Developments in the technique of sphincter of Oddi manometry
and investigation of sphincter of Oddi dysfunction

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by

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Medicine and Dentistry
This thesis is dedicated to my wife, Sarah.
ABSTRACT

The hazardous technique of endoscopic manometry precludes the investigation of “normal volunteers” required to advance our knowledge of the physiology and pathophysiology of the sphincter of Oddi, a suitable animal model is required. Large and small animal models have been proposed, as yet no one model has been accepted as being representative of the human sphincter of Oddi. Furthermore no animal model of sphincter of Oddi dysfunction has been developed.

In this thesis a porcine animal model of sphincter of Oddi function has been developed. The importance of selecting the appropriate anaesthetic agent, enflurane, has been proven. The effect of cholecystectomy on the porcine sphincter of Oddi is shown to have no overall significant effect on sphincter motility when compared to a sham laparotomy group. However, two of the seven pigs after cholecystectomy showed a paradoxical rise in sphincter basal pressure after cholecystokinin infusion, these animal may represent porcine sphincter of Oddi dysfunction.

Although substance P is found throughout the intestinal tract including the sphincter of Oddi of man and pig its action was hitherto unknown. In this thesis exogenous substance P was shown to stimulate the sphincter of Oddi in vivo.

In this thesis the first development in sphincter of Oddi manometry catheter design in nearly twenty years is presented. A superior nine
lumen catheter has been evaluated in porcine model and subsequently used to assess sphincter of Oddi asymmetry in man.

Two retrospective studies are reported in this thesis; an audit of the largest U.K. series, and a study assessing the relationship of sphincter of Oddi motility and duodenal activity. Tachyoddia dissociated from the duodenal migrating motor complex was associated with a raised sphincter of Oddi basal pressure and may be a part of sphincter of Oddi dysfunction.
ACKNOWLEDGEMENTS

I am indebted to Dr David Evans who supervised the production of this thesis from the time of inception to completion. In addition to helping formulate the ideas for the project, he had given unending support and freely made available the use of the facilities within the department.

I am also deeply indebted to Dr Colin Ainley who along with Dr Evans pioneered the technique of sphincter of Oddi manometry at the Royal London Hospital, and without his encouragement and perseverance in teaching me this technique this work would not have been possible.

I would also like to express my appreciation of Tony Price and Grant Stratford (Animal Technicians) for all their work with the animals and especially in their expertise in porcine anaesthesia.

I would like to thank Mr Clive Hepworth who advised me in the surgical procedures required for this project.

I would also like to thank my colleague Dr A Piotrowicz who likewise has waded through this difficult area.

Finally, I would like to thank my wife, Sarah, who has "lived" with this thesis and has managed to give all the support, encouragement and advice where required.
DECLARATION

This work was performed during my tenure of research fellowship in the GI science department at the Royal London Hospital between April 1996 and April 1998.

I performed all the surgical procedures and measurements in the development of the porcine animal model of sphincter of Oddi function and dysfunction.

With the help and advice of Dr D Evans the new nine lumen catheter was developed and produced by Mediplus, U.K..

I was assisted by Dr A Millar in collating the audit data of sphincter manometries performed at the Royal London Hospital.

I believe that this work represents a new contribution to medical knowledge. The work in this thesis has not already been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed

Dr E.A.Stoner MB. ChB. M.R.C.P.
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INTRODUCTION

1.1 Historical Aspects

Francis Glisson was the first to describe the sphincter choledocus in 1681 (Hendrickson 1989, Van Gulik 1990). However this sphincteric muscle was later named in 1887 after the Italian physiologist Dr. Ruggero Oddi who was the first to demonstrate its presence in several animals and to measure and document its action, to resist bile outflow from the bile duct into the duodenum.

1.2 THE SPHINCTER OF ODDI IN MAN

1.2.1 Embryology

The liver begins its development during the third week of gestation as an outgrowth of the endodermal epithelium at the distal end of the foregut. It is initially known as the liver bud or hepatic diverticulum. The common bile duct develops by the narrowing of the connection between the foregut and the liver bud. The gallbladder and cystic ducts develop as outgrowths from the ventral aspect of the bile duct.

The pancreas starts to develop at about day 30 as two endodermal buds on from the dorsal mesentery, the dorsal pancreatic bud, and one close to the bile duct the ventral pancreatic bud. The duodenum
rotates to become C-shaped and at this time the ventral pancreatic bud migrates dorsally and finally the ventral bud comes to lie immediately below and behind the dorsal bud. During the sixth week of development the dorsal and ventral parts fuse. The ventral bud forms the uncinate process and the inferior part of the head of the pancreas. The remaining part of the gland is derived from the dorsal bud. The main pancreatic duct (of Wirsung) forms from the distal part of the dorsal pancreatic duct and the entire ventral pancreatic duct. The proximal part of the dorsal pancreatic duct forms the accessory duct (of Santorini).

The sphincter of Oddi is formed at the site where the bile duct and pancreatic ducts enter the duodenal wall.

1.2.2 Anatomy

The biliary and main pancreatic ducts enter the duodenum at the Ampulla of Vater located in the second part of the duodenum in man. At autopsy the three commonest anatomical variations of the ampulla of Vater are (Plate A), the Y-shaped orifice (61.2%) with the pancreatic and bile ducts joining to form a long common channel ending in a single orifice, the U-shaped where the pancreatic and bile ducts remain separate with separate orifices (22.4%) and the V-shaped (14.3%) with a short common channel (Flati et al 1994). There are three distinct areas of muscle thickening: the sphincter choledochus
Plate A: schematic presentation of the anatomical variations found in the Ampulla of Vater (Morganroth et al 1991)

A: The V-shaped orifice neighboring ducts joining to form a short common channel

B: The U-shaped orifice with the ducts remaining separate.

C: The Y-shaped orifice with the neighboring ducts forming a longer common channel.
around the common bile duct, sphincter pancreaticus around the pancreatic duct, and sphincter ampullae around the Ampulla of Vater together these constitute the sphincter of Oddi.

When there is incomplete fusion of the dorsal and ventral ducts during development, the main pancreatic duct develops from the whole of the dorsal duct and drains into the duodenum via the accessory sphincter, this is known as pancreas divisum. This is the most common congenital abnormality and has been reported in 3.4-12.9% of patients undergoing endoscopic retrograde pancreatography (Gregg 1977, Mitchell et al 1979, Cotton 1980 and Bernard et al 1990). However as these patients represent a selective group, the true incidence in the population not undergoing any upper abdominal investigation is unknown.

1.2.3 Neuro-humoral control

The biliary tract is subject to both neuronal and hormonal control. The latter predominates in most species. More is known about the hormonal control of the sphincter of Oddi especially in animals, but our knowledge is far from complete in man.

Cholecystokinin appears to be the most important hormone regulating sphincter of Oddi and gallbladder function. The action of cholecystokinin
differentiates species into two groups, type 1: species with a pump like sphincter of Oddi which show increased activity to expel bile in response to cholecystokinin and type 2: species with a resistor like sphincter of Oddi which relax in response to cholecystokinin allowing bile flow. Historically authors disagreed on the action of cholecystokinin in man (fig. 1), however it is now taken that its action is to relax the sphincter (Toouli et al 1982, Tokunaga et al 1993).

The gallbladder and sphincter of Oddi are parasympathetically innervated by the vagus nerve, sympathetic innervation originates in the coeliac ganglion and spinal routes T7 to T10 (Becker et al 1993, Becker 1993). Evidence, based on animal studies in the Australian possum (Takahashi et al 1988) (a type 1, pump-like sphincter), has shown that vagotomy does not alter resting activity or the synchronization of sphincter phasic activity with the duodenal migrating motor complex. Vagotomy does lead to an alteration in the response to a meal, with a resulting reduction in sphincter of Oddi spike activity and diminished gallbladder emptying in the possum. Vagotomy also results in a more marked response to intravenous cholecystokinin-octapeptide and motilin.

There is also evidence that the right and left thoracic branches of the vagus may have differing actions (Funch-Jensen et al 1981). Funch-Jensen found that in dogs electrostimulation of the right and left branches of the thoracic vagus increased bile flow, by relaxing the
sphincter of Oddi and contracting the gallbladder. Stimulation of the right vagus alone produced a more complex response, the initial inhibition of the sphincter of Oddi was followed by an increase in motor activity. Although animals studies suggest an important role for the vagus in sphincter control the action of the vagus in man has not been fully elucidated.

Sympathetic innervation originates in the coeliac ganglion and spinal routes T7 to T10 (Becker et al 1993). Noradrenaline acts on pre and postsynaptic receptors as an inhibitory neurotransmitter in the guinea-pig sphincter of Oddi. The action in man is again unknown.

Alterations in gallbladder or duodenal distension lead to changes in sphincter of Oddi motility thus suggesting that direct local neural connections between the gallbladder, sphincter of Oddi and duodenum may exist (Saccone et al 1994, Simula et al 1997). Electrical stimulation of the duodenal ampulla provokes gallbladder contraction and this effect can be abolished by transection of the bile duct (Becker et al 1993).
**Fig. 1** The action of Intestinal Hormones on the Sphincter of Oddi

*Sarles 1986*

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<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>0</td>
<td>↓ or 0</td>
</tr>
<tr>
<td>In vivo</td>
<td>↑ or ↓</td>
<td>↑ or ↓</td>
<td>↓</td>
<td>0</td>
<td>↓ or 0</td>
</tr>
<tr>
<td>Man</td>
<td>↑ or ↓</td>
<td>↑ or ↓</td>
<td>↓</td>
<td>↑?</td>
<td>↓ or 0</td>
</tr>
</tbody>
</table>

**Key:**
- ↑ contraction of the sphincter of Oddi
- ↓ relaxation of the sphincter of Oddi
FIG. 2 IMMUNOREACTIVITY OBSERVED IN SMOOTH MUSCLE LAYER OF SPHINCTER OF ODDI IN SUBJECTS WITH NO PANCREATEOBLIARY DISEASES AND IN PATIENTS WITH GALLSTONE DISEASE, PANCREATITIS, OR PERIAMPULLARY CARCINOMA (SAND ET AL 1994)

<table>
<thead>
<tr>
<th></th>
<th>No Pancreatobiliary diseases</th>
<th>Organ donors (n=5)</th>
<th>Autopsies (n=3)</th>
<th>Gallstone disease (n=5)</th>
<th>Periampullary Carcinoma without gallstones (n=3)</th>
<th>Pancreatitits (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PHI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NPY</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CGRP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galanin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sub P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enkephalin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bombesin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: +, immunoreactivity observed
- , no immunoreactivity observed.
Anatomical evidence of direct pathways between the gallbladder and duodenum and the gallbladder and the sphincter of Oddi have been shown using retrograde labeling (Mawe et al 1997).

Immunohistochemical studies have located neural networks connecting the duodenum with the gallbladder and the sphincter of Oddi, and the sphincter of Oddi with the gallbladder (Padbury et al 1993, Simula et al 1997, Simula et al 1997). This implies that enteric nerve circuits participate in coordinating duodenal and biliary functions.

Many peptide neurotransmitters have been elucidated on immunohistochemical staining of the sphincter of Oddi (fig 2), however their action is unknown. Recently the non-adrenergic-non cholinergic transmitter, nitric oxide has been a source of much interest (Allescher et al 1993, Kaufman et al 1993, Baker et al 1993, Mourelle et al 1993, Manci et al 1995, Thune et al 1995, Konturek et al 1995, Altdorfer et al 1996, Sand et al 1997 and Shima et al 1998). The sphincter of Oddi in both pigs and humans have endogenous nitric oxide synthetase activity and immunoreactivity. Inhibition of endogenous nitric oxide production, enhances contractility while exogenous nitric oxide decreases sphincter contractility and electrical activity (Sand et al 1997). In view of this effect, glycerl-trinitrate has been used as a nitric oxide donor to aid cannulation of the biliary ampulla at endoscopic retrograde cholangiography (Luman et al 1997) and has also been
suggested as a therapeutic option in the treatment of sphincter
dyskinesia (Staritz et al 1985 and Velosy et al 1997).

1.2.4 Function

The role of the sphincter of Oddi is to regulate the flow of bile and pancreatic juice into the duodenum.

Bile is produced by the liver and is an isotonic aqueous mixture consisting mainly of electrolytes, proteins, bile salts, cholesterol, phospholipids, and bilirubin. Secretion across the canalicular membrane of the hepatocyte accounts for about two thirds of total bile flow. The remaining one third is an alkaline fraction generated by the epithelial cells lining the bile ducts. The total volume of bile produced daily is between 500 and 800 mls. During the fasting phase bile is stored in the gallbladder, with a capacity of 60 millilitres. The gallbladder also acts to concentrate bile salts by the rapid and selective absorption of fluid and electrolytes. The average bile salt content is a mixture of chenodeoxycholate, cholate, deoxycholate, ursodeoxycholate and lithocholate. Bile salts aid fat absorption in the small intestine by forming micelles. 95% of bile salts are reabsorbed throughout the intestine, bound to albumin in the portal blood and then removed by hepatocytes, this constitutes the enterohepatic circulation.
The sphincter of Oddi in man acts as a resistor to bile flow and assists in the collection of bile in the gallbladder and also prevents the reflux of duodenal contents into the biliary pancreatic ducts. In response to a meal, a combination of parasympathetic innervation via the vagus nerve and the humoral effect cholecystokinin leads to contraction of the gallbladder and relaxation of the sphincter of Oddi occurs, propelling bile into the duodenum.

1.3 SPHINCTER OF ODDI DYSFUNCTION

1.3.1 Background

It was Dr. Ruggerio Oddi himself who first recognized that the sphincter of Oddi might be responsible for clinical problems “It is possible that the sphincteric function of the distal choledochal musculature explains some clinical problems that are still obscure....” (Oddi 1887). To this day the clinical syndrome remains poorly defined, incompletely understood and difficult to diagnose.

A number of terms have been used to describe the same clinical entity e.g. papillary stenosis, sclerosing papillitis, biliary spasms, biliary dyskinesia and postcholesyctectomy syndrome. Sphincter of Oddi dysfunction is now regarded as falling into two types, sphincter of Oddi stenosis; an abnormal degree of narrowing of the sphincter
of Oddi canal and sphincter of Oddi dyskinesia; abnormal motility of the sphincter of Oddi, the present diagnostic criteria being a raised basal pressure (> 40mmHg) (Lans et al 1991). Since Oddi's description clinicians have attempted to elucidate the relationship between the sphincter of Oddi and clinical conditions suggestive of sphincter of Oddi dysfunction. Currently sphincter of Oddi dysfunction has been implicated in three clinical conditions post cholecystectomy pain syndrome, pancreatitis and right upper quadrant pain with the gallbladder in situ.
1.3.2 Post cholecystectomy pain syndrome

Cholecystectomy is the commonest abdominal operation performed in the United Kingdom and the United States of America. The introduction of laparoscopic techniques in the late eighties' led to a dramatic rise in the number of cholecystectomies being performed per annum (Lam et al 1996) (fig. 3).

Fig 3 The cholecystectomy rate in Scotland 1977-1993 (Lam et al 1996)
Hospital admission data for England shows that there were 35,772 patients admitted for cholecystectomy in 1994 compared to 19,617 in 1992. A number of studies have looked at the symptomatic outcome after cholecystectomy (Stefani et al 1974, Ros et al 1987). Stefani’s group retrospectively assessed the outcome of 800 of the 1,716 cholecystectomies performed at their centre between 1960 and 1973. The male to female ratio was 1:2 and the age range was 13 to 84 years. A good result from cholecystectomy with maintained symptomatic relief of their right upper quadrant pain was achieved in 68.9% whilst 31.1% continued to have symptoms postcholecystectomy. The prospective study by Ros et al 1987 reported 46% of patients continued to be symptomatic 2 years after cholecystectomy. The reported outcome from laparoscopic cholecystectomy is somewhat better with 77-95% of patients obtaining satisfactory symptom relief (Peters et al 1991, Velpen et al 1993, McMahon et al 1995, Luman et al 1996). The commonest post cholecystectomy symptoms include early satiety, post-prandial pain, flatulence, nausea, eructation and fatty food intolerance. Between five and thirty percent of patients with postcholecystectomy pain syndrome are thought to have sphincter of Oddi dysfunction (Bar-Meir et al 1984, Brandstatter et al 1991).

As with gallbladder disease post cholecystectomy pain syndrome is more common in females, 2: 1 females: males (Donovan 1996). There is a significant impact on quality of life with an associated impact on
degree of absenteeism from work, disability and healthcare demands (Drossman et al 1993).

Patients with postcholecystectomy syndrome have been classified into three types depending on their clinical presentation, the Milwaukee classification (Hogan et al 1987) (fig. 4).

**Fig. 4 The Milwaukee Classification (Hogan et al 1987)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Biliary Pain</th>
<th>Liver Biochemistry</th>
<th>Common Bile duct Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Abnormal</td>
<td>Increased</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Abnormal/ Normal</td>
<td>Increased/ Normal</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

The types are differentiated on the basis of the presence of biliary pain with or without abnormal liver biochemistry and or common bile duct dilatation. This classification is not only useful in indicating which patients warrant further investigation but also for comparing the outcome from different therapeutic techniques. The common feature for types 1, 2 and 3 is the presence of biliary type pain. Biliary pain has been defined by a panel of experts at the National Institutes of Health Consensus Conference on Laparoscopic Cholecystectomy (1993).
The characteristics of the pain are:

1. It is severe, steady right upper quadrant or epigastric pain which is gradual in onset and lasts 2-5 hours.
2. It customarily exceeds 3 month's duration and a single episode rarely lasts more than 24 hours.
3. It is associated frequently with nausea, sweating and restlessness. The patient may walk about during periods of distress in an effort to gain relief.
4. It occurs predominantly after meals and at intervals of weeks to years.
5. That it may awaken the patient, periodically, from sleep in the early morning hours.

Sphincter stenosis results in the triad of symptoms found in type 1 and therapeutic sphincterotomy is often performed without further investigation. In types 2 and 3 further investigation is warranted to confirm or exclude the diagnosis of sphincter of Oddi dysfunction Lans et al 1991, Hogan et al 1997).
1.3.3 Pancreatitis

In Western countries the most common known causes of acute pancreatitis are an excessive intake of alcohol or the presence of gallstones (Ranson 1982). In a significant proportion (10-40%) the cause is not found and these cases are classified as idiopathic pancreatitis (Ranson 1982). In idiopathic recurrent pancreatitis, sphincter of Oddi dysfunction has been found to be present and assumed to be the causal factor in a proportion of patients (figures 5 and 6).

**Fig. 5 Sphincter of Oddi manometry in patients with acute recurrent idiopathic pancreatitis.**

<table>
<thead>
<tr>
<th>Author (ref)</th>
<th>No. of Patients</th>
<th>No. with SO dysfunction (%)</th>
<th>Good outcome to Treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raddawi et al 24</td>
<td>7(29)</td>
<td>-</td>
<td>1991</td>
</tr>
<tr>
<td>Sherman et al 168</td>
<td>89(53)</td>
<td>-</td>
<td>1991</td>
</tr>
<tr>
<td>Toouli et al 28</td>
<td>25(89)</td>
<td>-</td>
<td>1985</td>
</tr>
<tr>
<td>Toouli et al 26</td>
<td></td>
<td>88</td>
<td>1985</td>
</tr>
<tr>
<td>Venu et al 116</td>
<td>17(15)</td>
<td>94</td>
<td>1989</td>
</tr>
</tbody>
</table>
Fig. 6 Various aetiological factors identified in patients with idiopathic recurrent pancreatitis
Pancreatic pain differs from biliary pain in that it is constant severe pain which is located in the epigastric and left upper quadrant pain, or the entire abdomen and in 50% of patients it radiates to the back and occasionally to the left anterior chest wall, left shoulder, or the lower abdomen. In pancreatitis the pain in the lower abdomen is believed to be due to pancreatic exudate spreading via the transverse mesocolon to the caecum along the left colon. The pain is usually more intense in the upper than the lower abdomen (Banks PA 1979, Banks PA 1986).

Venu et al 1989 investigated 116 patients with idiopathic recurrent pancreatitis. Forty-four (38%) were found to have a demonstrable cause for their pancreatitis as shown in table F. Seventeen (15%) were found to have sphincter of Oddi dysfunction as characterized by a significantly elevated basal pressure at manometry. Ten of the seventeen had a paradoxical response to cholecystokinin-octapeptide provocation (a rise in basal sphincter of Oddi pressure). Treatment was undertaken in sixteen of the seventeen (12 with endoscopic sphincterotomy and four operative sphincterotomy). These sixteen remained symptom free for a period of 36 ± 4 months. Toouli et al 1996 reported a prospective study in a selected group of patients with idiopathic recurrent pancreatitis due to sphincter of Oddi dysfunction. Over a ten year period thirty-five patients were found to have sphincter of Oddi dysfunction at manometry following the exclusion of common causes of pancreatitis. A total of twenty-six underwent operative sphincteroplasty and septoplasty (21 as
initial therapy, 2 after failure of conservative therapy and 5 after failure of endoscopic sphincterotomy). Patients were followed-up for a median of twenty-four months (range 9-109) and fifteen (58%) patients reported no further symptoms and were deemed as being cured, eight (31%) patients had only mild symptoms and were treated with mild analgesics. three (11%) patients had no improvement in their symptoms. It was concluded from this study that division of the sphincter of Oddi is associated with good symptomatic outcome in patients with recurrent episodes of pancreatitis and documented sphincter of Oddi dysfunction.

Patients with pancreatic sphincter dysfunction have been classified in a similar way to the Milwaukee classification by Sherman et al 1991 (fig.7).

**Fig 7 THE CLASSIFICATION OF PANCREATIC TYPE SPHINCTER OF ODDI DYSFUNCTION**

<table>
<thead>
<tr>
<th>Type</th>
<th>Pancreatic pain</th>
<th>Recurrent Pancreatitis</th>
<th>Dilated Pancreatic Duct</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No/ Yes</td>
<td>No/ Yes</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

It is not clear how sphincter of Oddi dysfunction leads to intermittent recurrent episodes of pancreatitis. It is possible that a rise in main
pancreatic duct pressure leads to pancreatic secretions being forced back into the pancreatic acini in a similar way that acinarization occurs after contrast is over injected into the pancreatic duct at endoscopic retrograde pancreatography. Alternatively increased tissue pressure due to increased intraductal pressure may lead to intra-acinar cell activation of digestive zymogens which are believed to be the earliest events in the evolution of acute pancreatitis. These enzymes, once activated, cause acinar cell injury (Saluja et al 1989, Grady et al 1996).

The commonest cause of chronic pancreatitis is the excessive intake of alcohol, the increase in cases of chronic pancreatitis in industrialized countries parallels a marked increase in alcohol consumption (Worning 1990). There is a linear relation between alcohol consumption and the logarithmic risk for chronic pancreatitis (Durbec et al 1978). As well as alcohol exerting a damaging effect directly on the gland, it also acts on the sphincter of Oddi. Studies using T-tubes have shown that intragastric (Capitane et al 1971) and intravenous (Pirola et al 1968) alcohol increases sphincter of Oddi tone. A more recent study using a perfused catheter looked at the direct effect of alcohol (3mls of 40% alcohol) on the sphincter and showed a significant increase in basal pressure without a change in the phasic activity (Guelrud et al 1991). An abnormal sphincter response to secretin (a rise in basal sphincter pressure) has been found in patients who chronically abuse alcohol with a rise in main pancreatic duct and sphincter pressures (Bradley et al 1982). It is therefore possible that the direct effect of alcohol on the
sphincter of Oddi may be a significant factor contributing to the development of chronic pancreatitis (Bradley et al 1982). In up to 20% of cases of chronic pancreatitis there is no causal factor found, and it is possible that pancreatic sphincter dysfunction may be responsible for precipitating a proportion of these cases. In a recent study (Fogel et al 1997), 102 patients with chronic pancreatitis in whom 56% had undergone a prior sphincterotomy of either or both ducts were investigated. The frequency of sphincter of Oddi dysfunction was 62% in the patients classified as having mild chronic pancreatitis, 27% in those patients with moderate chronic pancreatitis and 27% in those with severe chronic pancreatitis.

There have been several studies suggesting that the pain of chronic pancreatitis may be a result of increased pancreatic ductal pressure in some patients (Bradley et al 1982, Carr-Locke et al 1985 and Ebbehoj et al 1990). This has been supported by the finding that surgical decompression of the pancreatic duct in these patients is effective in relieving abdominal pain and that pain relief is associated with normalization of the pancreatic duct pressure.

In summary, these studies all suggest that sphincter of Oddi dysfunction may play a part in the pathogenesis of chronic pancreatitis whether as a direct effect or one mediated by the chronic abuse of alcohol. However there is, as yet, only preliminary data to support this
supposition and these effects may prove to be a secondary phenomenon.

1.3.4 Right upper quadrant pain with the gallbladder in situ

The presence and the role of sphincter of Oddi dysfunction with the gallbladder in situ is even more controversial. Two small studies (Choudhury et al 1993 and Catalano et al 1991) treated patients with right upper quadrant pain with intact gallbladders and elevated sphincter of Oddi basal pressures with endoscopic sphincterotomy. Both studies described good initial pain response rates (77\% and 83\%, respectively) but there was a high relapse rate in both studies and a high incidence of procedure-related pancreatitis. A more recent study (Catalano et al 1998) again with only limited numbers but stratified according to the Milwaukee classification reported a more favorable outcome initially, but at final follow-up, 46.4 months (range 10-111 months), the outcome was less favorable with resolution of symptoms in only 54\% of type 2 and 36\% of type 3 patients.

It has also been noted that abnormalities of gallbladder and sphincter of Oddi motility may occur concomitantly, though there is no clear-cut common aetiological factor (Ruffalo et al 1994). Toouli et al 1986 compared sphincter of Oddi motility in patients undergoing elective cholecystectomy for gallstones with a control group. In the control group, their sphincter of Oddi exhibited predominantly antegrade.
contractions whereas the sphincter of Oddi of patients undergoing cholecystectomy had a predominance of retrograde contractions. This suggests that a primary sphincter of Oddi motility disorder has resulted in the development of gallstones or that the presence of gallstones has led to sphincter dysmotility.

Gallbladder dyskinesia is equally as controversial a subject as sphincter of Oddi dysfunction. It is possible that the problem with patients with right upper quadrant pain and an intact gallbladder in the absence of stones still lies with the gallbladder rather than the sphincter of Oddi and is due to gallbladder dyskinesia (also known as acalculous cholecystitis). This diagnosis is difficult to make as it has been found that 66% of patients diagnosed as having acalculous cholecystitis by ultrasound are in fact found to have stones on further imaging (Ekberg et al 1991). There has also been growing recognition of the clinical importance of biliary microcrystals. The standard diagnostic modality was cholecystography after an intravenous bolus of cholecystokinin. The finding of reduced gallbladder contractility being indicative of gallbladder dyskinesia, however this test has a rather mixed reputation due to the inherent subjectivity. (Goldstein et al 1974, Berk et al 1977, Davis et al 1982, Rhodes et al 1988). More recently a quantitative method of measuring the gallbladder ejection fraction by DISHIDA (Digital subtraction imaging HIDA scan (99mTc-labelled dimethyl-acen tanilide iminodiacetic acid)) with concurrent cholecystokinin injection, quantitative cholecintigraphy, has been developed (Yap et al 1991). Yap et al's
randomized study of eighty-seven patients with chronic right upper quadrant pain, twenty-six were found to have an abnormal gallbladder ejection fraction (less than 40%±3 SD). These twenty-six patients were subsequently randomized to operative or conservative therapy. All thirteen patients with abnormal ejection fractions randomized to cholecystectomy had confirmed histological evidence of chronic cholecystitis and these patients did significantly better than those patients treated conservatively. The remaining fifty-nine patients with normal ejection fractions showed no significant or predictable outcome difference whether treated by cholecystectomy or conservatively. There are other studies to support quantitative cholescintigraphy (Peng et al 1994, Ruffolo et al 1994) however, there has been at least one study questioning its efficacy (Westlake et al 1990). A single study has shown that acalculous cholecystitis, assessed by quantitative cholescintigraphy, and sphincter of Oddi dysfunction, assessed by sphincter of Oddi manometry, may coexist (Pasricha et al 1993). Of the twenty-five patients studied with right upper quadrant pain and intact gallbladders, five patients were found to have decreased gallbladder ejection fractions and had acalculous cholecystitis demonstrated at cholecystectomy. Five patients were found to have raised basal sphincter of Oddi pressures and symptoms resolved following sphincterotomy. Two patients were found to have both abnormal gallbladder ejection fractions and raised sphincter of Oddi basal pressures, they were both treated with endoscopic sphincterotomy with satisfactory outcomes.
1.4 METHODS OF MEASUREMENT OF SPHINCTER OF ODDI

FUNCTION AND DYSFUNCTION

Invasive and non invasive methods have been developed to assess sphincter of Oddi function. This section will discuss advantages and disadvantages of each diagnostic modality and compare them against endoscopic manometry (the present "gold standard" method).

Non Invasive

1.4.1 The Morphine-Neostigmine/Prostigmine Provocation test.

The basis of this test is the action of each component, morphine and prostigmine on the sphincter of Oddi. In one subgroup of patients with suspected sphincter of Oddi dysfunction, morphine has been found to aggravate biliary-type pain, particularly after cholecystectomy via its contractile action on the sphincter of Oddi. As long ago as 1936 a study by McGowan reported that morphine given subcutaneously increased common bile duct pressures in patients with a T-tube in situ and this was associated with the onset of abdominal pain. Neostigmine (Knight et al 1959) and morphine (Burke et al 1950) have both been shown to lead to rises in serum amylase in a proportion of patients
with relapsing pancreatitis and biliary type pain. Following these observations the combination of morphine and neostigmine were promoted as a provocation test in patients with abdominal pain due to inflammatory disorders of the sphincter of Oddi (Nardi et al 1966). This test, as first described by Nardi et al 1966, was performed by giving the patient an intramuscular injection of morphine sulphate 10mg and neostigmine 1 mg, followed by four, hourly blood samples for the measurement of serum amylase and lipase. In a positive result a fourfold rise in amylase and lipase was observed along with the development of abdominal pain. Subsequent modifications on this protocol have been made along with inferences to their diagnostic implications (Milano et al 1973, Nardi et al 1974, Raskin et al 1977, Lonmire et al 1977, Gregg et al 1977 and LoGiudice et al 1979). A positive result was suggested as a twofold enzyme rise and a reproduction of abdominal pain (Milano et al 1973, Nardi et al 1974). Other groups have suggested that the enzyme rise alone without the reproduction of pain is of paramount importance (Lonmire et al 1977, Raskin et al 1977). Gregg et al 1977 found that a five fold increase in lipase after a morphine-neostigmine is associated with a difficult papilla to cannulate at endoscopic retrograde cholangiopancreatography due to papillary stenosis. Buntain et al 1986 found this provocation test to be useful in defining the cause of some cases of chronic relapsing pancreatitis in children.
Unfortunately controlled studies have failed to validate the morphine-neostigmine test as a useful clinical tool for the diagnosis of sphincter of Oddi dysfunction (LoGuidice et al 1979 and Steingberg et al 1980). In LoGuidice’s study, endoscopic manometry and operative assessment showed good correlation, but the morphine-prostigmine test failed to correlate with either of these parameters. Moreover three of the six healthy controls also had a significant enzyme rise. Similarly the morphine-prostigmine test was shown to give a significant false positive result in healthy volunteers and failed to provide reproducible results in patients studied by Steinberg et al.

We can conclude from these studies that the morphine-neostigmine test is not a useful clinical tool for the investigation of sphincter of Oddi dysfunction.

1.4.2 Ultrasonographic diagnosis of sphincter of Oddi dysfunction

Simple sonographic measurement of the common bile duct diameter (Thatcher et al 1987) and dynamic measurement studies following a fatty meal (Simone et al 1982) or CCK (Fein et al 1984) have been proposed as methods of diagnosing sphincter of Oddi dysfunction.

After cholecystectomy, a dilated common bile duct detected using ultrasonography has been suggested as a feature of sphincter of Oddi dysfunction that has a positive predictive value in the outcome for
therapy with sphincterotomy (Thatcher et al 1987). However dilatation of
the common bile duct in asymptomatic patients after cholecystectomy is
not an uncommon finding (up to 16%) (Garham et al 1987). It has also
be shown that the common bile duct diameter varies with age (Coehlo
et al 1996). Therefore common bile duct diameter cannot be used as
the sole diagnostic indicator for the diagnosis of sphincter of Oddi
dysfunction.

The dynamic response of the sphincter of Oddi and the resulting
dilatation of the common bile duct is another proposed diagnostic tool.
The fatty meal ultrasound involves the measurement of the common
bile duct diameter by ultrasound 45 minutes after ingestion of a
standard meal of lipomul (1.5ml/lb body weight). In normal patients the
common bile duct diameter remains unchanged following a fatty meal
or intravenous cholecystokinin in spite of the resulting increased bile
output. This is because the sphincter of Oddi relaxes and allows bile
flow into the duodenum. An increase in common bile duct diameter
greater than 2mm resulting from failure of relaxation or possibly
contraction of the sphincter in response to a fatty meal or
cholecystokinin has been observed in patients with sphincter of Oddi
dysfunction (Fein et al 1984). One study of forty-four controls and forty-
seven patients with suspected partial common bile duct obstruction
showed a specificity of 100% and a sensitivity of 74% (Darweesh et al
1988). In this study fatty meal ultrasound correctly identified seven of
the eight cases with manometrically proven sphincter of Oddi
dysfunction. A subsequent study failed to show a significant correlation between fatty meal ultrasound and endoscopic manometry in thirty-one patients with suspected sphincter of Oddi dyskinesia (Milwaukee type 3) (Dean et al 1991). Fatty meal ultrasound may be a reasonable non-invasive dynamic screening test, particularly in patients with right upper quadrant pain and an intact gallbladder.

Dynamic studies have also been used to investigate the pancreatic sphincter (Bolondi et al 1984). The diameter of the pancreatic duct in normals is $1.2 \pm 0.4$ mm. After secretin injection (1CU/Kg to a maximum of 75CU) there is increased secretion from the pancreas and a subsequent increase in pancreatic duct diameter that lasts for less than thirty minutes. If the pancreatic duct remains dilated after thirty minutes (>1.5mm) then it is suggestive of pancreatic outflow resistance. A dynamic test based on this observation has been proposed as a useful screening test to alert the clinician to the possibility of sphincter of Oddi dysfunction (Toouli et al 1996, Tzovaras et al 1998).
1.4.3 Hepatobiliary scintigraphy

Hepatobiliary scintigraphy or cholescintigraphy is performed by computer analysis of the excretion of a radioisotope-labeled tracer (usually $^{99m}$Technetium labelled dimethyl-iminodiacetic acid) using a gamma camera. For regions of interest i.e. the right lobe of the liver, liver hilum and the common bile duct, the computer generates time activity curves. This method has been standardized, and can give quantitative and qualitative information by measuring a significant delay in hepatic uptake and washout (Steinberg et al 1988). Abnormal results suggestive of sphincter of Oddi dysfunction are determined by the elevated hilar time-activity curve and reduced 45 minute clearance value (Fullarton et al 1988). Fullarton et al’s findings showed a statistically significant difference in scintigraphic analysis in patients with sphincter dysfunction (confirmed by endoscopic manometry) compared with asymptomatic controls, and it was proposed that this could be a useful non-invasive screening test for sphincter of Oddi dysfunction, however, subsequent studies have been less convincing (Lisbona et al 1992). The main problem with this technique is the difficulty in differentiating between parenchymal liver disease, intrahepatic cholestasis and extrahepatic bile duct pathology (Steinberg et al 1988, Coehlo et al 1996). This has led to the development of techniques involving prestimulation with cholecystokinin (Sostre et al 1992). Cholecystokinin acts as a functional stimulus to accentuate differences in bile flow rates between a normal post cholecystectomy population and those with
sphincter of Oddi dysfunction. A scoring system has been developed incorporating all the variables previously demonstrated to be of significance in the diagnosis of sphincter dysfunction i.e: time of peak liver activity, time at which the intrahepatic biliary tree is first visualized, prominence or dilatation of the biliary tree, time at which the bowel is first visualized, percentage of common bile duct emptying and common bile duct:liver ratio. This scoring system is superior to previous diagnostic criteria with a sensitivity of 100% and specificity of 100% for the diagnosis of sphincter dysfunction (Sostre et al 1992). This conclusion is limited by the fact that these results were obtained from with small patient (n=12) and control (n=14) populations. No subsequent confirmatory studies have been performed and more importantly this scoring system has not been used to assess its predictive merits in indicating a favourable outcome from therapeutic techniques such as sphincterotomy.

In conclusion modified quantitative Hepatobiliary scintigraphy using cholecystokinin pretreatment may be a suitable non invasive screening test for sphincter of Oddi dysfunction. It usefulness may be limited by the necessary exposure to gamma irradiation.
1.4.3 Endoscopic manometry

Manometry is regarded as the "gold standard" for the evaluation of sphincter of Oddi function. There are two methods, a perfused catheter technique and a microtransducer technique.

1.4.4 Perfused Catheter Technique.

The first description of perfused catheter endoscopic manometry was in 1974 from Germany (Vondrasek et al 1974). Subsequent perfused catheter manometric methods were reported in the literature but their focus was on measuring pressure within the biliary or pancreatic ducts rather than the sphincter of Oddi (Vondrasek et al 1974, Nebel et al 1975, Rolny et al 1991).

The first method for measuring actual sphincter activity was described by Geenen in 1978. Pressure recordings were obtained using two Teflon catheters each of an internal diameter of 0.8 mm, an outer diameter of 1.6 mm and length of 200 cm. A lateral recording orifice was cut into each catheter 3 mm from the end, and the catheter lumen distal to this orifice was sealed with glue. One catheter (the ductal catheter) was introduced into the biliary or pancreatic sphincter via the biopsy channel of a fiberoptic duodenoscope. The ductal catheter was
slowly withdrawn from the bile or pancreatic duct through the respective sphincters, a pull-through technique. The second catheter was attached to the outside of the duodenoscope and continually measured duodenal pressure. Both catheters were perfused with bubble free water at a rate of 0.25ml/min, by a minimally compliant hydraulic-capillary infusion (Arndorfer et al 1977) system driven by a constant reservoir pressure of 750mmHg (Geenen et al 1980, Geenen et al 1995). Pressure measurements were obtained when the sphincter compressed the catheter resulting in partial occlusion of the measuring orifice, the occlusion pressure was transmitted back up through the catheter via the column of perfused water to an external transducer. The external transducer transformed the readings into an electrical signal which was then amplified, displayed and recorded.

Subsequently the commercially made and now widely used Lehman (Wilson-Cook, USA) catheter was developed. This is a triple lumen five french catheter with two measuring ports orientated 180 degrees apart and 2mm apart in distance, the third central channel opens at the catheter tip (Plate B). Water perfusion and pressure transduction is performed as before. The central lumen facilitates decompression of the duct being measured by continual aspiration, this is known as the modified aspiration technique. The modified aspiration technique has been shown significantly reduce the complication rate from procedure related pancreatitis (31% to 4%) without significantly altering the pressure recordings (Sherman et al 1990, Sherman et al 1990).
Plate B: The Lehman triple lumen catheter

The catheter tip marked in 10 2mm sections

The central aspiration channel

The proximal and distal measuring channels

Line drawing of the Lehman catheter tip

The risk of procedure related pancreatitis has been shown to be greatest when the pancreatic duct is cannulated and pressures are recorded from the pancreatic sphincter. High perfusion flow rates is
another independent risk factor for inducing pancreatitis (Staritz et al 1991). The Lehman catheter enables easy differentiation between the ducts cannulated, the presence of bile as the aspirate confirms that the biliary sphincter is being assessed whereas clear aspirate indicated pancreatic duct cannulation. Thune et al 1991 and others (Geenen et al 1989, Smithline et al 1993, Guelrud et al 1990) have confirmed that this technique gives reproducible results.

The "pull-through" technique (through ten 2mm apart stages) has now been standardized, two recordings are made to demonstrate the sphincter pressure profile and to localize the point of peak basal pressure for subsequent pressure recordings and an additional pharmacological challenge with cholecystokinin. (Hogan et al 1997).

Computers rather than chart recorders are now used to capture and display the output from the pressure transducers. Software is commercially available for performing oesophageal manometry and these programs can be easily adapted to perform sphincter of Oddi manometry. An example of a typical normal recording taken from our own laboratory is shown in Plate C. Duodenal pressure is shown by channel 1 and the proximal and distal ports of the manometry catheter are displayed as channel 2 and 3 respectively.
Plate C: A "normal" sphincter of Oddi manometry recording from our laboratory.
The sphincter of Oddi pressure is denoted by the rise above the bile duct baseline in the proximal port (channel 2), followed by the distal (channel 3) port 2mm later. Regular phasic activity can be seen as the catheter is withdrawn. The sphincter of Oddi basal pressure is calculated from the net of the sphincter pressure and duodenal pressure. A summary of the pressure measurements from studies on "normal volunteers" (fig. 8).

Basal pressure is the most important parameter measured and most authorities agree that an abnormal basal pressure is greater or equal to 40mmHg (two standard deviations above the median basal pressure) (Gandolfi et al 1986, Funch-Jensen et al 1982, Gennen 1980). The direction of the phasic activity can be seen by following a wave from distal through the proximal port, normal contractions have an amplitude between 50-150mmHg with mostly antegrade propagation. The manometric abnormalities ascribed to sphincter of Oddi dysfunction other than a raised basal pressure are an increased amplitude of phasic activity (> 240mmHg), an increased frequency (> 6 per minute) of phasic contractions and an increased percentage of retrograde contraction (>50%) (Barinagarremen et al 1991, Lans et al 1991, Thune et al 1991, Blades et al 1993). However a raised basal pressure appears to be the most consistent, reliable and frequently used variable for the diagnosis of sphincter of Oddi dysfunction (Gilbert et al 1992, Blades et al 1993). From cumulative historical data, standardized
normal and abnormal reference ranges for basal pressure and phasic activity have been defined (Toouli et al 1990) (fig. 9).
<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>CBD Pressure (mmHg)</th>
<th>Basal SO Pressure (mmHg)</th>
<th>Amplitude (mmHg)</th>
<th>Frequency (per min)</th>
<th>Duration (s)</th>
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<tr>
<td>Csendes 1979</td>
<td>12</td>
<td>11.4 ± 1.3</td>
<td>12.4 ± 1.5</td>
<td>110 ± 10.6</td>
<td>7.5 ± 0.7</td>
<td>4.3 ± 1.5</td>
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<tr>
<td>Geenen 1980</td>
<td>26</td>
<td>4 &gt; CBD</td>
<td>3 ± 2.5</td>
<td>101 ± 50</td>
<td>4.1 ± 0.9</td>
<td>8 ± 0.6</td>
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<td>Carr-Locke 1981</td>
<td>25</td>
<td>15.2 ± 8.2+</td>
<td>8 ± 0.6</td>
<td>52.7 ± 10.7†</td>
<td>4 ± 0.5†</td>
<td>8 ± 0.6</td>
</tr>
<tr>
<td>Toouli 1982</td>
<td>20</td>
<td>17 ± 4</td>
<td>140 ± 13†</td>
<td>113 ± 8.6</td>
<td>6.89 ± 0.2</td>
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<tr>
<td>Gregg 1984</td>
<td>43</td>
<td>13.4 ± 6.2†</td>
<td>2.0 ± 1.7</td>
<td>118 (75 - 330)</td>
<td>4.3 (3 - 6)</td>
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<tr>
<td>Meshkinpour 1984</td>
<td>9</td>
<td>14.9 ± 0.99</td>
<td>8.6 ± 1.02</td>
<td>113 ± 8.6</td>
<td>6.89 ± 0.2</td>
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<tr>
<td>Toouli 1985*</td>
<td>10</td>
<td>17 (10 - 35)</td>
<td>188 (75 - 330)</td>
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† Bile duct sphincter measurements
‡ Proximal port (most cephalad in bile duct) measurements

* Except expressed a mean (range)
MISSING

PAGE

NOT

AVAILABLE
### Fig 9 “Normal” values for sphincter of Oddi manometry (Toouli et al 1990)

<table>
<thead>
<tr>
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<th>Normal</th>
<th>Abnormal</th>
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<tr>
<td>Basal Pressure (mmHg)</td>
<td>Median 15</td>
<td>Range 5 - 35</td>
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<tr>
<td>Amplitude (mmHg)</td>
<td>135</td>
<td>95 - 195</td>
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<td>Frequency (n/min)</td>
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<td>Sequences</td>
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<tr>
<td>Antegrade (%)</td>
<td>80</td>
<td>12 - 100</td>
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<tr>
<td>Simultaneous (%)</td>
<td>13</td>
<td>0 - 50</td>
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<tr>
<td>Retrograde (%)</td>
<td>9</td>
<td>0 - 50</td>
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<tr>
<td>Cholecystokinin 20ng/kg</td>
<td>Inhibits</td>
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</tr>
</tbody>
</table>

The sphincter pressure profile is similar, when recording from the biliary or pancreatic sphincters in normal subjects (Carr-Locke et al 1981, Raddawi et al 1991) by contrast, there are many reported instances in which differing pressures are recorded from each sphincter in symptomatic patients (Toouli et al 1985, Stone et al 1988, Funch-Jensen et al 1987, Okazaki et al 1988, Dodds et al 1990, Raddawi et al 1994, Geenen et al 1995, Berezny et al 1985). Raddawi et al reported eight of nineteen patients with biliary symptoms had elevated sphincter of Oddi pressure and in five (62.5%) of the eight it was
confined exclusively to the biliary sphincter. Seven of twenty-four patients with acute recurrent pancreatitis had an elevated sphincter of Oddi basal pressure and in five (72%) of the seven the pancreatic sphincter pressure only was elevated. It is therefore important that the catheter is positioned in the appropriate sphincter reflecting the clinical suspicion of biliary or pancreatic disease.

As with the non-invasive techniques the dynamic response to intravenous hormones has been investigated with some very interesting findings. Hogan et al reported in 1982 that five patients with suspected sphincter of Oddi dysfunction had a paradoxical increase in biliary sphincter pressure in response to exogenous cholecystokinin. Evans et al 1995 found in a group of patients with post cholecystectomy pain syndrome a paradoxical rise in sphincter of Oddi basal pressure in response to cholecystokinin in a group of patients with associated irritable bowel syndrome, although there were no other differences in basal pressure or phasic activity between those with or without irritable bowel syndrome (Evans et al 1995). The response to sphincterotomy in patients with a paradoxical response to cholecystokinin has only been assessed by one group (Rolny 1986). Eight of ten such patients experienced symptomatic relief following sphincterotomy with a follow-up period of 11-16 months.

Secretin has been used to assess the dynamic response of the pancreatic sphincter. Secretin acts to relax the pancreatic sphincter but
has no effect on common bile duct or biliary sphincter pressure (Carr-Locke et al 1985). Patients with chronic pancreatitis have been shown to have a prolonged elevation in main pancreatic duct pressure after secretin (1CU/Kg) compared with controls (Toouli et al 1985). More recently alcoholic patients (n=27) without evidence of pancreatic disease have been found to have a paradoxical elevation in sphincter basal pressure in response to secretin (1CU/kg) but not to cholecystokinin (75ng/kg) (Laugier et al 1998). It is possible that this abnormal response is a factor in the aetiology of chronic pancreatitis.

There is some debate about which patients should be investigated with endoscopic manometry. Sixty-five to ninety percent of the patients who have post cholecystectomy pain syndrome and fall into type 1 of the Milwaukee classification (table D) have abnormal sphincter of Oddi manometry (Stone et 1988, Sherman et al 1991) and endoscopic manometry is therefore regarded as an optional diagnostic modality. Approximately sixty percent of the patients within biliary type 2 have abnormal manometry and therefore manometry is regarded as essential (Sherman et al 1991, Lans et al 1991). A raised basal sphincter pressure is less common in biliary type 3 (12-35 %) (Lans et al 1991) and hence manometry is regarded as critical. There are no studies reporting the outcome of patients within Sherman classification pancreatic types 1 to 3 and pancreatic sphincter dysfunction but manometry it is recommended to make the diagnosis in pancreatic
types 2 and 3 but is not essential for patients classified as type 1 (Lans et al 1991).

Endoscopic manometry as with endoscopic retrograde cholangiopancreatography is performed with the patient sedated. Clearly the action of any sedative or analgesic drug on the sphincter of Oddi must be known prior to sphincter of Oddi manometry. Diazepam and midazolam are the commonest sedatives used and these do not alter the sphincter profile (Ponce Garcia et al 1988, Rolny et al 1993, Cuer et al 1993). In some centres endoscopic manometry is performed without the addition of a analgesia (Allescher et al 1993) but the patient is often less cooperative, which, may prolong the study, jeopardize the accurate recording of sphincter pressures, lead to the use of excessive sedation and may potentially increase the risk of post procedure complications (Hogan et al 1997). Pethidine is the analgesic, it does not alter sphincter basal pressure but phasic activity is increased (Elta et al 1994, Sherman et al 1996). More recently propofol has been proposed as an effective alternative to benzodiazepines and both canine (Baron et al 1994) and human (Goff 1995) studies have not shown an adverse effect sphincter motility. The action of general anaesthetics on sphincter activity in humans or large animals is not known. Pharmacological agents that are commonly used to aid cannulation at endoscopic retrograde cholangiography such as buscopan and glucagon clearly will alter sphincter motility and are therefore avoided.
1.4.5 Microtransducer endoscopic manometry

This technique has largely been utilized in Japan and Eastern countries (Okazaki et al 1984, Tanaka et al 1983, Tanaka et al 1984, Tanaka et al 1985, Okazaki et al 1988, Okazaki et al 1998, Okazaki et al 1988, Nishimori et al 1990, Yamasaki et al 1993). The microtransducer method has a miniature strain gauge pressure transducer mounted at the tip of a catheter. This technique was pioneered and data published in the same year as the perfused catheter, 1974 by von Vandrasek and Eberhardt. Subsequently two types of strain gauge catheters were developed (Yamada et al 1980, Kobayashi et al 1985, Mori et al 1989), one to record ductal pressure and the other to record sphincter of Oddi contractions. With the progressive developments in endoscopic instruments and techniques for cannulation, Tanaka et al 1981 succeeded in measuring pancreatic and common bile duct pressures in many patients with an intact papilla for the first time, using a 4 French diameter microtransducer catheter. This group are unique in their success in placing a catheter within the common bile duct and removing the duodenoscope thus allowing prolonged recordings (Tanaka et al 1983, Tanaka et al 1988).

The drawback of this method is that it is difficult to position and maintain the transducer within the high pressure zone of the sphincter of Oddi and therefore most studies have assessed biliary or pancreatic...
duct pressures rather than sphincter of Oddi pressures (Okazaki et al 1984, Okazaki et al 1988, Okazaki et al 1988, Okazaki et al 1988, Okazaki et al 1989, Tanaka et al 1992). The strain gauge microtransducer catheter is extremely fragile and easily damaged when passed through the duodenoscope. As there is often only one microtransducer per catheter it is not possible to measure wave propagation (Tanaka et al 1981). This method is not favoured in the West. In general the pressure measurements from the microtransducer catheter are lower than from a perfused catheter (Tanaka et al 1981).

In summary, the perfused catheter is the most widely used and preferred technique in the Western world and Australia, whereas Japanese groups prefer the solid state microtransducer catheter. The perfused catheter has a number of advantages over the solid state catheter, it is more robust and wave propagation can be evaluated. The advantage of the microtransducer is that it is a smaller catheter and the scope can be removed over the catheter allowing longer studies to be performed.
1.5 THERAPY FOR SPHINCTER OF ODDI DYSFUNCTION

The aim of treatment for sphincter of Oddi dysfunction is to lower basal pressure and facilitate drainage of biliary or pancreatic secretion into the duodenum. There are a number of invasive and non-invasive methods to lower basal pressure but endoscopic sphincterotomy is regarded as the principal therapeutic technique.

Invasive therapeutic techniques

1.5.1 Endoscopic sphincterotomy.

Endoscopic biliary sphincterotomy was first described in 1974 by Demling et al and Nakajima et al four years after the first diagnostic endoscopic retrograde cholangio-pancreatography. The technique rapidly became popular for the management of bile duct stones, especially in elderly and frail patients judged to be poor candidates for operation. A typical sphincterotomy is demonstrated in plate D.
PLATE D: AN ENDOSCOPIC BILIARY SPHINCTEROTOMY USING A BOWSTRING SPHINCTEROTOME
Endoscopic sphincterotomy has been shown to lower basal biliary sphincter pressure (Tanaka et al 1981). The response to sphincterotomy in patients with sphincter of Oddi dysfunction and a raised basal pressure is variable. Patients classified as Milwaukee type 1 often do not need to undergo biliary manometry and undergo endoscopic sphincterotomy as a primary diagnostic and therapeutic manoeuvre (Lans et al 1991, Classen et al 1981, Weitemeyer et al 1982). However, restenosis is not uncommon and occurred in four (25%) of the twenty-five in Lans et al’s study.

The most important and unique study to assess the efficacy of biliary endoscopic sphincterotomy was performed by Geenan et al 1989. In this double blind study forty-seven post cholecystectomy patients with type 2 sphincter of Oddi dysfunction were randomly assigned to undergo either endoscopic sphincterotomy or sham sphincterotomy (performed in exactly the same way as a true sphincterotomy, except that the sphincterotome was positioned in the duodenal lumen during the activation of the electrocautery unit). Sphincterotomy resulted in improvement in pain scores (p=<0.05) at one year follow-up in ten of the eleven patients with elevated sphincter pressure. In contrast, there was improvement in only three of the twelve patients who underwent the sham procedure (figures 10 and 11). In those patients with normal sphincter pressures there was no difference in response regardless of treatment. After one year sphincterotomy was performed in twelve
Fig. 10 CLINICAL OUTCOME OF BILIARY TYPE 2 PATIENTS AT 1 YEAR FOLLOW-UP BASED ON THEIR CLINICAL AND OBJECTIVE ASSESSMENT (GEENEN ET AL 1989)

![Graph showing clinical outcome of biliary type 2 patients at 1 year follow-up.]

Fig. 11 CLINICAL OUTCOME OF BILIARY TYPE 2 PATIENTS AT 4 YEAR FOLLOW-UP (GEENEN ET AL 1989)

![Graph showing clinical outcome of biliary type 2 patients at 4 year follow-up.]
symptomatic patients who had undergone the sham procedure. At four year follow-up seventeen of the eighteen with elevated sphincter pressures benefited from sphincterotomy. This outcome has now been shown to persist at 12 year follow-up (Kaikaus et al 1995). Subsequent prospective studies have confirmed the efficacy of sphincterotomy in type 2 patients but to date have failed to show benefit in type 3 patients (Bozkurt 1996, Wehrmann 1996).

Although sphincterotomy is efficacious it carries with it significant risks of pancreatitis, bleeding and perforation. The complication rate from sphincterotomy, performed for all diagnoses, is about 10% with an overall mortality of 1.5% (Cotton et al 1991, Freeman 1997, Sherman et al 1997). The complication rate is highest in patients with sphincter of Oddi dysfunction (Freeman 1997) and in patients without a dilated biliary tree (Chen et al 1994). It is thought that the elderly patient with poor pancreatic function is protected against pancreatitis after sphincterotomy. In comparison, patients with sphincter of Oddi dysfunction are often young, with good pancreatic function and therefore have greater risk of developing pancreatitis. The technique of precut biliary sphincterotomy without prophylactic stenting of the pancreatic duct is an independent risk factor for developing a sphincterotomy complication (Freeman 1997). In the past, endoscopic pancreatic sphincterotomy without stenting has been performed in patients with sphincter of Oddi dysfunction (Weitemeyer et al 1994, Sherman et al 1991, Sherman et al 1994) and has been shown to be
even more hazardous in patients with pancreatic sphincter hypertension (Tarnasky et al 1997). The risks may be reduced if the sphincterotomy is performed as a needle knife cut down onto a pancreatic stent, following the cut the stent is left in situ to allow pancreatic drainage (Sherman et al 1997, Tarnasky et al 1997). For this reason it has been proposed that the pancreatic duct should be prophylactically stented prior to all procedures, even biliary sphincterotomy in patients with sphincter of Oddi dysfunction (Sherman et al 1997, Tarnasky et al 1997). More recently, Elton et al 1998 reported that pancreatic sphincterotomy with overnight nasopancreatic drainage was as safe as prophylactic stenting, negated the need for repeat duodenoscopy to remove the stent and confirmed that pancreatic sphincterotomy was safer with a drainage procedure than without (pancreatitis rate 12.5% with no drainage procedure compared with 0.7% with p=<0.003). At present the efficacy of lone pancreatic sphincterotomy is poor, some evidence exists showing selected patients may benefit (Kozarek et al 1994), but this study was limited by its size. More surprisingly is the finding that Idiopathic recurrent pancreatitis may respond to endoscopic or surgical biliary sphincterotomy (Venu et al 1989, Toouli et al 1996, Lahoti et al 1996).
1.5.2 Surgical sphincteroplasty

Transduodenal sphincteroplasty with transampullary septectomy has been well standardized over the last two decades and is designed to provide adequate drainage of both bile and pancreatic juice. The original procedure included division of the entire length of the sphincter of Oddi with suturing of the mucosa of the duct to the duodenal mucosa to prevent narrowing to the opening and subsequent extravasation of biliopancreatic secretions (Jones et al 1969). This technique was improved by the addition of septectomy (Nardi et al 1966, Nardi et al 1983) and then with use of a carbon dioxide laser (Davis 1988). There are theoretical advantages of the surgical procedure over endoscopic sphincterotomy. Biliary endoscopic sphincterotomy generally does not affect the transampullary septum. The transampullary septum if inflamed or fibrotic may block the outflow of bile or pancreatic juices with resulting obstruction (Moody et al 1983). Although there are reported successful endoscopic septotomy (Fuji et al 1989, Grimm et al 1989). A study by Moody et al 1990 reported that septotomy is beneficial as twelve of seventeen patients with previously unsuccessful but adequate sphincteroplasty obtained pain relief after excision of the septum. The second advantage of surgical therapy over endoscopic is that a mucosa to mucosa apposition can be obtained. This is important in avoiding further scarring and restenosis of the ampulla (Moody et al 1990 and Watanapa et al 1992). The risk from complications of surgical sphincteroplasty is 2%
(Negro et al 1984). Even with the lower complication rate, surgical sphincteroplasty is not commonly performed in the United Kingdom and endoscopic sphincterotomy dominates.
1.5.3 **Endoscopic stenting**

Endoscopic stenting has been shown to lower common bile duct pressure significantly in animal studies and has been proposed as immediate therapy for the treatment of sphincter of Oddi dysfunction (Youngelman et al 1997). However while sphincter of Oddi patients do benefit in terms of symptomatic relief following biliary stenting there is an unacceptably high incidence of pancreatitis (38%) (Goff et al 1995) and this method is therefore not recommended. No studies have assessed the role of pancreatic stenting alone for pancreatic sphincter dysfunction.

1.5.4 **Endoscopic balloon sphincteroplasty.**

This technique involves positioning an "Olbert" balloon into the sphincter over a guidewire and then inflating the balloon to 10mm or 15mm for 1 minute. This procedure may be repeated to ensure adequate dilatation and muscle disruption. Many regard endoscopic balloon sphincteroplasty as an unsafe procedure for the treatment of sphincter of Oddi dysfunction (Kozarek et al 1988) due to the high rate of pancreatitis but really the evidence is not conclusive. Recent studies show that endoscopic papillary balloon dilatation is safe in patients with common bile duct stones, with a lower complication rate than endoscopic sphincterotomy (Sato et al 1997, Bergman et al 1997 and Mathuna et al 1995). Sato showed that balloon dilatation effectively
lowers sphincter basal pressure. Balloon sphincteroplasty has also been shown to be of therapeutic benefit in patients with biliary sphincter stenosis (MacMathuna et al 1983). Further studies are required to evaluate the efficacy of endoscopic balloon sphincteroplasty in the treatment of sphincter of Oddi dysfunction before a conclusive opinion can be determined.

1.5.5 Local injection of Botulinum Toxin.

Botulinum toxin A works as a potent inhibitor of the release of acetylcholine from nerve endings, by inhibiting calcium dependent release of acetylcholine, an effect mediated by the enzymatic cleavage of the synaptic vesicle protein SNAP-25 (Simpson et al 1981, Blasi et al 1993). It is an effective treatment for disorders of skeletal muscle spasm such as blepherospasm (Jankovic et al 1991). More recently it has been used in the gastrointestinal tract (Parisha et al 1994) to treat achalasia by lowering sphincter pressure. The therapeutic benefits are rather variable (Bhutani et al 1997). In animal studies Botulinum A toxin effectively lower sphincter basal pressure for up to 28 weeks and so is unlikely to provide long-lasting symptomatic relief. The first description of its use for sphincter of Oddi dysfunction was in 1994 by Parischa et al. In two patients with sphincter of Oddi dysfunction there was effective lowering of sphincter basal pressure but neither sustained symptomatic benefit despite these objective findings. In a prospective study of patients with type 3 sphincter of Oddi dysfunction only 50% of
the patients showed symptomatic improvement at six weeks (Wehrmann et al 1998), but such patients do not benefit from endoscopic sphincterotomy either (Thatcher et al 1987). Botulinum toxin may provide a safe and useful tool to determine which patients are likely to benefit from endoscopic sphincterotomy (Muehldorfer et al 1997)

**Non invasive - medical therapy**

1.5.6 Nifedipine

Nifedipine is a calcium channel antagonist which inhibits the movement of calcium into cells through voltage sensitive channels in the cell membrane. As with Botulinum toxin, it is used elsewhere in the gastrointestinal tract where there is failure of a sphincter to relax, such as the lower oesophageal sphincter in achalasia. Initial animal studies in the opossum showed that nifedipine reduces sphincter of Oddi motility (Coelho et al 1987). Simon and Kovacs in 1983 reported the first clinical use of nifedipine in patients with sphincter of Oddi dysfunction, four patients responded with an excellent clinical response, seven with a good response and three of fourteen with a poor response. A subsequent study using Nifedipine 20mg sublingually was shown to reduce sphincter basal pressure in normal healthy volunteers and in patients with sphincter of Oddi dysfunction (Guelrud et al 1988). A well designed prospective study (n=28) has shown that nifedipine is effective therapy in a selected group of patients with sphincter of Oddi dysfunction, those with elevated basal pressure and phasic contractions
that are mainly in an antegrade direction (Khruoo et al 1992). No long-
term study has been performed to assess the efficacy of Nifedipine
therapy or to assess its effectiveness in indicating subsequent response
to endoscopic sphincterotomy.

1.5.7 Nitrates

Nitrate therapy was first proposed as an alternative medical therapy for
as nitric oxide donors which relaxes the sphincter of Oddi in man and
animals with category 2 resistor like sphincters.(Gocer 1994, Luman
with suspected sphincter of Oddi dysfunction that glyceryl trinitrate
effectively normalised the quantitative parameters of the prostigmine-
morphine test in these patients. No prospective studies have been
performed to look at the efficacy of nitrate therapy in patients with
raised basal sphincter at endoscopic manometry.
1.5.8 Transcutaneous nerve stimulation

Transcutaneous nerve stimulation has been shown to reduce lower oesophageal pressure in patients with achalasia with an associated rise in levels of plasma vasoactive intestinal peptide. As has previously been discussed this neuropeptide is in abundance in the human sphincter of Oddi. Transcutaneous nerve stimulation has been assessed for the treatment sphincter of Oddi dysfunction in one study which showed a reduced sphincter basal pressure in patients with sphincter dysfunction but not in healthy volunteers. (Guelrud et al 1991).

In summary endoscopic and surgical sphincterotomy are effective modalities in patients with biliary type 1 and 2 sphincter of Oddi dysfunction. To date no satisfactory long-term outcome has been achieved with these therapies. Balloon sphincteroplasty has a bad reputation but further studies need to be performed. Botulinum toxin is an interesting technique but one which is unlikely to be a curative procedure. Nifedipine is the only medical therapy to have been evaluated systematically and may be suitable therapy for a select group of patients although no long-term outcome studies have been performed. Further studies are required before nitrates or transcutaneous nerve stimulation can be recommended, but may be used to determine responsiveness to future sphincterotomy. Pancreatic sphincter dysfunction may respond to biliary sphincterotomy, but there is little data on the outcome following pancreatic sphincterotomy. No medical
therapy has been investigated for the treatment of disorders related to pancreatic sphincter dysfunction.
Chapter 2
THE AIM

2.1 Introduction

The current state knowledge of sphincter of Oddi function and dysfunction have been discussed in chapter 1, and it is evident from this that there are many aspects of sphincter of Oddi physiology that are still unclear. The mechanism by which sphincter dyskinesia occurs with the gallbladder in situ or after cholecystectomy is not known. Sphincter of Oddi function in many animals has been studied, but there is limited data that can be extrapolated to man as many of the models used are animal with type 1 (pump-like) sphincters. The action of the many neuropeptides located within the sphincter of Oddi are not known, and the methods used for the measurement of sphincter of Oddi motility are not ideal in that they only provide a snap shot of sphincter activity. The measurements made with the present "gold standard" perfused catheter give the pressure profile of the sphincter at only two points and thus the presence and role of sphincter symmetry or asymmetry has not been adequately established.

The geographical distribution of the groups investigating the sphincter of Oddi illustrate the countries which recognize the existence of sphincter of Oddi dysfunction. In America, Japan and Australia sphincter of Oddi manometry is a common procedure in patients undergoing endoscopic
retrograde cholangio-pancreatography. There is some interest in France but little interest in this field within the United Kingdom.

2.2 Aims

1. To develop a suitable anaesthetic regime that does not interfere with sphincter of Oddi motility. (chapter 4)

2. To test the hypothesis that cholecystectomy leads to an alteration in neuro-humoral response of the sphincter of Oddi and in so doing develop an animal model of sphincter of Oddi dysfunction. (chapter 5)

3. To investigate the action of substance P on the porcine sphincter of Oddi in vivo and in vitro. (chapter 6)

4. To develop a new 8 channel catheter with a view to performing vector manometry of the sphincter of Oddi (chapter 7)

5. To audit the manometric measurements, diagnosis and outcome of consecutive patients studied at the Royal London Hospital. (chapter 8)

6. To determine the occurrence and relationship of tachyoddia and the duodenal migrating motor complex. (chapter 9)
THE DEVELOPMENT OF A SUITABLE ANIMAL MODEL FOR THE
INVESTIGATION OF SPHINCTER OF ODDI FUNCTION

3.1 Introduction

The use of animals for medical research involves many ethical issues and moral dilemmas, covered by both British and European Law, the Home Office being the governing body within the United Kingdom. Prior to any animal experimentation I completed modules one to five of the Animal Scientific Procedures Courses and a Large Animal Section to cover working with pigs. A Project Licence and relevant Personal Licence were granted by the Home Office for the studies carried out in chapter four, five six and seven and a relevant personnel license. Techniques cannot be practiced on any animals outside those included in the project. The personal licence also strictly defines each procedure that an individual is certified to perform.
To investigate a specific hypothesis in an animal model and to then extrapolate the findings and hence draw conclusions about man is regarded with caution. It is however often ethically and practically impossible to perform some studies in man. The development of suitable animal models which are representative of human physiology or disease are required to facilitate research. Animal models have been developed for the investigation of many aspects of gastrointestinal physiology and pathophysiology.

A number of considerations must be made in the development of an animal model that is representative of the human sphincter of Oddi:

1. The sphincter must respond in a similar way to hormones, primarily cholecystokinin.

2. The neuropeptide innervation must be representative of the human sphincter.

3. The anatomy should resemble the human anatomy to allow the development of new devices to measure sphincter pressure before use in man.

A large number of species have been used in the investigation of sphincter function including: the opossum, possum, guinea-pig, cat, dog and pig. Some species such as the rat and pocket gopher do not have gallbladders and have a rudimentary sphincter of Oddi, they are not therefore suitable models (Hallenbeck et al 1967).
3.1.1 The opossum and possum.

The opossum and possum have been widely used for in vitro (Baker et al 1992, Baker et al 1993 Simula et al 1997) and in vivo (Toouli et al 1981, Becker et al 1982, Toouli et al 1983, Parodi et al 1988, Takashashi et al 1988, Liu et al 1993) experiments for the investigation of sphincter of Oddi function. Unlike man, the opossum sphincter of Oddi is largely extraduodenal and the bile and pancreatic ducts join to form a common channel of entry into the duodenum. In the American opossum circular muscle encircles both ducts while in the Australian species the arrangement is that of a figure-of-eight. The extraduodenal position of the sphincter lends itself to easy access for resection, use in vitro, and also for electromyographic (Honda et al 1982, Takahashi et al 1984) experimentation. The opossum and possum sphincters respond in an excitatory manner to cholecystokinin (Becker et al 1982, Honda et al 1983) and are therefore type 1- pump like sphincters (Calabuig et al 1990). The response to caerulein, motilin, and pentagastrin is excitatory and therefore differs from that of man, whereas there are similar responses to man with other neuropeptides such as acetylcholine (excitatory), glucagon (inhibitory) and secretin (inhibitory) (Coehlo et al 1985, Helm et al 1989).
3.1.2 The guinea-pig

The guinea-pig sphincter anatomy is similar to man in that the bile duct and pancreatic duct enter a common ampulla which is situated within the duodenal wall. Little is known of many of the gastrointestinal hormonal actions on the guinea-pig sphincter but it does appear to relax in response to cholecystokinin and is a type 2, resistor like sphincter. Nitric oxide donors have been shown to relax the sphincter as in man (Gocer et al 1994). Interestingly a recent study has shown that cholecystectomy raises sphincter of Oddi opening pressure (Carvajal et al 1994). The guinea-pig does appear to be a valid small animal model for sphincter of Oddi function pressure but measurements cannot be made using the same apparatus as in man.

3.1.3 The cat

The anatomy of the cat resembles man with both bile duct and pancreatic ducts joining together in a short common channel which is surrounded by a sphincter that lies largely within the wall of the duodenum. The cat sphincter is a type 2 sphincter (Dahlstrand et al 1990). Enkephalin receptors act to contract the sphincter (Thune et al 1992) and motilin reduces sphincter of Oddi flow by increasing its motor activity (Behar et al 1988). The cat is a suitable model but again is limited by its small size relative to the methods of pressure
measurements and also by the ethical constraints in the United kingdom because this species is a "pet".

3.1.4 The dog

The anatomy of the dog differs from man with the pancreatic and bile ducts entering the duodenum separately but in close proximity; as with humans the sphincter lies within the wall of the duodenum. The dog sphincter exhibits a two-fold response to exogenous cholecystokinin; firstly, a contractile effect, probably mediated through a direct myogenic action and neural release of acetylcholine; secondly a relaxant effect, probably mediated by stimulation of postganglionic neurons (Pozo et al 1990). Motilin has been shown to contract the canine sphincter (Neya et al 1981). The dog is of a sufficient size that endoscopic measurement of the sphincter pressure is possible by means of a perfused or microtransducer catheter (Deng 1996) and has been used to investigate the interrelationship of the gallbladder, sphincter of Oddi and the duodenal migrating motor complex (Yokahata 1994, Funch-Jensen 1991). There are very strict criteria for the use of domestic animals for experimentation in Great Britain. Dogs must be obtained from licensed breeding establishments incurring considerable costs which often precludes their use.
3.1.5 The porcine model

The porcine sphincter anatomy is similar to the dog, and differs from man, the bile and pancreatic ducts opening separately into the duodenum. The biliary sphincter is located within the duodenal wall as in man, and opens in the first part of the duodenum. The pancreatic sphincter and opening is located approximately 10-15cm distal to the biliary sphincter. The porcine biliary sphincter of Oddi is a type 2 sphincter and relaxes in response to cholecystokinin (Pasricha et al 1995). The neural network in the porcine biliary sphincter closely resembles that in man with a similar group of neuropeptides represented (fig. 12) (Harling et al 1991, Sand et al 1993, Sand et al 1994, Sand et al 1997). Despite the differences in anatomy the porcine model has been proposed as a unique endoscopic model for teaching and research. It is possible to pass a duodenoscope and cannulate both the biliary and pancreatic ducts (Pasricha 1995).
Fig. 12 Immunoreactivity observed in nerves of pig sphincter of Oddi, gallbladder, common bile duct, and duodenum

<table>
<thead>
<tr>
<th>Duodenal Mucosa</th>
<th>Sphincter of Oddi</th>
<th>Gallbladder</th>
<th>Common Bile duct</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>++++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>NPY</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Galanin</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PHI</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Bombesin</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Sub P</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Serotonin†</td>
<td>-- (+)</td>
<td>--(++)</td>
<td>--</td>
</tr>
<tr>
<td>Somatostatin†</td>
<td>-- (+)</td>
<td>--(++)</td>
<td>--</td>
</tr>
</tbody>
</table>

Immunoreactivity grading ++++ very strong, +++ strong, ++ moderate, + borderline, — no observed immunoreactivity. † Immunoreactivity for serotonin or somatostatin was not observed in the nerves, but instead in the epithelium of the sphincter of Oddi, gallbladder, and duodenum, although no in the epithelium of common bile duct. NPY: neuropeptide Y, Sub P: substance P, PHI: peptide histidine-isoleucine, CGRP: calcitonin gene-related peptide.
In summary the cat, dog and pig appear to be suitable large animal models for investigating sphincter of Oddi function. The cat cannot be investigated endoscopically or with the catheters used for human studies. The use of the cat and dog for medical experimentation is strictly controlled and the animals are only available from licensed centers at considerable expense. Pigs may be purchased for medical research from any farm source and are therefore economically attractive. However as with all animal experiments the Home Office sets stringent limits to the number of animals that can investigated.
3.2 EXPERIMENTAL METHODS

3.2.1 Animals

Adolescent large white pigs (Sus-scrofus domesticus) initially weighing between 20-30kg were studied. The pigs were supplied by the Royal Veterinary College, Hertfordshire. The animals were housed within an authorised specialized large animal unit. The pigs were fed a standard dry food diet twice daily and occasional apples. Daily husbandry was carried out by animal technicians and I visited the animals most days.

3.2.2 Cholecystectomy

The animals were anaesthetized with a combination of halothane, nitrous oxide and oxygen after a twelve hour fast. Sterile gloves, gowns and instruments were used. Using an aseptic technique the abdomen of each animal was prepared with betadine. A Kocker's right subcostal incision was made through the skin. The muscle layers were incised using cutting diathermy and spread manually to expose the peritoneum. The peritoneum was grasped with forceps and careful palpation made to ensure that no loops of bowel were lying superficially before entry was made into the peritoneum with a scalpel. The liver and gallbladder were then easily identified. Packs were used to displace the bowel away from the liver. The gallbladder fundus was grasped with forceps and carefully retracted. The gallbladder was then
dissected from the liver bed using sharp and blunt dissection techniques. Haemostasis was maintained using coagulating diathermy. When the gallbladder was free from the liver and Cabot's triangle was easily identified, the cystic duct and cystic artery were ligated and cut. A full inspection of the gallbladder bed and ligatures were made prior to closure of the wound. The peritoneum and abdominal muscles were closed in layers using 2/0 Vicryl, sutures (Ethicon Ltd., U.K.). The skin was then closed using single subcutaneous 2/0 Ethilon suture (Ethicon Ltd., U.K.). Finally the wound was infiltrated with 2% lignocaine local anaesthetic to provide local post operative pain relief as commonly used in man. On recovery the animals were immediately mobile and hungry. Post operative antibiotics (intramuscular twice daily Cefuroxime 750mg, Galaxo pharmaceuticals) were given for forty-eight hours.

3.2.3 Sham Laparotomy

Each pig was starved for 12 hours and then anaesthetised as above. A Kocker’s incision was made, the muscle layer and peritoneum were divided. The gallbladder and liver were identified. The gallbladder was manually manipulated for five minutes and the wound was closed as above. The animal was allowed to recover and given post operative antibiotics for forty-eight hours (intramuscular twice daily Cefuroxime 750mg, Galaxo pharmaceuticals).
3.2.4 Standard sphincter of Oddi manometry

Manometry was either performed endoscopically or via a duodenotomy. A triple lumen Lehman catheter (Wilson-Cook, USA) was used as shown in plate A. The catheter has a central channel and two recording channels. Ten lines, 2mm apart are marked on the catheter tip to facilitate the station pull through. The two measuring channels open 2mm in distance and 180 degrees apart at the red marks seen. The catheter was perfused using a low compliance Arndorfer pneumohydraulic pump (mean pressure 0.2 bar) as shown in plate E. Water was perfused at a rate of approximately 0.1ml/minute per lumen. The external pressures transducers were connected to the catheter via anaesthetic connection tubing. The pressures transmitted proximally to external transducers were record by a computer system. The recordings were displayed and analyzed using a modified oesophageal manometry program (Phoenix, U.K.).

During the prolonged studies described in chapter four, five and six the sphincter high pressure zone was located by a pull-through manometry. The catheter was then positioned in the mid point of the high pressure zone and sutured to maintain this position. Baseline control and pharmacologically stimulated studies were performed.

Pharmacological dynamic studies were performed using cholecystokinin-octapeptide (Sigma chemicals) and Morphine sulphate. Cholecystokinin-
octapeptide was infused intravenously at a rate of 10ng/kg/min. Although this flow rate produces supra-physiological plasma levels, similar doses have been used to investigate gallbladder and sphincter of Oddi motility in other species (Grace et al 1987, Muller et al 1987, Cox et al 1990, Hanyu et al 1990, Pozo et al 1990, Richard et al 1993, Elbrond et al 1994, Muller et al 1987, Chen et al 1997). An infusion period of 30 minutes was used to allow gallbladder emptying to occur and to enable equilibrium of sphincter of Oddi motility. Analyses were made of the data obtained over the final ten minutes of each infusion.

Morphine Sulphate stimulates contraction of the human sphincter of Oddi (Madura et al 1981, Nardi et al 1983) but its action on the porcine sphincter of Oddi is not known. A bolus dose of 5mg Morphine sulphate was given intravenously and observations recorded for five minutes in each study.
3.2.5 Bench test: The dynamics of the Lehman triple lumen catheter

Ideally all manometric systems should be capable of recording absolute values of the pressure wave with accuracy. Both pressure rise rates and baseline offset are indicators of catheter fidelity (Omari et al 1996, Chen et al 1998).
Bench studies were performed to determine the fidelity of two five 
french diameter triple lumen Lehman catheters. Flow rates were also 
measured with different pressures and calibration studies were 
performed.

The baseline pressure offset was measured for perfusion pressures of 
0.1 bar, 0.2 bar and 0.3 bar. The mean baseline pressure offset for 
the anaesthetic extension tubing which connected the catheter to the 
pressure transducers was 1mmHg at all perfusion pressures. The 
pressure offset for two Lehman triple lumen catheters was measured 
by calibrating the system including the anaesthetic extension tubing to 
air and zeroing level to the pressure transducers, a primed catheter 
was then connected to the anaesthetic extension tubing and the 
pressure recorded was taken as the baseline pressure offset. The 
baseline pressure offset for the two Lehman catheters is shown in 
figure 13.

![Fig. 13 The baseline pressure offset for two Lehman catheters](image-url)
The pressure rise rate was determined for two Lehman catheters with perfusion pressures of 0.1, 0.2 and 0.3 bar by manual occlusion of the measuring ports three times to a pressure of 100mmHg above baseline.

The pressure rise rates were calculated and expressed in mmHg sec\(^{-1}\) and are shown in figure 14.
Both the baseline offset and the pressure rise rate rose almost linearly with increased perfusion pressures. In order to achieve accurate pressure measurements within the sphincter of Oddi the pressure rise rate must be sufficient to record peak amplitude. This criteria is determined by the equation below

\[ \text{Pressure rise rate} > 10 \times (\text{amplitude} \times \text{frequency of the waves to be measured}) \]

For the sphincter of Oddi this would mean be:

\[ \text{Pressure rise rate} > 10 \times (195 \times \frac{6}{3600}) = 3.25\text{mmHg sec}^{-1} \]

Therefore at all perfusion pressures this is achieved.

Baseline offset is also a determinant of manometric fidelity and reliability of recordings. High baseline offsets augment the degree of baseline fluctuations that occur with minor changes in reservoir pressure that are characteristic of pneumohydraulic pump systems. High baseline offsets also reduce the pressure differential between the manometric assembly and the transducer; this attenuates the net perfusion rate and hence the pressure rise rate and therefore fidelity. Baseline offset pressures less than 50mmHg are acceptable for the measurement of the lower oesophageal sphincter and the sphincter of Oddi (Chen et al 1998).
The flow rate was measured for perfusion pressures of 0.1, 0.2 and 0.3 bar by collecting the perfusate over a five minute period and the results are shown in figure 15 below.

**Fig. 15 Flow rates for a given perfusion pressure**

- **Flow rate per lumen (mls/min)**
  - 0.3
  - 0.25
  - 0.2
  - 0.15
  - 0.1
  - 0.05
  - 0

- **Perfusion pressure (bar)**
  - 0
  - 0.2
  - 0.4

- **Legend**
  - Catheter A
  - Catheter B
Finally calibration studies were performed for the same perfusion pressures with the Lehman catheter connected to a sealed system incorporating a sphygmomanometer. The results of these studies are shown in figures 16, 17 and 18. The measured pressure matched the actual pressure in nearly all cases.
Fig. 17 Calibration of the Lehman catheter with a perfusion pressure of 0.2 bar

Fig. 18 Calibration of the Lehman catheter with a perfusion pressure of 0.3 bar
In the experiments performed below a perfusion pressure of 0.2 bar was used because high perfusion flow rates resulting from high perfusion pressures have been shown to increase the risk of procedure related pancreatitis (Meshkinpour et al 1992). At this pressure the above bench studies show that there is an acceptable baseline offset and pressure rise rate to measure human and porcine sphincter of Oddi pressures. The catheter was shown to accurately measure known pressures.
3.3 Statistical methods

The experiments contained in this thesis generated a large amount of numerical data. In order to make valid statements on the groups and comparisons between groups, appropriate statistical analysis was used.

Data can be divided into two major types: one in which the data conforms to a "normal" distribution (where "normal" refers to a mathematical description of the data defined by its mean and standard deviation), and the other in which the data is not "normally" distributed. For "normal" data parametric statistical test are appropriate, whereas for "non-normal" data non-parametric tests must be used.

When repeated measurements of the same parameters were made on the same animals following different stimulus, and for analysis of physiological data the paired t-test was employed, with each subject acting as his own control.

Where comparisons were made of the results from different animals (sham group versus cholecystectomy group) non parametric methods were employed. For each parameter, the median, range and interquartile ranges were calculated and the Mann-Whitney U test employed to assess changes in baseline recordings and pharmacological stimuli. The Fisher's exact test was used to compare the patients with tachyoddia in chapter nine.
In all the statistical tests a level of 5% or less was used to indicate statistical significance, though due weight was also given to results approaching but not meeting that cut-off.
4.1 Introduction

Human sphincter of Oddi manometry is commonly performed under sedation only. The commonest agents used are the benzodiazepines: diazepam or midazolam, which do not interfere with or alter sphincter of Oddi motility (Garcia et al 1988, Cuer et al 1993). Unfortunately it is not possible to perform sphincter of Oddi manometry in large animal studies under sedation. It is not thought appropriate by the Home Office in view of the nature of the procedure. In addition the endoscopic equipment is costly and could be easily damaged if a lightly sedated animal were to bite down onto the endoscope. In view of this it is necessary to perform manometry on animals that are under general anaesthesia.

It is important that the effect of the anaesthetic agents used do not interfere with sphincter motility. There is little known about the action of halothane on type 2 animals. Only one study to date has looked at the possible effects of halothane on the sphincter of Oddi, in the Australian possum, a type 1 pump like sphincter. In this study trans-sphincteric flow was measured as the indicator of sphincter tone, it was found that halothane reduced trans-sphincteric flow in a dose
dependent manner, suggesting that halothane caused an increase in sphincter of Oddi tone (Lui et al 1993).

4.2 Aim

The aims of this study were to determine the effects of two commonly used anaesthetic agents (halothane and enflurane) on porcine sphincter of Oddi motility.

4.3 Method

4.3.1 Pilot Study

Two adolescent pigs (Sus-scrofus domesticus) which had previously undergone a sham laparotomy 8 weeks before were studied. The first animal was anaesthetised with a combination of halothane 2-5%, nitrous oxide and oxygen, it was then intubated and anaesthesia maintained with the same combination of gases. The second animal was induced using a combination of midazolam, enflurane, nitrous oxide and oxygen. Enflurane is not an easy agent for induction of anaesthesia because it causes respiratory irritation, therefore Midazolam was used to provide adequate depth of anaesthesia for intubation. In both animals sphincter of Oddi manometry was performed through a duodenotomy. A baseline sphincter of Oddi pressure recording was
made for 30 minutes. Then cholecystokinin (Sigma chemicals, U.K.) 10ng/kg/min was infused for thirty minutes with continuous monitoring. The baseline basal pressure, wave amplitude and wave frequency were compared between both animals and with published data. (Sand et al 1997).

### 4.3.2 Main study

Four pigs were studied, they had either undergone previous sham laparotomy (n=2) or cholecystectomy (n=2) as described in chapter 3. Induction and subsequent maintenance of anaesthesia was initially with an enflurane, midazolam, nitrous oxide, oxygen combination.

Sphincter of Oddi manometry was performed through a duodenotomy. Each animal followed this schedule of recordings:-

1. Baseline recordings for 15 minutes.
2. Cholecystokinin 10ng/kg/minute was infused for 30 minutes.
3. Washout period of 15 minutes.
4. Baseline recordings for 5 minutes.
5. 5mg of morphine sulphate was then given intravenously and recordings made for 5 minutes.
6. Washout period of 15 minutes.
7. Enflurane was stopped and halothane 2-5% commenced, recordings taken for 15 minutes.
8. Morphine sulphate 5mg given intravenously and recordings taken for a final 5 minutes.

4.4 Statistical methods

The results were analysed comparing the last ten minutes of the baseline recordings when a steady state was observed and the five minutes after morphine sulphate injection. Therefore the data is matched because it arises from the same animal statistical analyses were performed using the paired t test.

4.5 Results

4.5.1 Pilot study

The sphincter of Oddi of the pig anaesthetised with halothane appeared to be completely relaxed with no basal pressure, no evidence of phasic activity and no change in activity during or following the infusion of cholecystokinin. The pig anaesthetised with enflurane/midazolam had a sphincter of Oddi basal pressure of 10mmHg, the sphincter also showed phasic activity, with a wave frequency was 1cycle/minute with an amplitude of 5mmHg.
4.5.2 Main study

The results are displayed (fig. 19) as mean values (standard error of the mean) and graphs overleaf (figures 20 and 21). The motility index was calculated as the result of frequency multiplied by the amplitude.

Halothane significantly reduced sphincter of Oddi response to morphine sulphate stimulation compared with enflurane anaesthesia (basal pressure rise of 5.8mmHg under enflurane compared with a fall of 1.2mmHg under halothane (p=0.029), motility index: rose by 23.7 mmHg/c/min under enflurane compared with a rise of 6.2 mmHg/c/min under halothane anaesthesia (p=0.098)). Sample recordings after morphine sulphate using enflurane and halothane anaesthesia are shown in Plates F and G respectively. Although halothane also appears to reduce sphincter of Oddi resting basal pressure and motility the differences did not reach statistical significance (basal pressure 3.5mmHg under halothane compared with 4.2mmHg under enflurane anaesthesia (p=0.59), motility index 0mmHg/min under halothane compared with 4.7mmHg/min under enflurane (p=0.095)).

Regular phasic activity (frequency 1cycle/min, amplitude 4.7 mmHg) was observed whilst under anaesthesia with enflurane.
**Fig. 19 The effect of the anaesthetic agent on sphincter of Oddi motility**

<table>
<thead>
<tr>
<th>Basal Pressure (mmHg)</th>
<th>Motility index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre Morphine Sulphate</td>
</tr>
<tr>
<td>Enflurane + Midazolam</td>
<td>4.2 (2.92)</td>
</tr>
<tr>
<td>Halothane + Midazolam</td>
<td>3.5 (1.93)</td>
</tr>
<tr>
<td>p value paired t test</td>
<td>0.59</td>
</tr>
</tbody>
</table>
Fig 20 THE EFFECT OF ENFLURANE/ HALOTHANE ANAESTHESIA ON BASAL PRESSURE

<table>
<thead>
<tr>
<th></th>
<th>Pre Morphine Sulphate</th>
<th>Morphine Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p=0.59</td>
<td>p=0.062</td>
</tr>
<tr>
<td>Enflurane &amp; midazolam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halothane &amp; midazolam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=0.59</td>
<td>4.2</td>
<td>10.2</td>
</tr>
<tr>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

114
Fig 21 THE EFFECT OF ENFLUANE /HALOTHANE ANAESTHESIA ON THE MOTILITY INDEX

<table>
<thead>
<tr>
<th>Pre Morphine Sulphate</th>
<th>Morphine Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enflurane &amp; midazolam</td>
<td>5.8</td>
</tr>
<tr>
<td>Halothane &amp; midazolam</td>
<td>0</td>
</tr>
<tr>
<td>Enflurane &amp; Midazolam</td>
<td>28.5*</td>
</tr>
<tr>
<td>Halothane &amp; midazolam</td>
<td>6.2*</td>
</tr>
</tbody>
</table>

p=0.095

p=0.028
PLATE F: PORCINE MANOMETRY UNDER ENFLURANE AND MIDAZOLAM ANAESTHESIA
PLATE G: PORCINE MANOMETRY UNDER HALOTHANE AND MIDAZOLAM ANAESTHESIA

Phoenix Oesophageal U2.12

Analysis

Body motility

16:58:35

Patient

Investigation

Analysis

File

Setup

Quit

P3

morphine

11

2 mm Hg

P4

11 mm Hg

P5

0 mm Hg

Sulphate can be used to induce premature phase 3 activity (Yokohata et al. 1994) of the duodenum. In this study, morphine sulphate induced spasticity of Oddi phase activity under enflurane anaesthesia but there was little change in sphincter of Oddi activity under halothane.
4.6 Discussion

This study shows that halothane and enflurane have different effects on sphincter of Oddi motility. This difference may be a direct one on the sphincter of Oddi alone or be a more generalised effect on intestinal motility.

Enflurane does not appear to interfere with sphincter of Oddi motility, the baseline pressure recordings were comparable to that found by Sand et al 1997 (13.3 ± 4 mmHg). Halothane appears to inhibit sphincter of Oddi activity.

Animal studies have shown that sphincter of Oddi activity closely follows the phases of the migrating motor complex of the duodenum with maximum activity during phase 3 of the migrating motor complex (Yokohata et al 1994). In rats halothane has been shown to reduce or even abolish duodenal phase 3 activity whereas enflurane was found to have no effect on phase 3 activity but did reduced phase 2 activity (Wright et al 1982) halothane also reduces antral contractility the frequency of contractions in phase 3 of the duodenal migrating motor complex in man (Schurizek et al 1989). Certain drugs such as morphine sulphate can be used to induce premature phase 3 activity (Yokahata et al 1994) of the duodenum. In this study morphine sulphate induced sphincter of Oddi phasic activity under enflurane anaesthesia but there was little change in sphincter of Oddi activity under halothane
anaesthesia. Halothane suppressed the induction of phase 3 activity, as it does in rats and man, this effect is likely to be part of a more generalized effect on gut motility rather than specifically directed at the sphincter of Oddi. Halothane is therefore not a suitable agent for the induction or maintenance of anaesthesia in animal studies of sphincter of Oddi motility.

Five percent of patients require general anaesthesia to undergo endoscopic retrograde cholangiography or sphincter of Oddi manometry (Etzkorn et al 1998). In these cases, there is often of a history of substance abuse and thus a tolerance to sedatives. Halothane should be avoided if sphincter of Oddi manometry and enflurane would seem an acceptable alternative. Conversely for Endoscopic retrograde cholangio-pancreatography alone the reduction in antroduodenal and sphincter activity induced by halothane would be advantageous by relaxing the sphincter and theoretically easing cannulation of either duct. The effect of other volatile anaesthetic such as isoflurane have not been assessed in animal or human studies and therefore cannot at present be recommended.
5.1 Introduction

As discussed in chapter 1 sphincter of Oddi dysfunction is a recognised complication of cholecystectomy and occurs in up to 20% of patients post-cholecystectomy (Henry 1983, Drossman 1993). It is not clear whether sphincter of Oddi dysfunction predates cholecystectomy or whether disruption of neural networks lead to some alteration in sphincter control (Grace et al 1987, Thune et al 1998). The disruption of connecting neural networks is believed to lead to an alteration in responsiveness to cholecystokinin (Grace et al 1987, Evans et al 1995).

Between seventeen and nineteen percent of patients with sphincter of Oddi dysfunction (41% with associated irritable bowel syndrome) exhibit a paradoxical response to pharmacological doses of cholecystokinin (Rolny 1986, Evans et al 1995). The actions of cholecystokinin on the gallbladder and sphincter of Oddi are complex and mediated by myogenic and neuronal receptors. There are at least two mechanisms of action on the gallbladder. Postganglionic cholinergic neurons are stimulated in the gallbladder and these can be blocked by atropine (Behar et al 1980). Secondly direct stimulation of the gallbladder smooth
muscle occurs and this effect is unaltered by complete denervation with tetrodotoxin. The sphincter of Oddi is inhibited by cholecystokinin via the stimulation of postganglionic non-adrenergic, non-cholinergic presumably nitric oxide inhibitory neurones. This action is inhibited by tetrodotoxin but not by atropine (Behar et al 1980, Behar et al 1987). A direct effect is also mediated by stimulating excitatory receptors on the smooth muscle of the sphincter, this action is not inhibited by tetrodotoxin (Behar et al 1980).

Three animal studies have been performed to look at the effect of cholecystectomy on sphincter function (Grace et al 1987, Thune 1988 Carvajal et al 1994). Grace et al assessed the effect in the prairie dog of cholecystectomy (n=10) compared with sham laparotomy (n=8). The prairie dog has a type 1 sphincter which contracts in response to cholecystokinin. Eight months after cholecystectomy there was no alteration in sphincter resting motility but the response to cholecystokinin and duodenal fat infusion were blunted in the cholecystectomy group. A study in cats also failed to show any difference in resting activity after cholecystectomy but found an abnormal failure of relaxation of the sphincter in response to cholecystokinin (Thune et al 1988). Carvajal et al 1994 reported a study in twenty male Hartley guinea-pigs (a type 2 sphincter) and found a significantly higher sphincter opening pressure in the cholecystectomy group (n=10) compared with the sham group (n=10). The manometric
recordings in all these studies were of short duration and not obtained by methods that are used in human studies.

A single human study has been reported to date (Luman et al 1997). In this study of five patients with uncomplicated cholelithiasis, sphincter of Oddi manometry was performed before and six months after laparoscopic cholecystectomy. There was no change in basal sphincter of Oddi pressure or motility after cholecystectomy. However the dynamic response to cholecystokinin (a bolus intravenous injection of 1 Ivy Dog unit/kg) was altered, with a failure to suppress phasic activity after cholecystectomy. The findings from this study are limited by the short duration of the manometric recordings, approximately 10 minutes, also sphincter of Oddi dysfunction occurs in at most 20% of post cholecystectomy patients and therefore a study of such size is unlikely to show significant results. A larger study or even a repeat of this study would be difficult to perform for the ethical reason, that there a significant risk of pancreatitis after manometry making it unjustifiable to perform in patients that are symptom free.

5.2 Aims

The aims of this study were to determine:

1. If porcine sphincter of Oddi resting pressures are altered by cholecystectomy using prolonged manometric measurements.
2. To assess the response to cholecystokinin and morphine sulphate provocation following cholecystectomy.

3. To determine the action of morphine sulphate on the porcine sphincter of Oddi.

4. To determine if the post cholecystectomy porcine model is a suitable animal model of sphincter of Oddi dysfunction.

5.3 Methods

Sixteen female adolescent large white pigs (Sus-scrofus domesticus) were studied, with a starting weight of 20-25kg. Eight pigs underwent sham laparotomy and eight underwent cholecystectomy as described in chapter 3. Eight weeks after the initial procedure each animal was anaesthetised using a combination of enflurane, midazolam, nitrous oxide and oxygen. Sphincter of Oddi manometry was performed using a perfused catheter via a duodenotomy as described in chapter 3. Pressure recordings and dynamic studies were performed according to steps 1 to 5 of the schedule described in chapter 4.

5.4 Statistical methods

The results were analysed to compare:

1. The final ten minutes of the cholecystokinin infusion in each animal.

2. The five minutes after morphine sulphate injection.

Statistical analysis was performed using the Mann Whitney U and paired student's t test where appropriate.
5.5 Results

Two animals had to be terminated early (within two weeks of their initial laparotomy) due to deteriorating health from presumed porcine influenza. Post-mortem examination of these pigs revealed lung consolidation. Porcine influenza virus is endemic in domestic pigs and can lead to pneumonia and reduced fertility.

5.5.1 Baseline results: Sham v cholecystectomy

The baseline results illustrated below (figures 22, 23 and 24) show the sham group; median basal pressure of 9mmHg (range 2-13mmHg), median amplitude of 8mmHg (range:0-17) and median wave frequency 1cycle/minute (range 0-5cycles/ minute), a typical recording is shown in plate H. The baseline results of the cholecystectomy group were not statistically different (by Mann Whitney U) with a median basal pressure of 10mmHg (range 3-20mmHg), wave amplitude of 4mmHg (range 0-16mmHg) and wave frequency of 1cycle/minute (range 0-8cycles/minute).  

**Fig 22 A comparison of the baseline basal pressures**
Fig 23 A comparison of the baseline wave amplitude

mmHg

Sham group

Cholecystectomy group

p = 0.24 by Mann Whitney U test

Fig 24 A comparison of the baseline wave frequency

C/min

Sham group

Cholecystectomy group

p = 1.0 by Mann Whitney U test
Sham group: "Normal Values"

Basal pressure - 9 mmHg (2-13)
Wave amplitude - 8 mmHg (0-17)
Wave frequency - 1 c/min (0-5)
5.5.2 Results of the response to cholecystokinin infusion.

The effect on Basal pressure

The typical response of a sham animal sphincter to cholecystokinin infusion is shown in plate I. The median basal pressure of the sham laparotomy group fell significantly (p=0.028 by paired t test), from 9mmHg (25th-75th centiles; 5-9 mmHg) to 3mmHg (25th-75th centiles; 0-18mmHg) during cholecystokinin infusion (figures 25 and 26). The median basal pressure for the cholecystectomy group did not fall significantly (p=0.49 by paired t test), from 10mmHg (25th-75th centiles; 3-18mmHg) to 9mmHg (25th-75th centiles; 3-10mmHg). There was no significant difference (p=0.55 by Mann Whitney U test) in the pressure change between the two groups in response to cholecystokinin. Interestingly however two of the cholecystectomy group exhibited a paradoxical response to cholecystokinin with a rise in basal pressure and overall the inhibitory effect of cholecystokinin on basal pressure was less in the cholecystectomy animals.
Plate I: The response of the porcine sphincter of Oddi to cholecystokinin
Fig. 25 THE EFFECT OF CHOLECYSTOKININ INFUSION ON BASAL PRESSURE IN THE SHAM LAPAROTOMY GROUP

![Graph showing the effect of Cholecystokinin infusion on basal pressure in the sham laparotomy group.]

$p = 0.028$ by paired t test
Fig 26 THE EFFECT OF CHOLECYSTOKININ INFUSION ON BASAL PRESSURE IN THE CHOLECYSTECTOMY GROUP

mmHg

25
20
15
10
5

Pre CCK

CCK

p= 0.49 by paired t test
The effect on wave amplitude

In response to cholecystokinin median wave amplitude fell in the sham group from 7mmHg (25th-75th centiles; 0-8mmHg) to 1mmHg (25th-75th centiles; 0-4mmHg) ($p=0.056$ by paired t test) (fig. 27). The wave amplitude remained unaltered in the cholecystectomy group (pre 4mmHg (25th-75th centiles; 0-10mmHg) and post 4mmHg (25th-75th centiles; 3-8mmHg ($p=0.61$ by paired t test)) (fig. 27). Cholecystokinin dampened the wave amplitude in the sham group more compared to the cholecystectomy group (change in wave amplitude (pre -post )for the sham group compared with the change in wave amplitude (pre- post) for the cholecystectomy group $p=0.051$ by Mann Whitney U test).

![Fig 27 The comparison of the effect on wave amplitude](image)

**Key**
- **Sham group**
- **Cholecystectomy group**
The effect on wave frequency

In response to cholecystokinin wave frequency for the sham group rose from a median of 2 c/min (0-3) to 3 c/min (0-4) (p=0.56 by paired t test) and in the cholecystectomy animals from 1 c/min (0-3) to 3 c/min (3-4) (p=0.50 by paired t test) (Fig.28). There was no difference in pre CCK wave frequency between the two groups (p=1.0 by Mann Whitney U test) and there was no difference in frequency post CCK (p=0.58 by Mann Whitney U test). There was also no difference in the change in frequency in response to CCK between the two groups (difference in pre-post CCK frequency for the sham group v pre-post CCK frequency cholecystectomy group p=0.79 by Mann Whitney U test).

**Fig. 28 The comparison of the effect on wave frequency**

<table>
<thead>
<tr>
<th>C/min</th>
<th>Sham group</th>
<th>Cholecystectomy group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Key
- Sham group
- Cholecystectomy group
5.5.3 Results of the response to morphine sulphate provocation.

Two of the sham group were excluded from analysis because the catheter required relocation in the sphincter during the study and so the baseline and study measurements were not comparable.

The effect on basal pressure

The sphincter of Oddi in all animals was stimulated by morphine sulphate, a typical response is shown in plate J. The rise in basal pressure in response to morphine sulphate nearly reached significance in the sham group (\( p=0.063 \) by the paired t test); a rise from 6mmHg (25th-75th centiles; 2-12mmHg) to 12mmHg (25th-75th centiles; 10-13mmHg)(fig. 29). The rise in basal pressure in the cholecystectomy group reached significance (\( p=0.026 \) by paired t test); a rise from 9mmHg (25th-75th centiles; 1-13mmHg) to 11mmHg (25th-75th centiles; 7-16mmHg)(fig. 30). The change in basal pressure in response to morphine sulphate for both groups was not significantly different ((sham pre-post basal pressure v cholecystectomy group pre-post basal pressure) \( p=0.86 \) by Mann Whitney U test)
Fig. 29 The effect of morphine sulphate on basal pressure in the sham laparotomy group

mmHg

25
20
15
10

p=0.063 by paired t test

6
12

Pre Morphine sulphate

Morphine sulphate
Fig. 30 The effect of morphine sulphate on basal pressure in the cholecystectomy group

$p=0.026$ by paired t test

Pre Morphine sulphate

Morphine sulphate
PLATE J: THE RESPONSE OF THE PORCINE SPHINCTER TO MORPHINE SULPHATE
The effect on wave amplitude

The sphincter of Oddi motility was also increased in both groups in response to Morphine sulphate. There was an increase in wave amplitude from 4mmHg (25th-75th centiles; 4-7mmHg) to 10mmHg (25th-75th centiles; 4-12mmHg) in the sham group (p=0.099 by paired t test) and from 4mmHg (25th-75th centiles; 0-4mmHg) to 7mmHg (25th-75th centiles; 4-12mmHg) in the cholecystectomy group (p=0.11 by paired t test)(fig. 31). The change in wave amplitude after morphine sulphate was not significantly different between the two groups (sham pre-post amplitude v cholecystectomy pre-post amplitude p=0.93 by Mann Whitney U test).

**Fig. 31 The effect of morphine sulphate on wave amplitude**

<table>
<thead>
<tr>
<th>mmHg</th>
<th>Sham v cholecystectomy</th>
<th>p=0.60 by Mann Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
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<tr>
<td>20</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>mmHg</th>
<th>Sham v cholecystectomy</th>
<th>p=0.80 by Mann Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td></td>
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<tr>
<td>15</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td></td>
<td></td>
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</tbody>
</table>

**Key**
- **Sham group**
- **Cholecystectomy group**
The effect on wave frequency

Wave frequency increased from 1 cycle/minute to 3 cycles/minute in the both groups (fig. 32). There were no significant differences in response between the two groups (sham pre-post wave frequency v cholecystectomy pre-post wave frequency p=0.51 by Mann Whitney U test).

**Fig 32 The effect of morphine sulphate on wave frequency**

<table>
<thead>
<tr>
<th>Sham v cholecystectomy</th>
<th>Sham v cholecystectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>p= 0.8 by Mann Whitney U test</td>
<td>p=1.0 by Mann Whitney U test</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>C/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

**Pre morphine sulphate**

<table>
<thead>
<tr>
<th>Sham group</th>
<th>Cholecystectomy group</th>
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<tr>
<td>1</td>
<td>1</td>
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</table>

**Morphine sulphate**

<table>
<thead>
<tr>
<th>Sham group</th>
<th>Cholecystectomy group</th>
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<tbody>
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<td></td>
<td></td>
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</tbody>
</table>
5.6 Discussion

The basal pressures of our sham group were similar to those reported by Sand et al 1997. Interestingly we have found that in the majority of animals (9/14 (64%)) the sphincter of Oddi exhibited regular phasic activity (1/cycle/minute) a feature previously unreported. Sand et al failed to show phasic activity, probably because their observations were of short duration (pull-through recordings). Cannulation of the ampulla does lead to trauma and spasm of the sphincter and in fact this is believed to be important in the development of post endoscopic retrograde cholangiopancreatography pancreatitis (Sherman et al 1994). By observing a fifteen minute baseline recording the sphincter was allowed to settle and recover from any spasm induced by cannulation and thus our recordings were probably more physiological. The human sphincter of Oddi exhibits regular phasic activity (2-6cycles/minute) and therefore another characteristic of the porcine sphincter of Oddi has been found that strengthens its validity as a suitable animal model of sphincter of Oddi function.

In this study we have found as in previous human (Luman et al 1997) and prairie dog (Grace 1987) studies basal activity of the sphincter of Oddi is not significantly altered after cholecystectomy. Luman et al reported an abnormal response to cholecystokinin after cholecystectomy with a failure of suppression of phasic activity. In this study the sphincter of Oddi in the cholecystectomy group failed to relax in
response to pharmacological doses of cholecystokinin in contrast to the sham group where a significant fall in basal pressure was observed. The mechanism by which this has occurred is unexplained, but as was postulated ((Grace et al 1987, Thune et al 1998, Luman et al 1997) it is possible that during cholecystectomy the division of nerves connecting the gallbladder and sphincter of Oddi, results in altered responsiveness to cholecystokinin. An alternative explanation is that the sphincter of Oddi relaxes in response to large surges of bile flow. It is possible that the sudden increase in bile flow after gallbladder contraction, brought about by cholecystokinin in response to a meal, leads to common bile duct distension which directly relaxes the sphincter of Oddi via local reflexes. Bile flows continuously through the sphincter of Oddi following cholecystectomy and therefore sphincter of Oddi relaxation may not occur after cholecystokinin injection due to a lack of common bile duct distension. More interestingly two of the seven cholecystectomy animals exhibited a paradoxical rise in basal pressure in response to cholecystokinin as has been seen in some patients with sphincter of Oddi dysfunction (Hogan et al 1982, Rolny et al 1986). The paradoxical rise in basal pressure supports the theory that denervation of the sphincter of Oddi may be the cause of sphincter of Oddi dysfunction (Helm et al 1989, Saccone et al 1994, Simula et al 1997, Simula et al 1997) and loss of the described cholecysto-sphincter-of-Oddi reflex (Muller et al 1984, Webb et al 1988). This finding also supports the theory that cholecystectomy is a causal factor of sphincter of Oddi dysfunction and that sphincter of
Oddi dysfunction does not necessarily predate cholecystectomy. However factors other than just cholecystectomy must be involved in the aetiology of sphincter of Oddi dysfunction because sphincter of Oddi dysfunction has been described in patients with an intact gallbladder (Catalano et al 1991, Choudhry et al 1992, Lahoti et al 1997, Catalano et al 1998) and because not all humans develop sphincter of Oddi dysfunction post cholecystectomy (5-30% of those patients with post cholecystectomy pain syndrome (Bar-Meir et al 1984, Brandstatter et al 1991)).

The finding of a paradoxical rise in basal pressure in response to cholecystokinin in two pig raises the possibility that these animals may represent sphincter of Oddi dysfunction in pigs. This raises the exciting possibility that the goal of defining an animal model of sphincter of Oddi dysfunction has been achieved, further studies are warranted to confirm this.

To date the action of morphine sulphate on the porcine sphincter was not known. The findings reported is this study show that intravenous morphine sulphate produces rapid stimulation of the porcine sphincter, resulting in a rise in basal pressure, as it does in man (Coehlo et 1986, Helm et al 1988), thus lending further support for porcine model of sphincter of Oddi function. Helm et al showed that the effects of morphine on the sphincter of Oddi were not mediated by cholinergic nerves. In this study the response to morphine sulphate was not
altered by cholecystectomy, therefore opiate receptors and pathways are unaltered by the disruption of neural networks between the gallbladder, sphincter of Oddi and duodenum following cholecystectomy.
THE IN VIVO EFFECT OF SUBSTANCE P ON THE SPHINCTER OF ODDI.

6.1 Introduction

Substance P is a 11-amino acid oligopeptide which was first recognised as a vasoactive agent by Von Euler and Gaddum in 1931. It is found throughout the body but particularly in the central nervous system and gut (Holzer et al 1982, Pernow et al 1983). Immunohistochemical studies have shown that almost all intestinal substance P is present in neurons, and only a minor portion is contained in endocrine cells of the mucosa (Bartho et al 1985). It is for this reason that substance P has been regarded as a neurotransmitter or neuromodulator (Pernow et al 1983, Bartho et al 1985). Substance P has been shown by radioimmunoassay to be present in the gallbladder and bile duct tissue of the dog and rabbit (Brodin et al 1981, Heitz et al 1977). Immunohistochemical studies have located substance P in the neural networks of both the porcine and human sphincter of Oddi (Sand et al 1993, Sand et al 1994).

Substance P has been shown to stimulate gallbladder contraction in the dog (Lembeck et al 1972) and guinea-pig (Bjurstedt et al 1940). More recently substance P has been shown in vitro to lead to contraction of
the dog sphincter of Oddi by direct binding to substance P receptors (Guo et al 1989) and also to increase myoelectric activity in the opossum sphincter (Parodi et al 1988). While substance P can be found in the neural network of the human and porcine sphincter of Oddi it action is not known.

6.2 Aim

The aim of this study was to determine the effect of substance P on the porcine sphincter of Oddi.

6.3 Methods

Seven large white female pigs (Sus-scrofus domesticus) were studied. Eight weeks previously two pigs had undergone a sham laparotomy and five pigs had undergone cholecystectomy. Manometry was performed using a triple lumen perfused catheter via a duodenotomy incision. The animals had all completed steps 1 to 5 of the study protocol detailed in chapter 4. A 15 minute washout period was observed after the morphine sulphate injection and then 0.5 mg of Substance P was given intravenously through an ear vein and manometric measurements were recorded for 5 minutes.
6.4 Statistical analyses

Sphincter basal pressure and phasic activity were compared for the 5 minutes before and after substance P. The results are therefore paired and statistical analysis was performed using Students paired t test.

6.5 Results

The sphincter of Oddi was seen to contract in all cases and visible gastric emptying was observed (gastric contents was seen to enter the duodenum). The results are tabulated in figure 33. The mean basal pressure rose significantly \((p=0.008 \text{ by paired t test})\) from 4.3mmHg (range 0-18mmHg) to 14.4 (range 5-33mmHg)(fig. 34). Phasic activity significantly increased, wave frequency from 1.1cycles/minute (range 0-4cycles/minute) to 4.1cycles/minute (0-9cycles/minute) \((p=0.014 \text{ by paired t test})\)(fig. 35) and mean wave amplitude rose from 3.4mmHg (range 0-14mmHg) to 12.1mmHg (range 0-25mmHg) \((p=0.018 \text{ by paired t test})\)(fig. 36). The results table O, graphical representation of the data points and a typical manometry recording; plate K are shown overleaf.
**Fig. 33** THE IN-VIVO EFFECT OF SUBSTANCE P ON THE PORCINE SPHINCTER OF ODDI

<table>
<thead>
<tr>
<th></th>
<th>Basal pressure</th>
<th>Wave Amplitude</th>
<th>Wave Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmHg</td>
<td>mmHg</td>
<td>MmHg</td>
</tr>
<tr>
<td>Before substance P</td>
<td>4.3 (2.34)</td>
<td>1.1 (0.63)</td>
<td>3.4 (1.99)</td>
</tr>
<tr>
<td>After Substance P</td>
<td>14.4 (3.34)</td>
<td>4.1 (1.16)</td>
<td>12.1 (3.11)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before v After (paired t test)</td>
<td>p = 0.008</td>
<td>p = 0.018</td>
<td>p = 0.014</td>
</tr>
</tbody>
</table>
Plate K: The response of the porcine sphincter of Oddi to substance P
Fig. 34 The in vivo effect of substance P on the porcine sphincter of Oddi basal pressure

\[ p = 0.008 \]
Fig. 35 The in vivo effect of substance P on the porcine sphincter of Oddi wave frequency

$p = 0.018$

$p = 0.014$

Before

After

Substance P

Substance P
Fig. 36 The in vivo effect of substance P on the porcine sphincter of Oddi wave amplitude.

Substance P stimulates the sphincter of Oddi, where it has a dual effect, to raise intramural pressure and to increase phasic activity. This is also the first study to investigate the action of substance P in vivo, in a porcine animal model.

Studies in rats have shown that substance P stimulates peristalsis via the NK1 receptor (Holzer et al. 1997, Holzer et al. 1992). Studies in non-human primates show that substance P stimulates small intestinal motility (Pelletier et al. 1997, Holzer et al. 1997), and it has also been shown to cause canine gallbladder contraction in vivo (Boo et al. 1997). It is therefore likely that the action of substance P on the sphincter is part of a widespread stimulation of gut motility; indeed Ou et al. found enhanced gastric emptying occurring shortly after the injection of substance P. It is possible that substance P and the NK1 receptor have a role in coordinating small bowel, gallbladder and sphincter of Oddi motility.

More recently there have been advances in the understanding of the function of substance P and the NK1 receptor within the pancreas. Substance P appears to be involved in the development of acute pancreatitis (Bhatia et al. 1996). Studies in NK1 receptor knock-out mice show that in these mice lacking the receptor have an attenuated
6.6 Discussion

This study reports the first description of the action of substance P on the porcine sphincter of Oddi, where it has a dual effect, to raise sphincter pressure and to increase phasic activity. This is also the first study to investigate the action of substance P in vivo, in a category 2 (resistor type sphincter) animal.

Studies in rats have shown that substance P stimulates peristalsis via the NK1 receptor (Holzer et al 1985, Holzer-Petsche et al 1987, Lecci et al 1997). Studies in man have shown that substance P stimulates small intestinal motility (Lordal et al 1997, Holzer et al 1997), and it has also been found to invoke canine gallbladder contraction in vitro (Guo et al 1989). It is therefore likely that the action of substance P on the sphincter of Oddi is part of the more widespread stimulation of gut motility, indeed Guo et al found enhanced gastric emptying occurring shortly after the injection of substance P. It is possible that substance P and the NK1 receptor have a role in coordinating small bowel, gallbladder and sphincter of Oddi motility.

More recently there have been advances in the understanding of the function of substance P and the NK1 receptor within the pancreas. Substance P appears to be involved in the development of acute pancreatitis (Bhatia et al 1998). Studies in NK1 receptor knock-out mice show that in these mice lacking the receptor have an attenuated
inflammatory pancreatic and intrapulmonary response to caerulein induced pancreatitis.

There is also evidence that substance P receptors are pivotal in the transmission of pain from visceral sensory nerves to the spinal cord and central nervous system (Cao et al 1989, Cao et al 1998 and De Felipe et al 1998). Domschke et al 1988 and Giorgio et al 1993 have shown that substance P receptors may be involved in the inflammatory process of chronic pancreatitis. Buchler et al 1992 suggested that substance P receptors may be responsible for the long-lasting pain syndrome in chronic pancreatitis.

In summary, neurotransmitter substance P and its receptor NK1 may have a number of functions within the pancreaticobiliary system; as a neurotransmitter involved in sphincter of Oddi motility; in the modulation of the inflammatory response of acute pancreatitis; and in inflammatory and pain pathways of chronic pancreatitis.
CHAPTER 7
7.1 **Introduction**

In chapter one the two catheters for endoscopic sphincter of Oddi manometry namely, the Lehman perfused catheter and the solid state microtransducer were discussed. Since their development approximately 20 years ago there has been no change or advances in their design despite concurrent advances in catheter technology. The microtransducer catheter only measures the mean sphincter of Oddi pressure. Whereas the Lehman catheter is capable of measuring the pressure profile in two opposite directions. In ours and others experience the manometric pressure measurements taken from the two channels of the Lehman catheter may differ, possibly indicating that the sphincter of Oddi pressure profile is asymmetric in these cases. Examples from our lab of a normotensive symmetrical sphincter (Plate L), an asymmetric normotensive sphincter (Plate M) and an asymmetric hypertensive sphincter (Plate N) pressure profiles are shown. Agreed criteria for the interpretation of such recordings have not been devised (Hogan et al 1997), it is not clear whether to report the mean from the two channels, the lowest or highest basal pressure. A single study (n=288) has attempted to define sphincter asymmetry using the Lehman...
PLATE L A NORMOTENSIVE SYMMETRICAL SPHINCTER MANOMETRY USING THE LEHMAN CATHETER

Basal Pressure = 30 mmHg

Basal Pressure = 28 mmHg
PLATE M: A NORMOTENSIVE ASYMMETRIC SPHINCTER MANOMETRY USING THE LEHAMN CATHETER

Basal pressure = 35 mmHg

Basal Pressure = 20 mmHg
PLATE N A HYPERTENSIVE ASYMMETRIC SPHINCTER MANOMETRY

USING THE LEHMAN CATHETER

Basal pressure = 130mmHg

Basal pressure = 75mmHg

1. The lumen of each channel must remain separate from the adjacent channel and be patent.

2. The distal measuring port hole must remain separate and not coalesce with adjacent holes. Cutting of these holes is the most difficult step.
catheter by measuring the pressures in one plane and then changing the orientation of the catheter by ninety degrees and repeating the station pull-through (Patel et al 1998). Patel et al found that there is sphincter asymmetry in 88% (253/288) and this was more marked in patients with hypertensive biliary or pancreatic sphincters. However it seems unlikely that this group managed to orientate the catheter truly in two perpendicular planes because cannulation of the sphincter of Oddi is not easy at the best of times using the Lehman catheter and the preformed curve at the catheter tip does not facilitate changes in catheter orientation. The other problem with this study is that the Lehman catheter measuring ports are two millimetres (see plate B) apart in distance, the results therefore were manipulated to compensate for this.

Catheters are manufactured by the extrusion of a larger diameter multi lumen tube down to the required diameter. There are a number of difficult steps in this technique and key specifications which must be adhered too, namely:

1. The lumen of each channel must remain separate from the adjacent channel and be patent.

2. The distal measuring port hole must remain separate and not coalesce with adjacent holes, cutting of these holes is the most difficult step.
3. The walls of each channel must be non compliant so that the pressures in each channel is not transmitted across to adjacent channels.

4. The catheter material must be rigid enough to allow guidance of the catheter through the endoscope channel and into the sphincter.

Catheter diameter has been one of the key limiting factor in developing a catheter with more than three lumens because increasing the catheter diameter adversely affects the pressure measurements (Funch-Jensen et al 1984). Large diameter catheters also are more difficult to, pass down the endoscope, cannulate the biliary or pancreatic ducts with and are more likely to obstruct duct outflow, theoretically increasing the likelihood of pancreatitis.

In order to measure asymmetry a catheter needs as many lumens as possible, and the measuring ports to open uniformly around the 360 degrees of the catheter at a single level. The Lehman catheter clearly fails to achieve this objective. However catheters have been developed for the assessment of the lower oesophageal sphincter and anal sphincter asymmetry using a method termed as vector manometry (Williams et al 1994, Zbar 1999, Bombeck et al 1987, Stein et al 1991, Sluss et al 1995, Stein et al 1995). Vector manometry by definition defines a three dimensional contour map of sphincter function. In the assessment of these sphincters it is commonly performed using
a perfused multi-lumen catheter which is pulled through the sphincter at a fixed rate using a mechanical puller unit. Vector analysis computer programs are utilised to interpret the results and determine the radial pressure profile at the point of peak pressure from which three dimensional contour maps can be drawn. These computer programs are able to calculate the vector volume and also the radial asymmetry. The latter is calculated according to this formula:

\[
\text{Radial asymmetry} = 1 - \left( \frac{P_1 + P_2 + P_3 + \ldots + P_n}{n \times \text{maximum of } P_1, \ldots, P_n} \right) \times 100%
\]

\(n\) = the number of recording channels.

A perfectly symmetrical sphincter has a radial asymmetry of 0%.

Within the anal canal this technique has been used to assess obstetric sphincter damage (Donnelly et al 1998, Donnelly et al 1998). Sluss et al reported that patients with low pressure lower oesophageal sphincters were more likely to have asymmetric sphincters and it was speculated that in some patients with low pressure sphincter the degree of asymmetry in the sphincter could be an important factor in reflux events. At present a suitable catheter for sphincter of Oddi vector manometry is not available and therefore vector manometry of the human sphincter of Oddi has not been performed.

In conjunction with Medi-plus, U.K a company which has previously specialized in the manufacture of uro-dynamic catheters, we have developed a new 9 lumen manometry catheter as shown in Plate O.
This catheter of seven French diameter made of an ultra-low compliant PVC, has one central lumen which opens at the tip of the catheter and eight recording channels which terminate in side openings equidistantly spaced and radially arranged in 45° intervals 5mm for the distal end of the catheter.
Plate O: The new nine lumen biliary manometry catheter

All eight measuring ports open 5mm from the tip.
7.2 Aims

1. To bench test the new nine lumen catheter to determine the catheter's fidelity.

2. Using a porcine animal model to assess if the new nine lumen catheter recordings are reproducible.

3. Using a porcine animal model to assess agreement of the new nine lumen manometry pressure recordings with the Lehman triple lumen catheter.

4. To assess the feasibility of assessing human sphincter of Oddi asymmetry using the new nine lumen catheter.

7.3 Methods

7.3.1 Bench test of the new nine lumen catheter.

As discussed in chapter 3, ideally all manometric systems should be capable of recording absolute values of the pressure wave with accuracy. Both pressure rise rates and baseline offset are indicators of catheter fidelity (Omari et al 1996, Chen et al 1998). Pressure rise rate was determined for the new nine lumen catheter with perfusion pressures of 0.1, 0.2 and 0.3 bar by occlusion of the measuring hole and determining the mean pressure rise per second. The baseline offset was determined for the same perfusion pressures by calibrating and zeroing to air the perfusion system and then connecting the
primed catheter and the pressure recorded as the baseline offset. Flow rates were determine by collecting the perfusate over a five minute period for each perfusion pressure and calibration studies were performed using the same sealed system described in chapter 3.

7.3.2 The assessment of the new nine lumen catheter using a porcine animal model

Seven female 20-25kg pigs were studied according to the protocol below.

1. Each animal was anaesthetised with a combination of enflurane, nitrous oxide and oxygen by inhalation and midazolam as an intravenous infusion.

2. Endoscopic sphincter of Oddi manometry was performed using the new nine lumen catheter (only 6 of the 8 measuring channels were used due to limitations of the computer software available at the time). The catheter was introduced into the sphincter and positioned so that the measuring ports were located 1cm within the duct. A station pull through was then performed using five stations, 2mm apart. Two pull-throughs were performed in each case.

3. A single station pull-through manometry was then performed using the Lehman catheter from 1cm within the duct with five 2mm apart stations.
4. The results of each pull-through with the new nine lumen catheter were compared to assess reproducibility.

5. Results were analyzed to compare the mean pressure recordings of the new nine lumen catheter with the mean pressure from the Lehman catheter for the distal 1cm of the bile duct where the sphincter of Oddi is sited.

7.3.3 The feasibility of assessing human sphincter of Oddi pressure asymmetry using the new nine lumen catheter

Six patients with suspected sphincter of Oddi dysfunction, determined on clinical grounds, were studied according to this standard protocol:

1. Patients were sedated with diazemuls (10-30mg) and pethidine (50-100mg).

2. Video duodenoscopy was performed using a diagnostic duodenoscope (Olympus, U.K).

3. The new nine lumen catheter was perfused with bubble free water at a pressure of 0.2 bar from an Arndorfer pneumohydraulic pump at a rate of 0.16ml/channel/minute.

4. Endoscopic manometry was performed using the new nine lumen catheter by a station pull-through technique with 0.5cm apart stations.

5. The central lumen was continually aspirated to decompress the duct and reduce the incidence of procedure related pancreatitis.
6. The data was captured and analysed using a computer based manometry program (Albyn Medical, U.K.).

Two-dimensional radial pressure profiles were drawn from the mean basal pressure in each channel at each station. The radial profiles were then combined from the distal common bile duct through to distal sphincter orifice to form a three-dimensional pressure profile. As yet a suitable pull apparatus has not been developed and therefore manual pull-throughs were performed it is therefore not possible to accurately determine the vector volume from this data and the assessment of radial asymmetry is also limited. However from this data the radial asymmetry was at each station was estimated using the formula described above and then the mean radial asymmetry was calculated for each patient. The mean radial asymmetry was compared with the visual appearance of the 2 dimensional and 3 dimensional pressure profiles.

7.4 Statistical methods

Linear regression analysis was used to determine the correlation coefficient to assess agreement of two pull-throughs with the new multi-lumen catheter. To compare the new catheter with the Lehman catheter the plot of the difference against the average of the standard and new method was determined as described by Martin et al 1995.
7.5 Results

7.5.1 Bench testing of the new nine lumen catheter

The pressure rise rate increased with perfusion pressure with a mean pressure rise rate of 51 mmHg/sec at a perfusion pressure of 0.2 bar. The pressure rise rate against time is illustrated below (fig. 37).

![Fig. 37 Pressure rise rate for the new nine lumen catheter](image-url)
The baseline offset also rose with increased perfusion pressure although the baseline offset at 0.2 was the same as at 0.1 bar, 36mmHg. The graph below (fig. 38) illustrates the baseline offset against perfusion pressure.

![Fig 38 Baseline offset for different perfusion pressures]

The flow rate also increased with perfusion pressure (fig. 39). The recorded catheter pressures appeared to correlate well with the actual pressures at all perfusion pressures (figures 40, 41 and 42). The flow rates and calibration graphs are shown overleaf.
Fig. 39 Flow rates for different perfusion pressures

![Graph showing flow rates for different perfusion pressures.](image-url)
Fig. 40 Calibration of the new nine lumen catheter perfused at 0.1 bar

Fig. 41 Calibration of the new nine lumen catheter perfused at 0.2 bar
Fig. 42 Calibration graph for the new nine lumen catheter perfused at 0.3 bar.
7.5.2 The assessment of the new nine lumen catheter using a porcine animal model

Cannulation of the bile duct was achieved in all six animals in which the biliary ampulla was located. One study was excluded from analysis because in this animal the ampulla was not located due to a deformed duodenum and therefore cannulation was not achieved. The results are displayed below (fig. 43).

The two pull-throughs performed with the new multi-lumen catheter showed significant correlation ($r=0.92, \ p=0.0025$), the linear plot is shown below (fig. 44).

The comparison of the mean manometric pressures of the Lehman and the new nine lumen catheters showed poor correlation when comparing the average difference against the average measurement of the two methods ($r=0.53, \ p=0.09$), as shown below (fig. 44).
The results of porcine sphincter of Oddi manometry using the new nine lumen catheter and the Lehman catheter

<table>
<thead>
<tr>
<th>PIG No.</th>
<th>Mean basal pressure (mmHg)</th>
<th>The new 9 lumen catheter</th>
<th>Lehman Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pull-through 1</td>
<td>Pull through 2</td>
</tr>
<tr>
<td>304</td>
<td>7.0</td>
<td>7.0</td>
<td>11.5</td>
</tr>
<tr>
<td>305</td>
<td>1.0</td>
<td>4.5</td>
<td>11.2</td>
</tr>
<tr>
<td>306</td>
<td>17.0</td>
<td>16.0</td>
<td>9.0</td>
</tr>
<tr>
<td>307</td>
<td>7.0</td>
<td>9.0</td>
<td>13.5</td>
</tr>
<tr>
<td>308</td>
<td>9.3</td>
<td>8.0</td>
<td>10.6</td>
</tr>
<tr>
<td>309</td>
<td>13.8</td>
<td>12.0</td>
<td>13.3</td>
</tr>
</tbody>
</table>
Fig. 44 The assessment of the new nine lumen catheter in a porcine animal model.

### Pull-through 1 vs pull-through 2 with the new multi-lumen catheter

![Graph showing pull-through comparison between two catheters.](https://example.com/graph1.png)

- Pull-through 1 vs pull-through 2 with the new multi-lumen catheter.

### Difference against average of the new multi-lumen catheter and the Lehman catheter

![Graph showing pressure difference.](https://example.com/graph2.png)

- Difference against average of the new multi-lumen catheter and the Lehman catheter.

---

176
7.5.3 The feasibility of assessing human sphincter of Oddi pressure asymmetry using the new nine lumen catheter

It was possible to cannulate the bile duct and perform a satisfactory biliary sphincter pull-through in all patients with the new nine lumen catheter. No procedure related episodes of pancreatitis occurred.

A standard eight lumen recording is shown in plate P, phasic activity and a rise in basal pressure denoted the position of the sphincter of Oddi. Examples of the two-dimensional plot and three dimensional sphincter profiles are shown in plates Q, R and S. The radial asymmetry varied tremendously from 21% to 66% as shown in the table below (fig. 45).

Fig. 45 The mean basal pressure and calculated radial asymmetry

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Mean Basal Pressure (mmHg)</th>
<th>Radial asymmetry %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>SB</td>
<td>14</td>
<td>45</td>
</tr>
<tr>
<td>MC</td>
<td>15</td>
<td>53</td>
</tr>
<tr>
<td>CC</td>
<td>52</td>
<td>27</td>
</tr>
<tr>
<td>SBG</td>
<td>54</td>
<td>32</td>
</tr>
<tr>
<td>CB</td>
<td>32</td>
<td>21</td>
</tr>
</tbody>
</table>
PLATE P: A TYPICAL 8 CHANNEL RECORDING USING THE 9 LUMEN CATHETER

The sphincter of Oddi
PLATE Q: 2D and 3D pressure profile diagrams

Radial asymmetry 27%
Plate R: 2D and 3D pressure profile diagrams

Radial asymmetry = 32%
Plate S: 2D and 3D pressure profile diagrams

Radial asymmetry = 21%

The bench studies for the new nine lumen catheter show that its fidelity compares to the standard Lumen catheter reported in chapter 1. However, the new catheter has a higher level of pressure recorded in the greater number of the nine lumens compared with 0.2 mbar. Potentially this may increase the risk of pressure gradients.

Station 5

Station 1
7.6 Discussion

The bench studies for the new nine lumen catheter show that its fidelity compares favourably with the Lehman catheter reported in chapter 3. The pressure rise rate for the new nine lumen catheter was higher than the Lehman catheter for a given perfusion pressure and the back pressure was lower for a given pressure. The catheter recorded set pressures accurately. The total flow rate was higher for the new nine lumen catheter due to the greater number of lumens (1.3mls/minute for the new nine lumen catheter compared with 0.38mls/minute for the Lehman catheter at a perfusion pressure of 0.2bar). Potentially this may increase the risk of procedure related pancreatitis because the incidence of procedure related pancreatitis increases with increases in catheter flow rate (Meshkinpour et al 1992). However in this albeit small group there were no episodes of pancreatitis.

Results from the porcine study show that measurements made by the nine lumen catheter are reproducible (r=0.92, p=0.0025). However results from the new nine lumen catheter when compared with the pull-through by the Lehman catheter did not agree. The statistical method used in this study is superior than by simple correlation analysis and is the recommended statistical method for comparing methods of measurement (Martin et al 1995). However, Martin et al realise that it is unlikely that any method will agree exactly and with
small sample groups, small differences in measurements will have significant impact on the statistical analysis. This study of six animals (due to constraints from the Home Office) is regarded as small, and this has an impact on the conclusions drawn. A larger study comparing the recordings of the two catheters in normal human volunteers would be the ideal however ethical constraints due to the risk of complications are unlikely to make this possible.

In contrast to the Lehman catheter the new nine lumen catheter provides a complete 360 degree pressure profile of the sphincter of Oddi. Firstly it was possible to locate the catheter within the bile duct in all subject, this is a significant accomplishment because sphincter of Oddi manometry is regarded as one of the most difficult techniques to master for any experienced endoscopist. The measurements made by the nine lumen catheter indicate that the human sphincter of Oddi is asymmetric. Therefore measurements using the Lehman catheter are seriously flawed due to the limited sampling from two channels.

The high pressure zone was consistently shown to be directed upwards towards the transverse fold. This is to be expected because the anatomy of the sphincteric muscle is of a figure of eight around the pancreatic and biliary ducts and therefore the thickest muscle layer can be expected to be located between the two sphincters i.e. in an upward direction when assessing the biliary sphincter. This method of assessing sphincter of Oddi symmetry may improve the efficacy of
endoscopic sphincterotomy, which although proven in Milwaukee type 2 patients, is unproven in type 3 patients with hypertensive sphincters (Bozkurt 1996, Wehrmann 1996). It may be possible to direct the sphincterotomy towards the defined high pressure zone of the sphincter for example, in plate R the sphincterotomy should be directed towards 11-12 o'clock, which is away from the orthodox 12-1 o'clock biliary sphincterotomy direction. (Danilewitz et al 1984, Neoptolomos et al 1984, Neoptolomos et al 1988, Wehrmann et al 1996, Cotton et al 1998). Studies are required to evaluate the manometric and symptomatic efficacy of a manometrically directed sphincterotomy.

This study was a feasibility study to assess whether it was possible to record sphincter of Oddi pressures endoscopically using the new nine lumen catheter we have therefore achieved what we set out to do. However as indicated it was not possible to perform vector volume analysis and the radial asymmetry calculations are of limited value using the apparatus employed, predominantly due to the lack of mechanical pulling device and the limitations of the computer recording and display package. Further work is required to develop an apparatus in which the endoscope could be fixed once the catheter is located within the duct after which evaluation of a mechanical pulling device could be performed. The essential feature of the mechanical pulling device is that it must be able to withdraw the catheter through the sphincter at a constant and set rate. To achieve this the pulling device must be able to overcome the resistance of the catheter within the
biopsy channel of the endoscope and the rate of pull must be fast enough to minimise the effect of respiratory movement. Potentially, mechanical pulling devices used for oesophageal and ano-rectal studies could be used. There are already computer programs (Synectics Ltd, U.K.) available to assess the oesophageal and anal sphincter and these could also be easily adapted to measure the sphincter of Oddi. It is theoretically possible, now that this new nine lumen catheter has been developed, to perform true vector manometry of the sphincter of Oddi.
Chapter 8
AN AUDIT OF SPHINCTER OF ODDI MANOMETRY PERFORMED AT
THE ROYAL LONDON HOSPITAL.

8.1 Introduction

Sphincter of Oddi manometry is common practice in the U.S.A, Australia and Japan, but within Britain there are only a few centres carrying it out and none have published audit data of their outcomes.

The clinical service at the Royal London Hospital was established in 1993 by Dr Colin Ainley (the endoscopist) and Dr David Evans (the physiologist). I have subsequently joined this team and carry out the endoscopic procedure as well as data interpretation and reporting. The London Hospital receives many tertiary referrals from the North East Thames region and it is from this patient group that the referrals for sphincter of Oddi manometry arise. Most referrals are from consultant Gastroenterologists and a few from consultant Surgeons.

8.2 Aim

The aim of this study was to audit the outcome of 52 patients following who underwent sphincter of Oddi manometry between August 1993 and August 1996 at the Royal London Hospital.
8.3 Methods

All manometries were performed by a single endoscopist Dr. C. Ainley. Sphincter of Oddi manometry was performed by a standard technique:

1. Patients were sedated with diazemuls (10-30mg) and pethidine (50-100mg).
2. Endoscopic manometry was carried out by a station pull-through technique using two of the three channels of the Lehman (Wilson-Cook, USA) catheter. The catheter was perfused with bubble free water from a low compliance Arndorfer pneumohydraulic pump at a rate of 0.2ml/minute/lumen.
3. The third central lumen of the Lehman (Wilson-Cook, USA) catheter was continually aspirated to reduce the incidence of pancreatitis (Sherman et al 1990).
4. A second single lumen catheter was attached to the outside of the duodenoscope to record duodenal pressure. This catheter was perfused and transduced as above.
5. Attempts were made to achieve manometric measurements of both the pancreatic and biliary sphincters in all cases.
6. Manometry data was captured and analysed using a computer based manometry system. (Albyn Medical, U.K.).

Audit data was collected by obtaining the manometric records and the medical records of the patients. These were systematically reviewed by
Dr. A. Millar and Dr. E. A. Stoner. Patients were classified according to results of manometry; namely a hypertensive sphincter of Oddi (basal pressure >40mmHg) with or without tachyoddia (phasic activity >8 cycles per minute), tachyoddia alone, or normal.

8.4 Results

Sufficient follow up data was available for 37 of the 52 patients and is represented below (fig. 46).

**Fig. 46** The results of the audit of sphincter of Oddi manometry performed at the Royal London Hospital from August 1993 to August 1996

<table>
<thead>
<tr>
<th>Hypertensive ± Tachyoddia</th>
<th>Tachyoddia</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>36 (25-79)</td>
<td>43 (32-37)</td>
</tr>
<tr>
<td>Sex (F:M)</td>
<td>11:02</td>
<td>03:00</td>
</tr>
<tr>
<td>Post-cholecystectomy (%)</td>
<td>5 (38)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>RUQ pain (%)</td>
<td>5 (38)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Pancreatitis (%)</td>
<td>3 (23)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Most of the patients investigated were female (78%) and most of the patients (25(67%)) were classified into Milwaukee biliary type 3. No episodes of procedure related pancreatitis occurred.

Twenty-one (57%) of the thirty-seven had normal basal sphincter of Oddi pressure. Thirteen patients (35%) had a hypertensive sphincter of Oddi basal pressure with or without associated tachyoddia. Three patients (8%) were found to have tachyoddia alone. Of the thirteen patients with a hypertensive sphincter of Oddi, ten had undergone endoscopic sphincterotomy at the time of this review. Symptom resolution was achieved in seven (70%) (follow-up 3-20 months). Of the twenty-one with normal sphincter of Oddi manometry, eleven (52%) have subsequently been given alternative diagnosis and symptom resolution has occurred spontaneously in two of the remaining ten.

8.5 Discussion

Satisfactory patient follow-up was achieved in only 71% of patient. Contact was carried out by writing or telephoning the referring consultants secretary and depended on good will to forward the appropriate information. Information gathering and data collection could have been improved upon, by visiting the appropriate hospitals and seeking appropriate authorization to view patient records.
Since the introduction of this clinical service there has been a steady referral rate of patients with symptoms suggestive of sphincter of Oddi dysfunction. According to the Milwaukee classification our patients are predominately type 3 and a few type 2. By definition type 3 patients have only biliary type pain and as in other studies relatively few patients were found to have a hypertensive sphincter of Oddi (Hogan et al 1987, Wehrmann et al 1996, Hogan et al 1997). This mixture of type 2 and 3 patients explains why symptomatic relief was achieved in only 70% following sphincterotomy (Wehrmann et al 1996). The low rate of referral of patients categorised as Milwaukee type 1 patients was expected because in these patients after diagnostic endoscopic retrograde cholangiopancreatography they often go on to therapeutic sphincterotomy as a therapeutic trial rather than being referred for manometry. This course of management for type 1 patients is acceptable under current recommendations (Lans et al 1991, Hogan et al 1997). It is possible that type 2 patients have likewise undergone sphincterotomy as a therapeutic trial because referral for sphincter of Oddi manometry is regarded by many clinicians as a "last resort" investigation in patients with posing a diagnostic dilemma.

Of the patients found to have normal sphincter pressures half have been given alternative diagnosis, mainly irritable bowel syndrome or gastro-oesophageal reflux. Exclusion of sphincter of Oddi dysfunction was probably useful in the future management of these patients.
Surprisingly there were no episodes of pancreatitis which may be due to the aspirating technique employed and the low water perfusion rate used. (Sherman et al 1990, Meshkinpour et al 1992). The aspirating technique has also been proved to be useful in collecting pancreatic juice for other studies.

At this time dynamic studies were not part of our standard protocol. It is now our current practice to perform dynamic studies with both cholecystokinin (Kinevac, Bracco Diagnostics, USA) (20ng/kg bolus) and secretin (GIH, Kabi Diagnostica, Sweden) (1CU/kg bolus) with the catheter located midway in the high pressure zone.
TACHYODDIA INDEPENDENT OF THE DUODENAL MIGRATING MOTOR COMPLEX MAY BE A PART OF SPHINCTER OF ODDI DYSFUNCTION.

9.1 Introduction

During fasting in most mammals, the gastrointestinal tract exhibits cyclical changes in activity called the migrating motor complex. The migrating motor complex consists of four phases, phase 1 a period of quiescence; phase 2 shows irregular intermittent contractions; phase 3 is characterized by periods of short bursts or regular high-amplitude contractions; and phase 4 is a brief transition back to another quiescence (Sarna et al 1985). The phasic activity of the sphincter of Oddi of opossums (Honda et al 1982, Tanaka et al 1990) and dogs (Scott et al 1984, Scott et al 1984, Yokahata et al 1994) have been shown to vary with the phasic activity of the duodenum, with maximal activity at the time of phase 3 of the migrating motor complex. In man the phasic activity sphincter of Oddi phasic activity varies in synchrony with the migrating motor complex (Torsoli et al 1986). Tanaka et al 1992 reported a study of ten post cholecystectomy patients, using a solid state catheter with two microtransducers one to measure sphincter of Oddi pressure and one to measure duodenal activity. There was a transient elevation in basal sphincter of Oddi pressure in concert with phase 3 of the migrating motor complex and it was suggested that this
could contribute to the development of intermittent post cholecystectomy pain in some patients. However Tanaka’s study did not have a control group and therefore a rise in basal pressure during the fast phase of the migrating motor complex may be a normal finding. The normal frequency for sphincter of Oddi phasic activity is 2-6 cycles per minute and above 8 cycles per minute is considered abnormal (Toouli et al 1990). The term tachyoddia has been applied to this state of abnormally high frequency activity (Toouli et al 1990, Hogan et al 1995). At present the pathological significance of tachyoddia is not known and its presence is not a recognised indication for endoscopic sphincterotomy (Geenen et al 1989, Lans et al 1991, Hogan et al 1995).

9.2 Aims

The aims of this study were to:

1. To determine the incidence of tachyoddia in our series of 65 consecutive sphincter of Oddi manometries.

2. To determine if tachyoddia is always synchronous with or may be independent to the fast phase 3 of the duodenal migrating motor complex.
9.3 Methods.

The sixty-five consecutive sphincter of Oddi manometries were retrospectively reviewed by Dr E.A. Stoner. All manometries were performed in a standard way and duodenal activity was simultaneously recorded using a perfused catheter attached to the outside of the duodenoscope (as described in chapter 8). Phasic activity of the sphincter of Oddi greater than eight cycles per minute was defined as tachyoddia. The recordings were analysed to determine the incidence of tachyoddia in our sample. If tachyoddia occurred at a time when there was marked duodenal activity then this was termed as synchronous tachyoddia. If tachyoddia occurred when there was no or little duodenal phasic activity, phase one of the migrating motor complex, the tachyoddia was defined as being independent.

9.4 Statistical methods

The Fishers exact test was used to whether tachyoddia was associated with from the migrating motor complex.
9.5 Results

Fifteen (23%) of the sixty-five recordings had at least one episode of tachyoddia. Ten (67%) of the episodes of tachyoddia were synchronous with the duodenal phasic activity and five (23%) were independent. Of the five that were independent most (4/5) were associated with a raised basal pressure and conversely tachyoddia (9/10) synchronous with the migrating motor complex was more commonly associated with a normal pressure. The results are tabulated below (fig. 47).

A raised basal pressure was significantly (Fisher’s exact test; two sided; p=0.0007) associated with tachyoddia independent from the duodenal migrating motor complex.

Examples of typical recordings with tachyoddia independent to and synchronous with the migrating motor complex are shown in plate T and U respectively.
Fig. 47 The relationship of tachyoddia to the duodenal migrating motor complex

<table>
<thead>
<tr>
<th>Basal Pressure</th>
<th>Tachyoddia synchronous with the migrating motor complex</th>
<th>Tachyoddia independent from the migrating motor complex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raised (&gt;40mmHg)</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Normal (&lt;40mmHg)</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>
PLATE T: TACHYODDIA INDEPENDENT TO THE DUODENAL MIGRATING MOTOR COMPLEX.
Plate U: Tachyoddia synchronous with the duodenal migrating 

**motor complex**
9.6 Discussion

This study is the first to show that sphincter of Oddi phasic activity may be independent to the duodenal migrating motor complex. This observation suggests that the sphincter of Oddi has its own intrinsic pacemaker activity. It is probable that the neural connections between the gallbladder, duodenum and sphincter of Oddi co-ordinate phasic activity, similar to the sino-atrial node, atrio-ventricular node and purkinje fibres of the heart, (Dahlstrand et al 1995, Padbury 1993, Simula et al 1997, Simula et al 1997). Disruption of these pathways at cholecystectomy could lead to independence of the sphincter of Oddi from the duodenal activity as occurs in the ventricles of the heart in complete heart block.

It is important to note that independent tachyoddia occurred more commonly in patients with hypertensive sphincters. Therefore independent tachyoddia may be a part of sphincter of Oddi dysfunction. The term biliary colic by definition is a fluctuant pain, and may be due to the fast high pressure phasic activity of a sphincter which is not only hypertensive but exhibits tachyoddia.

Nausea and bilious vomiting are common symptoms of postcholecystectomy syndrome (Drossman et al 1993, National Institutes of Health Consensus Conference on Laparoscopic Cholecystectomy (1993)) and may be due to altered antroduodenal activity following
cholecystectomy, reducing the duration of phase 3 of the migrating motor complex as compared to normals (Perdikis et al 1994). Perdikis et al 1994 found gastric pH>3 for a significantly longer time in the symptomatic post cholecystectomy group compared to asymptomatic patients. In this study duodenogastric reflux was also assessed using a gastric aspiration technique following an infusion of 99mTc-DISIDA. Significantly higher radioactivity of the aspirates was found in the symptomatic patients compared to normals (P<0.001) and asymptomatic controls (P<0.01). It has been suggested that removal of the gallbladder leads to loss of the bile reservoir and the disordered antroduodenal activity leads to duodenogastric reflux. From our findings it is also possible that the disordered sphincter of Oddi activity leads to pooling of bile in the duodenum and therefore promoting duodenogastric reflux.

The method of station pull through manometry only provides a snapshot of the sphincter activity and to further define the activity of the sphincter of Oddi more prolonged studies of the sphincter and duodenal pressures are required.

Morphine sulphate is known to induce phase 3 of the migrating motor complex and studies have shown a synchronous rise in basal sphincter of Oddi pressure in dogs (Yokohata 1994) and pigs (Stoner 1998) and in man (Ogawa 1992). It is possible that morphine sulphate could be used in a provocation study, devised to invoke phase 3 of the
migrating motor complex and phasic activity of sphincter of Oddi manometry and assess their relationship.
Chapter 10
CONCLUSION

10.1 Summary and discussion of work presented

The development of laparoscopic cholecystectomy has led to a significant rise in the annual number of cholecystectomies performed due to a lower threshold for surgery (Lam et al 1996). As a consequence, the prevalence of post-cholecystectomy pain syndrome is increasing, despite the more favourable outcome that laparoscopic surgery provides, 77-95% report satisfactory symptom relief (Peters et al 1996, Velpen et al 1993, McMahon et al 1995, Luman et al 1996)). Sphincter of Oddi dysfunction is believed to be one of a number of putative causes of post cholecystectomy pain (5-30% of patients with post-cholecystectomy pain syndrome (Bar-Meir et at 1984, Brandstatter et al 1991)). Although less invasive techniques have been used to diagnose sphincter of Oddi dysfunction, sphincter of Oddi manometry is regarded as the gold standard. The results of sphincter of Oddi manometry are proven to be predictive of the outcome of therapeutic sphincterotomy (Geenen et al 1990, Kaikaus et al 1995).

In view of the risk of pancreatitis (up-to 25% Albert et al 1988, Sherman et al 1992, Rolny et al 1992, Catalano et al 1998) it is no longer acceptable to perform manometry on normal individuals or experiment on symptomatic individuals. Therefore suitable animal models...
are important for the development of new techniques. The majority of studies to date have developed small animal models such as the opossum or cat and in these animals manometric measurements cannot be made with the same apparatus as in man and so are not ideal. Although the dog is suitably large to study endoscopically, its use is more strictly controlled by the Home Office and incurs considerable expense. In this thesis a porcine animal model has been developed. The advantages of the pig over other animal models are; the pig is large enough to enable endoscopic manometry to be performed, the porcine sphincter of Oddi responds similarly to man to cholecystokinin, and pigs are relatively cheap to purchase and keep (Pasricha et al 1995).

Animal studies often require the use of general anaesthesia not only to meet approval from the Home Office by ensuring no suffering is incurred but also to keep the animal still and provide a safe environment in which to perform endoscopic techniques. It is essential that the anaesthetic agents used do not influence sphincter of Oddi motility. In chapter four a combination of enflurane, nitrous oxide and oxygen by inhalation and midazolam by intravenous injection was found to be a suitable anaesthetic regime (Stoner et al 1998). Conversely halothane was found to suppress porcine sphincter of Oddi motility and should be avoided (Stoner et al 1998).
In chapter 5 attempts have been made to explore the aetiology of sphincter of Oddi dysfunction and to develop the porcine model with a view to developing an animal model of sphincter of Oddi dysfunction. Cholecystectomy when compared to sham laparotomy did not appear to alter resting basal pressure or phasic activity, however it did dampen the inhibitory response to pharmacological doses of cholecystokinin but the stimulatory effect morphine sulphate was unaltered. This finding supports the theory that cholecystectomy is a causal factor of sphincter of Oddi dysfunction and that sphincter of Oddi dysfunction does not necessarily predate cholecystectomy. However factors other than just cholecystectomy must be involved in the aetiology of sphincter of Oddi dysfunction because sphincter of Oddi dysfunction has been described in patients with an intact gallbladder (Catalano et al 1991, Choudhry et al 1992, Lahoti et al 1997, Catalano et al 1998) and because not all humans develop sphincter of Oddi dysfunction post cholecystectomy (5-30% of those patients with post cholecystectomy pain syndrome (Bar-Meir et al 1984, Brandstatter et al 1991)).

Two post-cholecystectomy animals exhibited an abnormal, paradoxical (rise in basal pressure) response to cholecystokinin. In man a paradoxical rise in sphincter of Oddi basal pressure is regarded as a diagnostic feature of sphincter of Oddi dysfunction and is seen more commonly in patients with associated irritable bowel syndrome (Hogan et al 1982, Rolny et al 1986). It is possible that this sub-group of post-cholecystectomy animals are representative of porcine sphincter of
Oddi dysfunction. This statement is limited by the small numbers studied in this project in order to conform to the restrictions set by the Home Office and larger confirmatory studies are now warranted. This study is the first to report phasic activity of activity of the porcine sphincter of Oddi (1 cycle/minute) probably due to the prolonged recordings observed in these studies. Morphine sulphate was found to stimulate the porcine sphincter of Oddi manometry with an increase in basal pressure and phasic activity. Therefore morphine sulphate may be a suitable agent to use when stimulation of the porcine sphincter is required. The characteristics of the porcine sphincter closely resemble the human sphincter and is a suitable model for the investigation of the sphincter of Oddi function, alleviating the risk of causing pancreatitis in human subjects.

Substance P is an important neuropeptide found in the visceral sensory nerves, the central nervous system and the enteric nervous system (Anonymous 1993, Costa et al 1982) Via the latter pathway it is believed to modulate peristalsis (Anonymous 1993, Costa et al 1982). Immunohistochemical studies have shown this oligopeptide to be present in both neural networks of the human and porcine sphincter of Oddi (Sand 1993, 1994) however its action was not known. In chapter 6 it was found that intravenous exogenous substance P leads to stimulation of the porcine biliary sphincter. This is the first description of the action of substance P on not only the porcine sphincter but on any animal with a type 2 (resistor like) sphincter of Oddi. It is possible
that substance P and the NK1 receptor may have a role in coordinating small bowel, gallbladder and sphincter of Oddi motility. Others have found that substance P may play a pivotal role in the development of acute pancreatitis and also in the pain pathway of chronic pancreatitis (Cao et al 1989, Cao et al 1998 and De Felipe et al 1998, Domschke et al 1988 Giorgio et al 1993, Buchler et al 1992). Future work should assess the action of substance P on the porcine pancreatic sphincter and the action of substance P receptor antagonist on both sphincters.

Sphincter of Oddi manometry has been performed endoscopically using two types of catheters, the Lehman (Wilson -Cook) triple lumen perfused catheter and the solid state catheter favoured by Eastern countries. To date one unsatisfactory study has assessed symmetry of the sphincter of Oddi (Patel et al 1998). In chapter seven a new nine lumen catheter has been developed and used in man. This catheter has eight recording channels which are evenly spaced around the circumference of the catheter terminate at one level. Bench studies have shown that this catheter's fidelity compares favourably with the Lehman catheter. The feasibility study in a group of patients with suspected sphincter of Oddi dysfunction was successful and it is now possible to obtain a 360 degree pressure profile of the human sphincter of Oddi for the first time. Interestingly the sphincter of Oddi was found to be asymmetric, this is a cause of concern when measuring sphincter pressures using the Lehman catheter due to its
limited bi-directional measurement. The efficacy of therapeutic sphincterotomy may be improved with detailed mapping of the sphincter pressure profile, by enabling directed cuts towards the high pressure zone. There were no episodes of pancreatitis with this method though larger studies are required to confirm it to be a safe technique. Further developments are required in order to perform true vector manometry and vector volume analysis, but with this new catheter design it seems possible.

The audit of the first fifty-two sphincter of Oddi manometries at the Royal London Hospital has given insight into the outcome of the largest series of cases in the United Kingdom. The relatively low incidence of hypertensive sphincters reflects the pre-selected group of patients investigated, those classified as Milwaukee type 2 or 3, because type 1 patients are likely to have undergone diagnostic/therapeutic endoscopic sphincterotomy. The practice of simultaneously measuring duodenal activity at the time of sphincter of Oddi manometry has interestingly shown that tachyoddia independent to the duodenal migrating motor complex is associated with a raised basal pressure and may be a part of sphincter of Oddi dysfunction.
The most important development yet to be achieved is a safe method of prolonged possibly ambulatory manometry. At present pull-through techniques provide only a snap-shot of sphincter activity and are therefore far from ideal to diagnose sphincter of Oddi dysfunction a condition with characteristically causes intermittent symptoms. Prolonged manometry will not only provide more information about sphincter physiology, but will provide valuable information about sphincter of Oddi dysfunction, and may dismiss the dubious stigma with which this diagnosis is regarded. It is likely that microtransducers rather than perfused catheters will be developed for prolonged manometry. The catheter will need a method of anchorage within the bile duct so that the recording device lies within the sphincter. A prototype has been developed for our own laboratory which has two microtransducers mounted on a seven french catheter with a pigtail catheter at the distal end. There is a central channel which allows passage of a guidewire to straighten the pig-tail catheter for placement purposes. However this catheter has proved not only expensive to purchase but also in its upkeep due to its fragility. The size of the catheter also prevents its use in humans because of the theoretical risk of inducing pancreatitis due to obstruction of the pancreatic duct. Advances in microchip technology are likely to enable smaller and potentially safer catheters to be developed, it may even be possible to place a passive microtransducer onto a biliary stent and place this within the sphincter. However any object that distends the sphincter or allows free passage
of bile through the sphincter is unphysiological. It is important that suitable animal models such as the porcine model, are developed so that progress in catheter design and manometric methods can continue without putting patients or volunteers at risk.

Advances in imaging techniques such as magnetic resonance imaging may provide acceptable non-invasive assessment of sphincter of Oddi function. To date no study has used this modality to assess sphincter function however with the development of magnetic resonant cholangiopancreatography it does seem feasible and it may be possible to perform studies similar to the fatty meal, cholecystokinin or secretin ultrasound. Endoscopic ultrasound may also provide information about the sphincteric muscle. An interesting comparison would be to compare manometric assessment of sphincter of Oddi symmetry using the new nine lumen catheter with anatomical examination using endoscopic ultrasound.

Psychological factors also need consideration as sphincter of Oddi dysfunction is often regarded as a functional disorder along with irritable bowel syndrome. Certainly both conditions have similarities, affecting young women who present with a diversity of symptoms. The closeness of these two conditions and their relationship to psychological parameters are currently part of a prospective study which although, instigated by me, is not yet complete and therefore not presented in this thesis.
Endoscopic sphincterotomy is the only proven technique for the treatment of sphincter of Oddi dysfunction. Botulinum toxin seems an attractive alternative and warrants formal clinical assessment. As a placebo-controlled trial is probably not ethical blinded comparison should be made with endoscopic sphincterotomy. Medical therapies at present are unsatisfactory but with the increased knowledge that prolonged ambulatory studies might provide, it is conceivable that non invasive treatments may yet prove clinically useful.


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