peristalsis, the prevalence of defective secondary peristalsis, triggered by the air and water boluses, was similar in those with dysphagia (3/5, 60%) to those without dysphagia (6/9, 67%).

The amplitude and velocity of successful secondary peristalsis induced by the air boluses in the normal subjects was similar to that in the reflux patients (Table 8.1). For the water boluses, the amplitude of secondary peristalsis induced was similar in the two groups while secondary peristaltic velocity was slower in the reflux patients.

The patterns of the motor responses to air and water injection are summarised in Figure 8.4. In the normal subjects, 52% of the air injections triggered a peristaltic response and 21% resulted in failed peristaltic sequences. Of the remainder, 4% resulted in synchronous responses and 23% in no response. The reflux patients showed a distinctly different pattern; only 14% of air injections resulting in complete secondary peristalsis and 63% producing no response. The percentage of failed (13%) and synchronous (10%) responses were similar to those in the normal group. The difference between the two groups was maintained even when the patients with abnormal primary peristalsis were excluded from the analysis. The pattern of responses to the water bolus injections were similar to those seen for the air injections in the two groups.

b) Balloon distension

As discussed in chapter 7, balloon distension produces a manometric response that is different from the responses produced by the air and water bolus injections (see Figure 7.4 in chapter 7). The proportions of secondary peristaltic responses seen below the balloon after deflation in the normal subjects and patients with gastro-oesophageal reflux disease are shown in Figure 8.5. The frequency of secondary peristalsis after balloon distension in the patients with reflux disease (20%) was similar to that in the control subjects (0%), ($p=0.95$). As with air and water boluses, the response rate was not influenced by the presence or absence of oesophagitis. The contraction amplitude and peristaltic velocity of the successful secondary peristaltic responses that occurred after balloon deflation were also similar in the normals and the reflux patients (Table 8.1).

The patterns of balloon responses are shown in Figure 8.6. There were no significant differences in the proportions of the motor responses either above or below the balloon between the two groups.

8.4 Discussion

The major finding from the study is that patients with reflux disease have markedly lower secondary peristaltic response rates than have age-matched controls, with most patients failing to trigger any peristaltic response at all. This finding supports and extends earlier observations on spontaneous reflux episodes which revealed that secondary peristalsis occurred less frequently after reflux in patients with reflux oesophagitis compared with normal subjects [110]. In this previous study it was also noted that the time from the onset of reflux to the first clearance motor event was prolonged in patients with reflux oesophagitis, a finding which could be explained, at least in part, by failure of secondary peristalsis. Although the findings are at variance

with other studies that found no difference in the frequency of secondary peristalsis between normal subjects and patients with reflux disease [63, 64], it is possible that differences in methodology account for the discrepancy as these previous studies used slow infusions rather than rapid bolus injections as used in the present study.

The mechanisms underlying defective secondary peristalsis remain to be defined but inferences are possible from the pattern of the responses and from previous observations. As secondary peristalsis is a reflex response to oesophageal distension, the defect may lie in the oesophageal motor nerves or muscles, in oesophageal sensation, in the central integrative mechanisms or in a combination of these. The majority of patients with abnormal primary peristalsis also had abnormal secondary peristalsis and in these patients it is possible that the defect lies in the efferent limb of the motor pathway. The majority of patients with abnormal secondary peristalsis, however, had normal primary peristalsis. Because secondary peristalsis appears to share a common motor pathway with primary peristalsis [305] this side of the reflex arc would seem to be intact, implying that the defect in secondary peristalsis is due either to an abnormality of oesophageal sensation or in the integration of sensory information with the motor component of the reflex. This hypothesis is supported by the observations of Williams and colleagues who noted that the distension threshold required to trigger a motor response was higher in patients with oesophagitis than in healthy controls [344]. Others, however, have found no difference in the threshold volume required to trigger oesophageal motor responses using slow (1ml/sec) infusions [63, 64]. Differences in the methods of these other studies make direct comparisons with our results difficult and further studies are needed to define more accurately the mechanisms responsible for defective secondary peristalsis in patients with reflux disease.

The importance of defective triggering of secondary peristalsis to the pathophysiology of reflux disease remains to be determined. Secondary peristalsis can effectively clear almost all of an injected acid bolus from the oesophagus leaving a negligible residual

volume [162]. The occurrence of secondary peristalsis after up to 57% of reflux episodes in normal subjects suggests a potential role in acid clearance after spontaneous reflux [110]. The substantially lower occurrence rate of 17% in patients with reflux oesophagitis further suggests that defective secondary peristalsis might prolong acid clearance. It has been argued that because secondary peristalsis does not restore oesophageal pH to above pH 4 after reflux, it was not of physiological importance [38]. However, as secondary peristalsis acts in the initial clearing of the bulk of the refluxate volume from the oesophagus, changes in oesophageal pH would be unlikely until neutralisation of the residual acid by bicarbonate rich saliva delivered by primary peristalsis [162]. Thus secondary peristalsis would not by itself be expected to restore oesophageal pH, but to complement and accelerate the effects of the primary peristalsis that follows. During the day when patients are awake, any potential effect of defective secondary peristalsis on acid clearance will be minimised by frequent primary peristalsis. Secondary peristalsis is likely to be more important, however, during sleep when the rate of primary peristalsis is substantially reduced [259]. This notion is supported by data presented in the study of the mechanisms of reflux in normal ambulant subjects presented in chapter 12. In this study it was noted that whilst primary peristalsis was the most common oesophageal clearance event overall, secondary peristalsis was the major initial motor event when subjects were supine and/or asleep.

Non-obstructive dysphagia is a common symptom in patients with gastro-oesophageal reflux disease and correlates with the severity of primary peristaltic abnormalities [181]. We hypothesised that a similar correlation would also exist for patients with dysphagia and abnormal secondary peristalsis. Instead, in patients with normal primary peristalsis, the prevalence of defective secondary peristalsis was similar in those with dysphagia (60%) to that in patients without dysphagia (67%). This finding would suggest that defective secondary peristalsis does not contribute significantly to non-obstructive dysphagia in patients with gastro-oesophageal reflux disease.

The nature of the distending stimulus determined the manometric pattern of the secondary peristaltic responses we recorded. The air and water boluses were distributed rapidly along the length of the oesophagus as indicated by the common cavity seen in the oesophageal body after injection. This pattern of distension triggers an integrated response that sweeps the length of the oesophagus. Distension with air or water produces a pattern of distension analogous to the oesophageal distension induced by reflux; the 10ml bolus producing a common cavity pressure rise that is similar to that seen after reflux events. As seen in the normal subjects presented in chapter 7, air and water boluses were more effective stimuli for inducing secondary peristalsis in age-matched normal subjects and discriminated these subjects from patients with reflux disease more clearly than did balloon distension. Fixed balloon distension, on the other hand, produces a manometric response more analogous to acute oesophageal obstruction [349], and the propagated response associated with balloon deflation occurs only below the site of distension. It is also a suboptimal stimulus as evidenced by the lower response rate compared with those to air and water in the normal subjects, and its failure to discriminate between normal subjects and patients with reflux disease.

In summary, there is a marked defect in the triggering of secondary peristalsis in patients with reflux disease. The mechanisms underlying this defect and its significance in the pathogenesis of reflux disease remain to be determined.

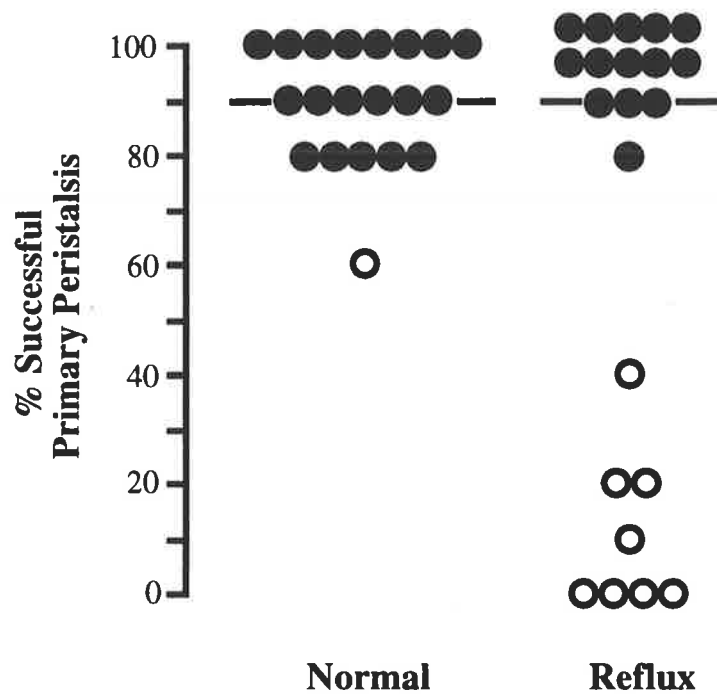


Figure 8.1: The percentage of complete primary peristaltic responses for each subject. Subjects defined as having normal primary peristalsis are represented by the filled circles and those with abnormal primary peristalsis with the open circles. The horizontal bars indicate the median values. There was no statistical difference in the response rates between the groups.

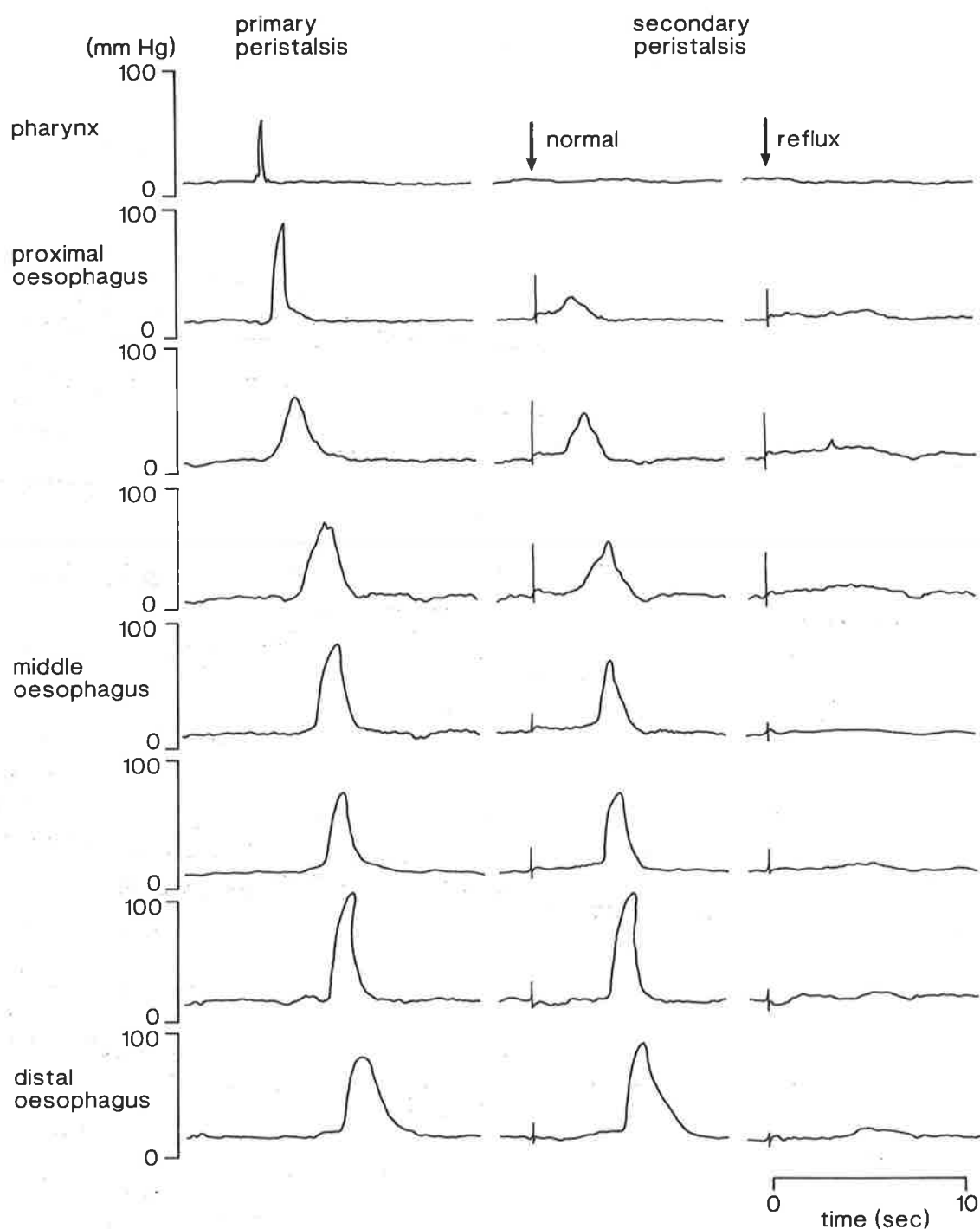


Figure 8.2: Pressure tracing showing primary peristalsis triggered by a water swallow and typical secondary peristaltic responses triggered by a 10ml air bolus injection in the normal subjects and patients with reflux disease. The arrow indicates the time of bolus injection. In the normal subjects, a secondary peristaltic wave would traverse the oesophageal body while in the reflux patients there was usually no response.

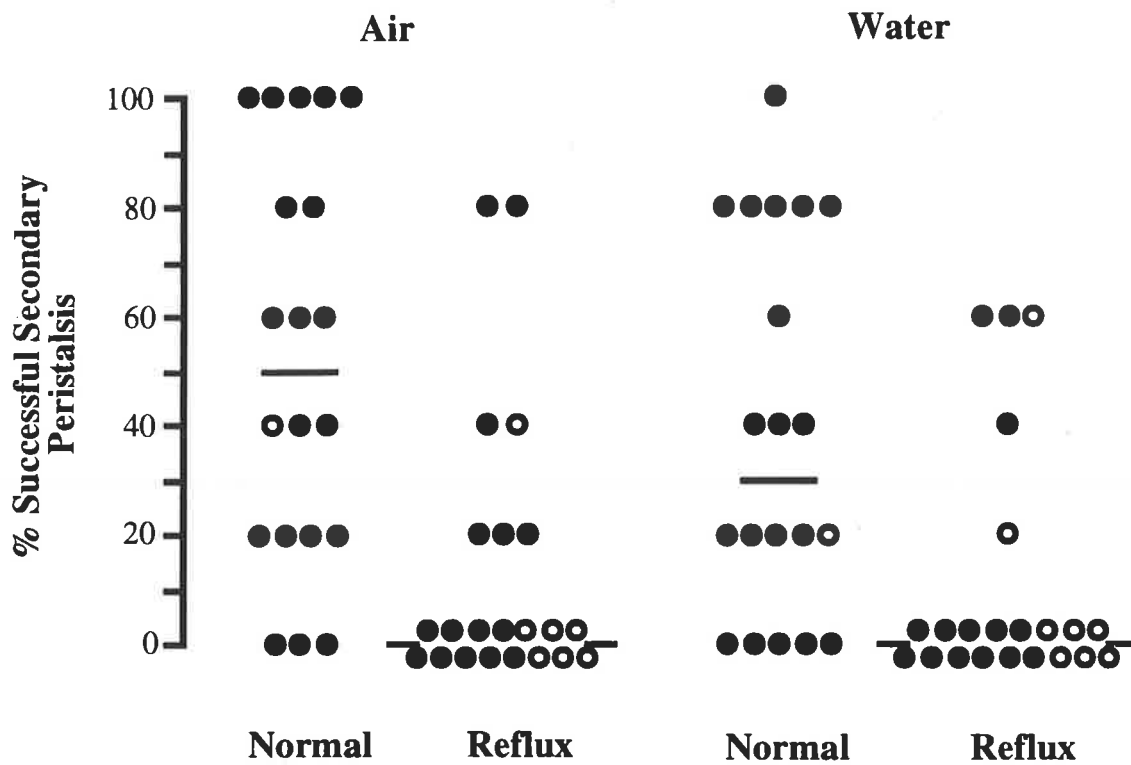


Figure 8.3: Percentage of secondary peristalsis with air and water bolus injections. Each point represents the proportion of normal secondary peristaltic responses (out of 5 tests) for each subject. Subjects with normal primary peristalsis are represented by the filled circles and those with abnormal primary peristalsis with the open circles. The horizontal bars indicate median values. The frequency of the responses for both stimuli were significantly less in the reflux patients compared with the normal volunteers ($p \leq 0.003$).

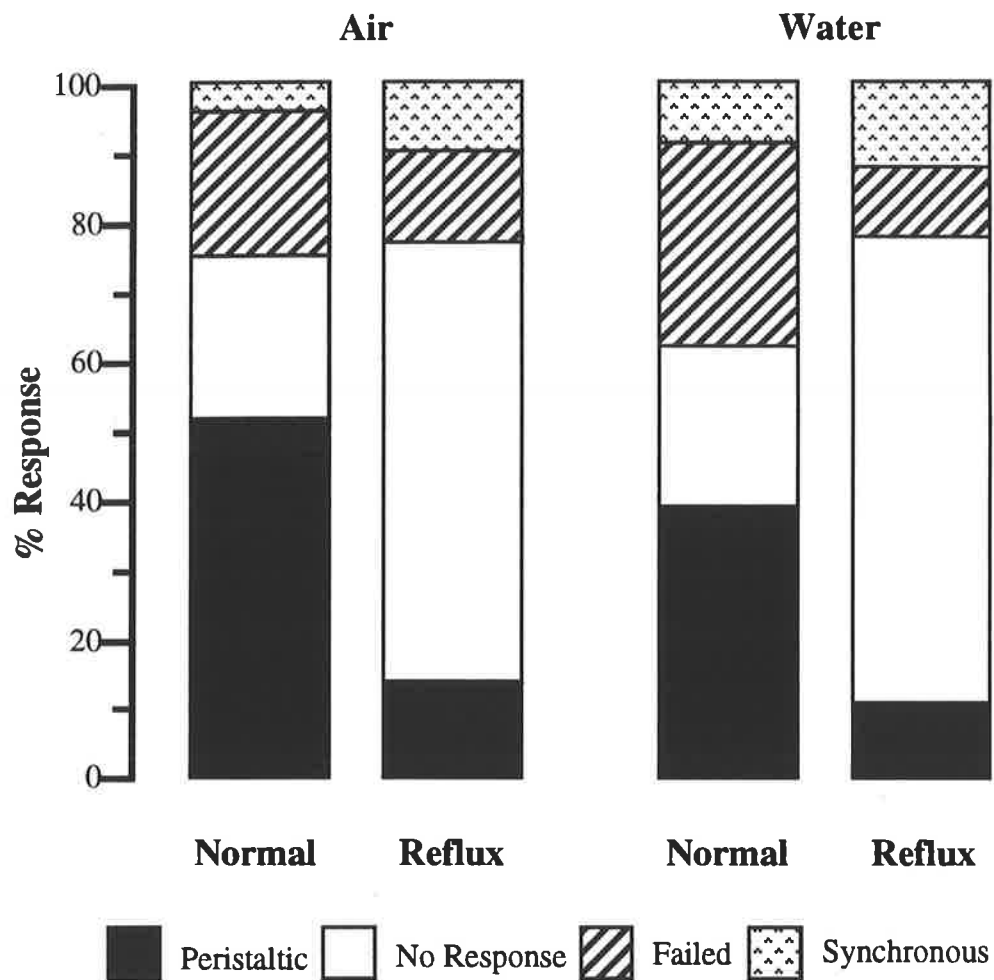


Figure 8.4: Patterns of manometric response to 10ml boluses of air and water. The proportions of the various patterns of manometric response are derived from pooled data of the individual responses. The most common pattern of failure of secondary peristalsis in the reflux patients was no response.

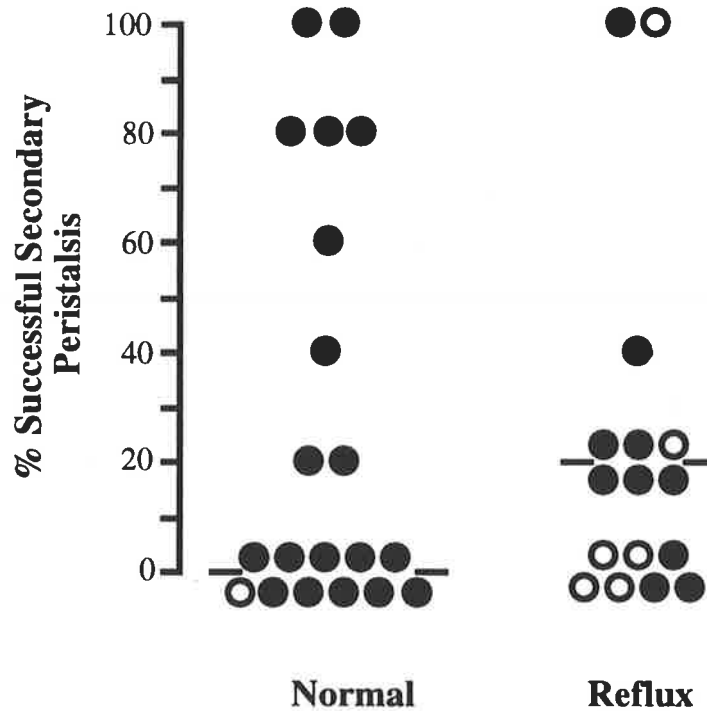


Figure 8.5: Percentage of secondary peristalsis with balloon distension. Each point represents the proportion of normal secondary peristaltic responses (out of 5 tests) for each subject. Subjects with normal primary peristalsis are represented by the filled circles and those with abnormal primary peristalsis with the open circles. The horizontal bars indicate median values. The response rates are similar in the two groups ($p=0.95$).

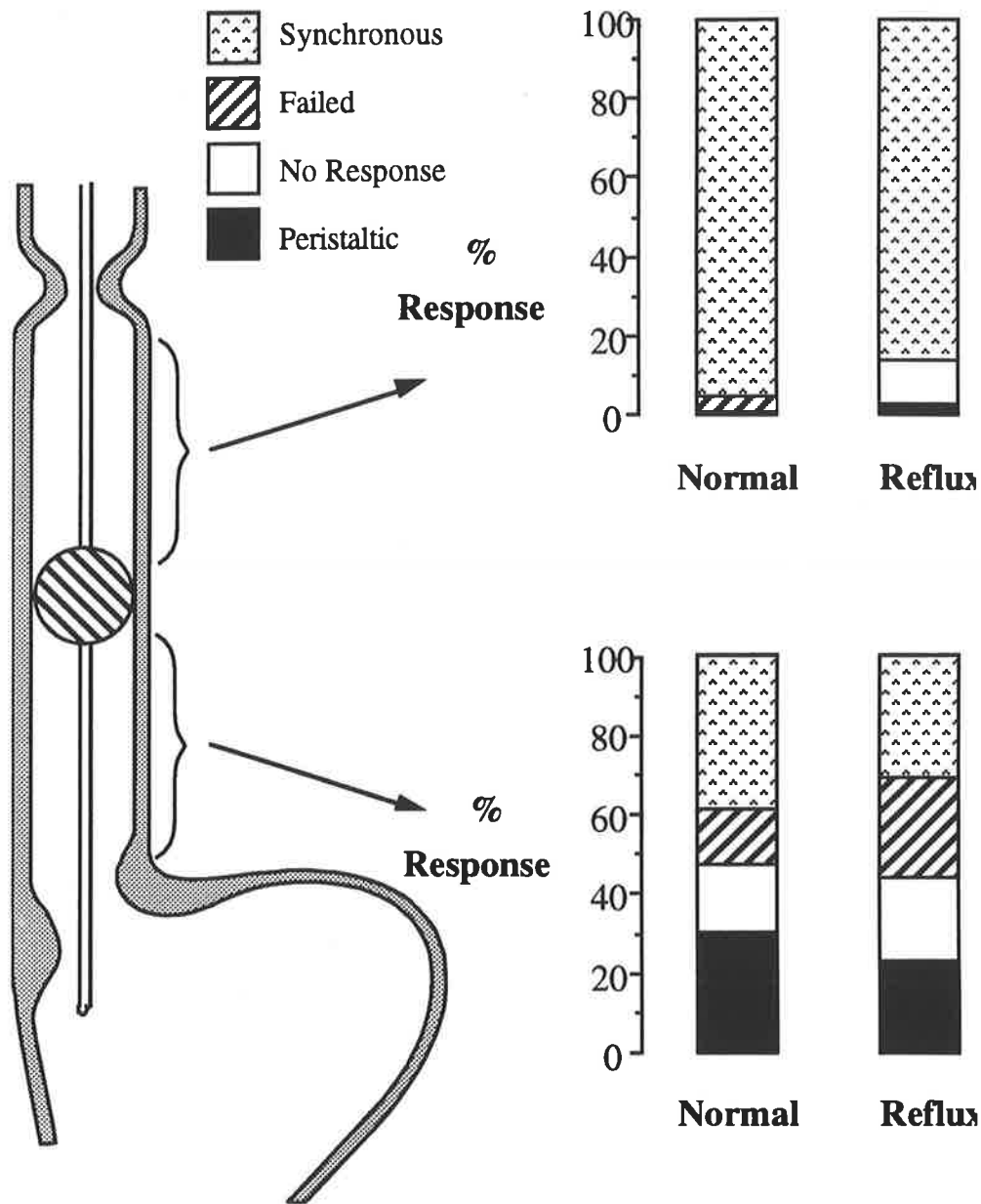


Figure 8.6: Patterns of manometric response to balloon distension. The proportion of various patterns of manometric response are derived from pooled data of the individual responses. Responses have been divided into those occurring above and below the balloon with the responses above the balloon occurred during inflation and the responses below the balloon occurred after deflation. The pattern of the responses was similar in the two groups.

Chapter 9

Secondary Peristalsis in Patients with Non-obstructive Dysphagia

- 9.1 Introduction
- 9.2 Methods
 - 9.2.1 Subjects
 - 9.2.2 Recording Techniques
 - 9.2.3 Protocol
 - 9.2.4 Data and Statistical Analysis
- 9.3 Results
- 9.4 Discussion

9.1 Introduction

Oesophageal dysphagia is an important symptom that suggests impaired transport of a swallowed bolus along the oesophagus. In patients with non-obstructive dysphagia, there is no evidence of mechanical obstruction and the symptoms are usually attributed to oesophageal motor dysfunction [181]. In a significant proportion of these patients, however, routine manometric assessment fails to reveal any abnormality of primary peristalsis and the aetiology of the symptom is unclear [16, 181, 197, 261, 333]. The likelihood of demonstrating a defect of oesophageal motor function may be improved with additional tests such as radionuclide measurement of oesophageal transit [203, 295] or by using solid boluses as a part of the manometric assessment [29, 176, 177, 201], but the mechanism of the delayed oesophageal transit or clearance detected by these methods are often not explained. The implication is, however, that there is an oesophageal motor abnormality. A possible explanation for these findings is an abnormality of secondary peristalsis. Abnormal oesophageal motor responses to prolonged balloon distension have been reported in patients with non-obstructive dysphagia and it has been postulated that this abnormality contributed to the pathogenesis of the symptoms [94, 345]. The secondary peristaltic responses triggered by oesophageal distension in such patients, however, have not been studied. The aim of this study was to examine the triggering, integrity and characteristics of secondary peristalsis in patients with non-obstructive dysphagia.

9.2 Methods

9.2.1 Subjects

Thirty patients with non-obstructive dysphagia (F17:M13), ranging in age from 26-74 years (median 59.5yrs), were studied. All patients complained of dysphagia as their predominant symptom. Nine patients also complained of chest pain. Mechanical obstruction of the oesophagus was excluded in all patients by either endoscopy (n=29) and/or barium swallow (n=26). Patients taking medication that may have affected oesophageal motility were asked to cease their medication at least 48 hour prior to the study.

Twenty age matched healthy subjects (F8 : M12) ranging in age from 23-76 years (median 50yrs) served as controls. Subjects were free of gastrointestinal symptoms, had no history of upper gastrointestinal surgery, were not taking antacids on a regular basis or took any medication known to alter oesophageal motor function. All patients and volunteers gave written informed consent and the study was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

9.2.2 Recording Techniques

The manometric recording techniques for this study have been described in detail in chapter 6 of this thesis. The specific details of the recording apparatus are described and illustrated in section 6.1. The recording configuration is identical to that described in chapter 8 for the evaluation of secondary peristalsis in patients with gastro-oesophageal reflux disease.

9.2.3 Protocol

An identical study protocol to that described for the evaluation of secondary peristalsis in patients with gastro-oesophageal reflux disease (Section 8.2.3) was used for this study. Primary and secondary peristalsis were both assessed. Primary peristalsis was

tested with 5ml water swallows and secondary peristalsis was tested by oesophageal distension with 10ml boluses of air and water and distension of a 3cm diameter balloon. Balloon distension was only performed in 23 of the 30 patients because of technical problems such as balloon rupture or intolerance of balloon distension due to chest discomfort.

9.2.4 Data and Statistical Analysis

The methods of data and statistical analysis used in this study are similar to those described in chapter 9 for the evaluation of secondary peristalsis in patients with gastro-oesophageal reflux disease. The reader is referred to section 9.2.4 for a complete description of the analysis methods used if required.

9.3 Results

Primary peristalsis

The median rate of successful primary peristalsis was significantly lower in the patients with dysphagia (50%) than in the normal subjects (90%, $p < 0.005$). Nineteen of the twenty normal subjects (95%) and 9 of the 30 dysphagia patients (30%) exhibited normal primary peristalsis (Figure 9.1). Conversely, 21 of the 30 dysphagia patients (70%) were defined as having abnormal primary peristalsis with a median response rate in this group of 40%. Eleven of the 21 patients were diagnosed as having diffuse oesophageal spasm [323] and ten had non-specific oesophageal motor disorders (NOMD) including two patients with high amplitude peristaltic contractions in the distal oesophagus [323]. The mean contraction amplitude and propagation velocity for the primary peristaltic responses are summarised in Table 9.1. Peristaltic amplitude was greater in the patients with dysphagia than the normal volunteers ($p < 0.0005$) but the primary peristaltic velocities were similar in the two groups ($p = 0.7$).

Table 9.1. Primary and secondary peristaltic amplitude and velocity

	NORMAL	NON- OBSTRUCTIVE DYSPHAGIA
AMPLITUDE (mm Hg)		
Primary Peristalsis	80.6 ± 2.1	94.6 ± 3.3 ^a
Secondary Peristalsis		
Air injection	77.1 ± 3.5	83.3 ± 9.0
Water injection	73.2 ± 3.2	87.4 ± 6.5 ^b
Balloon distension †	68.1 ± 3.2	94.0 ± 6.6 ^c
VELOCITY (cm/s)		
Primary Peristalsis	2.8 ± 0.04	2.9 ± 0.06
Secondary Peristalsis		
Air injection	2.9 ± 0.08	2.8 ± 0.15
Water injection	2.6 ± 0.11	2.5 ± 0.17
Balloon distension †	2.7 ± 0.19	3.2 ± 0.14

Data are expressed as mean ± SE; † Secondary peristaltic responses induced by balloon distension occurred below the balloon after deflation; ^a p=0.0003 vs normals; ^b p=0.03 vs normals; ^c p=0.005 vs normals

Secondary Peristalsis

a) Air and water boluses

Peristaltic response rates to air boluses were significantly lower in the patients with dysphagia as a group (median: 10%) than in the normal subjects (50%, p<0.002) (Figure 9.2). Response rates in the subgroups of patients with normal primary peristalsis (20%), diffuse spasm (20%) and non-specific oesophageal motor disorders (0%) were not statistically different and were each less than that in normal subjects. Using the fifth percentile in the normal subjects (20%) as the lower limit of normal,

secondary peristaltic responses to air were abnormal in 6 of 9 patients with normal primary peristalsis, 9 of 11 patients with diffuse spasm and 8 of 10 patients with non-specific oesophageal motor disorders.

Water boluses produced similar results with a median response rates of 0% in patients with dysphagia compared with 30% in the normal subjects ($p < 0.005$). Response rates in the subgroup of patients with diffuse spasm (20%) and non-specific oesophageal motor disorders (0%) were also less than those in the normal subjects, however, the response rate in the patients with dysphagia and normal primary peristalsis (20%) was similar. The response rates to air were similar to those for water in both the normal subjects and patients with dysphagia.

The mean amplitude for secondary peristalsis triggered by air boluses in the patients with dysphagia was similar to that in the normal subjects while for water boluses the amplitude was greater in the dysphagia patients than the normals ($p < 0.05$) (Table 9.1). Mean propagation velocities for the secondary peristalsis triggered by air and water injection were similar in the two groups (Table 9.1).

The pattern of the motor responses for the air and water boluses are shown in Figure 9.3. In the normal subjects, 52% of the air boluses triggered a peristaltic response while 21% failed to trigger any response at all. A small proportion of air boluses triggered failed or synchronous responses. In the patients with dysphagia, however, only 18% triggered secondary peristalsis and 43% of air boluses produced no response. The proportion of failed and synchronous responses were similar in the patients and controls, and among the three subgroups of patients. Water boluses resulted in a similar pattern of responses in the two groups.

b) Balloon distension

The frequency of secondary peristaltic responses seen distal to the balloon after deflation is shown in Figure 9.4. The response rate below the balloon varied widely among subjects and that in the patients with dysphagia (20%, 0-60%) was not

statistically different from that in the normal subjects (0%, 0-70%, $p=0.22$). In the normal subjects, the response rate to balloon distension was significantly less than that for air boluses ($p<0.04$) but similar to that for the water boluses. In the dysphagia patients, the secondary peristaltic response rate to balloon distension was greater than that for the air ($p<0.05$) or water boluses ($p<0.005$).

The contraction amplitude of secondary peristalsis elicited by balloon distension was greater in the dysphagia patients than the normal subjects ($p=0.005$) while the propagation velocities were similar in the two groups (Table 9.1).

The pattern of balloon responses are shown in Figure 9.5. There were no significant differences in the proportions of the motor responses either above or below the balloon between the two groups nor among the three subgroups of patients.

9.4 Discussion

Non-obstructive dysphagia is a common indication for referral for oesophageal manometry as this symptom is generally believed to be due to oesophageal motor dysfunction [181]. In a significant proportion of patients, however, assessment of primary peristalsis fails to reveal any abnormality and the cause of the symptoms remains unclear.[16, 181, 197, 261, 333]. One possible explanation for this discrepancy is that the routine testing of primary peristalsis is not sufficiently sensitive to detect subtle oesophageal motor abnormalities. This is supported by several studies demonstrating radiographic and scintigraphy abnormalities in patients with dysphagia and normal primary peristalsis [29, 176, 177, 201, 203, 295].

Abnormal secondary oesophageal peristalsis is a potential mechanism that might account for these discrepancies as secondary peristalsis is an important mechanism of oesophageal volume clearance [90, 162]. This study is the first assessment of secondary peristalsis in patients with non-obstructive dysphagia and shows that they

commonly have defective secondary peristalsis in response to oesophageal distension with boluses of air and water. The inference from this result is that these patients will similarly fail to respond to the distension induced by a retained bolus in the oesophagus and this may be an important mechanism in the development of their symptoms. Standard manometric assessment of primary peristalsis detected an abnormality in 63% of the patients studied but when the secondary peristaltic responses to air and water boluses is also considered, the proportion of patients with abnormal oesophageal motor function increased to 80%.

The patients with dysphagia in this study comprised a heterogeneous group including those with normal primary peristalsis, those with diffuse spasm and patients with non-specific oesophageal motor disorders. Nevertheless, secondary peristaltic response rates and the proportion of patients with defective secondary peristalsis were similar in the three subgroups suggesting that they share a common defect in triggering of secondary peristalsis. Whilst abnormal secondary peristalsis might be expected in patients with abnormal primary peristalsis, the finding of abnormal responses in patients with normal primary peristalsis indicates a specific abnormality of secondary peristalsis and a potentially important contributor to the pathogenesis of dysphagia in this subgroup of patients.

The mechanisms underlying defective secondary peristalsis in response to the air and water injection remain to be defined, but inferences are possible from the pattern of the responses, and previous observations. As secondary peristalsis is a reflex response to oesophageal distension, the defect may lie either in the oesophageal motor nerve supply or muscles, or in oesophageal sensation, or perhaps both. In patients with abnormal primary peristalsis, it is possible that there is a defect in the efferent limb of the reflex arc although the pattern of abnormal primary peristalsis did not have any significant bearing on the pattern of abnormal secondary peristalsis. Interestingly, in our study some patients with abnormal primary peristalsis had normal secondary peristalsis suggesting that the defect in primary peristalsis in these patients lies in the

central control mechanisms rather than in the peripheral neural pathways or oesophageal muscle. In patients with both abnormal primary and secondary peristalsis, however, the defect is more difficult to localise.

In patients with normal primary peristalsis and abnormal secondary peristalsis, the efferent pathways would appear to be intact and this suggests that there is a defect in the afferent limb of the reflex pathway with impaired oesophageal sensitivity to distension. This hypothesis has not been examined in patients with non-obstructive dysphagia. In patients with oesophagitis, however, Williams and colleagues noted that the distension threshold required to trigger a motor response to prolonged oesophageal distension was higher than that in healthy controls [344]. Deschner et al have reported that the distension threshold for symptoms was lower in patients with non-obstructive dysphagia [94]. The different results of these two studies is probably due to the different measured endpoints used with one examining the threshold volume required to elicit symptoms while the other documented the volume required to trigger a motor response. It is suggested in the former study that the volume required to elicit symptoms such as chest discomfort or dysphagia, may not be the same as the volume required to trigger an oesophageal motor response [344].

Secondary peristaltic responses to balloon distension were not significantly different between normal subjects and patients with dysphagia. This was due largely to a low response rate in the normal subjects. The findings in the patients with non-obstructive dysphagia are similar to those found in the study of secondary peristalsis in patients with gastro-oesophageal reflux disease reported in chapter 8. This again supports the conclusion that balloon distension is an ineffective method of testing secondary peristalsis. The apparent discrepancy between the balloon responses and those to air and water boluses most likely reflects differences in the nature of the distending stimulus, the balloon providing a focal and fixed distension in contrast to the more diffuse and mobile stimulus with air and water boluses.

Although secondary peristalsis has not been formally examined in patients with non-obstructive dysphagia, recent studies have shown that oesophageal motor responses to prolonged distension are abnormal in these patients [94, 345]. These studies revealed abnormal contractions in the distal oesophagus during balloon inflation in patients with non-obstructive dysphagia while the motor responses proximal to the balloon were similar to those in normal subjects. We also noted similar patterns of motor activity above the balloon in the normals and the patients, but failed to detect any motor activity in the distal oesophagus during balloon inflation. A possible explanation may be the greater balloon diameter used in our study leading to a greater degree of distal inhibition. Alternatively, we used sudden and relatively brief balloon distension rather than gradual distension (1ml / 3 seconds) which may have influenced the pattern of the response.

Evaluation of the manometric characteristics of the secondary peristaltic responses revealed a higher mean peristaltic amplitude in the patients with non-obstructive dysphagia compared to the normal volunteers while propagation velocity in the two groups was similar. The significance of this finding is not clear but it is interesting to note that a similar difference is seen for primary peristaltic amplitude and perhaps reflects a generally increased oesophageal contractile strength. Similar results were reported by Jacob et al who found higher primary peristaltic amplitudes in patients with dysphagia compared to patients without dysphagia [181].

In summary, we have demonstrated a marked defect in secondary peristalsis in patients with dysphagia. This defect may well result in delayed bolus transit along the oesophagus and contribute to the development of the patient's symptoms. Examination of secondary peristalsis may be useful in the diagnostic evaluation of patients with non-obstructive dysphagia.

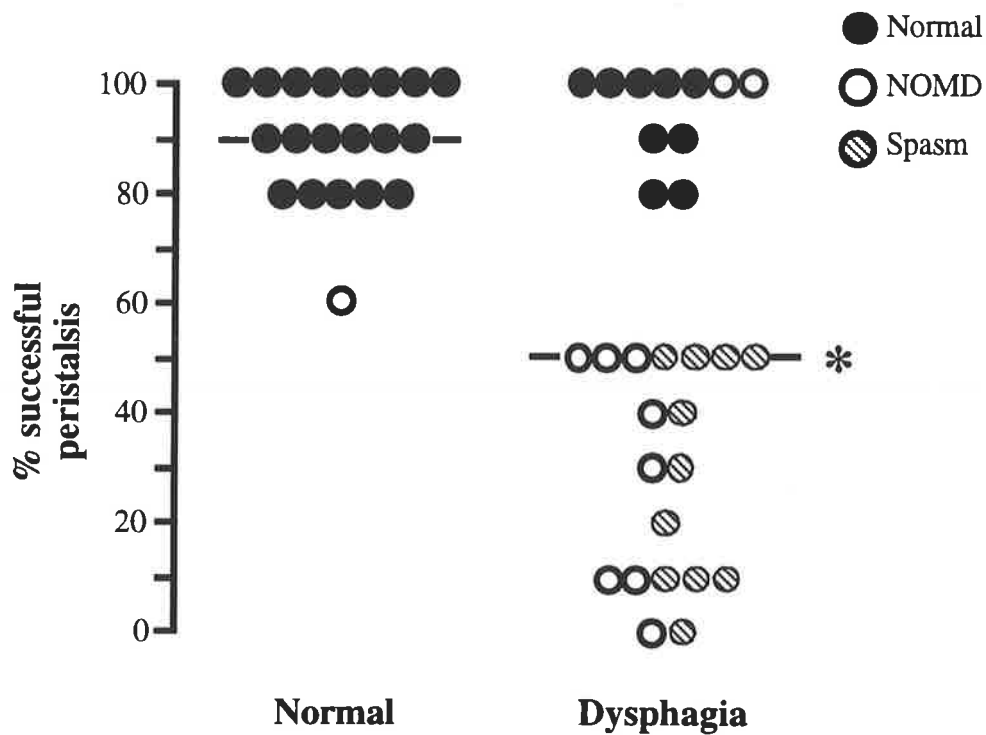


Figure 9.1: The percentage of successful primary peristaltic responses for each subject. Subjects defined as having normal primary peristalsis are represented by the filled circles and those with abnormal primary peristalsis with the open (non-specific oesophageal motor disorders; NOMD) or shaded circles (diffuse oesophageal spasm). The horizontal bars indicate the median values. * p=0.001 vs normal

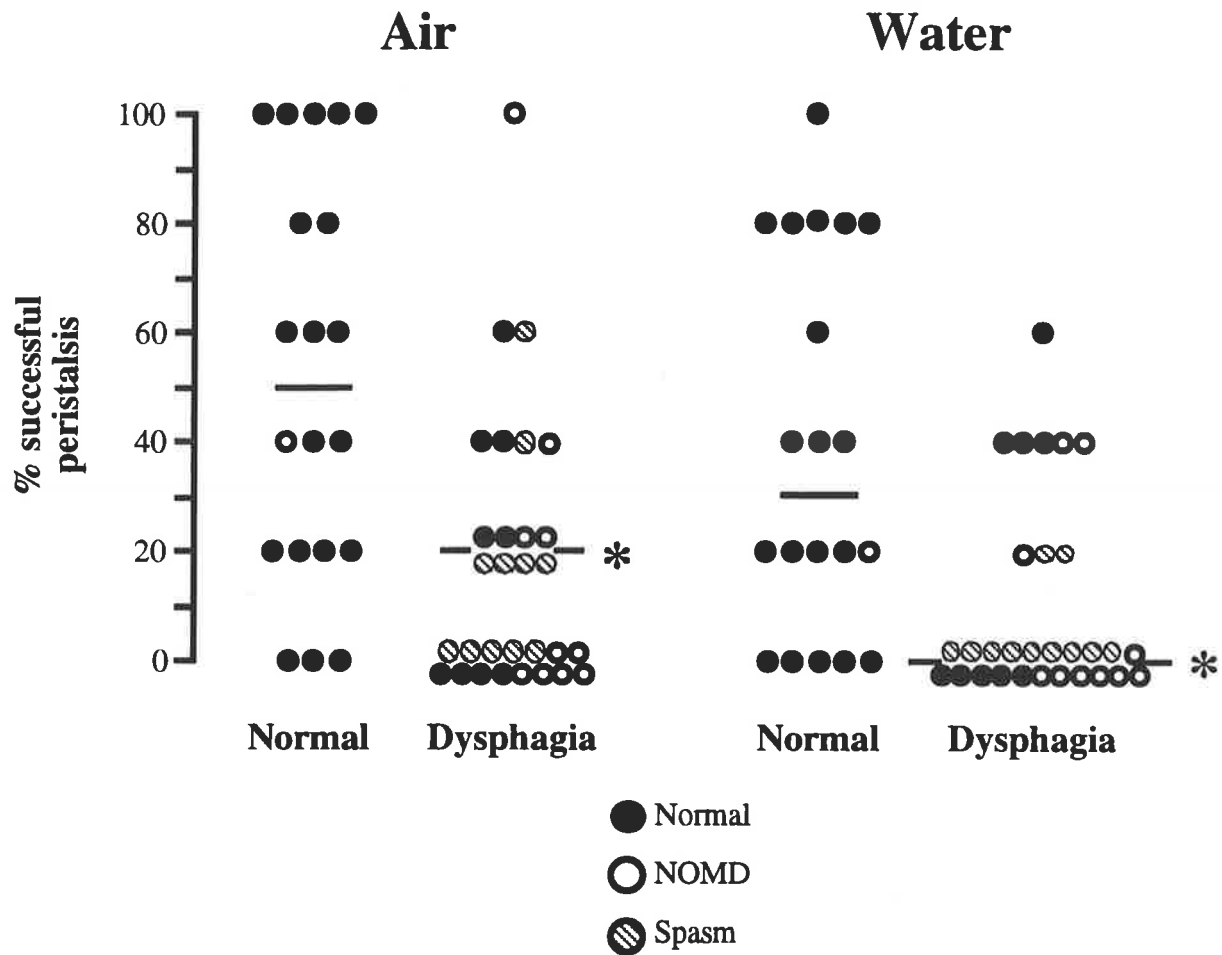


Figure 9.2: Percentage of successful secondary peristaltic responses after oesophageal distension with the 10ml air and water boluses. Each point represents the proportion of normal secondary peristaltic responses (out of 5 tests) for each subject. Subjects with normal primary peristalsis are represented by the filled circles and those with abnormal primary peristalsis with the open (non-specific oesophageal motor disorders; NOMD) or shaded circles (diffuse oesophageal spasm). The horizontal bars indicate median values. * $p \leq 0.003$ vs normal

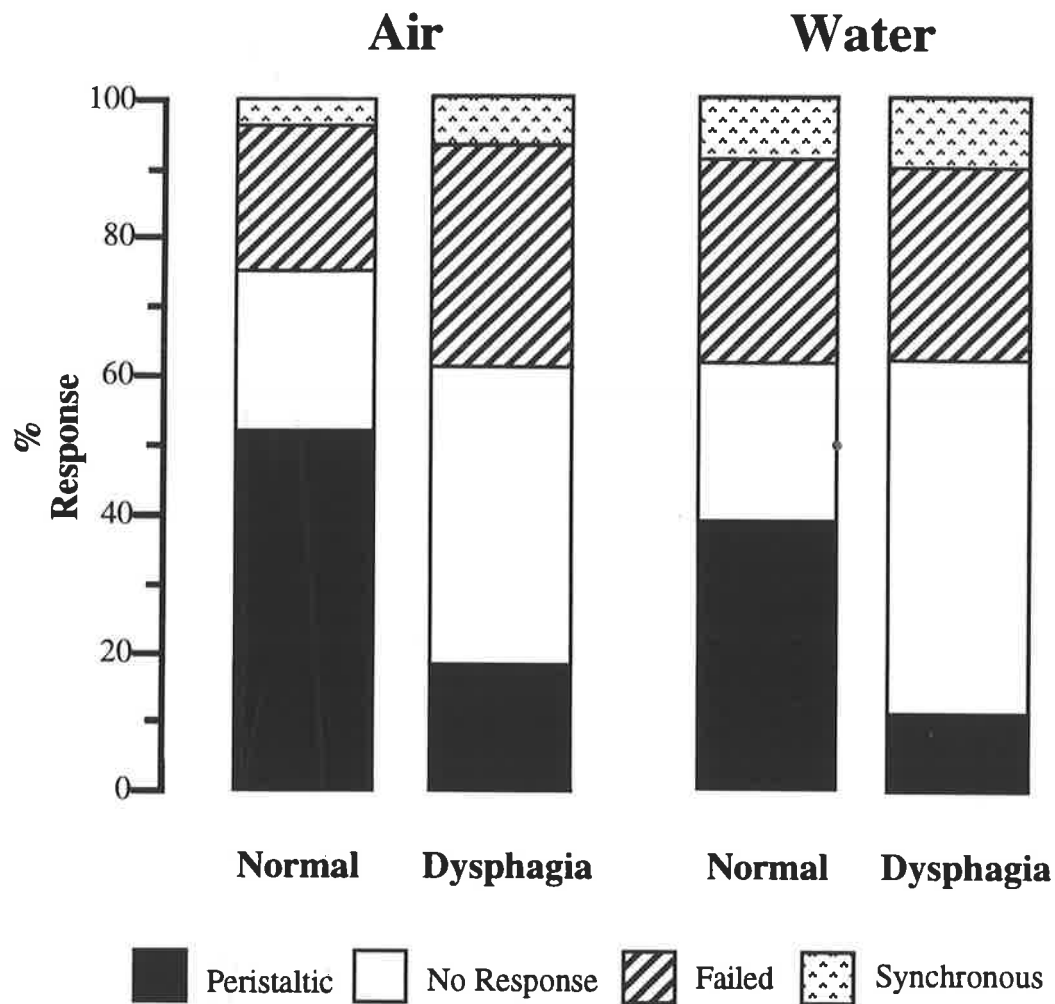


Figure 9.3: Patterns of manometric motor response to oesophageal distension with 10ml boluses of air and water. The proportions of the various patterns of manometric response are derived from pooled data of the individual responses.

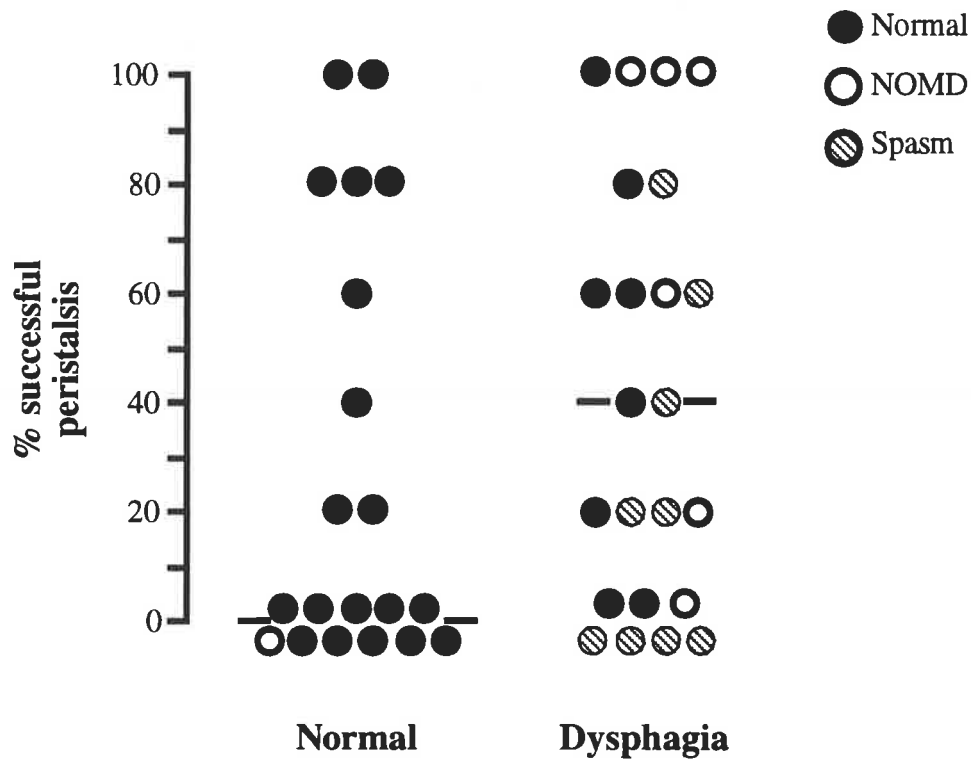


Figure 9.4: Percentage of successful secondary peristalsis with balloon distension. Each point represents the proportion of normal secondary peristaltic responses (out of 5 tests) for each subject. Subjects with normal primary peristalsis are represented by the filled circles and those with abnormal primary peristalsis with the open (non-specific oesophageal motor disorders; NOMD) or shaded circles (diffuse oesophageal spasm). The horizontal bars indicate median values. The response rates are similar in the two groups ($p=0.22$).

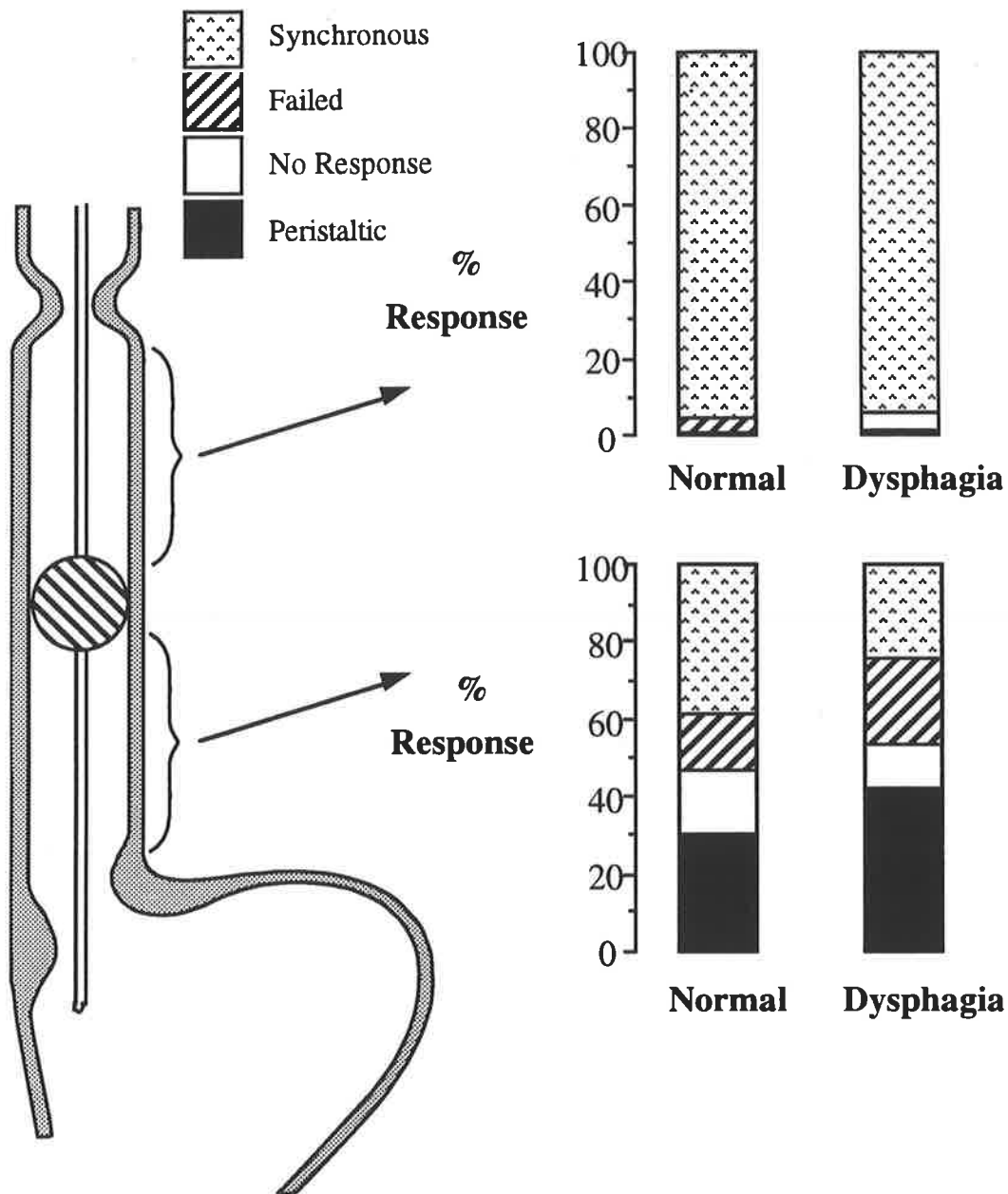


Figure 9.5: Patterns of manometric response to balloon distension. The proportion of various patterns of manometric response are derived from pooled data of the individual responses. Responses have been divided into those occurring above and below the balloon. The responses above the balloon occurred during inflation and those below the balloon occurred after deflation. The patterns of response are similar in the two groups.

Chapter 10

Lower Oesophageal Sphincter Responses to Oesophageal Distension

10.1 Introduction

10.2 Methods

10.2.1 Subjects

10.2.2 Recording techniques

10.2.3 Protocol

10.2.4 Data and statistical analysis

10.3 Results in young normal subjects

10.4 Effect of age on the sphincter responses to oesophageal distension

10.5 Results in patients with reflux disease

10.6 Results in patients with non-obstructive dysphagia

10.7 Discussion

10.1 Introduction

Abnormalities of lower oesophageal sphincter function are important in the pathogenesis of a number of clinical disorders. In gastro-oesophageal reflux disease, the major mechanism of reflux is prolonged and inappropriate sphincter relaxation [90, 93, 110] whereas in disorders associated with dysphagia such as achalasia or oesophageal spasm, there is evidence of incomplete sphincter relaxation [54, 99, 123, 198, 255]. Considerable data are available in the literature about sphincter function in response to swallowing but relatively little data exist for other triggers of sphincter relaxation such as oesophageal distension. In addition, previous studies that have investigated distension-induced sphincter relaxation have either been performed in anaesthetised animals [265, 268, 269, 272] or have used non-perfused side holes [68, 129, 135] which may record sphincter relaxation inaccurately due to potential displacement of the side hole out of the sphincter because of oesophageal shortening [113, 124]. Two studies have measured sphincter relaxation to oesophageal distension with a sleeve sensor but these did not investigate different stimuli or sphincter responses in disease states [190, 241].

As detailed in chapters 8 and 9 of this thesis, there is a marked defect in the triggering of secondary peristalsis in patients with gastro-oesophageal reflux disease and non-obstructive dysphagia. It is therefore possible that the lower oesophageal sphincter response to oesophageal distension is also defective and may contribute to the pathogenesis of these conditions. The aim of this study was to examine the response of the lower oesophageal sphincter to oesophageal distension in healthy volunteers, patients with gastro-oesophageal reflux disease and patients with non-obstructive dysphagia.

10.2 Methods

10.2.1 Subjects

All subjects and patients used in the studies of secondary peristalsis also had simultaneous measurement of lower oesophageal sphincter pressure responses to oesophageal distension. Recordings of the lower oesophageal sphincter responses in these subjects were included for analysis if there was a detectable lower oesophageal sphincter pressure ($\geq 5\text{mmHg}$) as sphincter relaxation could not be assessed adequately in subjects with low basal sphincter pressure. Two of the young healthy controls, four of the age-matched controls, five of the reflux patients and twelve of the patients with non-obstructive dysphagia were found to have sphincter pressures below 5mmHg and thus were excluded from the analysis. The study group therefore included 18 young healthy subjects (9F:9M) ranging in age from 19-36 years (median: 21 years); 16 age matched healthy subjects (F8 : M8) ranging in age from 23-76 years (median 50yrs); 17 patients with gastro-oesophageal reflux disease (6F:11M), ranging in age from 23-73 years (median 53yrs) and 18 patients with non-obstructive dysphagia (9F:13M), ranging in age from 26-74 years (median 56yrs).

10.2.2 Recording techniques

Lower oesophageal sphincter pressure was recorded using a perfused sleeve sensor as described in chapter 6. Pharyngeal, oesophageal and gastric pressures were recorded using perfused side holes as discussed in chapters 7, 8 and 9.

10.2.3 Protocol

The study protocol used for the different subject groups has been described in sections 7.2.3, 8.2.3 and 9.2.3.

10.2.4 Data and statistical analysis

The oesophageal body contractions and lower oesophageal sphincter response to oesophageal distension were analysed according to the methods described in Section 6.1.4. The variables recorded for each test were basal lower oesophageal sphincter pressure, the latency of the sphincter response from the onset of the stimulus to the onset of sphincter relaxation, nadir lower oesophageal sphincter pressure and the duration of the sphincter relaxation. The oesophageal body response to each stimulus was also examined and were classified as complete, failed or no response according to the previously stated criteria.

For each stimulus, sphincter responses were determined for each subject. The data for each test in each subject group were then combined. In the young normal subjects, the differences in response rates for each stimulus at the different sites and volumes were analysed using log-linear modelling techniques [28]. Comparisons between the stimuli in the young volunteers were analysed by analysis of variance.

In the patient studies, the differences in response rates for each stimulus for the age-matched controls and the patients were analysed using a Chi-squared test. The measured parameters of the sphincter response in each subject group were combined and comparisons made between groups using analysis of variance.

Data are presented as mean $\pm$ standard error of the mean unless otherwise stated.

10.3 Results in young normal subjects

Effect of site and volume on the frequency of sphincter relaxation

All subjects responded to oesophageal distension with sphincter relaxation. The effect of the site and volume of distension on the frequency of lower oesophageal sphincter relaxation is shown in Figure 10.1. The frequency of sphincter relaxation for all stimuli was proportional to the distension volume used but was not affected by stimulus site. The frequency of relaxation was significantly greater with the 10ml and 20ml air boluses than with the 5ml air bolus ($p < 0.01$) and greater with the 5ml and 10ml water boluses compared to the 2ml water bolus ($p < 0.01$). For balloon distension, the frequency of sphincter relaxation also increased significantly with increasing volume (1cm vs 2cm; $p = 0.001$, 2cm vs 3cm; $p = 0.01$). Response rates were similar among the different stimuli for comparable volumes.

Effect of site on the characteristics of sphincter relaxation

The influence of stimulus site on the sphincter relaxation is shown in table 10.1. There was no effect of site on the characteristics of sphincter relaxation for any of the stimuli with any of the volumes used.

Table 10.1 The effect of site of oesophageal distension on the measured parameters of lower oesophageal sphincter relaxation. There was no significant effect on the lower oesophageal sphincter response at any site with any of the stimuli.

		Basal Pressure (mmHg)	Latency (seconds)	Nadir Pressure (mmHg)	Relaxation (%)	Duration (seconds)	
AIR	low	18.30±2.15	2.63±0.59	3.85±1.23	78.51±6.59	5.02±1.10	
	5ml	mid	15.57±2.35	2.99±0.66	3.14±1.01	80.71±5.31	4.43±1.07
		up	13.33±0.99	1.45±0.43	3.08±0.81	78.12±6.00	4.88±1.25
		low	14.16±1.18	1.74±0.28	3.00±0.73	80.45±3.88	3.86±0.59
	10ml	mid	15.68±1.41	1.09±0.30	4.27±1.25	75.96±4.72	3.93±0.56
		up	16.83±1.65	1.85±0.23	4.08±1.31	82.47±4.33	4.58±0.53
		low	19.11±1.61	1.21±0.18	3.33±0.73	83.67±2.97	5.73±0.65
	20ml	mid	16.08±1.82	1.68±0.21	2.32±0.65	85.83±3.22	3.72±0.36
		up	13.44±0.99 *	1.36±0.25	1.96±0.50	85.78±3.82	4.20±0.65
WATER		low	20.50±4.50	2.80±0.20	6.50±1.50	65.00±15.00	2.05±0.35
	2ml	mid	19.25±2.50	1.20±0.80	8.50±0.96	52.77±10.48	4.23±1.23
		up	19.00±2.70	3.00±1.13	5.33±0.99	69.40±6.04	5.93±1.51
		low	16.93±0.89	3.79±0.67	5.20±0.81	68.61±4.86	6.56±1.46
	5ml	mid	19.44±1.59	2.15±0.41	4.94±1.13	71.54±6.30	4.17±0.45
		up	18.33±0.95	2.69±0.59	5.00±1.25	72.08±6.23	4.30±0.47
		low	18.65±0.92	2.64±0.52	2.87±0.60	84.13±3.38	7.19±1.01
	10ml	mid	17.40±1.13	1.67±0.27	3.28±0.60	81.53±2.97	4.05±0.79
		up	18.50±1.27	2.53±0.53	4.20±0.81	76.06±4.39	5.55±1.03
BALLOON		low	18.06±3.87	1.45±0.25	3.56±1.02	84.35±3.27	3.17±0.44
	2cm	mid	16.70±1.52	1.52±0.42	4.80±0.90	73.23±4.67	2.74±0.25
		up	17.6±2.04	1.30±0.32	3.85±0.71	81.27±2.92	4.16±0.52
		low	18.00±2.37	0.94±0.22	2.72±0.89	89.75±2.72	4.65±0.57
	3cm	mid	17.48±2.07	1.36±0.24	0.92±0.32	95.23±1.59	5.41±0.46
		up	14.75±1.49	2.14±0.57	0.55±0.21	97.17±1.13	5.38±0.76

* p=0.03 compared to the basal sphincter pressure for the lower 20ml air distension

Effect of volume on the characteristics of sphincter relaxation

Because site had no significant effect on the frequency or characteristics of the lower oesophageal sphincter response to distension, the data from the three sites were combined to examine the effect of volume (figure 10.2).

No consistent effect of volume was detected on the characteristics of the lower oesophageal sphincter relaxation induced by the air and water boluses. In addition, there was no clear effect of air and water bolus volume when the responses were analysed separately for those associated with a peristaltic or non-peristaltic oesophageal contraction. For balloon distension, the 3cm balloon induced a lower nadir pressure and longer duration of relaxation compared to the 2cm balloon. This effect was not seen for balloon distension associated with peristaltic oesophageal contractions but was seen for relaxations associated with non-peristaltic contractions. When equal volumes were compared between stimuli, the only significant difference found was between the 5ml air and water boluses and the 2cm balloon (Table 10.2). The 2cm balloon triggered a relaxation with a shorter latency and duration than the 5ml air and water boluses.

Table 10.2 Manometric characteristics of sphincter relaxation induced by different stimuli of equal volume

STIMULUS †	Latency (seconds)	Nadir Pressure (mmHg)	Duration (seconds)
5ml Air	2.4±0.34	3.4±0.59	4.8±0.64
5ml Water	2.8±0.33	5.0±0.59	5.0±0.52
2cm Balloon	1.5±0.21 *	4.2±0.54	3.3±0.26 *
10ml Air	1.6±0.16	3.8±0.64	4.1±0.32
10ml Water	2.2±0.26	3.4±0.38	5.6±0.56
20ml Air	1.4±0.13	2.6±0.37	4.6±0.34
3cm Balloon	1.4±0.21	1.5±0.36	5.1±0.34

† Stimuli have been grouped according to volume; * $p \leq 0.03$ compared to other stimuli of equal volume

Comparison of sphincter relaxation induced by swallowing and oesophageal distension

The characteristics of lower oesophageal sphincter relaxation triggered by swallowing and oesophageal distension are summarised in table 10.3. Because swallow-induced lower oesophageal sphincter relaxation was always associated with primary peristalsis, only the distension induced sphincter relaxations associated with oesophageal peristalsis were compared in this analysis. The latency of onset of sphincter relaxation was longer for distension induced lower oesophageal sphincter relaxation stimuli than for swallow induced lower oesophageal sphincter relaxation ($p < 0.0001$ for all volumes). Nadir pressures for sphincter relaxation induced by air and balloon distension were similar to that induced by swallowing whereas the nadir pressure with water distension was significantly higher ($p < 0.006$ for 5ml and 10ml volumes). The duration of sphincter relaxation with all distension stimuli was similar to that for swallowing.

Table 10.3 Manometric characteristics of sphincter relaxation triggered by oesophageal distension and swallowing

		Latency (seconds)	Nadir Pressure (mmHg)	Duration (seconds)
Water				
Swallows		0.5±0.07	1.2±0.13	4.1±0.12
Air	5ml	3.2±0.42 *	1.3±0.41	5.2±0.92
	10ml	1.6±0.21 *	2.2±0.31	4.4±0.41
	20ml	1.4±0.14 *	2.6±0.42	4.2±0.32
Water	2ml †	-	-	-
	5ml	4.2±0.70 *	3.9±0.87 *	4.5±0.47
	10ml	2.5±0.37 *	3.3±0.50 *	5.2±0.71
Balloon	1cm †	-	-	-
	2cm †	-	-	-
	3cm	1.6±0.27 *	1.1±0.54	5.2±0.48

† insufficient peristaltic responses for statistical analysis; * $p \leq 0.006$ compared to sphincter relaxation associated with primary peristalsis

10.4 Effect of age on the lower oesophageal sphincter responses to oesophageal distension

To evaluate the effect of age on sphincter relaxation, the sphincter responses to 10ml boluses of air and water and the 3cm balloon in the young normal subjects were compared to the data obtained from the group of older normal subjects who were used as control subjects for the patient studies. The frequencies of lower oesophageal sphincter relaxation in the two groups were similar for all stimuli except the air boluses which triggered sphincter relaxation less frequently in the older subjects (Figure 10.3).

The manometric characteristics of the sphincter relaxation induced by the different stimuli for the two groups are shown in Table 10.4. In the older subjects, the latency of onset of relaxation was longer for all stimuli. The nadir pressure was higher in the older subjects during sphincter relaxation induced by balloon distension but similar for air and water distension. The duration of sphincter relaxation was similar in the two groups.

Table 10.4 Manometric characteristics of sphincter relaxation triggered by oesophageal distension and swallowing in the young and aged-matched normal subjects

		Latency (seconds)	Nadir Pressure (mmHg)	Duration (seconds)
Air	young normals	1.6±0.16	3.8±0.64	4.1±0.32
	aged normals	2.2±0.17 *	3.8±0.58	5.3±0.56
Water	young normals	2.2±0.26	3.4±0.38	5.6±0.56
	aged normals	2.5±0.14	3.4±0.34	6.9±0.69
Balloon	young normals	1.4±0.21	1.5±0.36	5.1±0.34
	aged normals	2.5±0.17 *	2.7±0.39 *	5.1±0.32

* p≤0.02 compared to the young normal subjects

10.5 Results in patients with reflux disease

The frequencies of lower oesophageal sphincter relaxation in response to oesophageal distension are shown in figure 10.4. The frequency of sphincter relaxation with swallows and oesophageal balloon distension was similar in the two groups but significantly less for the air and water bolus distensions in the patients with reflux disease ($p<0.05$).

The manometric characteristics of lower oesophageal sphincter relaxation in response to swallowing and oesophageal distension in the two groups are shown in table 10.5. There were no major differences in the manometric characteristics of sphincter relaxation between the two groups. A comparison of swallow induced sphincter relaxation and that induced by oesophageal distension in the patients with reflux disease showed a similar pattern to that in the normal subjects with a longer latency of relaxation associated with oesophageal distension and a higher nadir pressure in relaxations triggered by air and water distension.

Table 10.5 Manometric characteristics of the sphincter relaxation induced by swallowing and oesophageal distension in normal subjects and patients with gastro-oesophageal reflux disease.

		Basal Pressure (mmHg)	Latency (seconds)	Nadir Pressure (mmHg)	Duration (seconds)
Water	Normal	14.4±0.67	0.6±0.08	2.0±0.20	3.6±0.15
Swallows	GOR	14.1±0.67	0.6±0.07	0.6±0.21	4.5±0.19 *
Air	Normal	12.2±0.74	2.2±0.17	3.8±0.58	5.3±0.56
	GOR	16.5±1.30	2.0±0.26 †	3.6±0.67 †	6.7±0.86 †
Water	Normal	13.8±0.73	2.5±0.14	3.4±0.34	6.9±0.69
	GOR	18.4±1.84	3.1±0.21 †	4.3±0.89 †	6.3±0.82
Balloon	Normal	14.6±0.83	2.5±0.17	2.7±0.39	5.1±0.32
	GOR	18.4±1.35	2.0±0.15 †	2.1±0.56	5.3±0.27

* $p<0.05$ compared to normal controls; † $p<0.05$ compared to swallow induced sphincter relaxation in reflux patients

10.6 Results in patients with non-obstructive dysphagia

The frequencies of lower oesophageal sphincter relaxation in response to oesophageal distension are shown in figure 10.5. The frequency of sphincter relaxation in the patients with dysphagia was similar to that in the controls with all stimuli except oesophageal distension with air boluses which triggered lower oesophageal sphincter relaxation significantly less frequently in the patients with non-obstructive dysphagia ($p<0.05$).

The manometric characteristics of sphincter relaxation in response to swallowing and oesophageal distension in the two groups are shown in table 10.6. There were no major differences between the two groups. A comparison of swallow induced sphincter relaxation and that induced by oesophageal distension in the patients with non-obstructive dysphagia showed a similar pattern to that in the normal subjects with a longer latency of relaxation and a higher nadir pressure in relaxations triggered by air and water distension. Basal lower oesophageal sphincter pressure was consistently higher in the patients with non-obstructive dysphagia than the normal subjects.

Table 10.6 Manometric characteristics of the sphincter relaxation induced by swallowing and oesophageal distension in normal subjects and patients with non-obstructive dysphagia.

		Basal Pressure (mmHg)	Latency (seconds)	Nadir Pressure (mmHg)	Duration (seconds)
Water	Normal	14.4±0.67	0.6±0.08	2.0±0.20	3.6±0.15
Swallows	NOD	17.7±0.84 *	0.6±0.07	1.7±0.16	4.5±0.16 *
Air	Normal	12.2±0.74	2.2±0.17	3.8±0.58	5.3±0.56
	NOD	18.8±1.92 *	2.6±0.29 †	5.2±1.07 †	4.8±0.55
Water	Normal	13.8±0.73	2.5±0.14	3.4±0.34	6.9±0.69
	NOD	20.5±1.56 *	3.3±0.22 †	4.5±0.87 †	7.1±0.73 †
Balloon	Normal	14.6±0.83	2.5±0.17	2.7±0.39	5.1±0.32
	NOD	20.4±1.39 *	2.3±0.15 †	2.3±0.44	5.4±0.32

* $p<0.05$ compared to normal controls; † $p<0.05$ compared to swallow induced sphincter relaxation in dysphagia patients

10.7 Discussion

Whilst there are a number of studies of lower oesophageal sphincter relaxation with oesophageal distension in animals [45, 221, 265, 268, 269, 272], studies in humans are few [68, 129, 190, 241, 346] and in many of these studies, technical or methodological deficiencies significantly limit the authority of the findings. Three of the studies used non-perfused side hole sensors to monitor lower oesophageal sphincter pressure [68, 129, 346]. This method is relatively insensitive for recording lower oesophageal sphincter pressure [271] and may over estimate the degree of lower oesophageal sphincter relaxation because of displacement of the side hole sensor out of the sphincter and into the stomach due to oesophageal shortening [113, 124, 346]. The two more recent studies have used recording methods similar to those used in the current study with a sleeve sensor to monitor lower oesophageal sphincter pressure [190, 241]. These studies did not, however, include a detailed investigation of the variables that might influence the sphincter relaxation response such as different stimuli or the site and volume of the stimuli. This study is the first detailed evaluation of lower oesophageal sphincter relaxation in response to oesophageal distension in humans using accurate and reliable recording techniques. The major findings are that: 1) complete lower oesophageal sphincter relaxation can be predictably induced by oesophageal distension and once triggered is a very well preserved and consistent motor response; 2) the response is influenced by the distension volume but not the site of distension; and 3) in patients with gastro-oesophageal reflux disease and those with non-obstructive dysphagia, the frequencies of the responses to some stimuli are reduced but the relaxation characteristics are similar to those found in normal subjects.

Findings in normal subjects

In the normal subjects, oesophageal distension predictably and uniformly induces lower oesophageal sphincter relaxation which is consistent with the previous findings in both humans [129, 190, 241, 346] and animals [45, 221, 265, 268, 269, 272]. In

contrast to these findings, Creamer and Schlegel reported that a 5ml water bolus induced sphincter relaxation after 31% of tests, contraction after 21% or a combination of the two [68]. In addition, for a 50ml water bolus, sphincter relaxation was only triggered after 11% of tests and sphincter pressure increased after 89%. The methodological deficiencies associated with the recording methods used in this study, as mentioned above, make interpretation of these apparently aberrant findings difficult.

The frequency and characteristics of lower oesophageal sphincter relaxation in response to oesophageal distension to all stimuli are volume dependent. A balloon diameter of 2cm (volume 4.5ml) appeared to be the distension threshold required to induce sphincter relaxation and this is similar to that reported previously by Mittal et al [241]. A similar threshold is also seen for air and water distension with the 5ml boluses producing relaxation with more than 50% of tests whereas the 2ml water bolus only triggered relaxation after 15% of tests.

The effect of distension volume on the characteristics of sphincter relaxation are less clear. The effect is most consistent for balloon distension, possibly because this stimulus is more defined and circumscribed, than with the air and water boluses which are able to diffuse along the length of the oesophageal body. The fluidity of the air and water boluses, therefore, probably mitigates against an effect of volume on the characteristics of sphincter relaxation for these stimuli. The duration of sphincter relaxation for the 2cm balloon was shorter than the duration of balloon distension and was less than that triggered by the 3cm balloon. This suggests that the sphincter relaxation "fatigues" with less intense stimuli. Mittal et al reported that sphincter relaxation lasted for the duration of a 30 second balloon distension when a balloon volume of 10ml was used but did not indicate if this effect was also seen for smaller distension volumes [241]. The 10ml balloon volume in Mittal's study is greater than the 2cm balloon (4.5ml) used in the current study and this probably accounts for the different duration of relaxation. The effect of balloon volume on the characteristics of lower oesophageal sphincter relaxation contrasts to the findings in chapter 7 where

distension volume influenced the frequency of secondary peristalsis but not the manometric characteristics. This suggests a more “direct” link between the distension stimulus and the inhibitory neurons triggering relaxation of the lower oesophageal sphincter compared with the neurons responsible for mediating secondary peristalsis for which the stimulus volume needs only to exceed a threshold before it triggers a pre-programmed response.

The manometric characteristics of the sphincter relaxation induced by all three stimuli were not different when associated with a peristaltic or non-peristaltic response. This might be expected for the duration of the relaxation for balloon distension as the duration of this stimulus is determined by the time that the balloon is inflated. For air and water, however, the stimulus remains if complete peristalsis does not occur and thus in such circumstances the duration of sphincter relaxation might be expected to be prolonged. The fact that this does not occur may again be due to distribution of the bolus along the oesophageal body with the degree of focal distension falling below the threshold required to maintain relaxation. The independence of the sphincter and oesophageal response to oesophageal distension suggests that the two events are triggered by separate pathways.

In the current study, site of distension had no effect on the frequency or characteristics of the sphincter relaxation. Whilst this effect may be expected for the air and water boluses due to their diffusion along the oesophageal body, this finding for balloon distension contrasts to the previous findings by Creamer and Schlegel [68] where distension of the proximal oesophagus induced relaxation more commonly than the middle or distal oesophagus. In the study in normal subjects reported in chapter 7, the middle balloon was more likely to trigger secondary peristalsis. However, if all responses were grouped together (ie. peristaltic, failed and synchronous) then the middle balloon was no more likely to induce an oesophageal body response than the distal or proximal balloons. In contrast to Creamer’s findings, it has been reported that the distension threshold to induce sphincter relaxation in the dog increases proximally

suggesting that the distal oesophagus is more sensitive in this species [272]. There are, however, clear species differences in the induction of sphincter relaxation by oesophageal distension as the response is mediated centrally in the dog and by peripheral pathways in the opossum [269]. These differences limit the comparisons that can be made with human studies. The two human studies that used comparable measurement methods to those in this study did not examine the effect of site on the frequency or characteristics of sphincter relaxation.

The only consistent difference in the characteristics of lower oesophageal sphincter relaxation in response to oesophageal distension and swallowing was a longer latency from the onset of the oesophageal distension stimulus to the onset of sphincter relaxation than from swallowing to swallow-induced sphincter relaxation. This is possibly because the intramural neural pathways, responsible for conveying distension induced inhibitory signals to the lower oesophageal sphincter, have a slower conduction velocity than the myelinated fibres that transmit the swallow induced inhibitory signals [48, 341]. The onset of sphincter relaxation induced by oesophageal distension, therefore, would be expected to be delayed when compared to swallow induced relaxations.

The effects of age on the frequency and characteristics of sphincter relaxation induced by oesophageal distension were small and not consistent among stimuli. There was a trend toward a decreased frequency and a prolonged latency of sphincter relaxation in the aged normals but these differences were not significant for all stimuli. There was also a higher nadir pressure in response to balloon distension in the aged normal subjects. There are no studies which examine the effect of age on sphincter responses to oesophageal distension but there are no significant effects of age reported on basal pressure or the nadir pressure and duration of swallow induced sphincter relaxation [72, 202, 287]. The differences that have been noted in the current study are minor and probably not of either clinical or physiological significance.

Patients with gastro-oesophageal reflux disease

In the patients with gastro-oesophageal reflux disease, there was a reduced frequency of lower oesophageal sphincter relaxation in response to distension with the air and water boluses but not for the balloon. This finding may be explained either by an alteration in oesophageal sensitivity to distension or to a disorder of the descending intra-mural inhibitory pathways. The first hypothesis is more likely to be true as the frequency of sphincter relaxation with balloon distension in the two groups are similar consistent with the notion that the 3cm balloon volume exceeded the distension threshold in the both groups whereas this was not the case for the air and water boluses which were of smaller volume. The hypothesis that there is an altered threshold to distension in patients with reflux disease is also supported by the observations of Williams and colleagues who noted that the distension threshold required to trigger an oesophageal motor response was higher in patients with oesophagitis than in healthy controls [344]. Once stimulated, however, the characteristics of the response were similar for all stimuli indicating that once the threshold for lower oesophageal sphincter relaxation is exceeded there is a similar degree of sphincter inhibition for each stimulus in the two groups. In the young normals, the degree and duration of lower oesophageal sphincter relaxation increased with increased balloon volume. It was not possible to assess such an effect in the patients with reflux disease as only one balloon volume (17ml; 3cm diameter) was tested.

The finding of an alteration in the distension threshold is similar to the data presented on secondary oesophageal peristalsis in patients with gastro-oesophageal reflux disease (chapter 8) with the air and water boluses mostly failing to trigger an oesophageal motor response and the balloon responses being similar in the patients and controls. An interesting difference between these two studies is that the frequency of sphincter relaxation in response to air and water distension is significantly higher in the patients than the frequency of secondary oesophageal peristalsis. In fact, in the

majority of the patients, oesophageal distension failed to trigger any oesophageal motor response while the triggering of sphincter relaxation is relatively well preserved. This suggests either that the distension threshold to induce these responses are different or that the neural pathways responsible for each event are different with a more pronounced defect in the neural pathway responsible for the oesophageal body response. The latter is probably more likely as it has already been shown that the stimulation and coordination of secondary oesophageal peristalsis involves central neural pathways [305] while sphincter relaxation induced by oesophageal distension is preserved after complete extrinsic denervation of the oesophageal body [221, 293].

The clinical significance of lower oesophageal sphincter relaxation in response to oesophageal distension in gastro-oesophageal reflux disease is unknown. In patients with gastro-oesophageal reflux disease, the major mechanism of reflux is an increased frequency of transient lower oesophageal sphincter relaxations [90, 93, 104]. It might be hypothesised that oesophageal distension induced by the gastric refluxate is one possible mechanism to account for this increased frequency in sphincter relaxation and if oesophageal clearance is delayed, prolong the duration of sphincter relaxation. The findings from the current study do not support this hypothesis and in fact suggests the reverse; namely that it is more difficult to induce sphincter relaxation by oesophageal distension and that the duration of sphincter relaxation with the air and water boluses is similar in the two groups.

Patients with non-obstructive dysphagia

The relaxation responses to distension in the patients with non-obstructive dysphagia were similar to those in the controls, the only significant difference being the reduced frequency of lower oesophageal sphincter relaxation in response to air distension. Given the similarity in the physical behaviour of the two boluses, the reason that this difference was not also seen with the water bolus distensions is unclear. In the patients with non-obstructive dysphagia, there is no evidence that sphincter relaxation is incomplete as a possible mechanism for symptoms as sphincter responses to

distension were reasonably normal. Another hypothesis to account for the symptoms in these patients might be a reduced frequency of sphincter relaxation in response to oesophageal distension. This hypothesis, however, was not supported by the data with a similar frequency of relaxation in the normal subjects and the patients with non-obstructive dysphagia.

One interesting finding is that basal sphincter pressure prior to the onset of oesophageal distension was higher in patients with non-obstructive dysphagia than the age-matched controls. This may be part of a generalised increase in smooth muscle contractility in these patients as the amplitude of both primary and secondary peristaltic contractions were also increased as reported in chapter 9. The increase in distal oesophageal contraction amplitude in these patients has also been reported by Jacob et al [181]. An alternative explanation for the higher basal sphincter pressure in the patients with dysphagia is that a significant number of patients were excluded from the analysis due to low basal sphincter pressure (12/30; 40%) and this may have introduced a selection bias where only patients with high sphincter pressure were examined.

In conclusion, lower oesophageal sphincter relaxation can be readily induced by oesophageal distension with the characteristics of the relaxation depending on the type and volume of stimulus used. Complete lower oesophageal sphincter relaxation can occur, provided that the distension volume is large enough. The distension threshold for sphincter relaxation in patients with reflux disease appears to be increased compared to the age-matched controls and it is postulated that this is due to dysfunction of the oesophageal afferent innervation in these subjects. In the patients with non-obstructive dysphagia, the sphincter response to distension is preserved and does not appear to be significant contributor to symptoms in these patients.

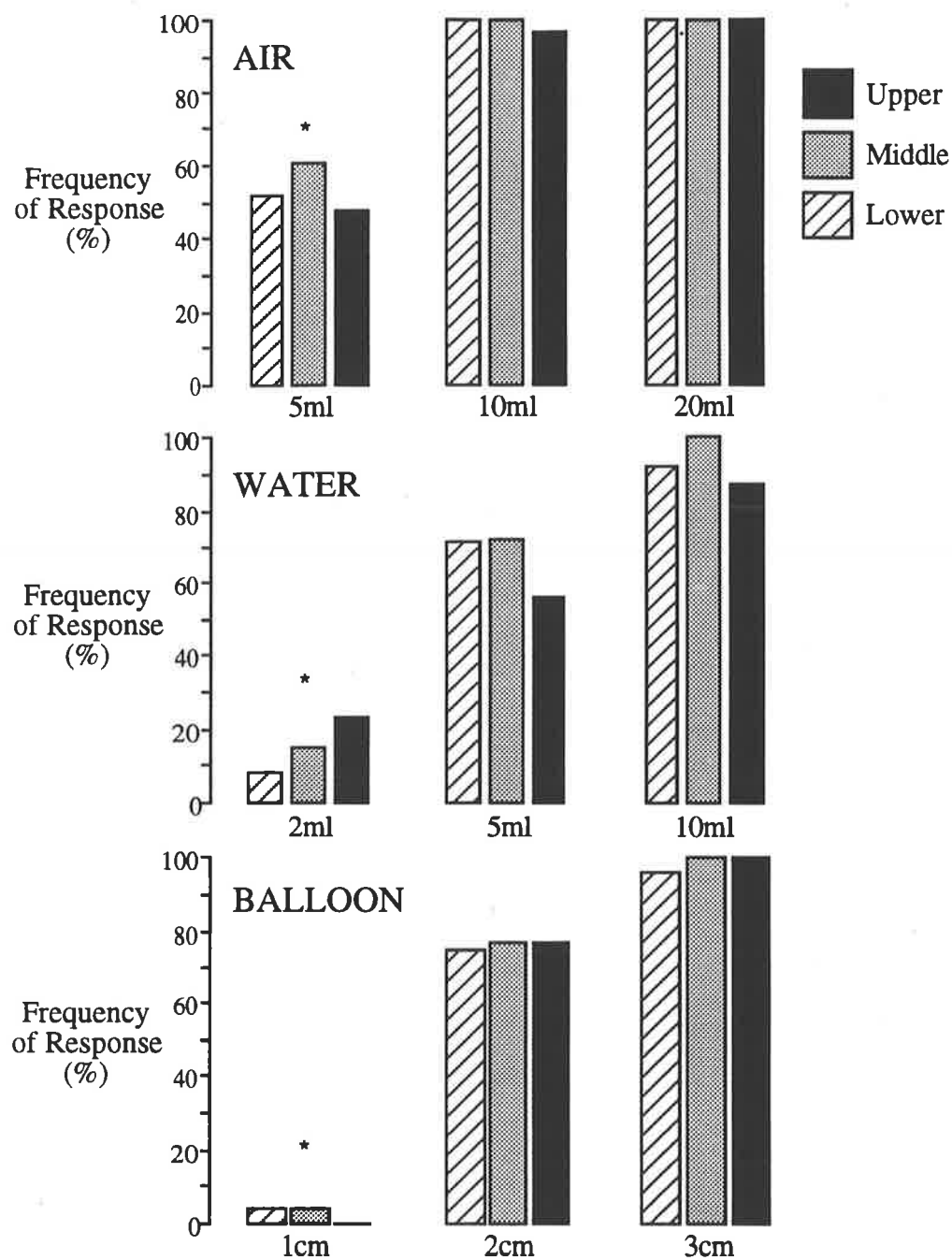
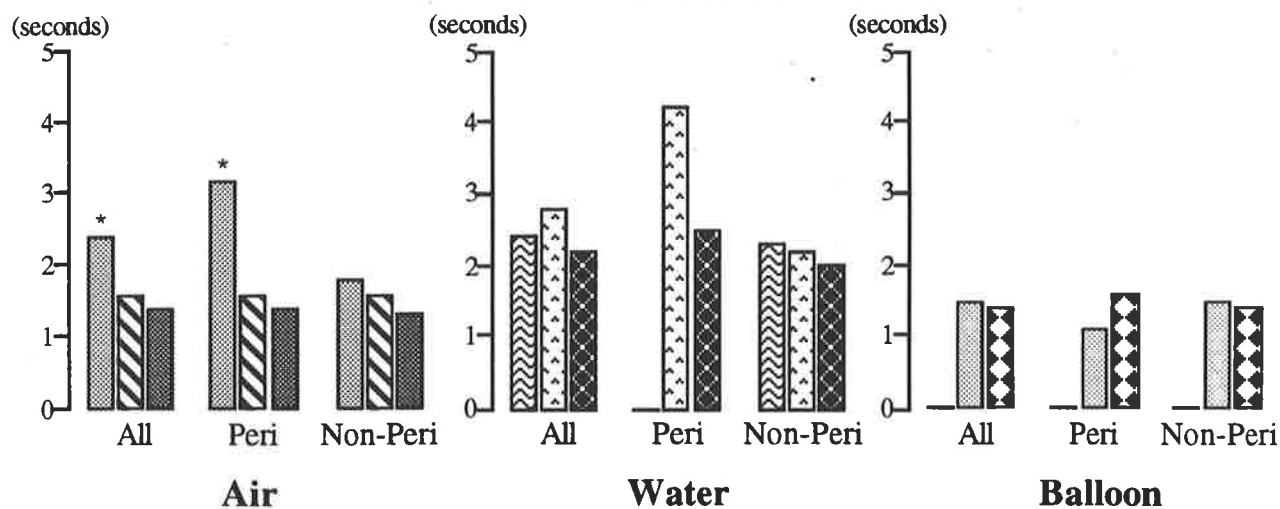
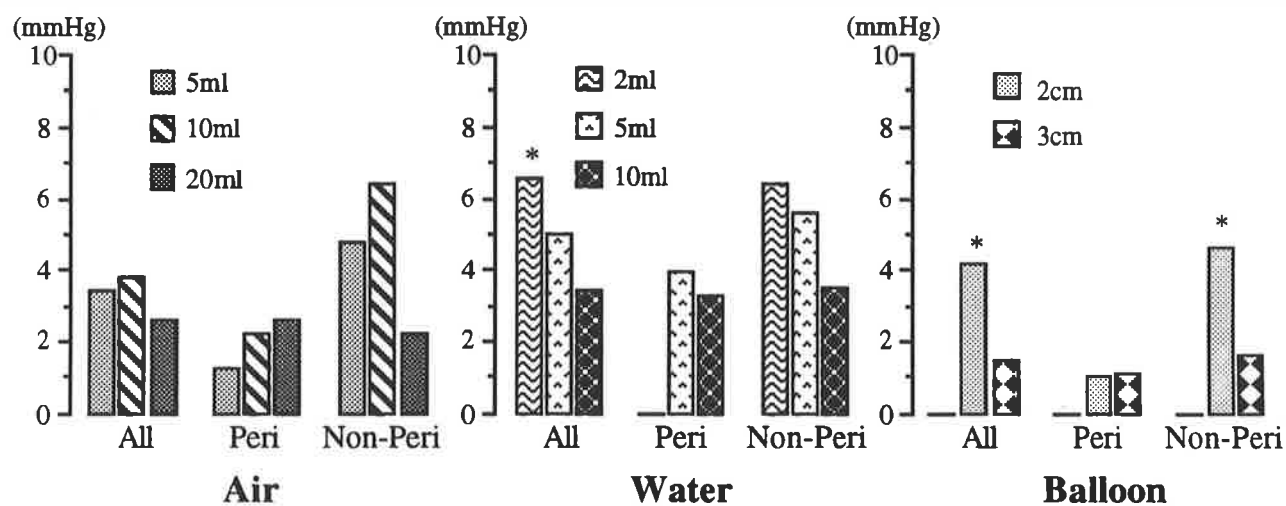


Figure 10.1 The effect of site and volume of oesophageal distension on the frequency of lower oesophageal sphincter relaxation. The frequency of the sphincter relaxation increased significantly with the volume of the stimulus but the site of distension had no effect.

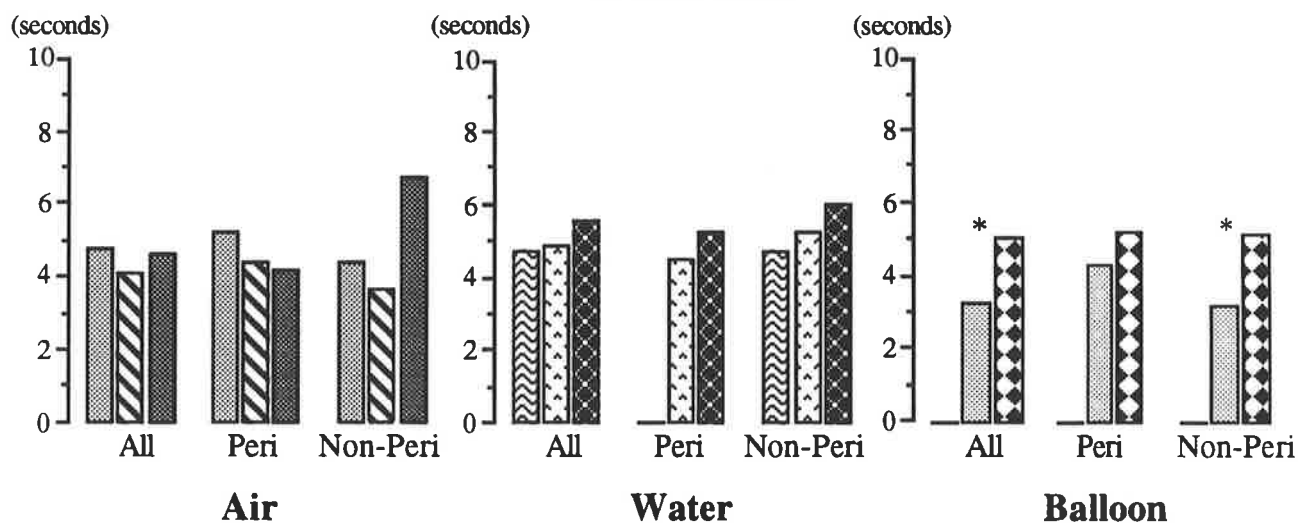
LATENCY



NADIR PRESSURE



DURATION



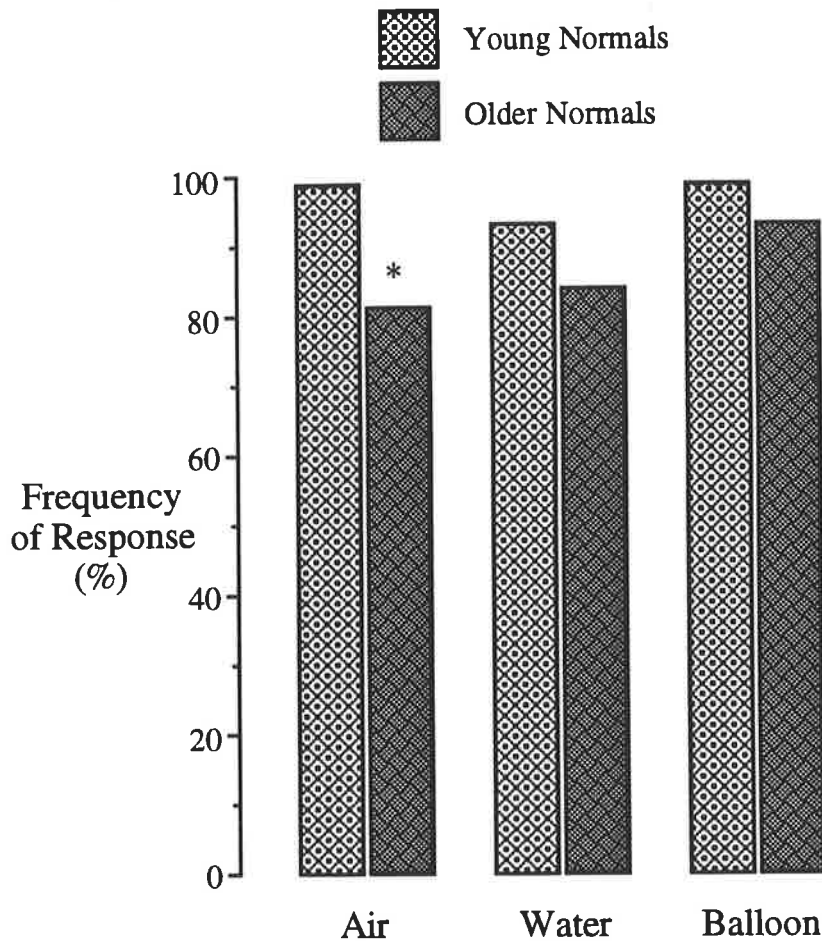


Figure 10.3 The effect of water swallows and oesophageal distension on the frequency of lower oesophageal sphincter relaxation in the two groups of normal subjects. For the air boluses, the frequency of lower oesophageal sphincter relaxation was significantly less in the older subjects ($p=0.001$) compared to the young normal subjects. The frequency of lower oesophageal sphincter relaxation was similar for the other stimuli in the two groups. * $p=0.001$ compared to the young normal group.

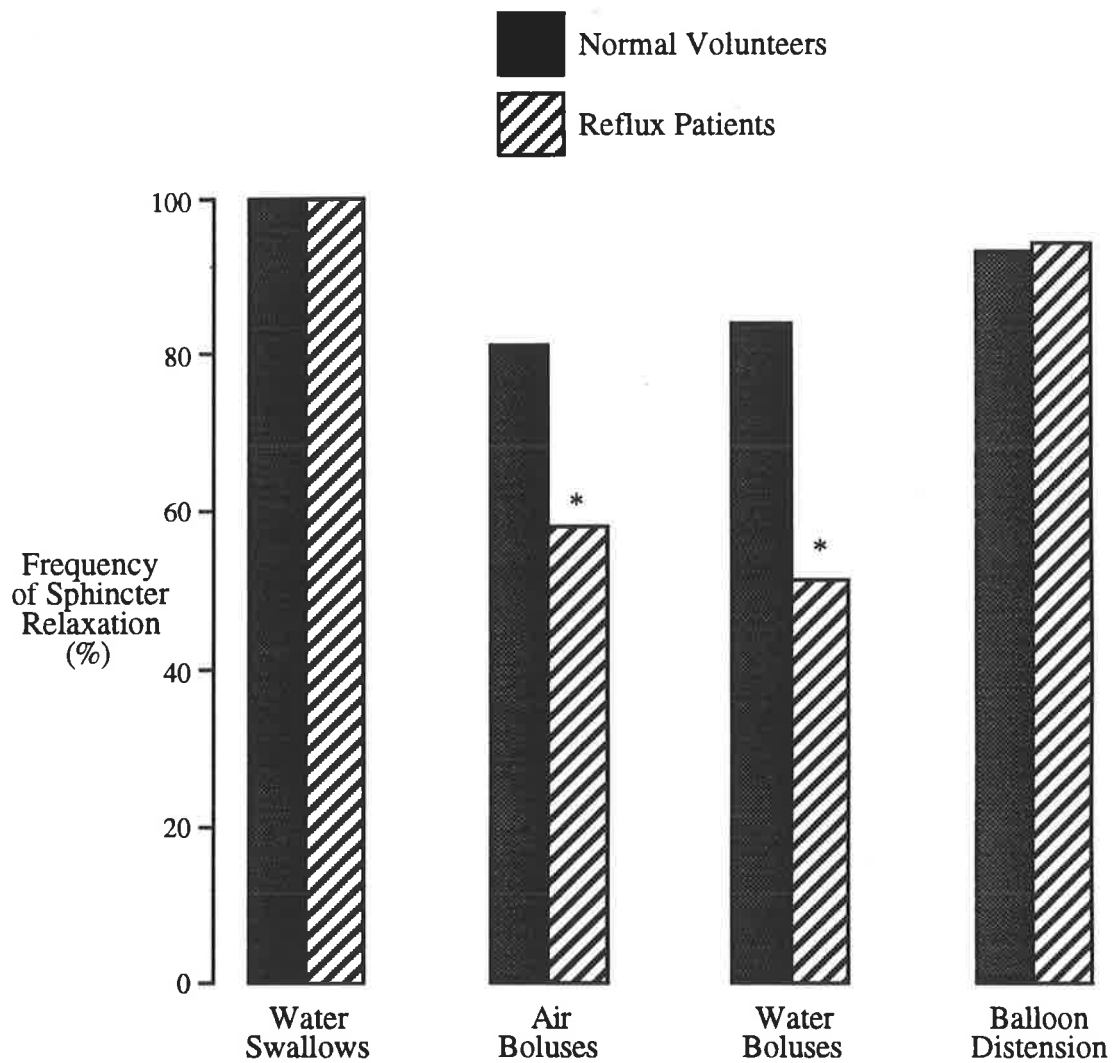


Figure 10.4 The effect of water swallows and oesophageal distension on the frequency of lower oesophageal sphincter relaxation in patients with gastro-oesophageal reflux disease. For the air and water boluses, the frequency of sphincter relaxation was significantly less in the patients (* $p < 0.005$) compared to the normal controls. The frequency of sphincter relaxation was similar in the two groups for water swallows and balloon distension.

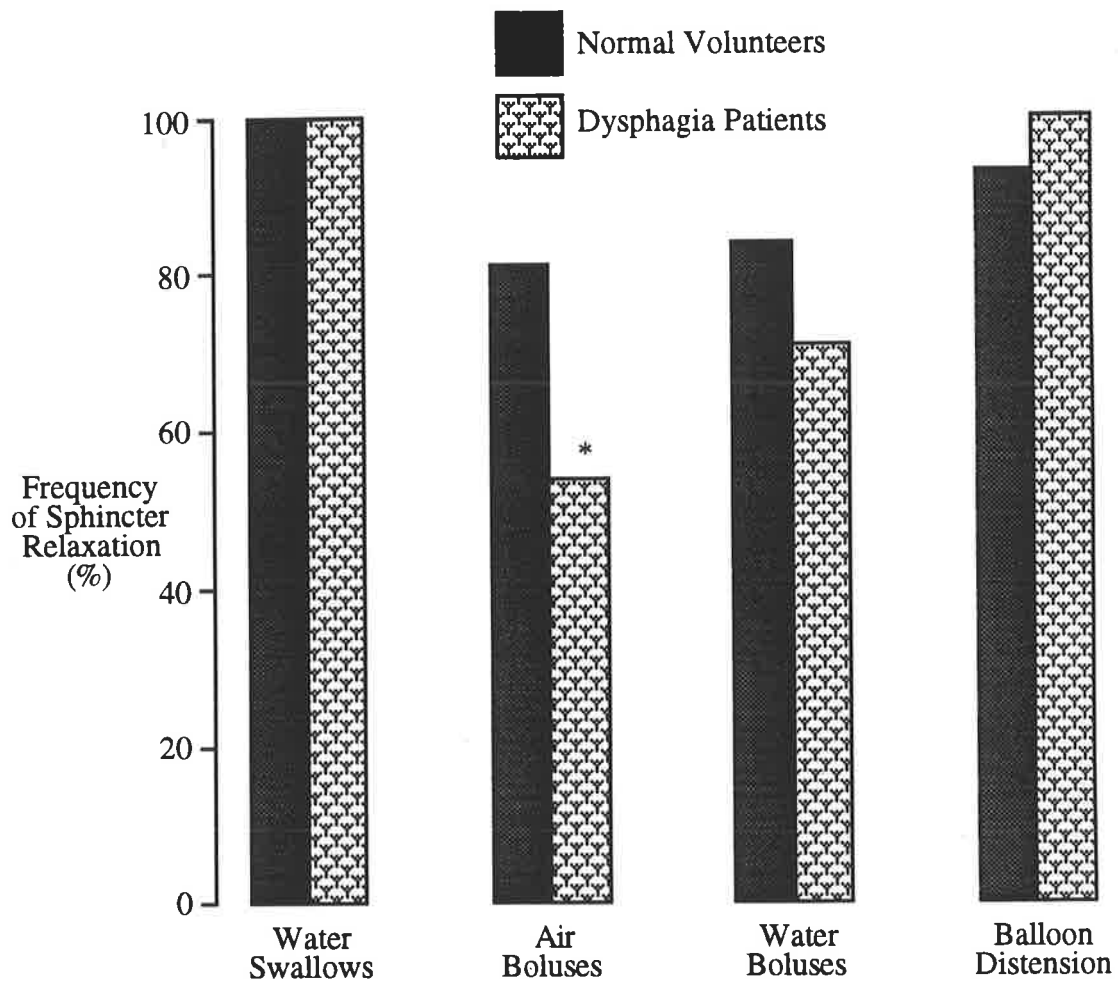


Figure 10.5 The effect of water swallows and oesophageal distension on the frequency of lower oesophageal sphincter relaxation in patients with non-obstructive dysphagia. For the air boluses, the frequency of lower oesophageal sphincter relaxation was significantly less in the patients (* $p < 0.001$) compared to the normal controls. The frequency of lower oesophageal sphincter relaxation was similar in the two groups for swallows, water boluses and balloon distension.

Chapter 11

Mechanisms of Gastro-Oesophageal Reflux in Ambulant Healthy Subjects

11.1 Introduction

11.2 Methods

- 11.2.1 Subjects
- 11.2.2 Recording Techniques
- 11.2.3 Protocol
- 11.2.4 Data and Statistical Analysis

11.3 Results

11.4 Discussion

11.1 Introduction

The physical limitations of standard manometric equipment has confined studies of the mechanisms of gastro-oesophageal reflux largely to resting, recumbent subjects [90, 93, 104, 133, 240]. These studies indicate that lower oesophageal sphincter relaxation is the major mechanism underlying reflux in both normal subjects and patients with gastro-oesophageal reflux disease. Reflux induced by abdominal straining is less common and, in the majority of cases, only important when sphincter pressure is also absent [91].

Physical activity or exercise are commonly reported to exacerbate reflux symptoms and exercise-induced reflux may be an important cause of non-cardiac chest pain [87, 302]. Ambulatory pH monitoring has shown an increase in oesophageal acid exposure during exercise [52, 207, 357] but the mechanisms responsible for this effect have not been examined adequately. A recent study [15] has suggested that the majority of reflux episodes in ambulant subjects are also associated with “visible” sphincter relaxation although quantified sphincter pressure measurements were not reported and the effects of exercise and physical activity were not examined. The aim of this study was to investigate the patterns of lower oesophageal sphincter function underlying reflux in healthy subjects and, to examine the effect of exercise and physical activity on reflux mechanisms. This aim was achieved after the development of an ambulatory manometric system that incorporated a perfused sleeve sensor capable of continuous ambulant measurement of lower oesophageal sphincter pressure.

11.2 Methods

11.2.1 Subjects

Ten healthy subjects (1F : 9M) ranging in age from 18-30 years (median 22 years) were studied. Subjects were free of any gastrointestinal symptoms, had no history of upper gastrointestinal surgery, were not taking antacids on a regular basis and did not take any medication known to alter oesophageal motor function. Each volunteer gave written informed consent and the study was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

11.2.2 Recording Techniques

Static manometry

Oesophageal motility was initially evaluated using a standard multi-lumen manometric catheter similar to that described in section 6.1. This catheter measured pressures from the oesophagus and stomach using perfused side holes and from the lower oesophageal sphincter with a perfused sleeve. A side hole in the pharynx recorded swallowing. The perfusion was provided by a low-compliance pneumohydraulic capillary infusion system. Pressures were sensed by external pressure transducers with output to a 12 channel polygraph recorder.

Ambulatory manometry

The development and performance characteristics of the ambulatory manometric system used in this study are described in section 6.2. Pressures were recorded from the stomach, lower oesophageal sphincter, oesophageal body and pharynx and oesophageal pH was monitored 5cm above the lower oesophageal sphincter. Signals from the pressure transducers and pH electrode were recorded by a portable datalogger and transferred on completion of each study to a computer for subsequent display and

analysis. The calibration pressures and the pressure measurements recorded during the study by the portable datalogger were in cmH₂O.

Events during the study such as meals, exercise periods and any time spent supine were recorded on the datalogger using one of five event markers. This information was also recorded in a detailed diary that was kept for each study and cross checked for accuracy with the event markers on the datalogger at the completion of the study.

11.2.3 Protocol

Each subject fasted for approximately eight hours and smoking and alcohol were prohibited for 12 hours prior to the study. Subjects initially underwent a static oesophageal motility study in order to assess primary peristalsis and to determine the locations of the upper and lower oesophageal sphincters. Oesophageal body peristalsis was tested with ten water swallows (5ml bolus) and was defined as normal if 8 or more of the 10 swallows resulted in complete peristalsis. Lower oesophageal sphincter pressure was defined as normal if basal pressure was >5 mmHg and relaxed completely with the water swallows.

After the standard motility study, the catheter was removed and the ambulatory assembly inserted. Formal recordings started at approximately 11.00 am and were continued for 24 hours. One hour after intubation the subjects were given a meal which consisted of minced meat, mashed potatoes, cooked vegetables, ice cream and 180 ml of full-cream milk (50% fat, 30% carbohydrate, 20% protein, 2395 kJ). After the meal, the subject remained sitting for a further hour to allow for acidification of the meal. Each subject then underwent a standardised exercise program consisting of three, ten minute periods of exercise beginning with vigorous cycling on an exercise bike, then walking at a steady pace on a manual treadmill and finally walking as fast as possible on the treadmill. The exercises were not assessed physiologically to determine degree of effort but all subjects were observed during all exercises to ensure their satisfactory completion.

On completion of the formal exercises, the subject returned to the laboratory to sit and rest for 15 minutes before being asked to take an unsupervised walk out of the hospital. During this unsupervised period, no protocol was observed, the subjects being allowed to walk at their own pace and sit and rest as they chose. Subjects recorded any rest time in the diary and signalled changes in activity with the datalogger event markers. The subject were instructed to remain upright at all times during this period.

At approximately 5.00 pm, the subjects returned to the motility laboratory and were again provided with the standardised meal described above. After the meal, the subjects spent the evening sitting in the laboratory watching television, reading or moving quietly about the room as necessary. At 11.00 pm, the subject went to bed and remained supine, except for visits to the toilet, until the following morning. Breakfast was provided at 7.00 am and consisted of corn flakes cereal, toast, margarine, jam, full-cream milk and 250 ml of tea (20% fat, 66% carbohydrate, 14% protein, 1893 kJ). The subject then spent the remainder of the study time sitting or moving quietly about the laboratory until the completion of the study at approximately 11.00 am. On completion of the study, the manometric and pH probe assemblies were withdrawn and calibrations and baselines checked for any drift.

11.2.4 Data and Statistical Analysis

A detailed description of the manometric and pH criteria used in the analysis of the data obtained during this study appears in section 7.2.4. The analysis concentrated on examining the motor events associated with gastro-oesophageal reflux and their relationship to different levels of physical activity and abdominal straining. The various manometric and pH events scored were all defined according to previously published criteria.

Data in the text are presented as mean $\pm$ standard error. Comparisons among the different activity periods were made using analysis of variance. The relationship

between straining and the occurrence of reflux during transient lower oesophageal sphincter relaxation was assessed by Chi-squared analysis.

11.3 Results

Static manometry

All subjects had normal oesophageal motor function. Mean basal lower oesophageal sphincter pressure was 18.7 ± 4.0 mmHg and mean peristaltic amplitude of 106.4 ± 10.2 mmHg.

Ambulatory manometry

The mean duration of each ambulatory study was 22.7 hours with a range of 21.6 - 23.4 hours. The recording times spent sedentary, ambulant, during exercise and in the two body positions are given in Table 1. Successful recordings were made in all subjects, but in five of the volunteers, sphincter pressure was not interpretable for part of the study (mean 169 min, range 90-315 min) due to either displacement or accidental disconnection of the recording apparatus. The cumulative loss of recording time when upright was 455 minutes (7.6 hours) and 390 minutes (6.5 hours) when supine constituting only 5.3% of the total upright recording time and 7.8% of the total supine recording time.

Reflux episodes

One hundred and twenty-three acid reflux episodes were recorded, of which 81 (66%) occurred in the first 3 hours post prandially and 42 (34%) occurred while fasting. Reflux occurred in all subjects but the number of reflux episodes recorded in each subject ranged widely from 3 to 22. The percentage time $\text{pH} < 4$, the mean rate of reflux episodes and acid clearance time during the different activities are shown in Table 1. The percentage time $\text{pH} < 4$ was similar during the ambulant and sedentary activities but

was less during the exercise period ($p=0.002$). Oesophageal acid exposure was significantly less when supine than when upright ($p=0.003$) because of the reduced frequency of supine reflux ($p=0.0004$). The acid clearance time for individual reflux episodes, however, was significantly longer in the supine position compared with upright ($p=0.0001$). The frequency and duration of reflux episodes was similar for the various activities in the upright position. The minimum pH during the reflux episodes was comparable for the different activities and positions.

Table 11.1: Characteristics of gastro-oesophageal reflux during various activities and postures

		Gastro-oesophageal reflux				
		Time	%	% Time	Reflux	Acid Clearance
		minutes (hrs)	Time	pH<4	Episodes/hour	Time (sec)
Activity	Ambulant	639 (10.7)	5.0	1.65±0.98	3.30±1.69	16.9±4.0
	Exercise	300 (5)	2.4	0.16±0.13	0.40±0.27	14.0±10.0
	Sedentary	7184 (119.8)	56.3	0.97±0.32 ^a	0.84±0.14	42.4±7.8
Posture	Upright	8128 (135.5)	63.0	0.93±0.29 ^b	0.85±0.14 ^c	38.7±6.7 ^d
	Supine	4629 (77.1)	36.3	0.37±0.36	0.08±0.06	173.7±64.8
Total		12757 (212.6)	100.0	0.71±0.23	0.57±0.09	46.3±7.7

The time that lower oesophageal sphincter pressure was not interpretable has been excluded from analysis; ^a $p=0.002$ vs exercise; ^b $p=0.003$ vs supine; ^c $p=0.0004$ vs supine; ^d $p=0.0001$ vs supine

Basal lower oesophageal sphincter pressure

Basal lower oesophageal sphincter pressure varied widely during the study (Figure 11.1). All subjects had a detectable sphincter pressure during most of the recording time. Overall mean lower oesophageal sphincter pressure for the entire study was 28 ± 3 cmH₂O (range 16-44 cmH₂O). Basal lower oesophageal sphincter pressure was

significantly higher when the subjects were supine (38.1 ± 4.3 cmH₂O) than when upright (24.0 ± 3.2 cmH₂O, $p < 0.001$). There was a significant reduction in basal lower oesophageal sphincter pressure post prandially (20.0 ± 2.2 cmH₂O) compared to when fasting (32.3 ± 3.5 cmH₂O, $p = 0.008$). Exercise was associated with a reduced lower oesophageal sphincter pressure, the mean pressure being 10.7 ± 3.0 cmH₂O during the three formal exercise periods compared to 14.6 ± 1.7 cmH₂O during the first post-prandial hour when the subjects were upright and sedentary ($p = 0.03$). There was no significant difference in lower oesophageal sphincter pressure, however, among the three types of exercise (bike: 9.3 ± 2.4 cmH₂O, slow walk: 11.6 ± 3.8 cmH₂O, fast walk: 11.1 ± 3.1 cmH₂O, $p = 0.86$). The occurrence of reflux was unrelated to basal lower oesophageal sphincter pressure (Figure 11.2).

Mechanisms of reflux

In 79% of reflux episodes, lower oesophageal sphincter pressure was absent (≤ 3 cmH₂O) at the onset of reflux and in 87% of episodes it was ≤ 5 cmH₂O (Figure 11.3). The patterns of lower oesophageal sphincter pressure associated with reflux are depicted in Figure 11.4. The mechanism of reflux was not able to be determined for 3 of the 123 reflux events due to movement artefact. The majority (82%) of reflux events occurred during transient lower oesophageal sphincter relaxations (Figure 11.5 and Figure 11.6) with most of the remainder (13%) occurring during swallow-induced lower oesophageal sphincter relaxation. Straining was associated with only 20% of reflux episodes, usually during coexistent lower oesophageal sphincter relaxation (18%) (Figure 11.7). In only 3 (2%) episodes did straining trigger reflux during periods of detectable lower oesophageal sphincter pressure. One reflux episode occurred during a period of sustained absence of lower oesophageal sphincter pressure, and a further 3 episodes (2%) occurred during periods of apparently stable lower oesophageal sphincter pressure in excess of 3 cmH₂O (range 9-59 cmH₂O). Eighty-one (66%) of the reflux events were accompanied by an oesophageal common cavity [90, 110, 190, 353]. The number of reflux episodes associated with straining or

the overall pattern of lower oesophageal sphincter pressure associated with acid reflux did not differ among the periods of exercise, ambulant or sedentary activity. Any potential effect of body position on the patterns of lower oesophageal sphincter pressure could not be determined because of the small number of reflux events when supine.

Transient lower oesophageal sphincter relaxations

A total of 584 transient lower oesophageal sphincter relaxations occurred in the 10 subjects. The frequencies of transient lower oesophageal sphincter relaxation during the different periods of the study are shown in Table 11.2. Reflux occurred during 197 (34%) of transient lower oesophageal sphincter relaxations. Straining significantly increased the likelihood of reflux during transient lower oesophageal sphincter relaxations (Figure 11.8) with reflux occurring during 39 (54%) of the 72 transient lower oesophageal sphincter relaxations associated with straining but only 158 (31%) of the 512 transient lower oesophageal sphincter relaxations not associated with strain ($\chi^2=16.1$, $p<0.001$).

The frequency of transient lower oesophageal sphincter relaxations was significantly greater while upright and awake than when supine ($p<0.001$) and more frequent in the post prandial period compared to fasting ($p<0.001$). Exercise did not influence the rate of transient lower oesophageal sphincter relaxations with similar frequencies occurring during ambulant, sedentary or exercise periods ($p=0.09$).

The mean duration of transient lower oesophageal sphincter relaxations that occurred with different activities are shown in Table 11.2. The duration of transient lower oesophageal sphincter relaxations was greater when upright than when supine ($p=0.04$), and greater in the post prandial 3 hours than during fasting ($p=0.01$). Physical activity, however, had no effect on the duration of transient lower oesophageal sphincter relaxations. Transient lower oesophageal sphincter relaxations

associated with reflux (16.3 ± 0.4 seconds) were significantly longer than those not associated with reflux (13.2 ± 0.3 seconds, $p < 0.001$).

Table 11.2: Effects of posture, feeding and physical activity on frequency of transient lower oesophageal sphincter relaxations

		Transient lower oesophageal sphincter relaxations		
		Number	No./hour	Duration
Posture	Upright	541	4.0 ± 0.3 ^a	14.4 ± 0.2 ^c
	Supine	43	0.5 ± 0.5	12.5 ± 0.6
Fed State	Post-prandial	377	4.3 ± 0.3 ^b	14.7 ± 0.3 ^d
	Fasting	207	1.7 ± 0.2	13.4 ± 0.4
Activity	Ambulant	49	5.7 ± 1.0	14.0 ± 0.8
	Exercise	15	3.0 ± 1.0	11.6 ± 1.0
	Sedentary	520	4.1 ± 0.3	14.4 ± 0.3
Total		584	2.8 ± 0.2	14.2 ± 0.2

^a $p < 0.0001$ vs supine; ^b $p < 0.0001$ vs fasting; ^c $p = 0.04$ vs supine; ^d $p = 0.01$ vs fasting

Mechanisms of oesophageal acid clearance

Overall, primary peristalsis was the most common initial oesophageal clearance event after acid reflux (57%) with secondary peristalsis following 40% of the reflux events (Table 11.3). Physical activity did not influence the pattern of the clearance events. Posture, however, had a significant effect with the majority (86%) of reflux episodes whilst supine being followed by secondary peristalsis ($\chi^2 = 5.6$, $p < 0.02$).

Table 11.3: Oesophageal clearance events

		Oesophageal clearance mechanism			
		Primary Peristalsis	Secondary Peristalsis	Other	Total
Upright	Sedentary	58 (59%)	38 (38%)	3 (3%)	99
	Ambulatory / Exercise	12 (71%)	5 (29%)	0	17
Supine		1 (14%)	6 (86%)	0	7
Total		71 (57%)	49 (40%)	3 (3%)	123

11.4 Discussion

Up to now, understanding of the mechanisms of gastro-oesophageal reflux has been based almost entirely on studies in resting subjects [90, 93, 104, 133, 240]. This study, using a novel ambulatory perfused sleeve sensor, reports the first detailed investigation of lower oesophageal sphincter function associated with reflux in ambulant healthy subjects. The findings establish the importance of transient lower oesophageal sphincter relaxation as the major lower oesophageal sphincter motor event underlying reflux in ambulant healthy subjects and indicate that straining, even during moderately vigorous exercise, is not an important precipitant of reflux except when lower oesophageal sphincter pressure is ablated by transient lower oesophageal sphincter relaxation. The study also demonstrates the importance of secondary peristalsis in oesophageal acid clearance when supine and asleep.

Accurate assessment of lower oesophageal sphincter function associated with individual reflux episodes necessitates the use of a sensor that can continuously and

accurately monitor lower oesophageal sphincter pressure as well as lower oesophageal sphincter relaxation. In this study, lower oesophageal sphincter pressure was monitored with a reverse-perfused sleeve sensor. This sensor has been validated by testing in a sphincter model and in humans [88, 92, 216, 310] and accurately records lower oesophageal sphincter pressures as low as 2 cmH₂O. The sleeve was reverse-perfused for two reasons. First, it allowed the embedded transducer to be positioned at the mid-point of the sleeve, thereby minimising differences in hydrostatic pressure between different body positions. Second, lower oesophageal sphincter pressure measurements during physical activity also include a component of diaphragmatic squeeze of the gastro-oesophageal junction. The use of reversed perfusion has been shown to improve the response rate of the sleeve during diaphragmatic contraction [310] and, coupled with the use of a narrower sleeve bed compared with that of a standard sleeve assembly, allowed the use of a low perfusion rate whilst maintaining an adequate rise rate.

A recent study [15] has also investigated mechanisms of reflux in ambulant normal subjects. This study, however, suffers from some methodological limitations. First, their study relied on a sphinctometer [146] to record lower oesophageal sphincter function. In contrast to the sleeve sensor, this device does not measure actual lower oesophageal sphincter pressure but a derivative of lower oesophageal sphincter pressure and lower oesophageal sphincter length, and its output is susceptible to pressure changes in both the stomach and oesophagus. Most importantly, however, the ability of the sphinctometer to measure lower oesophageal sphincter relaxation is undefined and it is not clear whether this method can recognise times when lower oesophageal sphincter pressure is absent. Interpretation of sphinctometer recordings in terms of lower oesophageal sphincter pressure and the anti-reflux barrier is therefore difficult. Significantly, in this previous study, lower oesophageal sphincter activity could not be assessed in 16% of reflux episodes and sphinctometer recordings allowed only qualitative assessment of lower oesophageal sphincter function in the remaining 84% [15]. In contrast, the perfused sleeve sensor measured and quantified lower

oesophageal sphincter pressure satisfactorily in 120 (98%) of 123 reflux events. Another important limitation of the sphinctrometer data reported by Barham et al is that swallowing was not monitored. Thus it is not possible to distinguish swallow-induced lower oesophageal sphincter relaxation from non-swallow related or transient lower oesophageal sphincter relaxation, and primary from secondary peristalsis.

This study establishes the importance of transient lower oesophageal sphincter relaxation as the major mechanism of reflux in ambulant as well as resting healthy subjects. The proportion of reflux episodes occurring by this mechanism was greater than that associated with "visible LOS relaxation" in the study by Barham et al [15] but consistent with previous observations in resting healthy subjects [90, 104]. As with resting subjects [132, 133, 170, 270], meals, sleep and posture were the major factors that influenced the rate of transient lower oesophageal sphincter relaxations. Walking or even moderately vigorous exercise had no significant effect on the rate of transient lower oesophageal sphincter relaxations which was similar to that reported previously under resting conditions [170, 235, 270].

Straining *per se* does not seem to be a major factor provoking reflux in healthy subjects, as only three reflux episodes occurred by this mechanism alone. However, straining was a significant factor when lower oesophageal sphincter pressure was ablated during transient lower oesophageal sphincter relaxation and almost doubled the likelihood of reflux at that time. There are several potential reasons for these findings. First, the degree of straining involved in the mild to moderate ambulatory activity that the subjects undertook may have been insufficient to overcome basal lower oesophageal sphincter pressure. Second, it is possible that straining was associated with diaphragmatic contraction that can prevent reflux even when lower oesophageal sphincter pressure is ablated by rapid swallowing or oesophageal balloon distension [91]. In contrast, transient lower oesophageal sphincter relaxations are associated with inhibition of the crural diaphragm [233], thereby removing all barriers to reflux. Specific measurement of crural diaphragmatic activity is needed to resolve this issue.

Basal lower oesophageal sphincter pressures of 4 to 5 mmHg appear to be sufficient to prevent reflux even during intervals of increased intra-abdominal pressure [91]. However, three reflux episodes occurred during periods of apparently stable lower oesophageal sphincter pressure. The mechanism underlying these episodes is not clear as they were not associated with pressure transients such as increased intra-abdominal pressure or deep inspiration that might overcome low levels of basal lower oesophageal sphincter pressure. It is possible that the sleeve was recording pressures coming from structures other than the lower oesophageal sphincter and thereby falsely registering a detectable lower oesophageal sphincter pressure.

Oesophageal common cavities occurred with 66% of reflux episodes, similar to that observed in resting recumbent subjects [90, 110]. In the previous study [15], this pattern of pressure change was assumed to represent belching. In this study, the subjects were not asked to indicate when they belched. However, whilst an oesophageal common cavity can be associated with belching, or more accurately gastro-oesophago-pharyngeal gas reflux [190, 353] it also occurs with reflux in the absence of belching and is merely an indicator of pressurisation of the oesophageal lumen by inflow of gastric contents. It is likely that some, if not most reflux episodes, result in trans-sphincteric flow of both gas and liquid. More importantly, however, the major underlying mechanism for reflux, whether gas or liquid or both, is transient lower oesophageal sphincter relaxation.

Ambulatory oesophageal pH monitoring records more gastro-oesophageal reflux than does static monitoring [37] and exercise has been reported to increase the rate of reflux episodes [302, 315, 357], particularly after food [52]. In the present study, an attempt was made to represent normal daily activities in the context of a standardised protocol. The results using this protocol showed that exercise had no significant effect on reflux even though the exercise was performed one hour after a substantial meal, a period when lower oesophageal sphincter pressure is at its lowest and the rate of reflux episodes is greatest [90, 93, 104]. Three factors may have contributed to this

discrepancy. First, apart from the periods on the treadmill and bicycle, the level of exercise in the present study is likely to have been lower than that used previously. In previous studies increases in reflux were seen only at sub-maximal exercise levels [315]. Such levels were unlikely to have been achieved in the present study except during the ten minutes of vigorous cycling which may have been too short a period to demonstrate an effect. Second, the subjects walked rather than ran and running appears to be a more provocative stimulus to reflux [52]. Third, the analysis was restricted to reflux episodes that conformed to more traditional criteria and resulted in a drop in oesophageal pH to below 4. It has been shown previously that reflux may be associated with smaller falls in oesophageal pH that do not drop below pH 4, particularly in the first hour after a meal [354]. Whilst the analysis in this study may have underestimated the frequency of gastro-oesophageal reflux during the post prandial exercise period, circumstances most favourable to the provocation of reflux, it would not have influenced significantly the analysis of lower oesophageal sphincter function at the time of reflux, as reflux episodes associated with lesser changes in oesophageal pH have similar patterns of lower oesophageal sphincter function and oesophageal motility as those seen during traditional reflux episodes [354].

Current evidence indicates that primary peristalsis is the principal mechanism of oesophageal clearance after reflux [90, 110], with secondary peristalsis playing an important but nevertheless minor role. Consistent with this notion, primary peristalsis was overall the most common oesophageal clearance event in the present study. An important additional finding, however, was that secondary peristalsis was the major mechanism of oesophageal clearance when the subjects were supine or asleep. It was not possible to separate the effects of sleep from those of the supine posture as sleep monitoring was not performed. Patients with reflux disease have impaired acid clearance, particularly when supine [80, 299]. In chapter 8 of this thesis, it has been shown that triggering of secondary peristalsis is defective in patients with reflux oesophagitis. This defect may therefore be an important factor in the pathogenesis of impaired nocturnal acid clearance in patients with reflux disease.

In conclusion, this study reports the development and application of a novel ambulatory perfused manometric system that is the first validated method for performing accurate measurements of lower oesophageal sphincter motor function in ambulant human subjects. This method will allow the important investigation of reflux mechanisms in patients with reflux disease under conditions that more closely approximate normal daily physical activity. Based on the findings from studies in resting patients with reflux disease, factors such as absent lower oesophageal sphincter pressure [91] and the presence of hiatus hernia [312] could potentially have a significant impact on the mechanisms of reflux and particularly on the effect of straining. The impact of these factors, as well as the evaluation of therapies designed to improve gastro-oesophageal competence such as increasing basal lower oesophageal sphincter pressure, can only be assessed adequately in ambulant patients and further studies in patients with reflux disease are therefore needed.

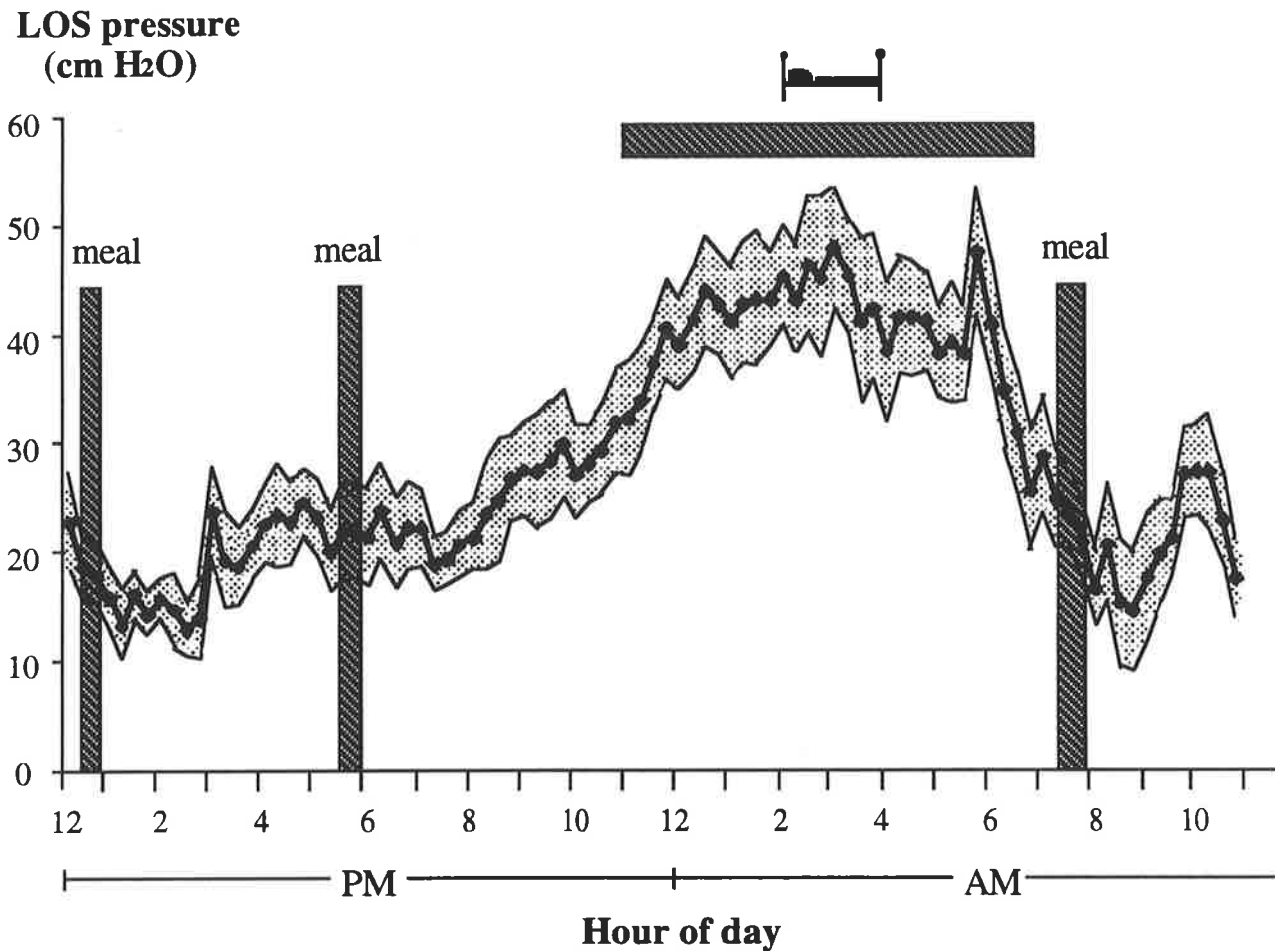


Figure 11.1: Basal lower oesophageal sphincter pressure during the study period. Each point represents the mean value for a 15 min interval with the hatched area indicating ± 1 SE. The vertical diagonally hatched bars indicate time of meals and the horizontal hatched bar the time the subjects were in bed. There is a significant reduction in lower oesophageal sphincter pressure after meals ($p=0.008$) and a significant increase in lower oesophageal sphincter pressure at night when supine ($p=0.005$).

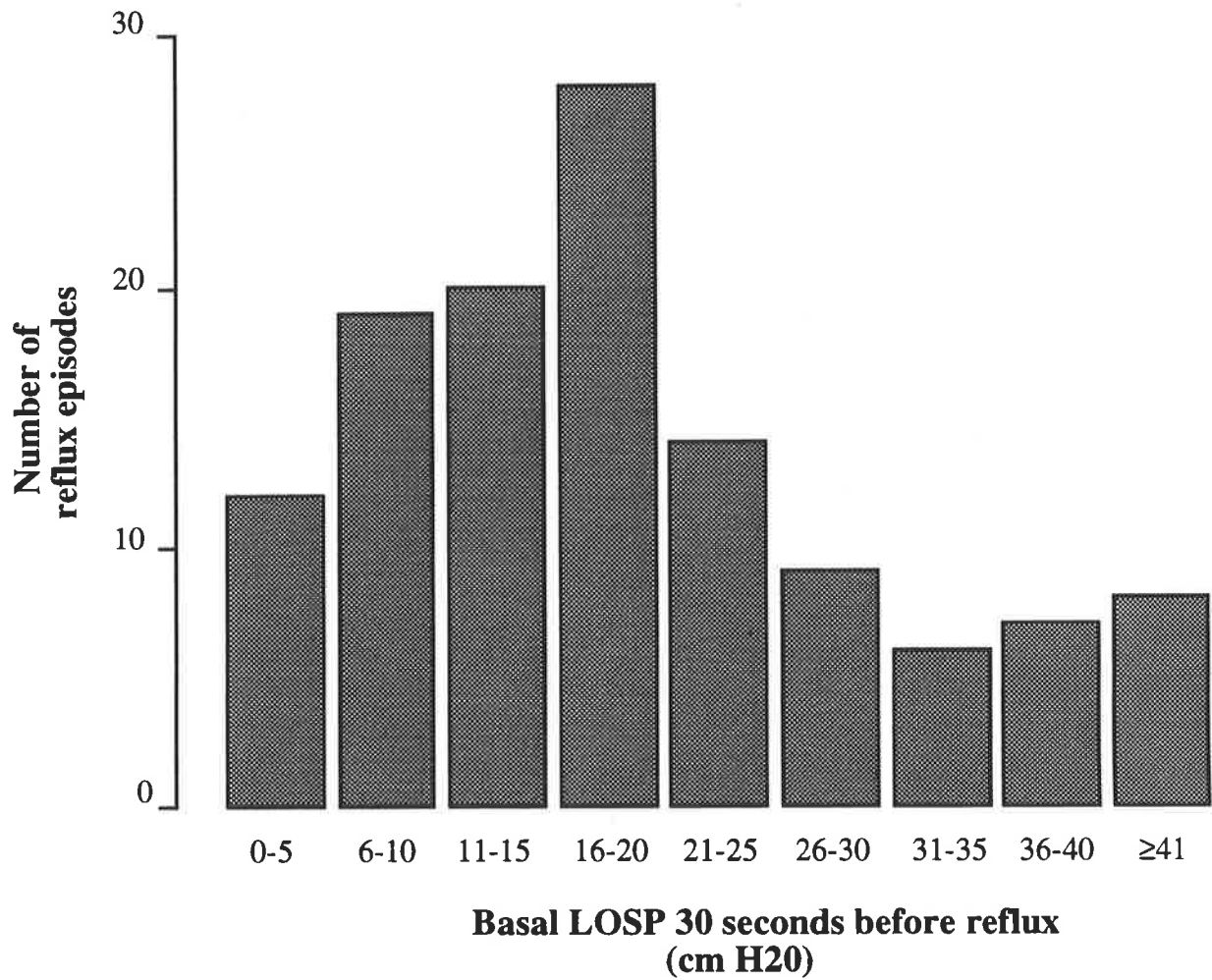


Figure 11.2: Relationship between basal lower oesophageal sphincter within 30 seconds of the onset of reflux and the occurrence of reflux. Data have been grouped into intervals of 5 cmH₂O. There was no significant correlation between basal lower oesophageal sphincter pressure and the frequency of gastro-oesophageal reflux.

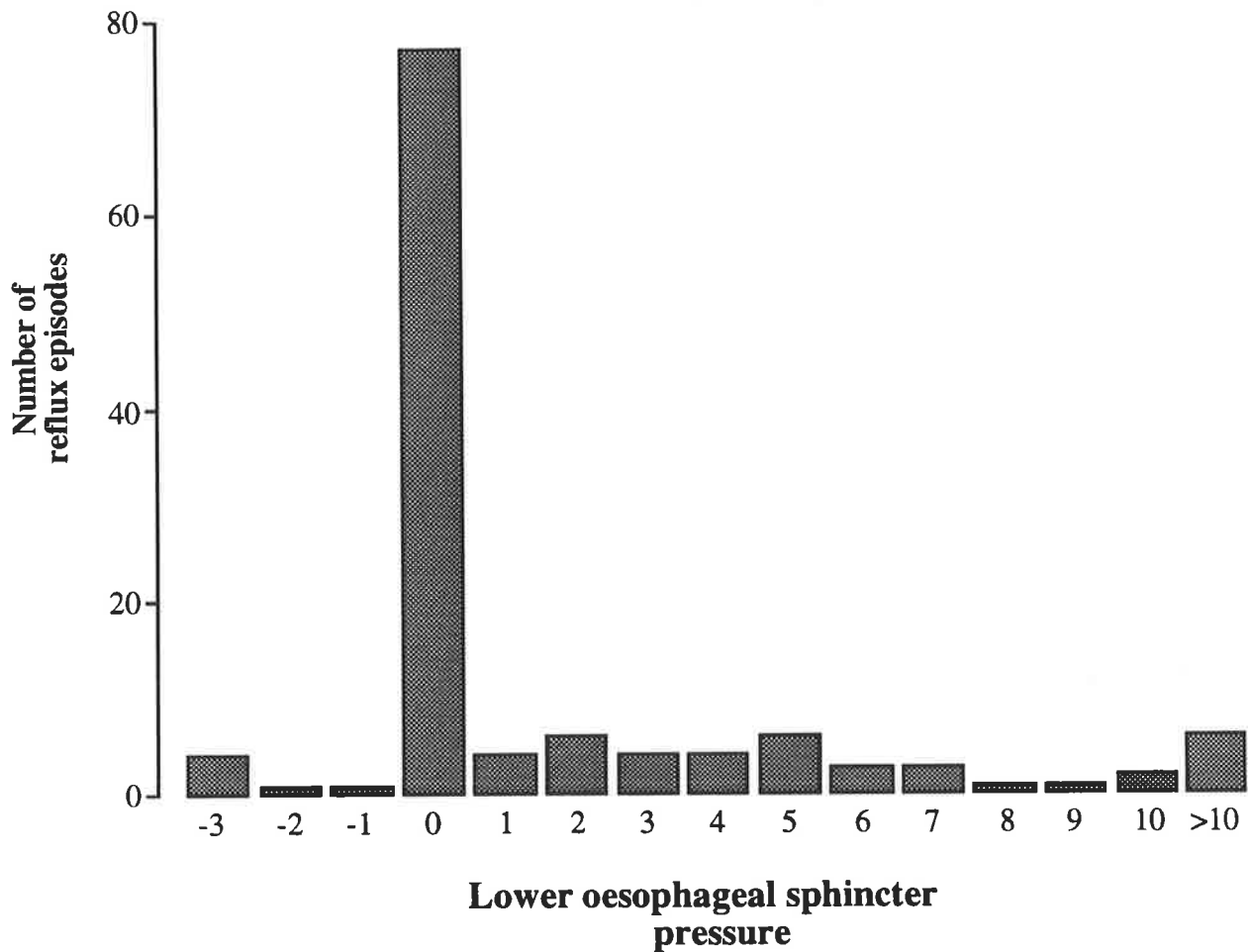


Figure 11.3: Lower oesophageal sphincter pressure at the onset of reflux. The data are plotted as the number of reflux episodes that occurred at the designated lower oesophageal sphincter pressure. In 79% of instances, lower oesophageal sphincter pressure was absent (≤ 3 cmH₂O) at the onset of reflux and 87% of instances it was ≤ 5 cmH₂O.

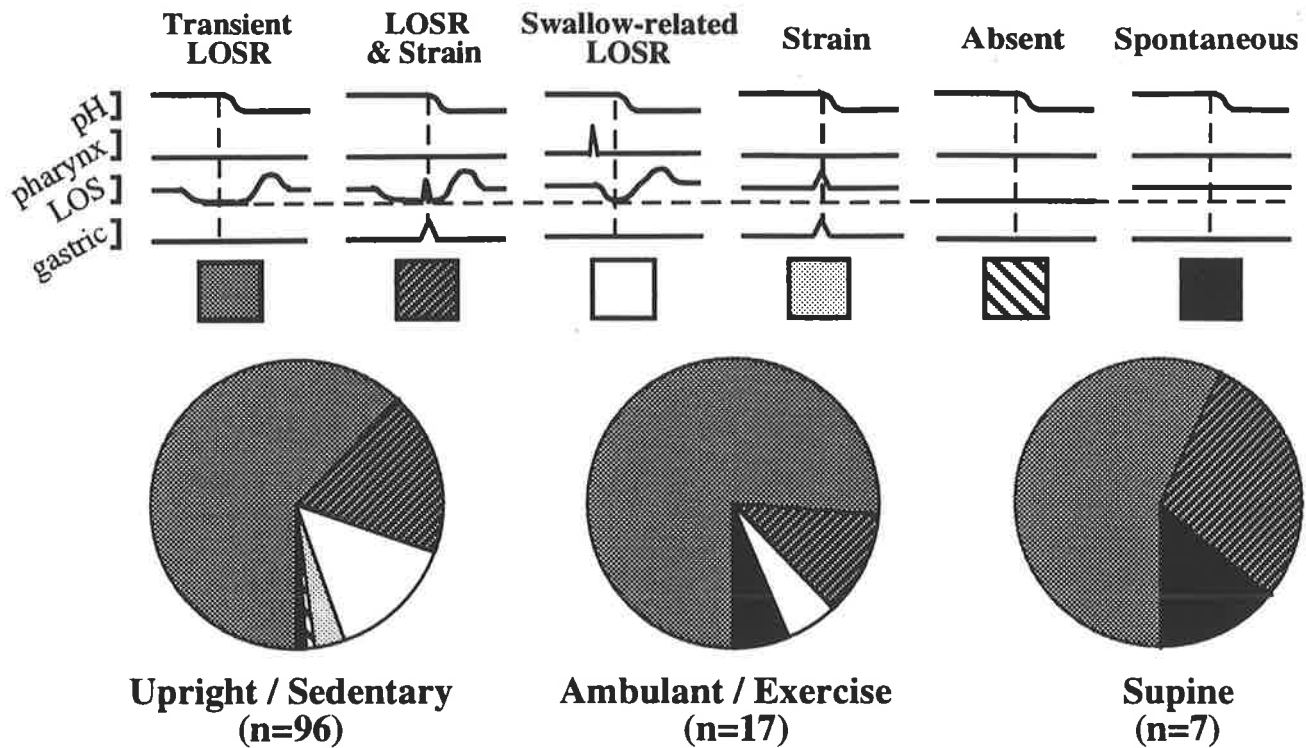


Figure 11.4: Patterns of lower oesophageal sphincter function associated with reflux. The different patterns are illustrated schematically at the top of the figure. The broken horizontal line in the LOS tracing indicates gastric pressure while the vertical broken lines indicate the onset of gastro-oesophageal reflux. The pie charts depict the proportion of reflux episodes associated with each pattern for the three major periods of activity. The numbers below each pie indicate the number of reflux episodes during each respective activity. The most important mechanism associated with gastro-oesophageal reflux, regardless of physical activity, was transient LOS relaxation (82%). The majority of the remainder (13%) occurring during swallow-induced LOS relaxation. Straining was an uncommon cause of reflux and was generally associated with reflux during coexistent LOS relaxation (18%). In only 3 episodes did straining trigger reflux during periods of detectable lower oesophageal sphincter pressure. One reflux episode occurred during a period of absent lower oesophageal sphincter pressure and 3 events (3%) occurred spontaneously during periods of apparently stable lower oesophageal sphincter pressure.

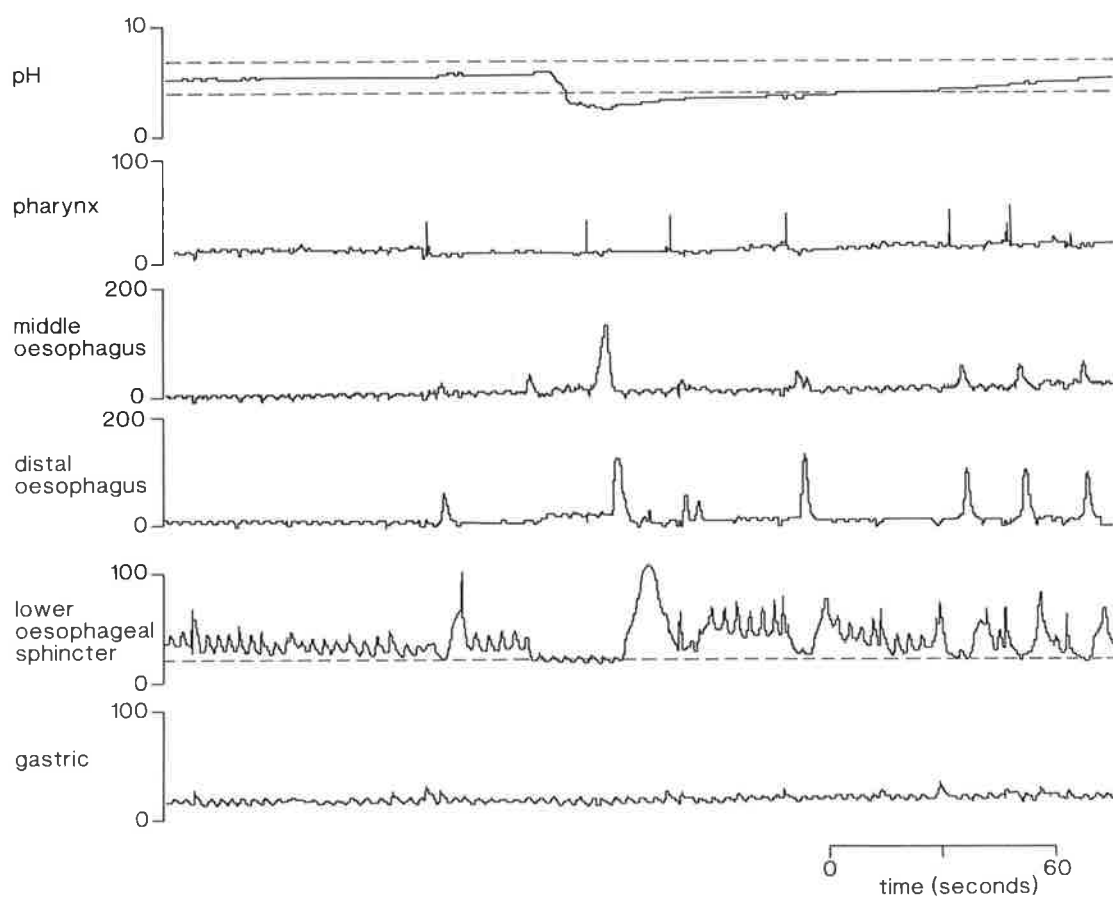


Figure 11.5 Example of a reflux episode occurring during a transient lower oesophageal sphincter relaxation. The broken horizontal line in the LOS pressure tracing indicates gastric pressure and the two horizontal broken lines in the pH tracing indicate pH4 and pH7.

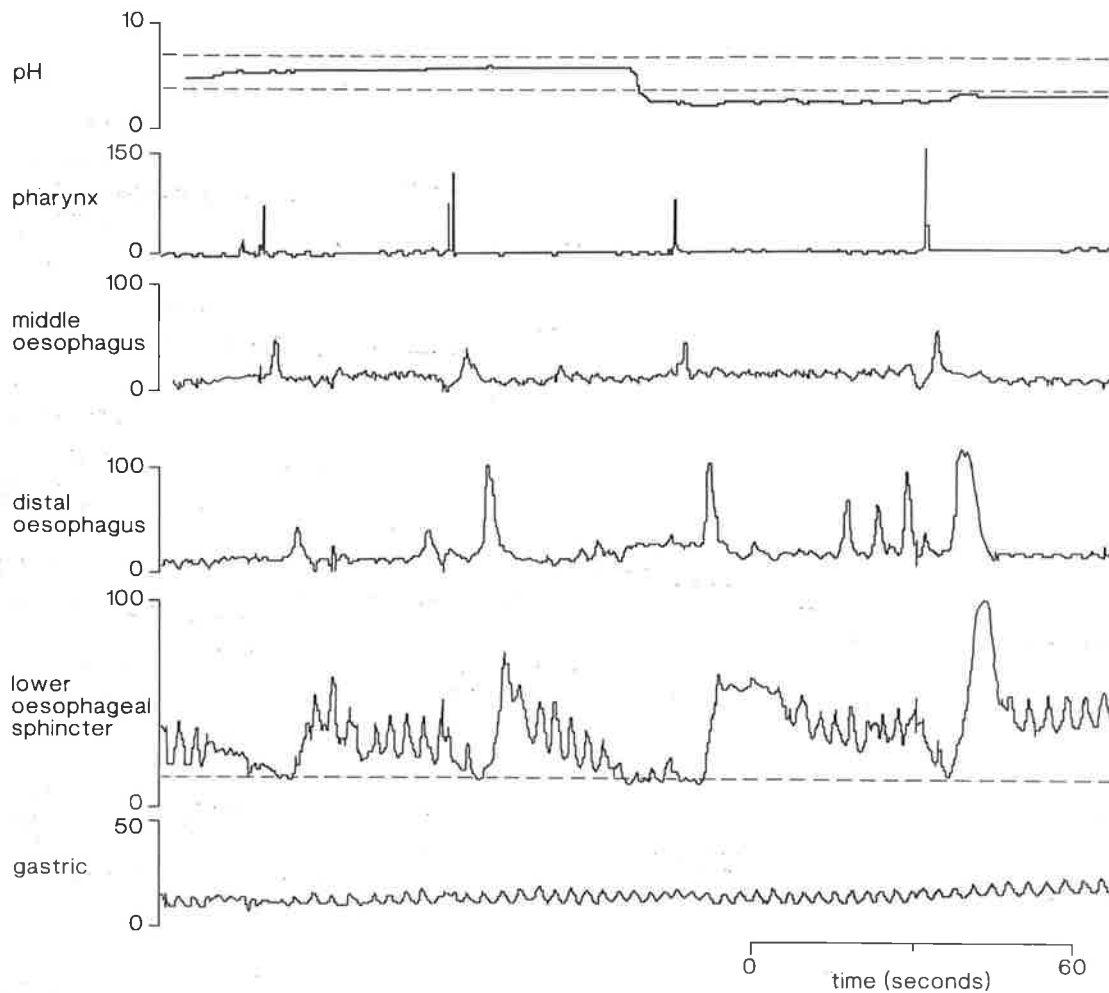


Figure 11.6 Example of a reflux episode occurring during a transient lower oesophageal sphincter relaxation. The broken horizontal line in the LOS pressure tracing indicates gastric pressure and the two horizontal broken lines in the pH tracing indicate pH4 and pH7.

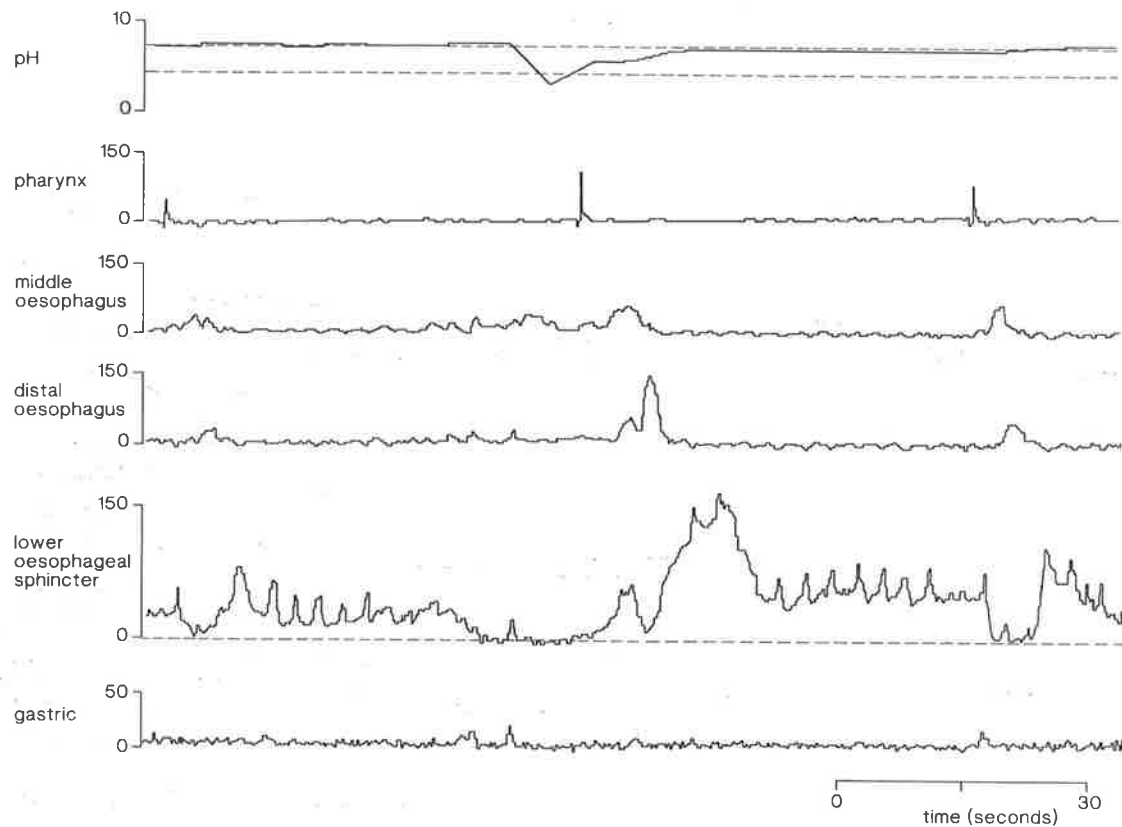


Figure 11.7 Example of a reflux episode occurring during a transient lower oesophageal sphincter relaxation accompanied by abdominal straining. The broken horizontal line in the LOS pressure tracing indicates gastric pressure.

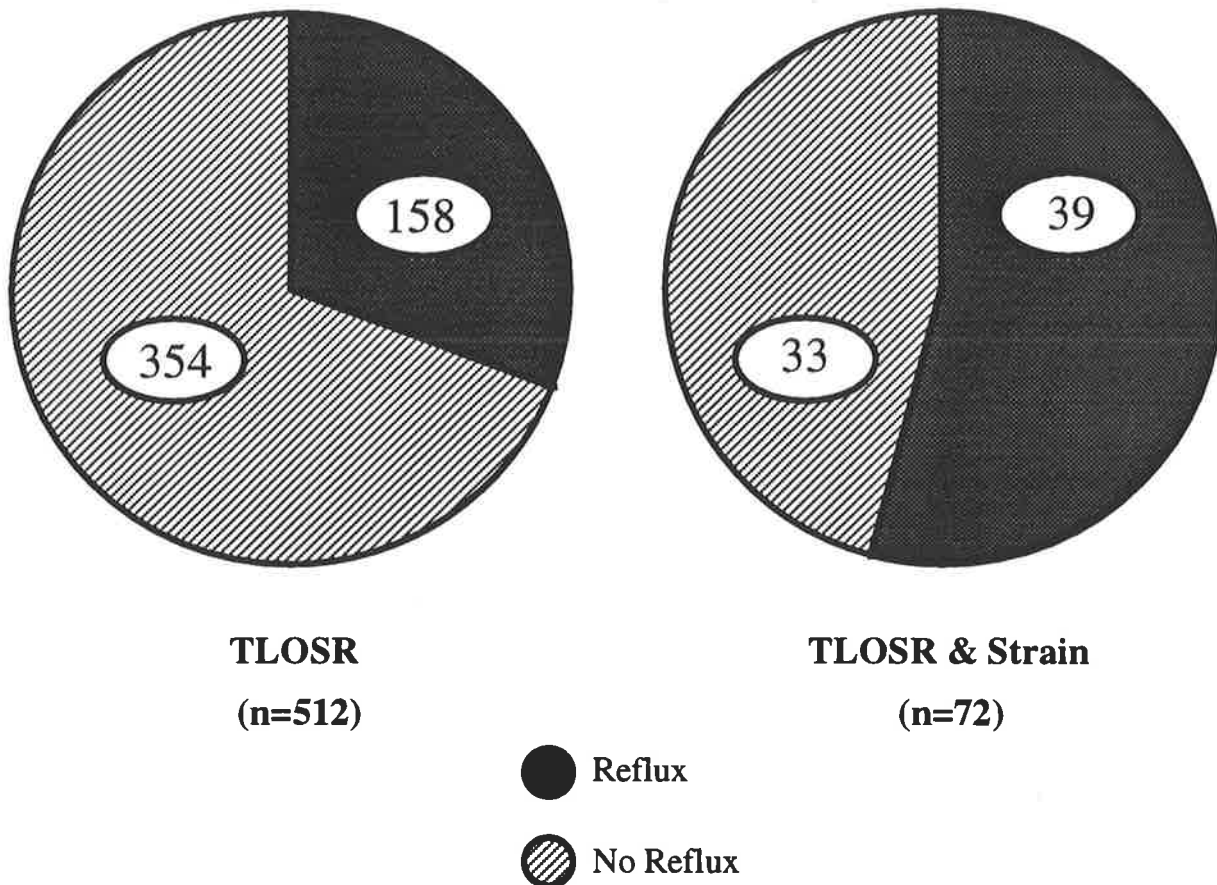


Figure 11.8: Effect of straining on the occurrence of reflux during transient lower oesophageal sphincter relaxations. Straining significantly increases the likelihood of reflux during transient lower oesophageal sphincter relaxation ($\chi^2=16.1$, $p<0.001$).

Chapter 12

Effect of Hiatus Hernia on Lower Oesophageal Sphincter Pressure and Gastro-Oesophageal Reflux During Straining

12.1 Introduction

12.2 Methods

12.2.1 Subjects

12.2.2 Recording Techniques

12.2.3 Protocol

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12.4 Discussion

12.1 Introduction

The role of a hiatus hernia in gastro-oesophageal reflux disease is controversial but epidemiologically, there is a strong correlation between the presence of a hiatus hernia and gastro-oesophageal reflux disease [23, 317]. The postulated explanation of this association is that the displacement of the lower oesophageal sphincter from the diaphragmatic hiatus results in reduced competence of the gastro-oesophageal junction and predisposes to reflux. In normal subjects, the crural diaphragm plays a significant role in the competence of the gastro-oesophageal junction [35, 157, 236-238, 242] but in patients with hiatus hernia, the deranged anatomy displaces and potentially reduces the contribution of the diaphragm, particularly when straining. This hypothesis is supported by the finding of a strong correlation between the size of the hiatus hernia, lower oesophageal sphincter pressure and the predisposition to reflux during straining manoeuvres in reflux patients [312].

Displacement of the lower oesophageal sphincter from the diaphragmatic hiatus and into the thoracic cavity results in a number of potential mechanical disadvantages to sphincter function. First, there is loss of the extrinsic support of the lower oesophageal sphincter by the crural diaphragm. Second, the sphincter is moved from the intra-abdominal to an intra-thoracic pressure environment and thus is exposed to potentially large negative intra-thoracic pressures which may be sufficient to overcome sphincter pressure and cause reflux. Third, compartmentalisation of the proximal stomach in the chest by diaphragmatic contraction may generate large intra-hernia pressures during straining that may "blow open" the sphincter. Finally, compression of the herniated stomach by the diaphragm may increase tension in the herniated gastric wall, resulting in triggering of transient lower oesophageal sphincter relaxations. Existing studies in patients with hiatus hernia have not examined these postulated mechanical disadvantages directly due to the inability of their recording methods to make valid concurrent measurements from the various regions of interest. Inferences can be drawn about diaphragmatic function from animal studies and studies in healthy volunteers but

these do not address the potential defects in function associated with a hiatus hernia. The aim of this study, therefore, was to determine the effect of abdominal straining on the pressure profile across the gastro-oesophageal junction in order to more accurately determine the mechanical effects of a hiatus hernia on gastro-oesophageal competence.

12.2 Methods

12.2.1 Subjects

Eight subjects (median age 72.5yrs) with a hiatus hernia identified at endoscopy were enrolled in the study (4F : 4M; age 65-77yrs). All patients had symptoms of gastro-oesophageal reflux disease and were endoscoped as part of either initial evaluation for this condition or for follow-up of response to therapy. At the endoscopy, the axial length of the hiatus hernia was measured as the distance from the gastro-oesophageal junction, defined as the point at which the gastric folds disappeared, to the mid-point of the impression made by the diaphragm on the proximal stomach. Only subjects with large (≥ 5 cm) hiatus hernias were included in the study as it was felt that large hernias were less likely to reduce during the manoeuvres to be performed during the study. In the 8 patients who took part in the study, the mean axial length of the hiatus hernia was 6cm (range 5-8cm). All patients had previously been documented to have gastro-oesophageal reflux disease at endoscopy but only 4 of the 8 patients had ulcerative or erosive oesophagitis at the time of the study (two patients were defined using a modified Savary Miller scale as having grade II oesophagitis and two patients with grade III). Patients taking medication that may have altered oesophageal motility ceased these at least 48 hours prior to the study. Treatment with H_2 antagonists or acid pump inhibitors was continued.

Data from the patients with a hiatus hernia were compared to those from ten healthy subjects (2F : 8M; age 19-58 yrs, median 28.5yrs). The normal subjects were free of gastrointestinal symptoms, had no history of upper gastrointestinal surgery and were

not taking antacids regularly or any medication known to alter oesophageal motor function. All patients and volunteers gave written informed consent and the study was approved by the Human Ethics Committee of the Royal Adelaide Hospital.

12.2.2 Recording Techniques

The manometric recording system and catheter used in this study are described in Chapter 6 and illustrated in Figure 6.4. In both groups, pressures were measured from the stomach, lower oesophageal sphincter, oesophageal body and pharynx. In the patients with hiatus hernia, additional pressures were measured from the diaphragm and hernia sac. The position of the sleeve sensor across the gastro-oesophageal junction was monitored by checking the pressure patterns measured by the side holes at the proximal and distal margins of the sleeve sensor and by the continuous recording of the trans-mucosal potential difference gradient that exists between the stomach and oesophagus. Reflux events were detected by a pH electrode located 5cm above the proximal margin of the lower oesophageal sphincter.

12.2.3 Protocol

Subjects were studied following an overnight fast. The catheter was passed pernasally and the subjects positioned supine in a 10° head down position in order to maximise the chance of reflux. After a 10 minute period of adaptation, primary peristalsis and lower oesophageal sphincter function were tested with ten, 5ml water swallows.

After assessment of primary peristalsis, the stomach was loaded with 200ml of 10% dextrose solution acidified to pH2 with hydrochloric acid. Intra-gastric volume was maintained by a constant infusion of acidified dextrose at a rate of 5 ml/minute [39]. Concurrent pressure and pH measurements were then made during a series of manoeuvres designed to either relax the lower oesophageal sphincter and/or to increase intra-abdominal pressure. Lower oesophageal sphincter relaxation was induced by either intra-oesophageal balloon distension to a diameter of 2.5cm for 20 seconds (using a volume of 12ml air) or rapid swallowing of 1ml boluses of water at a rate of

one swallow every two seconds for 15 seconds [91]. This swallow rate was sufficient to inhibit propagation of peristalsis in the distal oesophagus until after the last swallow. Intra-abdominal pressure was increased by either abdominal compression or straight leg raising. Abdominal compression was performed using an abdominal binder which was inflated so that intragastric pressure increased by approximately 15mmHg (or up to the maximum tolerated by the subject if less than 15mmHg). Pressure within the abdominal binder was not recorded. Leg raising was done for 15 seconds by elevation of the legs to 40° from the horizontal. Care was taken to prevent the generation of large, intra-thoracic pressure transients by unconscious performance of a Valsalva manoeuvre during leg raising by instructing the subjects to continue breathing quietly during the manoeuvre. A minimum of 10 seconds stable basal lower oesophageal sphincter pressure tracing was obtained before each manoeuvre was tested. Swallowing was not permitted during each test except when evaluating the effect of rapid swallowing. All pressure channels were checked at 30 minute intervals for baseline drift and the function of the pH electrode was tested before and after the study to exclude any significant drift (acceptable drift <1 pH unit during the 2.5 hour study; drifts of <1pH unit were assumed to be linear).

Balloon distension, rapid swallowing, abdominal compression and leg raising were each tested in triplicate, either alone or in paired combinations. The testings were: (1) abdominal compression, (2) leg raise, (3) rapid swallowing, (4) balloon distension, (5) abdominal compression and balloon distension (abdominal compression was first applied and then the balloon was inflated for 15 seconds), (6) abdominal compression and rapid swallowing for 10 seconds, (7) leg raising and balloon distension (the balloon distension was applied prior to the start of the leg raise and maintained until after leg raising ceased), and (8) leg raising and rapid swallowing (rapid swallowing was started after leg raising and continued for 10 seconds while leg raising was maintained). The order of testing was randomised, for a total of 24 test manoeuvres in each subject. Any test that was incorrectly performed was repeated.

Gastro-oesophageal reflux was judged to have occurred when the pH tracing showed an abrupt drop (>1 pH unit per second) of greater than 1 pH unit that lasted a minimum of 4 seconds. After the occurrence of reflux, the next test was not done until oesophageal pH had risen above 6.

12.2.4 Data and Statistical Analysis

Tracings were analysed manually. All pressure measurements were made at end-expiration and referenced to atmospheric pressure as zero. The pressure values so obtained are referred to as absolute pressures. These absolute pressures, however, represent a summation of three pressure influences. The first of these is the internal pressure of interest within the subject. The second is a constant pressure that represents the hydrostatic pressure difference between the subject and the external pressure transducers that is determined by the height of the manometric catheter relative to the transducers and the third is the perfusion resistance of the manometric catheter. In the analysis of standard manometric tracings using a similar recording system, pressures are conventionally referenced to an internal zero such as gastric pressure when calculating lower oesophageal sphincter pressure or to basal pressure such as when calculating oesophageal peristaltic amplitude. This cancels out the influence of hydrostatic pressure and perfusion resistance leaving the true pressure exerted by the structure of interest in the patient. In this study, lower oesophageal sphincter (LOS) pressure is referenced to intra-abdominal gastric pressure in the normal subjects while in the patients with hiatus hernia, LOS pressure is referenced to intra-hernia pressure (ie. intra-thoracic gastric pressure). The change in pressure induced by each manoeuvre at each recording site was calculated by subtracting the basal pressure in the channel of interest from the pressure recorded in that channel during the manoeuvre. The pressure value obtained after this subtraction is independent of the hydrostatic pressure difference between the patient and the external recording transducer and can be compared between subjects. A detailed description of the terms and their meaning, as used in this study, are provided below.

For each manoeuvre, the time that minimum lower oesophageal sphincter (LOS) pressure occurred during the test was noted and this time point was used to determine corresponding pressures recorded by the other channels. Only pressures referenced to an internal zero such as gastric pressure or changes in absolute pressure, as described above, were compared between subjects and subjected to statistical analysis.

Definition of terms used in this study

Normal subjects

LOS pressure (LOSP)	pressure recorded by the sleeve sensor located in the LOS referenced to intra-abdominal gastric pressure
Δ LOS pressure (Δ LOSP)	the change in absolute LOS pressure (the absolute LOS pressure recorded during each manoeuvre minus the absolute LOS pressure prior to the manoeuvre)
Δ gastric pressure (Δ GP)	the change in absolute intra-abdominal gastric pressure (the absolute gastric pressure recorded during each manoeuvre minus the gastric pressure prior to the manoeuvre)

Patients with hiatus hernia

LOS pressure (LOSP)	pressure recorded by the sleeve sensor located in the LOS referenced to intra-hernia pressure
Δ LOS pressure (Δ LOSP)	the change in absolute LOS pressure (the absolute LOS pressure recorded during each manoeuvre minus the absolute LOS pressure prior to the manoeuvre)
Δ Intra-hernia pressure (Δ HP)	the change in absolute intra-hernia pressure (the intra-hernia pressure recorded during each

	manoeuvre minus the intra-hernia pressure prior to the manoeuvre)
Diaphragmatic pressure (DP)	the pressure recorded by the sleeve sensor located across the diaphragm referenced to intra-abdominal gastric pressure
Δ diaphragmatic pressure (Δ DP)	the change in absolute diaphragmatic pressure (the diaphragmatic pressure recorded during each manoeuvre minus the diaphragmatic pressure prior to the manoeuvre)
Δ gastric pressure (Δ GP)	the change in absolute intra-abdominal gastric pressure (the absolute intra-abdominal gastric pressure recorded during each manoeuvre minus the absolute intra-abdominal gastric pressure prior to the manoeuvre)
Trans-diaphragmatic intra-gastric pressure difference	the pressure gradient across the diaphragm calculated by subtracting gastric pressure from intra-hernia pressure

Because of significant differences in basal lower oesophageal sphincter pressure between the controls and the patients with hiatus hernia, changes in lower oesophageal sphincter pressure with each manoeuvre were also expressed as a percentage change of the basal pressure so that comparisons between the two groups could be made.

Data are presented as mean $\pm$ SE of the mean unless otherwise stated. Group mean data were determined for each test. The pressure changes recorded in the different recording regions before and during each test within each group were compared using a paired Student's t-test and Wilcoxon Signed Rank Test. Patients were compared to

the controls using the Mann-Whitney U test. The frequency of gastro-oesophageal reflux associated with each test was analysed by the Chi-squared test.

12.3 Results

Basal pressure measurements

Basal lower oesophageal sphincter pressure in the normal subjects was significantly greater than that in the patients with hiatus hernia (21.7 ± 0.6 vs 11.7 ± 0.7 mmHg, $p < 0.0001$). In the patients with hiatus hernia, there were no significant differences among basal intra-hernia pressure, gastric pressure or the pressure exerted by the diaphragm. At rest, the diaphragm did not compartmentalise the stomach with a basal diaphragmatic pressure of -0.2 ± 0.2 mmHg and a trans-diaphragmatic intra-gastric pressure difference was 1.0 ± 0.2 mmHg.

Manoeuvres to increase intra-abdominal pressure

In both normal subjects and patients with hiatus hernia, the increase in gastric pressure (ΔGP) associated with leg raising was nearly double that induced by abdominal compression (Table 12.1). The increase in gastric pressure induced by each manoeuvre, however, was similar in the two groups.

Lower oesophageal sphincter pressures for each individual manoeuvre in each subject are shown in Figures 12.1 & 12.2 with mean values shown in Table 12.1. In the normal subjects, the mean lower oesophageal sphincter pressure (referenced to gastric pressure) during abdominal compression (17.8 ± 1.9 mmHg) was significantly less than basal sphincter pressure (22.6 ± 1.7 mmHg, $p = 0.03$). This was due to a greater increase in gastric pressure (ΔGP ; $+11.9 \pm 0.8$ mmHg) than in lower oesophageal sphincter pressure ($\Delta LOSP$; $+7.0 \pm 1.9$ mmHg). During leg raising, however, lower oesophageal sphincter pressure (LOSP) when referenced to intra-gastric pressure was unchanged (23.9 ± 2.0 vs 25.5 ± 1.9 mmHg, $p = 0.47$) with similar increases in lower oesophageal

sphincter pressure (ΔLOSP ; $+21.7 \pm 3.1 \text{ mmHg}$) and gastric pressure (ΔGP ; $+20.1 \pm 2.0 \text{ mmHg}$).

In the patients with hiatus hernia, the patterns of pressure change in the lower oesophageal sphincter (referenced to intra-hernia pressure) during abdominal compression and leg raising were similar to those in the normal subjects with sphincter pressure being reduced by abdominal compression (10.6 ± 2.0 vs $6.3 \pm 2.1 \text{ mmHg}$, $p=0.048$) but not leg raising (11.9 ± 2.1 vs $9.9 \pm 3.3 \text{ mmHg}$, $p=0.42$). However, whilst the pressure changes in lower oesophageal sphincter pressure induced by abdominal compression and leg raising are numerically similar in the two groups, the percentage change in the sphincter pressure during the two manoeuvres are statistically different (Figure 12.3). The mean percentage change in sphincter pressure was greater in the patients with hiatus hernia than in the normal subjects during both abdominal compression (-59% vs -13% ; $p=0.03$) and leg raising (-50% vs $+30\%$, $p=0.01$).

During leg raising in the patients with hiatus hernia, the increase in lower oesophageal sphincter pressure (ΔLOSP ; $+12.1 \pm 3.1 \text{ mmHg}$) and intra-hernia pressure (ΔHP ; $+14.1 \pm 2.2 \text{ mmHg}$) were similar but both were significantly less ($p \leq 0.04$) than the increase measured in gastric pressure (ΔGP ; $+22.9 \pm 3.2 \text{ mmHg}$) or diaphragmatic pressure (ΔDP ; $+25.7 \pm 3.7 \text{ mmHg}$). In addition, the increase in diaphragmatic pressure (ΔDP) during leg raising was greater than the increase in gastric pressure (ΔGP) ($p=0.03$). These differences between the various recording regions in the patients with hiatus hernia were not seen during abdominal compression. The changes in pressures induced by abdominal compression and leg raising in the various recording regions in both subject groups are also shown in Table 12.1.

Table 12.1 Pressures prior to (basal) and during (test) abdominal compression and leg raising in the two subject groups.

		Abdominal Compression	Leg Raise
Normal Subjects	Basal LOSP	22.7±1.7	23.9±2.0
	Test LOSP	17.8±1.9 †	25.5±1.9
	ΔLOSP	+7.0±1.9	+21.7±3.1
	ΔGP	+11.9±0.8	+20.1±2.0
Hiatus Hernia Patients	Basal LOSP	10.6±2.0 *	11.9±2.1 *
	Test LOSP	6.3±2.1 †	9.9±3.3
	ΔLOSP	+5.4±2.2	+12.1±3.1
	ΔHP	+9.7±0.9	+14.1±2.2
	ΔDP	+11.0±1.1	+25.7±3.7
	ΔGP	+11.2±0.8	+22.9±3.2

Pressures are expressed in mmHg. * $p < 0.05$ compared to comparable pressure in the normal subjects; † $p < 0.05$ compared to basal sphincter pressure.

Manoeuvres to decrease lower oesophageal sphincter pressure

The effects of balloon distension and rapid swallowing were similar in the normal subjects and the patients with hiatus hernia. In both groups, lower oesophageal sphincter pressure (LOSP), when referenced to intra-gastric or intra-hernia, was significantly reduced by both manoeuvres (Table 12.2). Balloon distension or rapid swallowing did not alter gastric pressure (ΔGP) in the normals or intra-hernia (ΔHP), diaphragmatic (ΔDP) or gastric pressure (ΔGP) in the patients with hiatus hernia.

Table 12.2 Pressures prior to and during balloon distension and rapid swallowing in the two subject groups.

		Balloon Distension	Rapid Swallowing
Normal Subjects	Basal LOSP	22.0±1.7	22.4±2.1
	Test LOSP	5.3±1.3 †	2.8±0.7 †
	ΔLOSP	-16.8±1.4	-20.0±1.9
	ΔGP	-0.2±0.1	-0.4±0.2
Hiatus Hernia Patients	Basal LOSP	12.6±1.7 *	12.3±2.0 *
	Test LOSP	2.3±1.4 †	0.4±0.6 †
	ΔLOSP	-10.6±1.9	-12.1±2.2
	ΔHP	-0.3±0.4	-0.3±0.4
	ΔDP	+0.6±0.4	+2.0±0.5
	ΔGP	-0.4±0.2	+0.8±0.3

Pressures are expressed in mmHg. * $p < 0.05$ compared to comparable pressure in the normal subjects; † $p < 0.05$ compared to basal sphincter pressure.

Manoeuvres to both decrease lower oesophageal sphincter pressure and increase intra-abdominal pressure

In both groups, the increase in gastric pressure (ΔGP) associated with abdominal compression or leg raising and the decrease in lower oesophageal sphincter pressure (ΔLOSP) due to balloon distension or rapid swallowing resulted in a reduction in the lower oesophageal sphincter pressure when referenced to intra-gastric or intra-hernia pressure (LOSP) (Table 12.3). The mean percentage reduction in lower oesophageal sphincter pressure was comparable in both groups (Figure 12.3). In the patients with hiatus hernia, abdominal compression associated with either balloon distension or rapid swallow resulted in similar changes in intra-hernia (ΔHP), diaphragmatic (ΔDP) and gastric (ΔGP) pressures. For leg raising, however, the change in intra-hernia

pressure (Δ HP) was significantly less than the change in diaphragmatic pressure (Δ DP) ($p=0.01$) and gastric pressure (Δ GP) ($p=0.005$) when associated with balloon distension but not for rapid swallowing.

Table 12.3 Pressures before and during the combined manoeuvres in the two subject groups.

		AC & BD	AC & RS	LR & BD	LR & RS
Normal Subjects	Basal LOSP	19.1 $\pm$ 1.4	22.1 $\pm$ 1.6	20.2 $\pm$ 1.4	21.2 $\pm$ 2.2
	Test LOSP	4.8 $\pm$ 1.6 †	6.4 $\pm$ 1.1 †	6.4 $\pm$ 1.6 †	6.1 $\pm$ 1.6 †
	Δ LOSP	-0.4 $\pm$ 1.5	+0.2 $\pm$ 1.7	+4.0 $\pm$ 1.7	+0.9 $\pm$ 2.5
	Δ GP	+13.6 $\pm$ 0.6	+16.3 $\pm$ 1.1	+17.9 $\pm$ 1.5	+16.8 $\pm$ 1.6
Hiatus Hernia Patients	Basal LOSP	10.2 $\pm$ 1.5 *	8.9 $\pm$ 1.8 *	13.0 $\pm$ 2.2 *	13.8 $\pm$ 2.3 *
	Test LOSP	2.3 $\pm$ 0.7 †	1.1 $\pm$ 0.5 †	2.2 $\pm$ 0.9 †	0.1 $\pm$ 1.4 †
	Δ LOSP	+2.5 $\pm$ 2.4	+2.5 $\pm$ 2.3	-0.6 $\pm$ 2.3	+1.0 $\pm$ 2.7
	Δ HP	+10.5 $\pm$ 1.5	+10.3 $\pm$ 1.5	+10.3 $\pm$ 1.2	+14.8 $\pm$ 2.2
	Δ DP	+12.8 $\pm$ 1.5	+11.7 $\pm$ 1.0	+19.3 $\pm$ 3.7 **	+17.9 $\pm$ 2.7
	Δ GP	+13.3 $\pm$ 1.5	+12.5 $\pm$ 1.1	+17.0 $\pm$ 3.1 **	+17.8 $\pm$ 1.8

Pressures are expressed in mmHg. * $p<0.05$ compared to comparable pressure in the normal subjects; † $p<0.05$ compared to basal sphincter pressure; ** $p\leq 0.01$ compared to Δ HP

Gastro-oesophageal reflux

The incidence of gastro-oesophageal reflux during each of the manoeuvres is shown in Figure 12.4. When applied alone, abdominal compression and leg raising induced significantly more reflux in patients with hiatus hernia than in the normal subjects (AC: 50% vs. 13%, $p<0.005$; LR: 21% vs. 0%, $p<0.01$). The percentages of events associated with gastro-oesophageal reflux during balloon distension and rapid swallowing were similar for the two groups.

For the combined manoeuvres, the frequency of gastro-oesophageal reflux was similar in the two groups except for leg raising and rapid swallowing (Figure 12.4) which provoked significantly more reflux episodes in the patients with hiatus hernia than in the normal subjects (29% vs 7%; $p < 0.05$). An example of gastro-oesophageal reflux induced by rapid swallowing during leg raising is shown in Figure 12.5.

Gastro-oesophageal reflux occurred only during periods of virtual or complete abolition of the pressure gradient across the lower oesophageal sphincter (ie. equalisation of LOS and gastric pressures). Lower oesophageal sphincter pressures at the time of gastro-oesophageal reflux during each manoeuvre are shown in Figures 12.1 & 12.2. The lower oesophageal sphincter pressure at the time of reflux was ≤ 3 mmHg in 91% of the normal subjects and in 89% of the patients with hiatus hernia. The incidence of gastro-oesophageal reflux during manoeuvres where the lower oesophageal sphincter pressure was ≤ 3 mmHg in the normal subjects (42%) was similar to that in the patients with hiatus hernia (35%).

Gastro-oesophageal reflux during lower oesophageal sphincter relaxation was favoured by a concurrent increase in intra-abdominal pressure. In the normal subjects, the proportion of sphincter relaxations (≤ 3 mmHg) during BD and RS associated with reflux rose from 21% to 53% when accompanied by an increase in intra-abdominal pressure ($p < 0.005$). In the patients with hiatus hernia, however, although the proportion rose from 22% to 38%, this increase failed to achieve statistical significance ($\chi^2 = 3.1$, $0.10 > p > 0.05$).

12.4 Discussion

In order to assess the relevance of a hiatus hernia to gastro-oesophageal reflux during straining, it is important to evaluate the role of the crural diaphragm and the effect of its displacement in these patients. In normal subjects and patients with reflux disease but without hiatus hernia, the sphincter-like effects of the crural diaphragm are

superimposed on the lower oesophageal sphincter. In this position, the diaphragm contributes to sphincter competence during respiration [3, 122, 206, 220, 236, 343] and also during transient increases in intra-abdominal pressure induced by straining [234]. In patients with hiatus hernia there are a number of potential mechanical disadvantages resulting from the altered anatomy which have been postulated as predisposing to gastro-oesophageal reflux. The widened diaphragmatic hiatus could potentially impair the sphincter function of the crural muscles by preventing lumen occlusive contraction of the diaphragm on the lower oesophageal sphincter. This would impair diaphragmatic function during abdominal straining or during large negative increases in intra-thoracic pressure. Conversely, the displaced contraction of the diaphragm on the proximal stomach might generate high pressure within the hernia sac that may overcome the often low basal sphincter pressure in patients with hiatus hernia and thus promote gastro-oesophageal reflux. In this study, the findings have revealed that neither of the above postulated mechanisms are entirely correct. During manoeuvres to increase intra-abdominal pressure and which are associated with diaphragmatic contraction, there is indeed compartmentalisation of the stomach but contrary to expectation, this diaphragmatic contraction protected the hiatus hernia from the full increase in intra-abdominal pressure. In manoeuvres that increased intra-abdominal pressure without diaphragmatic contraction, there was a decrease in sphincter competence in both normals and patients with hiatus hernia but the effect was greater in the patients, with a larger percentage reduction in sphincter pressure and an increased incidence of gastro-oesophageal reflux.

The relative contribution of the lower oesophageal sphincter and diaphragm during abdominal straining remains contentious with previous studies supporting either one of two schools of thought. In some studies, the rise in lower oesophageal sphincter pressure during straining exceeded the rise in intra-abdominal pressure and led to the concept of an intrinsic capability of the sphincter to respond to such pressure alterations [57, 183, 215]. In contrast, a number of other studies have shown that the change in sphincter pressure during abdominal straining is similar to the change in

abdominal pressure suggesting a purely passive response of the lower oesophageal sphincter to external forces [106, 136, 164, 248, 336]. There are a number of methodological limitations associated with these studies as they all used side holes to record sphincter and diaphragmatic pressures. The side holes were either placed in the area of interest or slowly withdrawn through the sphincter by employing a pull-through technique during the straining manoeuvre. Both techniques, however, are unreliable and limit the validity of the previous findings. A fixed side hole fails to allow for movement of gastro-oesophageal junction during straining and thus is unable to adequately record true pressure changes in the sphincter or diaphragm during straining. The pull-through technique, on the other hand, only provides a “glimpse” of the pressure profile across the lower oesophageal sphincter and is limited by factors such as respiratory movement artefact, the inability to monitor swallowing and inadvertent valsalva manoeuvre by the subject. In this study, pressure measurements during straining were simultaneously made from the various recording regions using methodology that was capable of accommodating sphincter and diaphragmatic movement. A standard sleeve sensor was used to record lower oesophageal sphincter pressure [88] and a reverse-perfused sleeve was used to optimise recording of diaphragmatic pressure [310]. Furthermore, catheter position was constantly checked during the study by monitoring the side hole pressures above and below each sleeve and by ensuring that the proximal sleeve was always located across the gastro-oesophageal junction by monitoring the transmucosal potential difference between the oesophagus and stomach. This methodology not only accurately records the various individual pressures but also allows comparison of the pressure profile across the gastro-oesophageal junction during straining.

The findings of this study are consistent with the second hypothesis mentioned above that the pressure changes seen across the gastro-oesophageal junction during straining are due to mechanical transmission of intra-abdominal and diaphragmatic pressures. During leg raising in the normal subjects, there was a greater increase in sphincter pressure than gastric pressure whereas for abdominal compression the reverse was

true. This difference can simply be explained by the effect of the diaphragm and is not due to an intrinsic capability of the sphincter to respond to changes in intra-abdominal pressure. In patients with hiatus hernia the effect of the diaphragm is displaced from the region of the lower oesophageal sphincter. During leg raising in these subjects, the increase in diaphragmatic pressure exceeded the increase in gastric pressure, thus protecting the hernia from the full increase in gastric pressure. During abdominal compression, however, the diaphragm was not tonically contracted and the increase in gastric pressure was similar to that measured within the hiatus hernia. Functionally this resulted in more reflux than when intra-abdominal pressure was increased with associated diaphragmatic contraction in the patients with hiatus hernia. This is also despite the fact that the increase in gastric pressure induced by leg raising was approximately double that seen with abdominal compression.

In both the normal subjects and patients with hiatus hernia, the combined manoeuvres resulted in an increase in intra-gastric pressure and a decrease in lower oesophageal sphincter pressure. Abdominal compression and balloon distension or rapid swallowing resulted in a similar frequency of gastro-oesophageal reflux in the two groups. During the manoeuvres combined with leg raising, however, the frequency of reflux in the normal subjects was different, with less reflux occurring during leg raising and rapid swallowing than during those associated with abdominal compression. This observation supports the concept that the diaphragmatic contraction induced by leg raising contributes to gastro-oesophageal competence. Inconsistent with this observation is the unexpected high frequency of reflux induced by leg raising and balloon distension in the normal subjects which is similar to that seen during manoeuvres associated with abdominal compression. There is no clear explanation for this discrepancy as it also contrasts to the findings during leg raising and balloon distension of a previous study using similar methodology in healthy volunteers [91]. It is possible that the difference is due to different effects of balloon distension and rapid swallowing on the voluntary diaphragmatic contraction induced by leg raising. This hypothesis needs further evaluation. In the patients with hiatus hernia, the frequency

of reflux was similar during the combined manoeuvres associated with abdominal compression or leg raising which suggests that there is reduced competence of the gastro-oesophageal junction during manoeuvres associated with diaphragmatic contraction.

Gastro-oesophageal reflux is more common during straining in the patients with hiatus hernia than in normal subjects [312]. This finding is similar to the present study with significantly more reflux seen during leg raising and abdominal compression in the patients with hiatus hernia than the normal subjects. Reflux is also favoured by the simultaneous occurrence of absent sphincter pressure and abdominal straining. It has previously been reported that a prerequisite for the occurrence of gastro-oesophageal reflux is a sphincter pressure to 3mmHg or less [91]. This is similar to the data from the current study where 89% of reflux episodes in the patients with hiatus hernia and 91% of the episodes in the normal subjects occurred when sphincter pressure was 3mmHg or less. The greater proportion of straining manoeuvres associated with reflux in the patients with hiatus hernia, however, is presumably due to the greater percentage reduction in lower oesophageal sphincter pressure induced by leg raising or abdominal compression in the patients compared to the normal subjects.

In conclusion, this study confirms the important role of the diaphragm in the competence of the gastro-oesophageal junction and the clinical impression that a hiatus hernia reduces the competence of the gastro-oesophageal junction. Importantly, however, this study shows that the diaphragm still contributes significantly to the prevention of gastro-oesophageal reflux during abdominal straining in patients with large hiatus hernias despite the displacement of the diaphragmatic hiatus. In this situation, crural diaphragmatic contraction on the proximal stomach provides a pressure barrier to help protect the hiatus hernia from changes in intra-abdominal pressure. It is possible to reconcile this apparent conflict in data if one considers that the manoeuvres performed in this study are likely to involve maximal diaphragmatic contraction and thus induce contractions that are likely to be lumen occlusive across the

proximal stomach. Lesser degrees of effort or patients with large diaphragmatic hiatal openings, may not achieve lumen occlusion and thus be at greater risk of gastro-oesophageal reflux. This hypothesis could be examined by using graded degrees of straining in patients with a large diaphragmatic hiatal openings compared to those with only small hiatal defects.

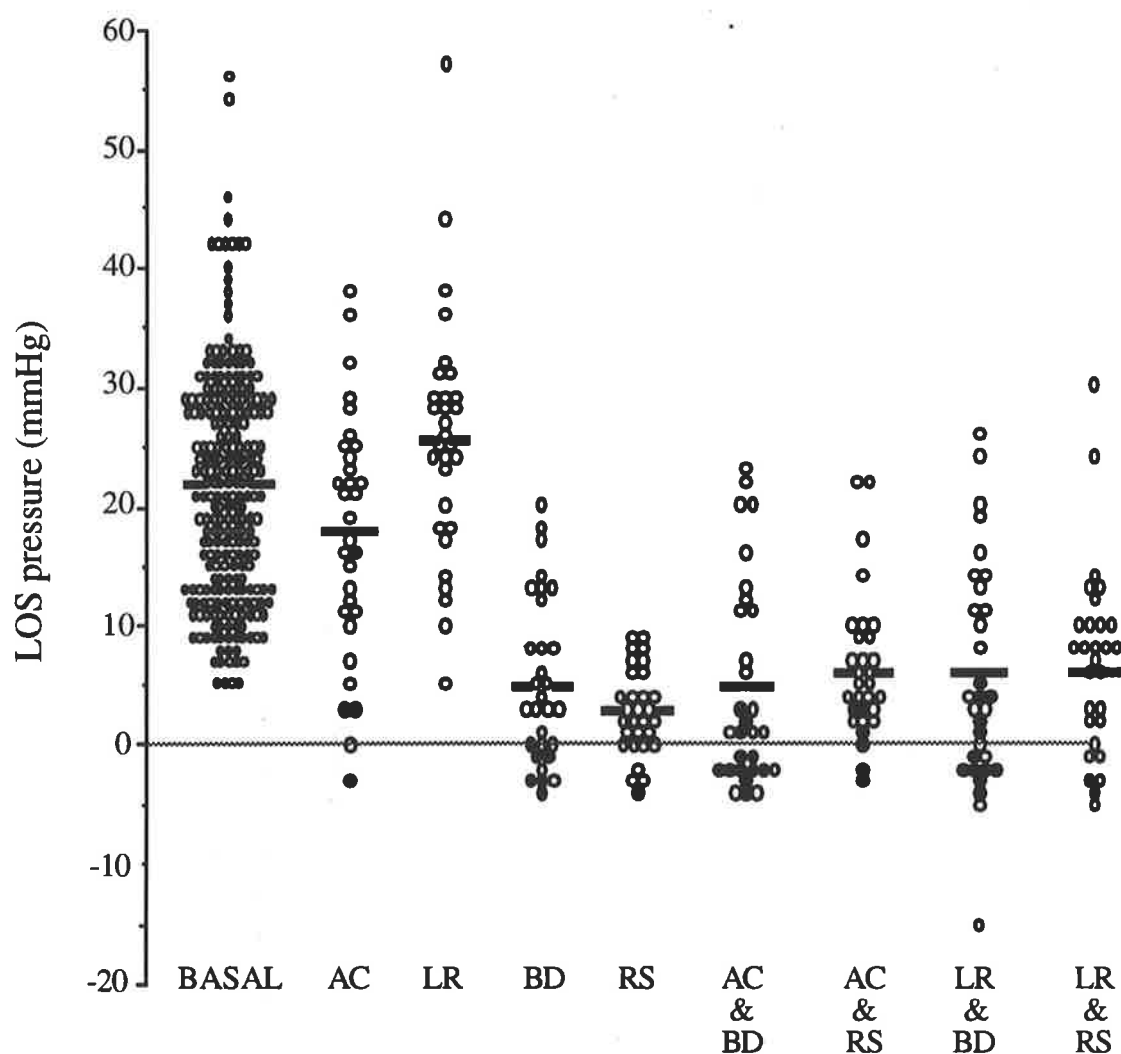


Figure 12.1: Each dot represents lower oesophageal sphincter pressure during each individual manoeuvre for each normal subject. The filled dots indicate the manoeuvres associated with gastro-oesophageal reflux. Mean sphincter pressure, indicated by the horizontal bars, was significantly less than basal during abdominal compression (AC), balloon distension (BD), rapid swallowing (RS) and unchanged during leg raising (LR). During the combined manoeuvres, there was also a significant reduction in sphincter pressure compared to basal.

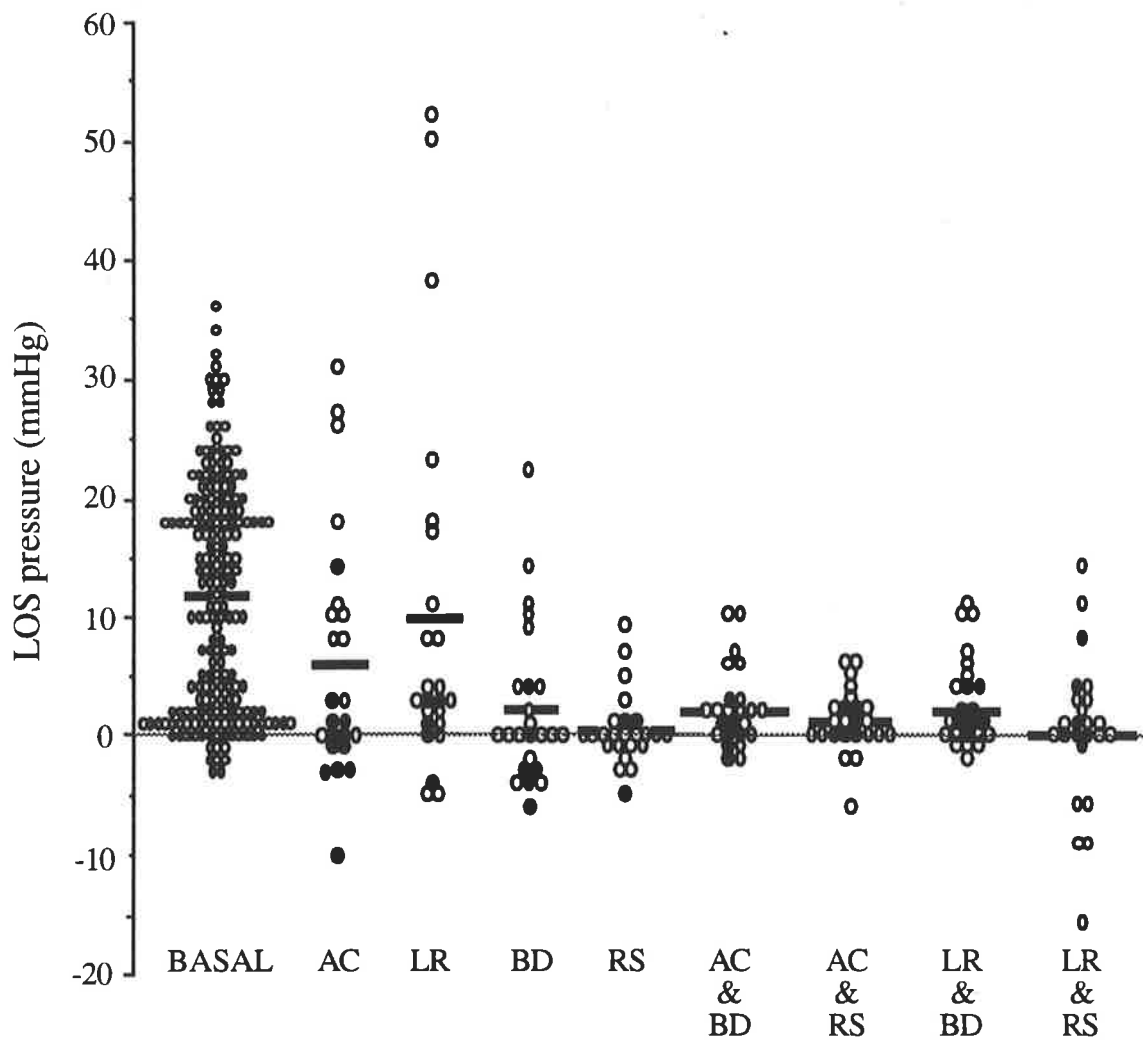


Figure 12.2: Each dot represents lower oesophageal sphincter pressure during each individual manoeuvre for each patient with a hiatus hernia. The filled dots indicate the manoeuvres associated with gastro-oesophageal reflux. Mean sphincter pressure, indicated by the horizontal bars, was significantly less than basal during abdominal compression (AC), balloon distension (BD), rapid swallowing (RS) and unchanged during leg raising (LR). During the combined manoeuvres, there was also a significant reduction in sphincter pressure compared to basal.

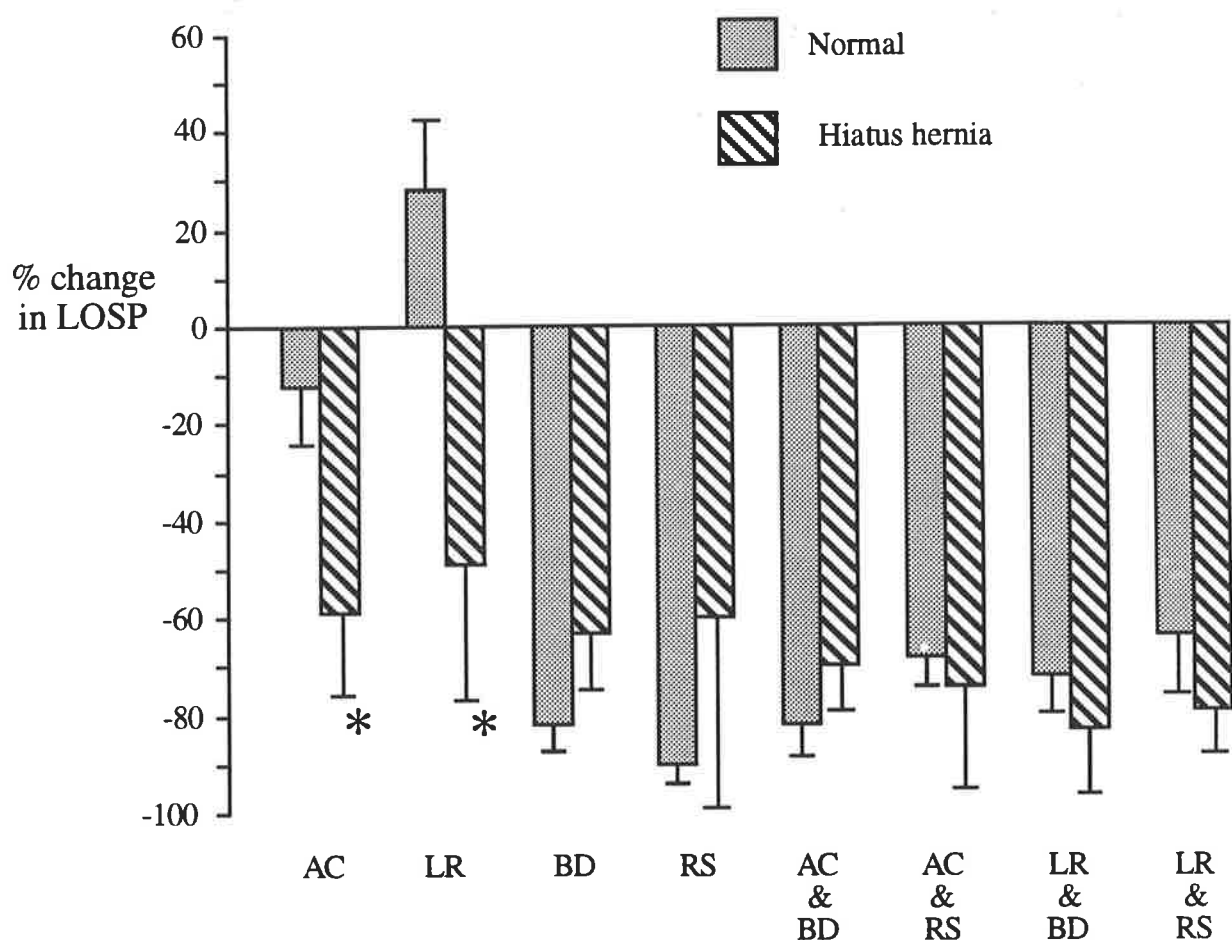


Figure 12.3: The change in LOS pressure during the various manoeuvres compared to basal sphincter pressure. Data are expressed as the mean percentage change in LOS pressure and the error bars indicate standard error. During abdominal compression, lower oesophageal sphincter pressure is reduced in both groups but the reduction is significantly greater in the patients with hiatus hernia (* $p < 0.05$). During leg raising, sphincter pressure increases slightly in the normal subjects but is reduced in the patients with hiatus hernia. This difference was also significant (* $p < 0.05$). During balloon distension, rapid swallowing and the combined procedures, sphincter pressure is reduced by a comparable percentage in both groups.

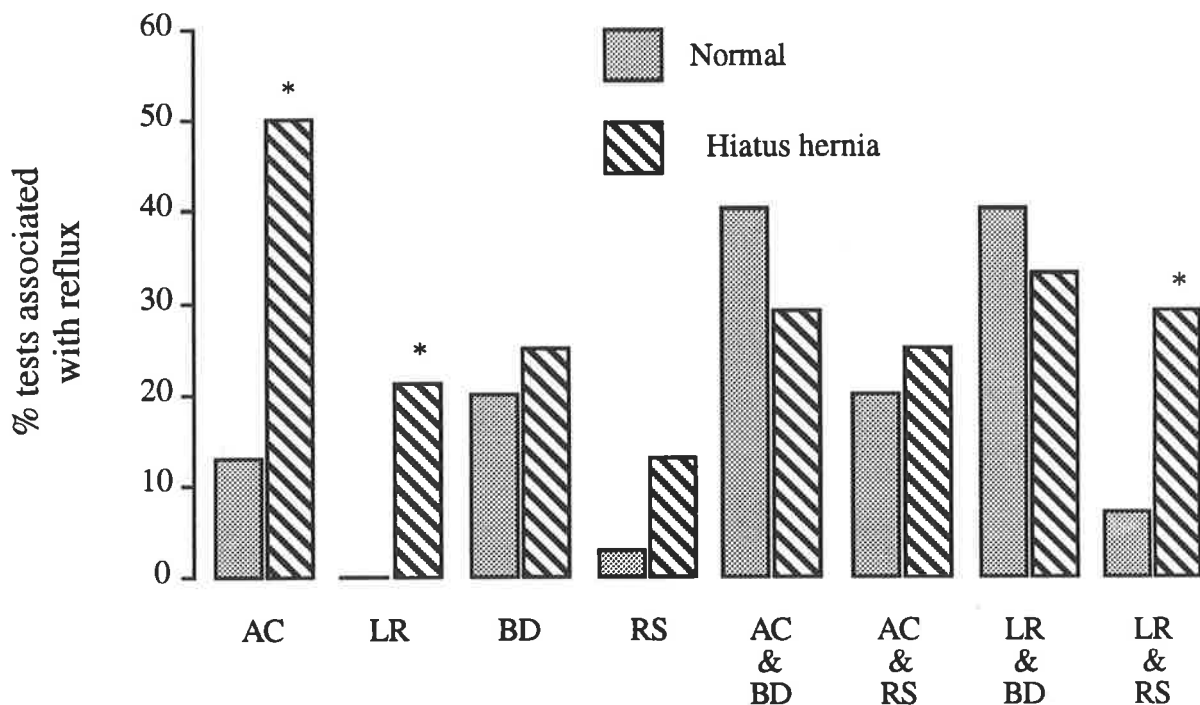
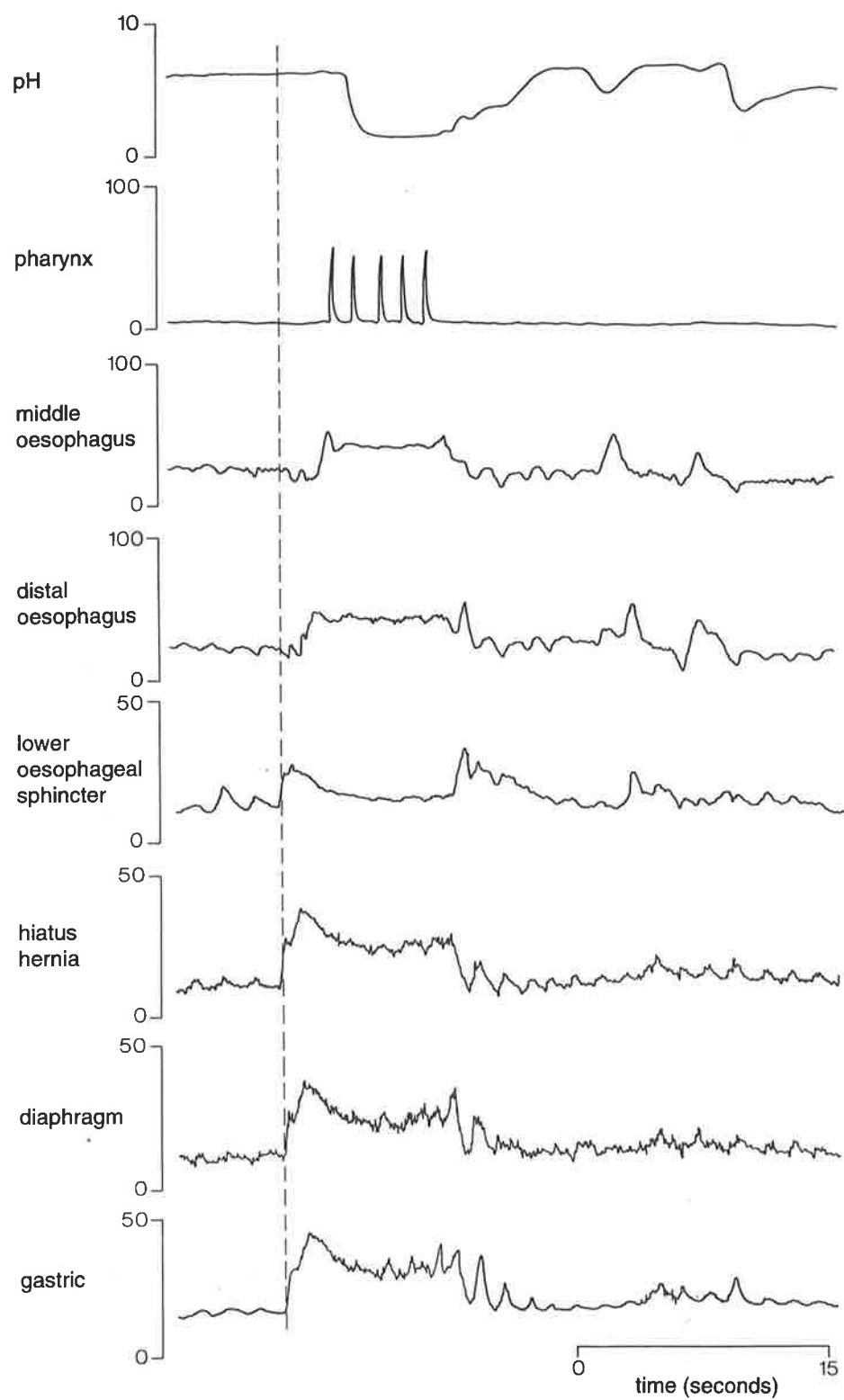


Figure 12.4: The number of manoeuvres associated with reflux are expressed as a percentage of the total number of tests performed. Abdominal compression, leg raising and the combination of leg raising and rapid swallowing resulted in significantly more reflux in the patients with hiatus hernia than in the normal volunteers. (* $p < 0.05$ cf normals)



Appendix

The material in this thesis formed the basis for the publications listed below:

Original Articles

Schoeman MN, Holloway RH. Stimulation and characteristics of secondary oesophageal peristalsis in normal subjects. *Gut* 1994; 35: 152-158.

Schoeman MN, Holloway RH. Integrity and characteristics of secondary peristalsis in patients with gastroesophageal reflux disease. Accepted for publication *Gut* 1994.

Schoeman MN, Holloway RH. Integrity and characteristics of secondary peristalsis in patients with non-obstructive dysphagia. Accepted for publication *Gut* 1994.

Schoeman MN, Akkermans LMA, Tippet M, Dent J, Holloway RH. Mechanisms of reflux in ambulant healthy volunteers. Accepted for publication *Gastroenterology* 1994.

Schoeman MN, Holloway RH. Lower oesophageal sphincter responses to oesophageal distension. Submitted for publication 1994.

Schoeman MN, Tippet M, Dent J, Holloway RH. Effect of hiatus hernia on lower Oesophageal sphincter pressure and gastro-oesophageal reflux during straining. Submitted for publication 1994.

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