ASPECTS OF COLONIC MOTILITY

IN

IDIOPATHIC SLOW TRANSIT CONSTIPATION

Volume I of a Thesis submitted to the University of Glasgow for the Degree of Doctor of Medicine.

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This work is dedicated to my wife and second son, from whom the time has been taken to research and present this thesis and to my father and first son, from whom life was taken that they were never able to read it.

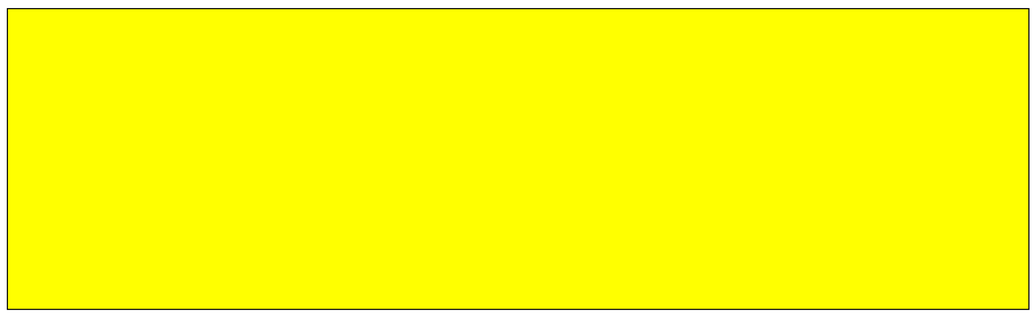
ARRANGEMENT OF THESIS

This thesis is arranged in two volumes for the benefit of the reader. Volume I contains the main text and references, which should be read in association with volume II which contains, in chronological order, the graphs, tables and diagrams which accompany this work. Volume I is divided into two parts; the clinical investigation of idiopathic constipation, and *in-vitro* studies of colonic smooth muscle function.

CERTIFICATE

This is to certify that, except where stated, the work contained in this thesis was carried out by the candidate. Colorectal manometry studies were carried out, exclusively, by myself. Radio-isotope and radiological marker studies were carried out in association with the Department of Nuclear Medicine and the Radiology Department of Glasgow Royal Infirmary and their assistance has been indicated in the acknowledgements.

In-vitro studies on colonic smooth muscle were performed with the assistance of Mr John Craig (Department of Pharmacology, Glasgow University), whose contribution has been acknowledged.



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PREFACE

The majority of patients with idiopathic slow transit constipation have pancolonic dysmotility which manifests itself as delayed colonic transit. For those patients who do not respond to conservative management, surgery usually involves colectomy and ileorectal anastomosis. This operation usually results in symptomatic relief in about 60% of patients. However, many of the residuum are not improved and indeed are often worse following surgery.

This thesis sets out to examine the hypothesis that some patients with idiopathic constipation, notably those who develop their symptoms following childbirth or hysterectomy, have regional as opposed to total colonic dysmotility. Such a group may be amenable to segmental rather than total colonic resection.

Several clinical studies are presented which establish postchildbirth/hysterectomy constipation as a distinct subgroup of idiopathic constipation. Studies of gastric emptying demonstrate that patients with postchildbirth/hysterectomy constipation have normal motility in the proximal gastrointestinal tract. In contrast, patients with idiopathic constipation have prolonged gastric emptying indicating that proximal GI

dysmotility may form a significant component of the presenting symptoms. Having identified that the proximal GI tract appears normal in patients with postchildbirth/hysterectomy constipation the next task was to identify in which region of the colon the dysmotility was most severe.

Segmental colonic transit studies, using radio-opaque markers, identify delayed transit in the left colon, while dynamic radio-isotope studies localise the area of abnormality to the sigmoid colon. Colonic manometry studies, using a water-perfusion catheter point to a region of hindgut dysmotility which manifests as an excess of low pressure waves at rest and a specific failure to generate high pressure propagative waves. The usefulness of prostigmine provocation testing is examined critically in this group of patients and the pitfalls of this technique are presented.

Following surgical resection of the sigmoid colon, a series of in-vitro experiments were carried out on the resected specimens. Nerve and receptor-mediated responses were identified in both constipated and control sigmoid taenia coli suggesting that the efferent neural pathways are intact. In a repeat of the experiments first described by Trendelenberg, peristalsis was demonstrated in segments of control sigmoid colon. However this activity could not be

produced in constipated sigmoid colon despite the addition of ganglion stimulating agents. This observation suggested that while the sigmoid colon of patients with postchildbirth/hysterectomy constipation is sensitive to the pharmacological stimuli which initiate contraction/relaxation of the human colon it is less sensitive to the afferent stimuli which normally mediate the peristaltic reflex.

The nature and pathophysiology of this subgroup of patients has not been fully identified by the work in this thesis but sufficient evidence is presented to propose traumatic childbirth and hysterectomy as predisposing factors in regional as opposed to pancolonic constipation. Idiopathic constipation is a heterogeneous condition and the investigation thereof requires a heterogeneous approach.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

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1.1 Introduction

At the beginning of this century Arbuthnot W. Lane, reported in a series of communications to the British Medical Journal (1-4) the results of colectomy and ileo-rectal anastomosis for severe constipation. He identified a group of patients, predominantly female, of child bearing age or younger, with severe constipation, whose symptoms were dramatically improved by removal of the colon. He also observed that the colon of these women with severe constipation, though macroscopically normal, was non-functional. The syndrome then became known as Arbuthnot Lane's Disease. In the absence of a pathonmonic test, whether or not the disease he treated with some success is the same as that which we now call idiopathic slow transit constipation (ISTC) remains unknown. Equally, in the absence of histology which identifies pathological abnormality, there is no certainty that the condition which we treat 80 years on, with no greater a success rate, is in fact Arbuthnot Lane's Disease (5,6).

ISTC is currently defined as a disorder of normal colon propulsion which results in severe constipation. By convention ISTC excludes megarectum and megacolon patients.

1.2 Pathophysiology

Constipation is a common clinical complaint but what the patient means by it is often unclear (7). Normal bowel function is difficult to define, but most people defaecate between three times a day and once every three days (8,9). Marginal infrequency beyond this may be attributed to poor diet and frequently responds to bulk laxatives (10). Other constipated patients, who do not respond to bulking agents, find that their symptoms improve with cisapride (11). Clinicians are increasingly recognising a group of patients with severe chronic constipation whose symptoms are not helped by bulking agents, prokinetic drugs or other laxative therapy. These patients who are invariably women, defaecate as infrequently as once every three to four weeks and seldom without the aid of enemeta or ano-rectal digitation. On taking a detailed history, two groups of patients are emerging. Firstly, there are those patients whose symptoms date from their teens or earlier and whose condition has progressively deteriorated with time (12-14). In contrast, the second group of women (to whom this thesis is addressed) attribute the onset of their condition to a recognisable event e.g. pelvic surgery or child birth. The first group is defined physiologically as having delayed colonic transit, (15-17) (idiopathic slow transit constipation) and the second by their inability to expel a balloon from the rectum

thus implying a rectal/pelvic floor dysynergia (outlet obstruction constipation) (18-21). The picture is confused by the observation that some patients, with evidence of slow transit constipation, also appear to have features of outlet obstruction constipation (OOC) (22,23). Patients with delayed colonic transit with or without outlet obstruction constipation should be classed as ISTC whilst those with OOC alone should not be included in this nosological category.

Patients who suffer from ISTC often complain of urological symptoms. Urodynamic studies performed in these patients demonstrate detrusor dysfunction, indicating that an abnormality of the autonomic nerve supply to the bladder may be present (24,25). Further studies have shown that denervation in the bladder often co-exists with rectal denervation (26), in each case accompanied by increased tissue sensitivity. This observation is consistent with Cannon's law of denervation hypersensitivity (27,28). In contrast, studies performed in those patients who attribute their symptoms to child birth (OOC) suggest that there is resulting obstructed urinary flow which cannot be accounted for on the basis of autonomic neuropathy (29). This might be explained by primary damage to the pelvic floor during a traumatic second stage in childbirth which could result in a dysinergia of rectal and urethral emptying mechanisms.

The pelvic floor and its nerve supply are at risk during prolonged labour and in some cases even normal child birth is associated with a permanent change in pelvic floor anatomy and physiology (30). The recent observation that a denervation injury can result following hysterectomy lends some support to the hypothesis that trauma can result in disordered rectal physiology (31). The inferior hypogastric plexus in the female runs along the side of the rectum, uterine cervix, vaginal fornix and posterior aspect of the bladder and then passes into the base of the broad ligament and it is perhaps at this point that nerve injury can occur during hysterectomy. Simple hysterectomy as perfomed for benign disease should, in theory, be less likely to injure the pelvic nerves. In contrast, a radical hysterectomy with dissection of the broad ligament and pelvic side walls would place the pelvic parasympathetic nerves at risk. Severe constipation appears to occur following both simple and radical hysterectomy (31,32). However, post hysterectomy constipation patients appear to be clinically indistinguishable from those in whom the condition arises, de-novo (32). In addition, other workers have shown that urological symptoms attributed to hysterectomy are often present prior to surgery but are given heightened significance following surgery (33). The same may be true of constipation.

The source and distribution of both the sympathetic and parasympathetic supply to the left colon and rectum is now more clearly understood. Injury to the autonomic supply to the pelvis and recto-sigmoid may occur during pelvic surgery (34,35). Experimental work has shown that following such injuries, subsequent imbalance of re-innervation with proportionally more sympathic nerve fibres may contribute to the dysfunction (36,37). In patients, resection of the rectosigmoid may interrupt the pelvic nerves in their course through the left colon (38,39) and rectal surgery is often associated with the development of constipation (40).

The aetiology of outlet obstruction is often apparent with patients attributing the onset of their symptoms to either hysterectomy or childbirth. However, it is more difficult to logically explain the aetiology of idiopathic slow transit constipation.

Childhood constipation is not sex specific and yet those presenting with ISTC are almost exclusively female. It is tempting to "blame it on the hormones." Perhaps the onset of menstruation is the "straw that breaks the camel's back." Progesterone, in pharmacological doses, relaxes both animal and human colonic smooth muscle (41,42). Similarly, the responsiveness of ileum and colon in vitro to acetylcholine is reduced in the presence of progesterone (43). A similar picture exists in the gallbladder and the gastrooesophageal

junction; this implies that progesterone may have an inhibitory effect on the whole gastrointestinal tract (44). Although some women suffer from constipation during pregnancy, the precise aetiology is unclear. Abnormally high concentrations of the sex hormones have been detected in the urine of women with severe constipation but this may be related to abnormal enterohepatic circulation and may not be the primary cause. Sex hormones have little effect on the colonic motility of non-pregnant, healthy women as colonic transit is unaffected by the menstrual cycle (45). Thus the role of the sex hormones in severe constipation is unclear.

Routine light microscopy has failed to identify a consistent structural abnormality in severely constipated colon. The first histological abnormality was reported by Dyer and colleagues in 1969. This comprised of a thickening of the circular smooth muscle, a reduction of the number of axons and neurones which were often mis-shapen, and Schawnn cell hyperplasia. Changes in the myenteric plexus of patients with idiopathic constipation, distinct from other pseudo-obstruction syndromes have been reported (47-51) viz, a reduction in the total number of agyrophylic neurones coupled with a morphological abnormality. The total number of axons was decreased and a variability in the size and chromatin content of the nuclei were found. Circular muscle hyperplasia and Schawnn cell hyperplasia previously

identified by Dyer et al were however not reported. These findings were distinct from previous reports in cases of megacolon which identified hypertrophy of the smooth muscle layer (52). A third study of four cases of organic constipation in adults supported the original findings (53).

The advent of special stains has now permitted the re-exploration of the morphology of the severely constipated colon (54-56). The absence of an extrinsic nerve supply in the colon of some patients with long standing constipation is notable (54), while reductions in vasoactive intestinal polypeptide (VIP) levels, the virtual absence of VIP containing nerves from circular smooth muscle and the lowered peptide histidine-methionine levels in a small series may be significant (55). As VIP is thought to be a nonadrenergic, noncholinergic (NANC) inhibitory transmitter, involved in peristalsis, its absence could point to the primary pathology in ISTC. Other evidence exists for a functional cholinergic deficit in patients with severe constipation (56). The extent of the pathology has not been clearly established. The proximal gastro-intestinal tract has not been adequately studied in ISTC but oesophageal dysmotility has been reported (47). As with the multiple aetiologies for this condition, there may be variable pathologies.

1.3 Diagnosis

Radiological marker studies and videoproctography form the basis of screening patients for severe constipation. Patients thought to suffer from ISTC have the diagnosis excluded on the basis of normal transit times. Any significant delay in colonic transit can be identified using the technique originally described by Hinton et al (8). The presence of 80% of radiological markers in the left colon five days after ingestion suggests the diagnosis and their persistence on day 7 and day 10 confirms it. Modifications of this technique, involving three different markers consumed on three consecutive days enable measurement of segmental transit (58). This modification may be helpful in identifying right and left sided colonic delay (59).

Videoproctography is sometimes preferred to balloon proctography by some clinicians for the diagnosis of OOC since controls sometimes have difficulty in expelling a balloon from the rectum. While failure to open the ano-rectal angle is of debatable significance, inability to evacuate the rectum of liquid barium is a reliable indicator of pelvic floor dysynergia. Electromyography is sometimes used by clinicians to examine contractility in the external anal sphincter and puborectalis muscle (60). While changes in electrical activity have been demonstrated in the sphincter mechanism during simulated defaecation, the same

changes may occur in normal volunteers (60). To assume that asymptomatic controls always behave in a physiological manner with needles in the perineum is perhaps unrealistic. More importantly, the results of electromyography do not always correlate with manometry, videoproctography or indeed symptoms. The value of any one of these measurements in isolation is questionable (60).

Colonic motility can be further examined in ISTC using colonic manometry. These studies in which a catheter is passed either nasally into the right colon or per rectum into the left colon have been used for research purposes (61-64). Reports reveal that dysmotility may be either total or regional, due to hypersegmentation as opposed to hyposegmentation, or appear as infrequent or absent mass movements. The value of colonic manometry is often limited by the infrequent nature of events being measured and the observation that segmental colonic contraction may fail to raise intra-luminal pressure due to pressure being dissipated by adjacent segmental relaxation. Abnormally high pressure recordings, as sometimes detected in the sigmoid colon may be due to kinking of the bowel as it descends into the pelvis. Likewise, high pressures in a particular region of the bowel may be due to their being recorded through a side channel recorder as opposed to an end port; the first being more likely to record wall

pressure and the second more likely to record true intraluminal pressure. The catheters which are placed in the colon endoscopically require the bowel to be prepared and this may influence the recordings which are obtained. In contrast, catheters which are placed into the right colon using the oral route, do not require prior bowel preparation. The disadvantage of the latter technique is the time required for the catheter to reach the colon which in constipated patients this may require several days hospitalisation. Cleansing of the bowel per se may alter the motility patterns recorded. Conversely, emptying the colon standardizes the environment in which the studies are taking place. One study suggested that a tap water enema three hours before measurements were made did not significantly alter the motility patterns seen (65). More information is needed before it can be assumed that prepared and unprepared colons behave in the same manner. The main advantage of a manometry catheter is that recordings can be made at several sites simultaneously.

An alternative approach uses a radiotelemetry capsule (66,67). Here, increases in colonic pressure move a metal diaphragm within the capsule. This causes changes in the inductance of an oscillating circuit which modifies the frequency of an emitted radio signal. Recordings are made from ariels placed externally on abdominal wall. The

capsule is usually labelled with a small disc of filter paper, impregnated with ^{111}Indium - DTPA. Transit of the capsule through the colon can then be followed using a handheld Geiger counter. Patient compliance is high and most patients can carry out normal activities for the duration of the study. The system has disadvantages; a) pressure can only be recorded at one site at one time, b) the radiocapsule is likely to be pushed forward infront of the main contractile wave and important data might not be recorded. The magnitude of pressure rises in any particular area of the colon requires careful assessment.

No matter how the colon contracts, one of its roles is to transport contents from the caecum to the rectum and much might be learned from observation of this task. The imprecise and infrequent nature of events makes continuous monitoring difficult. However, in a well designed study, Kamm et al, in a small number of patients artificially reduced the period of colonic transit by stimulating the caecum with bisacodyl and observing, with a gamma camera, the subsequent passage of radio-nucleotide through the colon (68). They demonstrated that some patients with severe constipation had motility patterns in the right colon and transverse colon which were comparable with normal controls while abnormal patterns were confined to the distal colon or rectum. Other patients appeared to have an abnormal motility

pattern throughout the colon. The former observation might support other studies (31) which have implicated pelvic denervation as the cause of left sided constipation. It is tempting to speculate that the latter observation may reflect the situation in pure ISTC.

1.4 Treatment

The choice of surgical approach for severe constipation has been based on the presumed underlying pathology. Colectomy and ileorectal anastomosis has remained the treatment of choice for ISTC, and until recently, has been preferred to other surgical options like limited resection (5). A satisfactory outcome has been achieved in the majority of patients with this procedure but a small number remain unable to defaecate after ileo-rectal anastomosis (5, 6, 69). The reason for this is unclear but, in some patients, there is spasticity of the rectum with pseudo-obstruction of the distal ileal segment. For this reason, proctocolectomy and ileo-pouch formation in the management of idiopathic constipation has been proposed (70). These two operations are not without their problems such as urgency, faecal incontinence and recurrent obstruction and this may result in the patient eventually having to have a permanent ileostomy created. The delivery of a fluid stool to the rectum may overwhelm the outlet

obstruction as is intended but if the sphincter mechanism has been damaged by repeated straining, surgery may have succeeded only in converting a chronically constipated patient into a faecally incontinent one. Thus, measurement of sphincter function prior to surgery is essential. Where the dysmotility is segmental rather than total, colectomy may appear as surgical overkill. Indeed, left hemicolectomy and excision of the upper rectum can be successfully employed where the dysmotility is confined to the distal large bowel (71).

In cases where the primary pathology was thought to have its origin in a spastic sphincter mechanism ("anismus"), surgery has been directed towards the local problem. Previous attempts to deal with outlet obstruction by division of the puborectalis muscle, thus decreasing the pressure in the anal canal during defaecation have met with mixed results. In practice, frequent incontinence of flatus and mucus have been serious disadvantages to its widespread use (72-74). Similarly, poor long term results of anorectal myectomy do not justify its 10% incontinence rate and therefore this operation has been abandoned (75). Attempts to treat anismus with locally acting Botulinum toxin have also been unsuccessful in the long term (76), The presence

of a strong psychological overlay in many patients has prompted some workers to treat this component using biofeedback techniques with some success (77-80). Anismus in these cases is thought to be a learned response, possibly secondary to painful defaecation. Contraction of the external sphincter during defaecation persists, despite the cause being removed. As only the striated muscle is involved, this should be amenable to modulation by biofeedback. This approach may be of benefit in the group of patients who are unable to empty a loaded rectum, but results are poor in ISTC in which the problem is not how to empty the rectum but that the rectum is empty.

1.5 Future Prospects

At present, the investigation and management of ISTC is based upon taking a thorough history and performing videoproctography and transit studies (either total colonic or segmental). Surgery is usually carried out with the most common operation being subtotal colectomy.

However, where some patients fail to benefit from this treatment, dysmotility of the proximal GI tract must be excluded. My own clinical impression supports the findings of previous workers in that some patients with ISTC have proximal disease, affecting to a varying extent, the oesophagus, stomach and small bowel (47). Colectomy and

ileorectal anastomosis may not be effective in these patients as proximal dysmotility may form the major component of their disease process. The measurement of gastric emptying and small bowel transit may identify these patients. Where significant proximal disease exists, the natural history of the disorder should be determined. Those patients who fail to evacuate following ileorectal anastomosis and in whom contrast studies show a spastic rectum with adjacent ileal obstruction, need to be identified. These patients might be diagnosed by a combination of anorectal manometry and prostigmine/carbachol sensitivity testing. The presence of rectal hypersensitivity might persuade the surgeon to remove the rectum in addition to the colon and leave the patient with an ileal pouch.

The advent of total colonic manometry, now available to measure motility over 24 hours, might help to elucidate the regional nature of ISTC, if indeed it exists. Responses to stimuli both natural and pharmacological can be measured on a regional basis and the possibility of localising areas of dysmotility offers the surgical option of more precise limited resection.

A group of patients who might conceivably benefit from limited resection are those young women who attribute the onset of their constipation symptoms to either childbirth or hysterectomy. Injuries to the pelvic nerves

would be expected to cause dysmotility affecting the distal colon. Constipation in these patients could result from delayed transit through the left colon with relative sparing of transit times through the small bowel and proximal colon. Identifying a regional basis for the pathophysiology would lead to a more conservative surgical approach. To remove the colon for all cases of severe constipation is a surgical option, but it does not have a rational scientific basis. This thesis aims to clearly define the nature and extent of the pathophysiology in constipation which results from childbirth or pelvic surgery. If this is not done, then the condition will continue to be known as Arbuthnot Lane's disease. Whilst this is less important in the majority of patients with idiopathic slow transit constipation in whom we get the diagnosis and management correct (like Arbuthnot Lane), it is important for the smaller group of patients who have an isolated hindgut neuropathy and who could be adequately managed by segmental as opposed to total colonic resection.

CHAPTER 2

OVERVIEW OF ANATOMY & PHYSIOLOGY

The human colon is a poorly understood organ which carries out a variety of complex functions. It is involved in the storage and mixing of ileal effluent, in the absorption and secretion of electrolytes and the transport and evacuation of faecal material. To perform these tasks it is influenced by neural, endocrine and paracrine factors as well as its own enteric mechanisms. When these overseers of colonic function work in harmony, reception, dehydration and evacuation of a solid stool preceed without difficulty. However, derangement of neurological control compromises colonic function and gives rise to a variety of clinically important conditions.

Before investigating the various factors which might contribute to colonic dysmotility in general and postchildbirth/hysterectomy constipation in particular, a review of the function of the normal colon based on an understanding of its anatomy and physiology is necessary.

2.1 Anatomy of the Colon

The colon is derived embryologically from the distal mid-gut and the hind-gut. Together, the caecum, ascending, transverse, descending and sigmoid colon form three and a half sides of a square. The caecum, which forms a reservoir for undigested food in animals is strictly speaking not part of the colon: an exclusion which it shares with the

rectum. Technically, the caecum is that segment of dependent ascending colon which lies below the ileo-caecal valve. Unlike the remainder of the right colon, the caecum is not a retro-peritoneal structure and is often mobile (81,82). The ileo-caecal valve opens on its postero-medial aspect and below this is the opening of the vermiform appendix. In the recumbent position, the caecum usually occupies the right iliac fossa, but descends into the lesser pelvis in the upright position. The ascending colon extends upwards from the caecum in the right paracolic gutter to the hepatic flexure. From there the colon leaves the posterior abdominal wall to traverse the great vessels of the abdomen. In order to achieve this, the transverse colon is suspended by a meso-colon. Towards its distal portion, the transverse colon turns upwards and posteriorly to the splenic flexure. At this point, the colon is more adherent to the posterior abdominal wall than at the hepatic flexure owing to the presence of fibrous bands. The absence of a liver also permits the splenic flexure to occupy the more superior site of the two flexures. The flexure then turns acutely down to the descending colon which meets the sigmoid colon at the level of the iliac crest. The sigmoid colon again leaves the posterior abdominal wall and arches medially on a mesentery of variable length and disappears beneath the pelvic peritoneum to form the rectum. The rectum widens below the

peritoneal reflection to form the rectal ampulla which bends sharply back to follow the curve of the sacrum. The lumen of the rectum is indented by three lateral folds, the upper and lower concave to the left and the middle concave to the right. The rectum then angles acutely downwards and backwards to form the anal canal which passes through the pubo-rectalis component of the pelvic floor and is surrounded by the external anal sphincter. The anal canal is between 2.5 and 3.5 cm in length and appears as an anterior-posterior slit which ends at the anus (83,84).

The colo-rectum is about 1.5 to 1.8 metres long with the greatest degree of variation in the transverse and sigmoid colon. The ascending colon measures 12-20 cm, the transverse 50 cm, the descending 25 cm and the sigmoid 40 cm. The rectum constitutes the remaining 12-15 cm.

The outer surface of the colon differs from the small bowel in three main features.

(i) <u>The presence of taenia coli.</u> The longitudinal muscle is gathered together in three main bundles from the caecum to the recto-sigmoid junction known as the taenia coli. Differences in length between these and the remaining longitudinal muscle fibres produce the second feature-

(ii) <u>Haustrations.</u> These give the colon its sacculated appearance and are evident from the ascending colon to the recto-sigmoid junction. Haustrations are caused

by the longitudinal muscle being shorter than the adjacent circular muscle thus throwing the circular muscle into sacculations.

(iii) Appendices Epiploica. These are elongated protrusions of fat adherent to the peritoneal surface of the colon.

The above three features are present throughout the colon to the junction of the sigmoid and rectum where the tri-fold arrangement of the taenia gives way to an anterior and posterior band. At this point, the posterior covering of peritoneum disappears, as do the appendices epiploica. The superior rectal artery divides into right and left branches at this juncture and the surface is marked by an external constriction at the level of the third sacral vertebra.

The blood supply to the colon (the superior and inferior mesenteric arteries) is derived from the embryological vessels of the mid gut and hind gut. The caecum is supplied by the ileo-colic artery, the ascending colon by the right colic artery, and the transverse colon by the middle colic artery. The descending and sigmoid colon receive a supply from the artery to the hind gut; the inferior mesenteric artery supplies the descending and sigmoid colon through its left colic and sigmoid branches respectively. The perfusion of the colonic wall is from the marginal artery of Drummond which travels the length of the

colon and is augmented at various intervals by the above mentioned arteries (85). The rectum and anal canal take their supply from four sources: inferior mesenteric, internal iliac and internal pudendal arteries and the aorta. The blood supply to this region can be considered in unison as all the vessels involved contribute to a rich anastomosis. The vessels concerned are the superior rectal, middle rectal, inferior rectal and median sacral arteries.

The veins and lymphatics generally follow the arteries and take their names from them. The superior and inferior mesenteric veins come together to form the portal vein which enters the liver. Porto-systemic anastomosis occur between the superior rectal and middle rectal veins thus linking the inferior mesenteric vein to the internal iliac vein. The lymphatic drainage of the proximal colon is via the superior mesenteric artery into mesenteric nodes then on via the intestinal trunk to the thoracic duct. In contrast, the distal colon drains via the inferior mesenteric artery to the left lumbar nodes and then to the thoracic duct. Whilst the upper rectum drains along with the distal colon, the middle and lower rectum drains into sacral, internal iliac and then common iliac nodes. The lymphatic drainage of the upper anal canal also follows this

route, whilst the lower anal canal drains via the superficial inguinal nodes to the external iliac and then common iliac nodes.

The nerve supply to the colon is derived from various sources (86). Four control centres are identified: the coeliac, superior mesenteric, inferior mesenteric and hypogastric (pelvic) plexuses. The parasympathetic supply to the ascending and transverse colon comes from the vagus nerve via the coeliac and superior mesenteric plexuses. The distal colon is supplied via the pelvic plexus by the pelvic nerves. The inferior mesenteric plexus contains no parasympathetic nerves. The sympathetic supply reaches the colon from the thoracic and lumbar splanchic nerves passing through the superior and inferior mesenteric plexuses. The colonic wall therefore contains two populations of extrinsic nerves: pre-ganglionic parasympathetic fibres which link with the ganglion cells in the intrinsic plexus of Auerbach; and post-ganglionic sympathetic nerves, the exact function of which has remained unclear.

In addition to the extrinsic nerves which modulate activity, there are populations of intrinsic nerves which have both sensory and motor functions. Two types of excitatory and two types of inhibitory nerves have been identified (87).

(1) Cholinergic Excitatory - Electrical stimulation of colonic muscle produces contractions which are potentiated by neostigmine. This drug which competitively inhibits naturally occurring cholinesterase exaggerates the effect of acetylcholine released from nerve endings. The effect can be reversed by cholinergic antagonists eg. atropine confirming that these nerves are cholinergic in nature (87-89). In addition, cholinesterase positive nerves have been demonstrated histologically in Auerbach's plexus (90) .

(2) Non-cholinergic Excitatory - Electrical stimulation of some populations of nerves in the circular muscle of sigmoid colon and taenia result in contraction even in the presence of anti-cholinergic drugs. The contractions are sensitive to tetrodotoxin confirming their neural origin (87). There is some evidence that non-cholinergic excitation in the proximal colon in animals is mediated by 5-hydroxytryptamine (5-HT) (91). However, this is unlikely to be the case in human colon as 5-HT usually causes relaxation.

(3) Adrenergic Nerves - These nerves have been identified in the colon using catecholamine fluorescence imaging (92,93). Most nerve endings terminate around the parasympathetic ganglia though some fibres enter the muscle

layer directly. They probably exert their effect by modifying the parasympathetic input to the colon. While most of the fibres end in the vicinity of the ganglia . The relaxation produced by stimulation of these nerves can be blocked by adrenergic antagonists (94-96).

(4) <u>Non-Adrenergic, Non-cholinergic Nerves (NANC)</u> - Where tone is raised, nerve stimulation produces relaxation of the colon followed by rebound contraction. Relaxations persist, in the presence of atropine and phentolamine indicating their independence of the cholinergic and adrenergic pathways. These inhibitions are again blocked by tetrodotoxin indicating their neural origin rather than direct stimulation of the muscle (87-89). Ganglion stimulating drugs, eg nicotine also produce these relaxations. Recent work has suggested that two neurotransmitters are involved in this pathway and there is strong evidence for nitric oxide (NO) being one of them (97,98). This is supported by the identification of the enzyme, NO synthase, in myenteric nerves. Nitric oxide is synthesised from L-arginine and in turn elevates the concentration of cyclic GMP by increasing the activity of guanylate cyclase (99,100). Cyclic GMP inhibits the release of calcium from intracellular stores and causes muscle relaxation (101). Production of NO from L-arginine can be

inhibited by L-arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA)(102) and N^G-nitro-L-arginine (103) but not their enantiomers, e.g. D-NMMA. Colonic muscle relaxation, induced by electric field stimulation can be blocked by the inhibitory analogues of L-arginine, indicating a role for nitric oxide (104). Similarly, inhibitory junction potentials in the proximal canine colon can be reduced by L-NMMA. Nitric oxide has been implicated in descending inhibition in the proximal colon (105), relaxation of the stomach (106) and NANC relaxation of the internal anal sphincter (107-109).

How these four innervations interact to produce an integrated colonic motility pattern is unknown. However, the overall input to the colon is inhibitory in keeping with its role as a reservoir. In contrast the small bowel has a predominately excitatory input in keeping with its mixing and digestive function. (110)

2.2 Response of the Colon to Various Transmitters

Acetylcholine, noradrenaline, 5-hydroxytryptamine, histamine vasoactive intestinal peptide and prostaglandins E and F all occur naturally within the colon and might therefore be thought to have a role in the control of colonic motility.

Acetylcholine contracts isolated colonic smooth muscle via muscarinic receptors. This is mediated through its cholinergic effect and is reversed by anticholinergics eg atropine. By contrast noradrenaline added to muscle strips causes profound relaxation through *a* and *b* receptors. The effect which this drug has on the post-ganglionic release of acetylcholine is swamped by its direct effect on the smooth muscle. However given the anatomical relationships previously described the likelihood is that noradrenaline acts by inhibiting the release of acetylcholine from postganglionic parasympathetic nerve endings. This view is supported by the observation that noradrenaline inhibits the electrically induced release of acetylcholine from human colon. (96) Its direct effect on smooth muscle appears to be a balanced one as blockage of *b* receptors by oxprenolol results in contraction through *a* excitatory nerves (111).

Although 5-HT and histamine are present in the bowel wall their role in the modification of colonic motility is unclear. 5-HT is present in large quantities throughout the gastro-intestinal tract, located within granulues in the basal aspect of enterochromaffin cells and is released in response to stretch or chemical stimului from the lumen of the gut. The distribution of enterochromaffin cells varies, but they are found in particularly high numbers in the

rectosigmoid. The effects of 5-HT vary from site to site depending upon the distribution and nature of 5-HT receptors. In the canine terminal ileum and ileal colonic junction, 5-HT causes excitation via 5-HT_1 like receptors located on the smooth muscle cell. Stimulation of neuronal based 5-HT_3 receptors produces both contraction and relaxation. The contractile response is mediated through acetylcholine whereas the relaxation is effected by an as yet unknown nonadrenergic, non-cholinergic neurotransmiter (112).

The newly described 5-HT_3 receptors may be involved in the induction of nausea since Ondansetron, a selective 5-HT_3 receptor antagonist and other anti-emetics with 5-HT_3 receptor antagonist properties have been used clinically to counter the nausea and vomiting induced by chemotherapy. Ondansetron has been shown to prolong the mean total colonic transit time in healthy volunteers (113). While the right colonic transit time was unchanged, transit through the left colon and rectosigmoid was prolonged. This may reflect the distribution of argentochromoffin cells and 5-HT_3 receptors in the sigmoid colon. The mechanism by which certain drugs, eg Cisapride affect gastrointestinal motility cannot be accounted for by their action on 5-HT_3 receptors and recently the existence of a 5-HT_4 receptor has been postulated (114). Stimulation of these receptors produces

contraction of the longitudinal smooth muscle of the guinea pig ileum (115). The enhancement of gastrointestinal motility produced by substituted Benzamides in man is well documented, but the underlying mechanism for this prokinetic activity is unclear. 5-HT_4 and 5-HT_3 receptors may interact to modulate the peristaltic reflex, rather than to initiate it (116).

Histamine is also a naturally occuring substance in the human colon and is a putative neurotransmitter. *In vitro* studies have demonstrated a role for histamine in mediating intestinal chloride secretion. Secretion of chloride was reduced in the presence of indomethacin, suggesting that histamine acts to promote prostaglandin release. The effects are also reduced in the presence of tetrodotoxin, indicating a nerve mediated response (117). Histamine also contracts colonic smooth muscle via H_1 receptors and amplifies the response to prostaglandins, vaso-active intestinal peptide and acetyl choline (117, 118).

Prostaglandins also occur naturally in the human colon. Prostaglandins of the "F" series contract both the circular and longitudinal muscle layers. In contrast, prostaglandins of the "E" series contract the longitudinal layer and relaxes the circular layer (119-121). The contribution of prostaglandins to normal motility has not

been established as prostaglandin synthetase inhibitors eg indomethacin appear to have little demonstrable effect on colonic transit. Prostaglandins may mediate the rebound contraction, which follows nonadrenergic non-cholinergic relaxation in human colon, as prostaglandin synthetase inhibitors reduce this.

Certain clinical conditions lead to alterations in colonic motility. In medullary carcinoma of the thyroid, prostaglandins released into the blood stream may be responsible for the diarrhoea which is often seen. Whether this response is mediated through a direct alteration in colonic motility or through an increase in colonic secretions is not known (122). Similarly, the altered bowel habit and abdominal pain which occur in some women at the time of menstruation may be due to release of prostaglandins from the endometrium.

Vaso-active intestinal polypeptide (VIP) is one of the most abundant neuropeptides in the human colon. Nerve fibres containing the peptide are particularly rich in the mucosa and muscle layers (123,124) and VIP has been proposed as one of the inhibitory neurotransmitters in the human colon. However, little is known about the anatomical and physiological basis of peptidergic control of the human colon. VIP-containing nerves from the submucous plexus project to the lamina propria and to the circular muscle

(125) whereas VIP fibres from the myenteric ganglia project to both the longitudinal and circular muscle layers. In guinea pig distal colon, VIP relaxes of the longitudinal and circular muscle in the proximal colon and the circular muscle alone in the distal colon. The VIP-induced contractions in the longitudinal muscle layer were converted to relaxations in the presence of tetrodotoxin and atropine, indicating that while the direct effect of VIP on colonic smooth muscle is inhibitory, it may also indirectly stimulate longitudinal muscle via cholinergic neurones in the myenteric plexus (126). Decreased levels of VIP-containing fibres have been identified in constipated colon. In contrast, levels of VIP appear to be elevated in diverticular disease. It is not clear whether the abnormal neural content of vaso-active intestinal peptide in the bowel wall in these two conditions initiates or is the consequence of the disease processes (127).

The remaining substances which might have a role in colonic motility are only now receiving attention. Substance P, the presence of which was identified in human colon as long ago as 1953 contracts the circular muscle of the sigmoid colon at a site which is unaffected by antagonists of acetylcholine, histamine and 5-HT (128). Unlike VIP fibres which run in a caudal direction in the myenteric

plexus of both the small and large intestine, substance P fibres run in the oral direction in large intestine and both proximally and distally in the small intestine (129).

Neurotensin is also present throughout the gastrointestinal tract. Its effects vary with contraction of the oesophagus, biphasic relaxation and contraction of the stomach, duodenum and ileum and relaxation of the colon (130,131). Gamma aminobutyric acid causes nerve-mediated relaxation of both circular and longitudinal muscle by inhibiting release of acetylcholine from cholinergic neurones in the muscle wall (132). Vasopressin increases colonic spiking and induces propagating colonic contractions through a mechanism which is unknown (133). Dopamine produces dose-dependant increases in the motility index of the sigmoid colon, which are unaffected by atropine, phentolamine and propanolol, suggesting the involvement of specific dopaminergic receptors. This is in contrast to its inhibitory role in the proximal gastrointestinal tract (134). In experimental animals, pelvic nerve stimulation induces colonic smooth muscle relaxation and vasoconstriction. This relaxation is inhibited by neuropeptide Y, which, in contrast, has no effect on receptor-mediated colonic contraction (135). This suggests that neuropeptide Y effects its inhibitory role by antagonising nerve-mediated relaxation of the colon.

Opioid peptides and opiod receptors are also present throughout the gastrointestinal tract. The effect of systemic opiates on colonic motility depends on the class of receptor involved and the relative activity of the agonist. Opiates with predominantly neuro agonistic activity inhibit gastrointestinal motility by a central mechanism in contrast to delta and kappa agonists, which are inactive when injected intravenously. Systemically administered opiates prolong gastrointestinal transit by action at both central and peripheral sites, enhancing tonic contractions, but inhibiting peristalsis (136). An infusion of morphine increases the spiking activity and contractility in the sigmoid colon. This response can be obliterated by pre-treatment with naloxone. (136) However, naloxone has no effect on the increase in electrical and mechanical activity produced by neostigmine (an anti-cholinesterase which potentiates the effect of acetylcholine) (136). This suggests that naloxone is acting independently of the cholinergic system to produce its effect.

Cholecystokinin also has an effect *in vivo* and *in vitro* on colonic smooth muscle. Intravenous infusion of the octapeptide of cholecystokinin causes an increase in distal colonic spike activity (137). This is not affected by atropine indicating that the cholinergic system is not involved in mediating this response. This result has been

confirmed in vitro in both the animal and human model (138). However, an intravenous infusion of naloxone inhibits the colonic electrical and mechanical activity produced by the octapeptide of cholecystokinin. This observation suggests that opiate receptors are important in mediating the colonic response to a fatty meal. Enkephalin receptors have been identified in the human colon and the octapeptide of cholecystokinin may have structural similarities to met-enkephalin and therefore may act at opioid receptor sites (139,140). Its effect may be produced by a non-specific action on opiate receptors in the colon.

An in depth description of the remaining neurotransmitters which are present in the human colon falls outwith the scope of this literature review. Substances like Adenosine triphosphate (ATP) and Adenosine monophosphate (AMP) clearly have a role in colonic motility. Although studies have shown that these substances relax colonic smooth muscle, it is not clear whether they act in a primary or facilitatory mode (141). No single neurotransmitter acts in isolation in the human colon. The action of every nerve is modified by the reaction of adjacent neural pathways. The role of this integrated computer circuit is to co-ordinate contraction and relaxation in human colon.

The anatomical and physiological properties of the human colon combine to transform the fluid effluent of the ileum to the solid faeces of the rectum. Studies from ileostomy patients would suggest that about 1500 ml of ileal fluid enter the colon every day (142). The contents of this vary but may contain 6 g of protein, 2 g fat and 28 g of absorbable carbohydrate (the typical contents of ileostomy fluid). This, in fact, may represent an underestimate as production from an ileostomy accommodates with time. Several factors then combine to mix and propel this slurry along the colon.

The colonic smooth muscle exhibits a basal electrical activity which is a property of the tunica muscularis (myogenic activity). This is overseen by the intrinsic and extrinsic nerve supplies. Contractions of the colon produce local increases in pressure which mixes colonic content and finally there is myogenic and neurogenic integration to produce propagation of colonic contents.

2.3 Myoelectrical Activity of the Human Colon

Human colonic smooth muscle is a naturally depolarising and repolarising tissue. Recordings made *in vitro* demonstrate an intrinsic rhythmicity which produces a "slow wave" pattern. Periods of slow wave activity have been recorded between 20-40 cycles per minute. Superimposed upon

this at the zenith of depolarisations is spiking activity which is the electrical correlate of colonic contraction. These electrical spikes are either of short or long duration. These electrical events synchronize to produce local and segmental contractions. Carbachol (1×10^{-6} M and above) increases slow wave and spiking activity and subsequently increases the amplitude and frequency of colonic contractions (128). This is mediated through muscarinic receptors, as the response is blocked by atropine. The increased activity persists in the presence of tetrodotoxin, confirming that the response is receptor-mediated and does not involve intrinsic cholinergic nerve fibres. Adjacent areas of colon electrically oscillate at different frequencies and phases without producing contraction. The effect of carbachol is to coordinate activity and produce a contraction. In contrast, isoproterenol, a sympathomimetic, abolishes slow wave activity and spikes causing circular muscle to relax. (128) Electrical and contractile activity can also be induced and modified by stretching the tissue which presumably depolarises the membrane. Thus myogenic activity can be modified in response to neurotransmitters and pressure changes within the colonic lumen.

In vivo recordings of myoelectrical activity have been made using suction electrodes. Interpretation of the observations is difficult because electrical activity from the circular muscle and adjacent taenia are inseparable. Similarly, electrical noise from the small bowel cannot be excluded. Certain patterns of activity are emerging. Long duration "spike bursts" lasting 12-45 sec have been recorded and have been seen to propagate distally. These prolonged periods of electrical activity are associated with large colonic contractions which move colonic contents from one segment to another. Periods of quiescence in colonic motility are associated with a reduction/absence of electrical activity.

2.4 Neural Control of Colonic Activity

The proximal colon is supplied by the vagus nerve and the distal colon by the pelvic nerves which emanate from the pelvic plexus. The precise point of cross over is not known but there is evidence for considerable overlap (35). The region where the two innervations merge is between the distal transverse colon and the splenic flexure. The dual innervation of the colon effectively divides it into two separate organs; the ascending and transverse colon which have a mixing and storing function and the descending and sigmoid colon which together with the rectum is concerned with evacuation. Colonic transit is not delayed by division

of the vagus nerve. Indeed vagotomy frequently results in speeding colonic transit. However, there is evidence that left colonic transit is dependent on the integrity of the pelvic nerves and damage to them during surgery can lead to constipation (31).

Stimulation of the parasympathetic input to the colon increases colonic motility. The sympathetic innervation does not directly affect colonic smooth muscle but rather it inhibits the parasympathetic input. In addition to the extrinsic nerve supply, non adrenergic, non cholinergic excitatory and inhibitory nerves exist which function independently of extraneous control. Thus the myenteric plexus has been described as the mini- brain of the gut (143). Indeed colonic motility, in laboratory animals at least, may be controlled at the level of the prevertebral ganglia, independently of the central nervous system. Afferent input from mechanoreceptors in the bowel wall are processed in the pre-vertebral ganglia (144-147). From there, sympathetic efferents exert an inhibitory effect on other regions of the colon. Thus, the motility of different regions of the colon are intimately related.

2.5 Colonic Contraction

The exact mechanism by which the colon responds to its contents to produce peristalsis is unclear. For peristalsis to occur there must be a co-ordinated contraction of the longitudinal and circular muscle of the colon. The taenia contracts ahead of the circular muscle thus pushing colonic contents along. In front of the contracting wave the colonic musculature must relax to allow the peristaltic wave to proceed. Two factors appear to be of importance. Firstly, distension of the colon stimulates a reflex arc, intrinsic to the muscularis propria, which results in colonic contraction (148). Secondly, the volume required to produce this peristaltic reflex varies depending upon the chemical make-up of the distending fluid. When fluid resembling ileal effluent is introduced to a partially distended caecum there is an increase in peristalsis which results in movement of colonic content from the right colon to the transverse colon (149). Therefore, initiation of right colonic activity may be dependent on ileal emptying. The ileo-colic junction exhibits the features of a sphincter, a property which it has in common with the smooth muscle of the distal segment of the oesophagus. Its ability to act as a sphincter mechanism is augmented by its anatomical site. When the caecum is distended with fluid, the back pressure creates a flap-valve effect on the

protruding terminal ileum. Ileal emptying is thought to occur in two phases. Firstly there is intermittent release of up to one third of the ileal contents. Secondly,there is a steady trickle of ileal effluent into the caecum (150,151). The exact mechanism by which ileal contents stimulates colonic motility remains unclear but the ileal sphincter mechanism appears to be important as right hemi-colectomy frequently results in an increase in stool frequency.

2.6 Colonic response to a meal

Following the ingestion of a meal there is an immediate and sustained increase in colonic electrical and mechanical activity. (137,152) The response is dose dependent; a 350 Kcal meal is unable to elicit an increase in activity, while a 1000 Kcal meal produces the "gastro-colic reflex". The fat content of the meal is a particularly important initiator of the response (152). The term " gastro-colic reflex " is a misnomer as the response can be demonstrated in patients who have undergone total gastrectomy. The reflex is however reduced and modified in patients who have undergone a vagotomy.

The exact mechanism by which ingestion of a fatty meal induces an increase in colonic activity is unclear but probably involves both neural and hormonal factors. The colonic response to a meal can be blocked by lavaging the

stomach and duodenum with procaine prior to the meal stimulus (136). As procaine is a locally acting anaesthetic which blocks chemoreceptors and pressure receptors in the submucosa, the afferent sensory limb is clearly important in initiating the gastrocolic reflex. A delayed response to the meal is observed following gastroduodenal lavage with procaine. This may be due to the release of peptides from the proximal gastro-intestinal tract which stimulate the muscle or myenteric plexus distally.

Following the sensing of a meal in the gastroduodenal area, there is an immediate increase in the electrical spiking from the sigmoid colon, although the slow wave pattern remains unchanged (136). The speed with which the increase occurs implicates a nerve mediated response. Spiking is blocked by atropine indicating involvement of the cholinergic system and by naloxone (an opiate antagonist) suggesting that opiate receptors in the bowel wall may also be important in mediating the response. The two systems appear to be independent of each other.

Gastrin and cholecystokinin have each been implicated in the colonic motor response (153-157). Serum gastrin levels rise after a fatty meal and infusions of gastrin in physiological doses increase the myoelectrical activity of the human colon (153). However, infusions of gastrin mimicking post-prandial levels do not produce the

same rise in spiking activity or colonic motility, suggesting that other mediators may be involved. Also serum gastrin levels continue to rise after the maximum colonic response to a meal. Gastrin is secreted in two forms (G_{17} and G_{34}) after a meal (158). G_{17} and G_{34} show distinct patterns of release. G_{17} which is released in the first 20 minutes after a meal is about six times more potent than its G_{34} form, which continues its rise beyond the period of maximum colonic response to a meal (137). It may be that G_{17} is responsible for the early response to a meal.

The post-prandial increase in colonic motility persists for up to 3 hours, but maximum activity occurs in the first hour. Following this period of activity, large contractions originating in the proximal transverse colon can be seen propagating distally. These colonic "mass actions" or migrating motor complexes propel colonic contents from one segment of the colon to another. These are infrequent events and may occur only once or twice a day. Little is known about the factors which combine to produce a migrating motor complex. Their increased frequency following a meal stimulus, suggests that proximal sensory input maybe an important factor in promoting colonic contraction. Similarly, increased postprandial serum levels of cholecystokinin and gastrin may produce the environment in which migrating motor complexes can be initiated.

Propagating contractions of the colon can also be produced by the luminal application of bisacodyl or other nonspecific colonic stimulants and therefore a sensory imput from the colonic mucosa is also important. Local distention of the sigmoid colon by a balloon induces colonic contractions (159) indicating that local colonic distention is also an important factor in promoting migrating motor complexes. The contribution of the extrinsic nerve supply to the frequency and magnitude of migratory motor complexes is not known. However, as colonic transit persists in patients who have undergone abdominal perineal resection of the rectum, one must presume that the integrity of the pelvic parasympathetic nerves is not essential for facilitating migrating motor complexes.

Migrating motor complexes which originate in the left colon and propogate into the rectosigmoid may initiate an urge to defaecate.

The human colon exhibits periods of prolonged quiescence. Sleep usually all but abolishes whereas wakening increases colonic activity.

Thus a variety of factors and mechanisms which are still poorly understood result in the conversion of fluid chyme in the caecum to solid stool in the recto-sigmoid. The arrival of solid stool in the rectum initiates a series of events which culminate in defaecation.

Before reviewing this we should briefly consider the anatomy and physiology of the pelvic floor.

2.7 Anatomy of the Pelvic Floor

The pelvic floor consists of a gutter shaped muscular diaphragm which embraces the midline structures of the anal canal, urethra and in the female,the vagina. Although the levator plate is comprised of three named parts; ischio-coccygeus, ileo-coccygeus and pubo-coccygeus it functions as a single structure. It arises from the spine of the ischium, the white line overlying the obturator fascia and the pubis. The fibres sweep downwards and backwards to the mid-line, inserting into the sacrum, coccyx and the ano-coccygeal raphe. The component parts successively overlap each other. The fibres of pubo-coccygeus which arise most medially are functionally different from the remainder of the muscle in that they are not inserted into the midline raphe. Fibres pass medially behind the opening for the anal canal and link with fibres from the opposite side and with the posterior fibres of the profundus part of the external sphincter. It is this structure, the " pubo-rectalis " which supports the ano-rectal angle. Fibres lying more medially, form the pubo-prostaticus and pubo-vaginalis. The perineal branch of the fourth sacral nerve courses between the coccygeus and the ileo-coccygeus supplying both from their

upper surface. The major part of the muscle is supplied on its undersurface by the inferior rectal and the deep branch of the common perineal nerve.

The anal canal is surrounded by two muscles. The internal sphincter is a 3 cm thickening of the distal circular smooth muscle. It occupies the upper two thirds of the anal canal down to Hilton's line. It is innervated by the inferior hypogastric plexus. Sympathetic stimulation contracts it and parasympathetic stimulation relaxes it. On its own this muscle is too weak to maintain continence and it therefore relies on the lower fibres of the external sphincter from which it is separated by a downward extension of fibres of the longitudinal muscle. The external sphincter is a tube of striated muscle which is made up of three named parts. The subcutaneous part lies immediately below the corrugator cutis ani muscle and it is separated from the unfortunately named superficial part by a fibrous band, the outward fibrous extension of the longitudinal muscle coat which has its medial attachment at Hilton's line. The superficial component is attached posteriorly to the tip of the coccyx and anteriorly to the perineal body. The deep external sphincter overlaps the lower third of the internal sphincter. Its posterior fibres merge with the puborectalis but anteriorly the muscle separates from this and fills the gap left by the two halves of the puborectalis.

The external sphincter is supplied by the inferior rectal nerve and the perineal branch of S_4.

Together, these muscles act to maintain continence but intermittently relax to allow the passage of flatus and faeces. The sensory arm of the rectum is less clearly understood. Abundant non-myelinated fibres lie within the rectal mucosa (160). It was previously thought that the receptors for detecting rectal distension lay solely within the rectum. However, recent work in patients who have undergone colo-anal anastomosis after resectional surgery suggests that the reflex is at least in part preserved or re-established. This observation points to there being further receptors lying outside the rectum, perhaps in the fascia of the pelvic floor musculature (161). Sensation for rectal distension travels in S_2 and S_3 whereas nociceptive information is conducted by both the sympathetic and parasympathetic systems via the superior and inferior hypogastric plexuses.

The lower rectum for the most part lies empty but the arrival of faeces is sensed by stretch receptors in the rectal wall and in the pelvic floor. If it is not suitable to defaecate at that point, the urge to defaecate can be overridden until the rectum is distended again. Infusion of liquid mass in the rectum results in contraction of the posterior pelvic floor which leads to an increase in the

ano-rectal angle from 90^{o} (162). Distension of the rectum also results in a reflex relaxation of the internal sphincter. This reflex is dependent upon the integrity of the myenteric plexus and is absent in Hirschprung's disease. Contraction of the posterior pelvic floor brings the rectum into the defaecatory position. There is then an increase in intra-abdominal pressure which precedes defaecation. The exact contribution of intra-abdominal pressure, colonic contraction and gravity to the process of defaecation is not known but in a recent study, evacuation of a rectal balloon was shown to occur as a result of raised intrapelvic pressure and not rectal contraction (163). Studies using barium indicate that not only the rectum but the sigmoid and descending colon are emptied during defaecation.

Despite the inroads which have been made into the understanding of colorectal physiology, it remains difficult to marry symptoms with physiological observations. Patients who present with colonic symptoms are usually at the end of the disease process and therefore any observed changes in physiology or immunohistochemistry may represent effect and not cause. Diverticular disease, the pathology of which is well described manifests itself in later life. Although a lack of dietary fibre has been put forward as the cause of the condition, little is known about the exact dysmotility process which leads to the condition. Indeed, asymptomatic

diverticular disease is often identified in elderly patients who are being investigated for other reasons. In contrast some patients describe severe left-sided abdominal colic in the presence of minimal diverticular disease.

Given the complex nature of the human colon and the limitations which the organ places on its investigation, the results and ideas presented in this thesis must be seen as a very small part of a large jigsaw.

AIMS OF THE THESIS

The aims of this thesis can be set down as a series of questions.

Part I

Do patients with postchildbirth/hysterectomy constipation have

(I) normal gastric emptying and small bowel transit?

(II) a regional basis for the slow transit observed?

(III) *in vivo* evidence of cholinergic denervation in the distal colon?

(IV) normal anorectal function?

Part II

Do patients with postchildbirth/hysterectomy constipation have

(I) excitatory and inhibitory nerves present in resected sigmoid taeni coli?

(II) evidence of denervation supersensitivity in resected sigmoid taeni coli?

(III) a demonstrable peristaltic reflex in resected sigmoid colon?

(IV) normal myo-electrical activity present in resected sigmoid taeni coli?

METHODS FOR CLINICAL INVESTIGATIONS

The methods employed in the clinical investigation of patients with postchildbirth/hysterectomy constipation are outlined in this section. The following procedures were performed.

3.1 Gastric Emptying Studies

Radio-isotope gastric emptying studies are a standard investigation of proximal gastrointestinal function. The transit of both solid and liquid components of a meal are usually examined simultaneously. Dual isotope gastric emptying is a well established procedure to assess patients with various gastric motility disorders.

After an overnight fast, both patients and controls receive a breakfast, which consists of cornflakes, sugar, and milk. The liquid marker ^{111} Indium DTPA, is added to the milk. The solid marker, ^{99m} Technetium colloid, is

impregnated onto paper and sealed with cellulose. It is added to the cornflakes as approximately 40 small pieces of paper.

8 MBq of Indium and 80 mBq of Technetium are used and screening is performed using an IGE gap 400A ring mounted gamma camera with a medium energy collimator.

An area of interest is drawn round the stomach to monitor the rate of gastric emptying. A radionucleotide marker is placed on the anterior superior iliac spine as a landmark for ileal emptying into the right colon. Anterior and posterior views of the stomach are taken, with the patient standing, every 15 minutes for a minimum of two hours. Subsequent views are taken at 6 hours, 24 hours and 36 hours. Imaging starts immediately after the breakfast. Screening for the ^{99m} Tc solid phase and ^{111} indium DTPA liquid phase is performed simultaneously. The geometric mean of the corresponding anterior and posterior images is then calculated for both the solid and the liquid phase of the meal. The ^{99m} Tc channel photopeak was set at 141 KeV +/- 11% window. The ^{111} indium DTPA photopeak is set at 245 KeV +/- 10%. The total body irradiation was 214 msV.

3.2 Radio-opaque Marker Studies

The conventional method of measuring colonic transit determines the position of 20 radio-opaque markers four days after their ingestion. Retention of 80% or more of the markers in the colon is indicative of slow transit and their persistence at day 10 is confirmatory. However, this method does not indicate the segment of the colon in which the slow transit occurs.

Using the method described by Metcalf et al, patients and controls swallow 20 radio-opaque plastic markers on three consecutive days (59). On the fourth day a plain abdominal x-ray is taken and transit through the right colon, left colon and rectosigmoid is calculated using a standard formula.

$$\text{Transit} = 1.2\ (N_1+N_2+N_3)$$

were N_1, N_2 and N_3 are markers of differing shape 1,2 and 3.

The vertebral column is used to identify the midline between right and left colon. Markers which lie below a line joining the anterior superior iliac spines are ascribed to the rectosigmoid. Any markers that are clearly within a particular region, despite their radiological site, are allocated to their anatomical position. Where transit is severely delayed a second abdominal x-ray is taken on day 10.

3.3 Radioisotope Colonic Transit Studies

Continuous monitoring of colonic transit is time-consuming. A more practical approach is to study the colon for a short period during which transit is more predictable. This involves stimulating the colon either enterically or parenterally using prokinetic agents.

A method for observing colonic transit following administration of the myenteric plexus stimulant, bisacodyl, to the left colon has been described previously (68).

Both patients and controls undergo cleansing of the bowel with 2 sachets of sodium picosulphate. 24 hours later a 2 metre PVC catheter was passed, colonoscopically, into the right colon. A further period of 24 hours elapsed before the study commenced. With the patient lying supine under the gamma camera, an infusion of 10 mls, ^{99}Tch labelled DTPA (9 MBq) was injected through the central port of the catheter into the right colon. Dynamic serial scanning of the abdomen commenced for a period of 45 minutes. This means that every minute, a picture of the radioisotope within the colon was built up. At the end of each minute, a new picture was generated. During this part of the study, 45 pictures were developed. After an initial observation period of 10 minutes, Bisacodyl 5.5 mg in 10 mls of saline was infused into the right colon. Movement of the radio-isotope through

the colon was observed during the 45 minute scanning period and at half hour intervals thereafter, for a maximum period of 2 hours. A radionucleotide marker was placed on the left anterior superior iliac spine as a reference point. The developed scintigrams of the colon were also superimposed on the late gastric emptying studies to confirm the region of the gastrointestinal tract the radionucleotide had reached.

The times for radioisotope to reach the splenic flexure, sigmoid colon and rectum were recorded. The total body dose of radioactivity was 0.22 msV.

3.4 Colonic Manometry Studies

The three previous studies are designed to examine gastrointestinal transit alone and provides no information on the frequency, duration and magnitude of colonic pressure changes which produce these movements. This can be done by making manometric recordings at several sites in the colon, simultaneously.

Patients are admitted to the ward 24 hours prior to colonoscopy for bowel preparation which is achieved using two sachets of sodium picosulphate, administered orally. Thereafter, all non-essential medicines are withdrawn and the patient maintained on fluids only. The following day, patients are taken to theatre for colonoscopy. Sedation not exceeding pethidine 50 mg, diazemuls 20 mg is administered intravenously. A full diagnostic colonoscopy is performed and any abnormal features noted. Prior to removal of the colonoscope, a 480 cm guide wire is passed into the proximal colon via its therapeutic channel. As the colonoscope is withdrawn excess air is sucked from the lumen of the bowel. Next, a 190 cm M_8 multilumen catheter (Arndorfer) is passed over the guide wire until about 110 cm are lying in the colon. The guide wire is then removed (fig 1).

The multilumen catheter is 190 cm in length with an outer diameter of 5 mm (fig 2) and central lumen diameter of 3 mm. There are 8 channels placed radially in the substance

of the catheter (fig 3). The perfusion points are placed 12 cm apart starting 5 cm from the tip. which gave a working length of catheter of 89 cm. Sterile water is perfused from a two litre reservoir via seven of the eight channels. Perfusion pressure is maintained at 6 lb/in^2 resulting in a flow of 0.6 ml/channel/min (fig 4).

Pressure is transferred via low compliance manometry tubing to a series of water-pressure transducers which are linked to a Gaeltec recording system (Version 3 Rel 0.3 920703 Gaeltec Ltd, Isle of Skye)(fig 5). The system reflected pressures up to a maximum at 230 cm H_2O. The system was calibrated to read 0 cmH_2O at atmospheric pressure and then checked against a mercury manometer. Before the patient was connected to the recording system, the low compliance water perfusion catheters were each thoroughly flushed with sterile water to remove air bubbles from the system. Following connection of the manometry tubes, the patient was asked to cough in order to demonstrate that all seven channels were recording accurately. The event marker was then pressed to indicate the start of a study period.

When the system is operating, variations in colonic pressure are relayed to the transducers as a series of oscillations in pressure. As this is a water-perfusion system which records not only colonic pressure but also

changes in the patients posture, coughing and sneezing, changes in recorded pressure had to meet certain criteria before being classed as waves. The parameters for wave classification are set out in appendix 1.

Prior to commencing manometry studies, the location of the catheter was ascertained by x-ray. The position of the catheter in both the study and control groups was recorded (appendix 2). Recordings of intraluminal pressure were performed 5 hours after colonoscopy and continued for a period of 20 hours thereafter.

After an initial observation period lasting one hour, the first of two 1,000 kcal meals was administered to the patient. The timing of each meal was recorded on the computer. Patients were required to consume the meal within 20 minutes. This meal had previously been shown to consistently elicit the gastrocolic reflex (137). A second 1,000 kcal meal was given 5 hours later, its timing recorded and 20 mins again allowed for its consumption. Recordings were continued throughout the night until mid-morning.

One hour after wakening (approximately 0800) a subcutaneous injection of neostigmine 0.01 mg/kg s.c. was administered. This acetylcholinesterase inhibitor enhances the activity of acetylcholine at nerve and indirectly gives

an estimate of the cholinergic nerve supply of the colon. The timing of the injection was again recorded via the event marker.

Data Analysis

The perfusion channels of the manometry catheter were allocated to areas of interest, as indicated by their position on an abdominal x-ray (fig 6). Perfusion points were therefore allocated to ascending, transverse, descending and sigmoid colon. Where two or more perfusion points lay within one segment, the pressure recordings were averaged. The average pressure, number of peaks over 5 cm H_20, number of peaks over 50 cm H_20, and area under the curve, were calculated for one hour before and after each meal and the neostigmine provocation test.

3.5 Ano-rectal Manometry Studies

Pressure measurements in the ano-rectum were made using pressure-tip transducers, linked to a Gaeltec/Aspen recording system. The system was set to read zero at atmospheric pressure. Patients and controls adopted the left lateral position for examination. Measurement of the basal anal canal pressure was made by passing the transducer

lubricated with soft paraffin through the anal canal into the rectum. The transducer was connected to a pulley which moves at 1 mm/sec. The transducer was then set at 8 pixels/sec giving a pressure sampling rate of 8 times every second. As the transducer moved from the low pressure area of the lower rectum into the anal canal, pressure rose and continued to do so, as long as the catheter tip remained in the anal canal. When the catheter tip reached the perineal skin, the pressure fell to atmospheric. Knowing the pulley speed and the period during which the catheter was in the high pressure zone, the anal canal length could be calculated. This procedure was repeated in each patient a minimum of three times as patient agitation sometimes caused an artificial rise in basal anal canal pressure. The lowest recorded pressure was taken as a measure of the basal anal canal pressure.

Next the catheter was placed in the rectum and the patient is asked to maximally squeeze the anal sphincter as the catheter was pulled quickly through the anal canal. The sampling rate was increased to 16 pixels/sec to make sure that maximum anal canal pressure was recorded. This procedure was again repeated at least three times and the highest recording taken as a measure of maximum anal canal pressure.

A balloon catheter was then lubricated with jelly and introduced into the lower rectum. This catheter was made by tying a Durex condom over a Jaques catheter (size 12). A condom was used instead of a standard balloon catheter for two reasons. First, the mode of enlargement more closely resembled a stool. Second, the condom demonstrated very high compliance, allowing a volume exceeding 400 ml to be infused before recording a pressure rise. A pressure tipped transducer was then introduced anteriorly alongside the rectal balloon to record rectal pressure. A second transducer was introduced posteriorly into the rectum and then slowly withdrawn until the catheter tip was lying in the high pressure zone of the anal canal. This catheter was placed to record the fall in anal canal pressure during filling of the rectum. The presence of this "recto-anal reflex" is normal and excludes the diagnoses of short segment Hirschsprung's disease. The rectal balloon was then filled with water at room temperature at a rate was 60 ml per min. The patient was asked to indicate first awareness of rectal filling and the volume which elicited a call to stool. Measurements were also made of anal canal pressure, rectal pressure and rectal volume. From the latter two, the rectal compliance during rectal filling was calculated.

3.6 Patients and Statistics

Thirteen patients underwent radio-opaque segmental marker studies. In the remaining studies, complete data was available for 10 patients. The same 10 patients underwent each of the clinical studies.

The age of the patients (n=10) was 35(8) years. Patients were selected on the basis of their clinical history and their failure to evacuate the colon of 80% of radiological markers by five days after their ingestion. The duration of symptoms ranged from 6 months to 8 years. All patients had been on long term laxatives and/or bulking agents without effect. None of the patients moved their bowels without the use of enemeta. Stool frequency ranged from 10 - 22 days. Patients with a history of postchildbirth/hysterectomy constipation who had undergone previous abdominal or pelvic surgery were excluded from these studies.

Female controls (n=13) (age - 32(5) years) were admitted to the segmental transit study if their bowel frequency was between one motion per three days and three motions per day (Hinton et al).Those with a stool frequency greater than three per day were excluded as were those who had undergone previous colonic surgery. Although no special diet was followed, both patients and controls were asked to refrain from consuming medicines or food stuffs, which

might artificially shorten or prolong the transit time, for 4 days before and during the period of the study. 11 of the 13 controls were parous.

Twelve healthy adult females (age - 29(12) years) acted as controls for the gastric emptying studies. Controls came from nursing and secretarial staff within the hospital. The same entry and exclusion criteria applied as in the previous study.

Controls for the anorectal manometry came from 10 female patients (age - 38(13) years) who were undergoing surgery for unrelated conditions. The entry and exclusion criteria were again applied to this group of controls. 9 of the 10 controls were parous and none of these controls had anorectal symptoms.

Ten controls (2 males) volunteered for the dynamic radioisotope transit studies. Controls (age - 41(16) years) were invited from patients undergoing colonoscopy for unexplained rectal bleeding or screening for colorectal cancer. Stool frequency again conformed to the entrance/exclusion criteria.

Controls (n=9, 2 males, aged - 43(10) years) for the colonic manometry studies were again invited from patients who were undergoing colonoscopy as a diagnostic or screening procedure; unexplained rectal bleeding or patients from high risk families who were being screened for colonic carcinoma.

All controls had normal bowel function and had no previous gastrointestinal surgery. In this and the previous studies the colonoscopies were normal.

While all ten patients underwent all clinical studies, controls were not duplicated. Although controls were not sex-matched, they were well matched for age. Written informed consent was obtained from all patients and controls prior to each study. Approval for each of the studies was obtained from the ethical committee of Greater Glasgow Health Board. In addition, permission for the use of radioisotopes in the gastric emptying and dynamic radioisotope transit studies was obtained from Administration of Radio-active Substances Advisory Committee.

All data is presented in the form of mean(standard deviation) except Table 17, Figure 28 which is also presented in the form of median(interquartile range) due to the excessive spread of the data. Except where stated, the Mann-Whitney U test for non-parametric data has been used for statistical analysis with $P<0.05$, significant.

CHAPTER 4

CLINICAL INVESTIGATIONS - RESULTS AND DISCUSSION.

4.1 Gastric emptying.

4.2 Radio-opaque marker studies.

4.3 Dynamic radio-nucleatide colonic transit studies.

4.4 Colonic manometry studies.

4.5 Ano-rectal manometry studies.

4.1 GASTRIC EMPTYING IN PATIENTS WITH POSTCHILDBIRTH/ POST HYSTERECTOMY CONSTIPATION

Ten patients with postchildbirth/hysterectomy constipation and 12 controls were studied. Gastric empyting was also performed on 10 patients with idiopathic slow transit constipation (ISTC) for comparison.

The results of this study are shown in tables 1,2, figs 7,8. An area of interest has been drawn on these plates to highlight the presence/absence of radionucleotide in the stomach. The right anterior superior iliac spine has also been marked.

Liquid phase empyting was normal in idiopathic slow transit constipation and postchildbirth/hysterectomy constipation, $t_{1/2}$=43(10)mins and 38(9)mins, controls 42(17)mins, $p<0.6$ (analysis of variance). Solid phase gastric emptying was profoundly delayed in idiopathic constipation, $t_{1/2}$=144(31)mins compared with controls, $t_{1/2}$=89(22)mins and patients with PC/PH constipation, $t_{1/2}$=83(24)mins, $p<0.006$ (analyses of variance) (figs 9, 10). Subsequent Tch 99m scanning identified the radio-isotope head in the caecum in 9 out of 10 controls, 9 out of 10 PC/PH, but only 1 out of 10 ISTC at 6 hours. At 24 hours, the isotope had reached the transverse colon in 9

of 10 controls, 8 out of 10 PC/PH patients and 0/10 ISTC still residing in the small bowel in 5 cases. At 30 hours the radio-isotope had reached the descending colon in 8 of 10 controls, 8/10 PC/PH and 0 of 10 ISTC. It persisted in the small bowel in 5 cases and in the ascending colon in 5 cases.

While there is some overlap between the results from PC/PH and ISTC patients and controls, PC/PH constipation patients clearly have normal proximal gastrointestinal transit and transit through the proximal colon. In contrast, some patients with idiopathic constipation have, in addition, proximal gastrointestinal dysmotility.

Constipation resulting from difficult childbirth or hysterectomy clearly represents a distinct clinical entity. In addition, identification of normal proximal gastro-intestinal transit in PC/PH patients suggests a favourable outcome from surgery (segmental colonic resection). In contrast, the profound delay identified in the ISTC patients suggests that a major component of their symptoms is related to proximal gastrointestinal dysmotility, which is unlikely to be improved by resection of the colon.

These results provide for the first time, evidence that patients with postchildbirth/hysterectomy constipation form a distinct subgroup of idiopathic slow transit constipation.

The demonstration of normal proximal colonic transit in these patients will be shown to be consistent with the results from segmental marker studies and dynamic radio-isotope scanning as outlined in sections II and III of this chapter.

4.2 RADIO-OPAQUE MARKER STUDIES

Segmental colonic transit times for left, right and rectosigmoid colon are summarised in table 3, fig 11. Right colonic transit was similar in both groups, patients - 19.4(24) hours, controls - 14(10.2) hours, $P<0.38$. Left colonic transit was prolonged significantly in patients at 26(14.7) hours when compared with controls, 13.5(10) hours, $P<0.024$. The measured transit through the rectosigmoid was normal in patients, 13.5(10) hours compared with controls, 10.9(7.8) hours, $P<0.64$. Markers were not present in the rectosigmoid in two patients. It is not possible to have a zero transit time for any given segment; these observations were thus discounted and the mean rectosigmoid transit time

was based on 8 observations. Similarly, in one control, markers were not seen in the right colon and in another, markers were absent from the rectosigmoid. The mean transit time for these areas was based on 12 observations.

Discussion

The calculation of segmental transit time is based on a regular distribution of markers throughout the colon and assumes that all markers are handled in exactly the same way. However, mass actions result in movement of colonic content, and presumably markers, from one segment to another in a few seconds. This perhaps explains the large variation in segmental transit times, even among controls. This problem was encountered in previous studies (58,59) and individual segmental transit times should be viewed in this context.

The finding of a normal transit time through the rectosigmoid (the area proposed as being the most affected) was unexpected. It may be due to relative failure of markers to enter the rectosigmoid. Apparent prolonged transit in the left hemicolon in this series may be due to the presence of an abnormal area, with respect to physiological function, within the rectosigmoid (fig 12). Not withstanding these limitations, the technique of segmental colonic transit is simple and well tolerated and provides more information on transit than previous methods (57). However the sizeable

overlap between segmental transit times in patients and controls means that clinical decisions should not be made on the basis of serial marker studies alone.

The results of this study suggest that there may be a regional basis to post-childbirth/hysterectomy constipation. As a group these patients appear to have delay confined to the hindgut, however given the reservations already expressed it is not possible to say, on the basis of segmental marker studies that this is an isolated hindgut problem.

4.3 DYNAMIC RADIO-ISOTOPE SCANNING IN PATIENTS WITH POSTCHILDBIRTH/HYSTERECTOMY CONSTIPATION

The mean response time following the instillation of Bisacodyl 5 mgs into the right colon was similar in patients and controls; 12.5(5.7)mins and 13.1(3.5)mins respectively, $p<0.9$. Transit of radionucleotide to the splenic flexure was similar in both groups, patients - 19.1(6.7)mins, controls - 20.0(4.3)mins, $p<1$. Radioisotope reached the proximal

sigmoid in the same time; 28.3(5.6) mins in patients, 29.9(4.7)mins in controls, p<0.38 (table 4, fig 13). In all 10 patients, radio-isotope arrested in the proximal sigmoid and failed to progress over the next 2 hours (fig 14). In contrast, radio-nucleotide reached the rectum in 9 of the 10 controls within 45 minutes (fig 15).

Discussion

The majority of patients with idiopathic slow transit constipation have pancolonic disease. Isolated damage to the pelvic nerves occurring during hysterectomy (31) or childbirth, may result in a pattern of dysmotility confined to the hind gut. Previous studies of dynamic radio-isotope scanning in severe constipation identified some patients in whom transit through the proximal colon appeared normal (68). However, this was a heterogeneous group of patients in whom the aetiology was unclear. This thesis examines a more homogeneous group of patients who have attributed the onset of symptoms to either traumatic childbirth or hysterectomy. The results of this study demonstrate that bisacodyl induced transit through the proximal colon was normal in PC/PH constipation and delay was confined to the rectosigmoid. Dynamic radioisotope scanning using bisacodyl is not a physiological test. Studying colonic transit with

radio-isotope under physiological conditions is time consuming while transit times through particular regions maybe inaccurate. Colonic mass movements, though infrequent, result in the movement of colonic content over whole segments of colon. During the intervening period the colon may be quiescent.

This simple technique reduces the observation period from 36-48 hours to a dynamic scan of 45 minutes and 3 further recordings of up to 2 hours.

If the results of this study are compared with the colonic marker studies we can see that all the patients are identified as having delayed transit through the rectosigmoid colon. In contrast, using segmental marker studies, the measured transit through the rectosigmoid was normal in patients with PC/PH constipation. The region of delay with marker studies appeared to be in the descending colon. Essentially these studies are reporting the same abnormality, ie delay is confined to the left colon. However, using marker studies only 4 of the 10 patients fell outside the normal range for transits through the left colon. In contrast, left sided delay is identified in all 10 patients using dynamic radio-isotope scanning. Radio-isotope scanning would therefore appear to be the more useful test of regional dysmotility.

Two patients subsequently proved to have a megacolon indicating that bisacodyl is able to overcome physiological obstruction in the proximal colon. Hence in those radioisotope studies which indicate normal proximal colonic motility, a false positive result must be considered. Larger numbers of patients need to be studied to assess the specificity of this procedure. Although dynamic radioisotope scanning indicates that the main area of delayed transit is in the left colon, this test should not be used in isolation for planning the appropriate surgical procedure.

4.4 COLONIC MANOMETRY STUDIES IN PATIENTS WITH POSTCHILDBIRTH/HYSTERECTOMY CONSTIPATION.

The effect of Neostigmine on Colonic Motility

1. Contractions > 5 cm H_2O
2. Contractions > 50 cm H_2O
3. Mean intracolonic pressure
4. Motility (area under the curve) cm H_2Omins.

The effects of Neostigmine on the frequency of contractions > 5cm H_2O

The results of this experiment are summarised in tables 5-7, figs 16-18.

In controls, neostigmine (0.01 mg/kg S.C.) significantly increased the number of contractions/hour from 64(45), to 96(67), $p<0.01$. In the descending colon, a significant increase from 60(51) to 112(32) was observed, $p<0.003$. In the sigmoid colon, the number of >5 mmHg contractions increased from 65(51) to 116(70), $p<0.01$.

In patients with PC/PH constipation, the frequency of contractions did not alter in response to neostigmine (0.1 mg/kg S.C.) in each of the regions studied; 105(70) to 124(60) in the transverse colon, $p<0.3$, 83(71) to 93(60) in the descending colon, $p<0.5$, 101(58) to 122(50) in the sigmoid colon, $p<0.2$.

These results demonstrate that neostigime significantly increases the number of >5 cm H_2O contractions in the transverse, descending and sigmoid colon of controls. In contrast, no effect is seen in PC/PH patients. A further analysis of the data summarised in table 7 shows that the number of contractions in the resting phase is significantly higher in the transverse and sigmoid colon in patients with PC/PH constipation than controls. Neostigmine (0.01 mg/kg S.C.), produced more contractions in the transverse colon of patients when compared with controls, $p<0.04$. In the descending and sigmoid colons, the total number of >5cm H_2O contractions produced by neostigmine was similar in patients and controls.

<u>The Effect of Neostigmine on Contractions >50cmH_2O.</u>

These results are summarised in tables 8-10, figs 19-21.

In controls, neostigmine (0.01mg/kg s.c.) increased significantly the number of large contractions/hour in the transverse colon from 0.1(0) to 1.5(2.5). $p<0.02$. In the descending colon, a change from 0.6(0.9) to 3.6(4.2) was noted, $p<0.06$. In the sigmoid colon, large contractions increased significantly from 0.5(0.8) to 2.2(2.4) following the administration of neostigmine, $p<0.003$.

In patients with PC/PH constipation, neostigmine (0.01mg/kg s.c.) did not affect the number of large contractions/hour in each of the regions studied: 2.3(4.6) to 5.3 (8.6) in the transverse colon, $p<0.15$; 2.5(5.6) to 7.1(14.5) in the descending colon, $p<0.5$; 2.3(5.4) to 3.7(6.6) in the sigmoid colon, $p<0.27$.

Analysis of the results summarised in table 10 show that there was no difference between the number of large contractions in the resting phase in patients and controls in the descending and sigmoid colon. However, the transverse colon generated more large wave contractions in the resting phase in patients, than controls, $p<0.01$. Similarly, following the administration of neostigmine, there was no statistically significant difference between patients and controls. The effect achieved by neostigmine in controls appears to be due to the low number of >50 cm H_20 contractions in the resting phase, which although not statistically different from the study group was sufficient to achieve statistical significance in the control group.

Effect of Neostigmine on the Mean Colonic Pressure

The results for this study are summarised in tables 11-13, figs 22-24.

In controls, neostigmine (0.01mg/kg s.c.) raised the intraluminal pressure significantly from 10.1(1.7)cm H_20 to 12.5(3.2)cm H_20, $p<0.004$. A significant increase in intraluminal pressure from 10.0(2.2)cmH_20 to 14.8(4.1)cmH_2O was observed in the descending colon, $p<0.002$. In the sigmoid colon the resting pressure was 14.5(15.2)cmH_2O, which changed to 14.3(4.0)cmH_2O, following the administration of neostigmine. This difference was significant at $P<0.004$. The similarity in the mean values in the sigmoid colon in controls is due to two excessively high values. This highlights a problem with water perfusion catheters which was discussed in the literature review, ie side recording channels may at times record wall pressure as opposed to true intraluminal pressure. Comparison of the other pressures recorded in the sigmoid colon of controls before and after the administration of neostigmine shows that there is a measurable and significant increase in mean colonic pressure in the sigmoid colon.

In patients with PC/PH constipation, neostigmine (0.01mg/kg s.c.) did not affect the intraluminal pressure in the two proximal regions studied: 12.3(4.0)cmH_20 to 15.2(7.6)cmH_20 in the transverse colon, $p<0.25$; 10.4(5.7) to 16.2(12.8)cmH_20 in the descending colon, $p<0.12$. An increase in intraluminal pressure from 11.8(4.6)cmH_20 to 14.5(5.0)cmH_20 was observed in the sigmoid colon, $p<0.01$.

The results in table 13 demonstrate a difference in resting pressure in the transverse colon between patients and controls, which almost reached statistical significance $p<0.06$. However, those of the descending and sigmoid colon did not. The pressure elevation produced by neostigmine in the transverse, descending and sigmoid colons was similar in patients and controls. This would suggest that the effect of the drug in controls may therefore be due to the lower resting pressure and not to a greater effect of neostigmine.

Effect of Neostigmine on Motility (area under the curve)

The three previous sections can be summarised by looking at the motility pattern produced by the administration of neostigmine. This has been done by measuring the area under the motility curve. The results for this section are summarised in tables 14-16, figs 25-27.

In controls, an increase in motility (AUC) was observed in the transverse colon, 131(55)cmH_20 compared to 194(94) cmH_20, $p<0.03$. In the descending colon, motility increased following the administration of neostigmine from 90(48)cmH_20mins to 178(54)cmH_20mins, $p<0.001$. The motility increased in the sigmoid colon from 116(77)cmH_20mins to 272 (102)cmH_20mins following the administration of neostigmine, $p<0.001$.

In patients with PC/PH constipation, neostigmine (0.01mg/kg s.c.) did not increase the overall motility in the transverse colon, 241(228)cm$H_2$0mins, compared with 244 (153)cm$H_2$0mins , p<0.48. Similarly, in the descending colon, motility was unchanged following the administration of neostigmine, 323(383)cm$H_2$0mins compared with 281(272)cm$H_2$0mins, p<0.44. Motility in the sigmoid colon was also unaffected by neostigmine, 183 (115)cm$H_2$0mins compared with 211(124)cm$H_2$0mins, p<0.53. Consideration of the results summarised in table 16 shows that the resting motility in the descending and sigmoid colon of controls was significantly less than in the patient group. The significant effect of neostigmine on the overall motility pattern in controls relies on the low values recorded in the resting phase. The effect of neostigmine on colonic motility in patients with PC/PH constipation, where resting motility is raised, is similar to that of a control group.

Discussion

An alternative method of analysing motility is to express the effect of a stimulus as a percentage of the motility during the resting phase (31). If the data examined in the last section is analysed in this way, then the results recorded in table 17, fig 28 are obtained. The relative increase in motility in the transverse colon of

patients and controls is similar, $p<0.59$. In the descending colon the relative increase in motility in the control group exceeds that of the patient group, but the increase is not significant $p<0.09$. In the sigmoid colon the relative increase in motility in the control group is significantly greater than in the patient group, $p<0.02$. Analysis of data using this technique suggests that there is a cholinergic deficit in the sigmoid colon. However, vide infra, this apparent deficit is due to the increased basal motility pattern in the sigmoid colon in constipated patients. It is not possible, therefore, to comment on whether cholinergic denervation is present on the basis of the raw data presented in this study. The motility profile represented in figure 29 indicates a motility gradient from right to left in the control group. However, this motility gradient is lost in patients with PC/PH constipation. On the basis of the data presented, it cannot be said whether the loss of motility gradient in patients with PC/PH constipation is due to a relative failure of the sigmoid colon to respond to neostigmine or an unusually large response in the transverse colon. In controls, the response to neostigmine was greater in the sigmoid colon when compared to the transverse colon, $p<0.01$. In contrast, in patients with PC/PH constipation

there was no difference between the motility in the sigmoid colon and the transverse colon in response to neostigmine, $p<0.55$. Figures 30,31 illustrate some of the above points.

Colonic Response to 1000 calorie Meal

For the purposes of this study, the motility indices were analysed from the second meal.

The same parameters were then recorded as in the neostigmine stimulation test.

1. Contractions over 5 cm H_2O
2. Contractions over 50 cm H_2O
3. Mean amplitude (cm H_2O)
4. Total area cm H_2O

The effect of 1000 calorie Meal on contractions >5mmHg

The results for this study are summarised in tables 18-20, figs 32-34.

In controls, a meal stimulus increased significantly the number of contractions/hour in the transverse colon from 67(54) to 227(129)cmH_20, $p<0.0001$. In the descending colon a significant increase from 46(24) to 136(93)cmH_20 was observed, $p<0.001$. The number of contractions in the sigmoid colon again increased significantly from 51(45) to 114(125)cmH_20 in response to meal stimulus, $p<0.001$.

In patients with PC/PH constipation, the meal stimulus increased significantly the number of >5cmH_20 contractions/hour in the transverse colon from 86(64) to 170(93), $p<0.002$. In the descending colon, a significant increase from 77(90) to 122(60) contractions/hour was observed, $p<0.01$. In the sigmoid colon the number of contractions/hour also increased significantly from 84(43) to 127(63), $p<0.02$. Analysis of the data presented in table 20 demonstrates that the number of contractions in the resting phase in the transverse and descending colons were similar in patients and controls. However, in the sigmoid colon there were more contractions >5cmH_20 in the PC/PH group when compared to controls, $p<0.01$. There was no

difference in the number of contractions produced by a 1000 calorie meal in the transverse, descending and sigmoid colons between patients and controls.

The effect of a 1000 calorie meal on contractions >50cmH_2O.

The results for this study are summarised in tables 21-23, figs 35-37.

In controls, the meal stimulus increased significantly the number of large contractions in the transverse colon from 0.14(0.64) to 2.3(3.2) contractions/hour, $p<0.0001$. In the descending colon, an insignificant trend was seen, 0.58(0.9) before and 2.6(2.8) contractions/hour after the meal, $p<0.07$. In the sigmoid colon, the number of contractions/hour increased from 0.17 (0.51) to 1.1 (1.6), following the administration of the meal, $p<0.009$.

In patients with PC/PH constipation, the number of large contractions/hour in the transverse colon did not change; 1.2(1.9) before and 4.4(9.4) after the meal, $p<0.42$. Similarly, the number of large contractions/hour in the descending colon did not increase, 1.1(1.9) compared with 3.8(6.3), $p<0.61$. In the sigmoid colon, there were no large contractions during the resting phase and only a minimal response following the meal, 0.2(0.5), $p<0.84$. On reviewing the data presented in table 23 one can see that

there were more contractions >50cm$H_2$0 in the transverse colon during the resting phase in patients with PC/PH constipation, p<0.002. The response to a meal in both the transverse and descending colons was similar in patients and controls. In contrast, there were significantly more large contractions induced in the sigmoid colon of controls, compared with patients, p<0.02.

<u>The effect of a 1000 calorie meal on the mean colonic pressure</u>

The results of this study are summarised in tables 24-26, figs 38-40.

In controls, the mean pressure in the transverse colon rose significantly from 10.0(1.9) to 11.9(2.0)cm$H_2$0, p<0.004. In the descending colon, pressure was unchanged from 10.1(2.4) to 11.6(3.5)cm$H_2$0, p<0.18. In the sigmoid colon, the mean pressure before colonic stimulation was 13.6(16.6) which changed to 13.1(5.8)cm$H_2$0, p<0.004. As in the neostigmine stimulation study, two of the pressures recorded in the sigmoid colon of controls were excessively high accounting for the large standard deviation. These were probably recordings of the wall pressure in the sigmoid colon and do not reflect true intraluminal pressure. However, they have been included in the analysis of the data.

In patients with PC/PH constipation, the mean pressure in the transverse colon remained unchanged, 11.4(3.4) before and 13.3(5.4)cm$H_2$0 and after the meal, p<0.2. Similarly, in the descending colon, no change in intraluminal pressure was noted, 11.2(4.6) compared with 13.3(6.0)cm$H_2$0, p<0.18. In the sigmoid colon, a 1000 calorie meal produced a significant rise in pressure from 9.5(1.5) to 10.9(2.2)cm$H_2$0, p<0.05. Analysis of the data summarised in table 26 shows that there was no difference in the mean pressure during the resting phase or following a 1000 cal meal in each of the transverse, descending or sigmoid colon in patients when compared with controls.

<u>Effect of a 1000 calorie meal on motility (area under the curve).</u>

The results of this study are summarised in tables 27-29, figs 41-43.

In controls, the 1000 calorie meal did not affect the area under the motility curve in each of the three regions studied: 172(86) to 189(105)cm$H_2$0mins in the transverse colon, p<0.59; 113(78) to 148 (107)cm$H_2$0mins in the descending colon, p<0.32, and 130(94) to 173(120)cm$H_2$0mins in the sigmoid, p<0.37.

In patients with PC/PH constipation, motility in each of regions did not change following a 1000 calorie meal: transverse colon - 167(97) compared with 239(238)cm$H_2$0, p<0.63; descending colon - 222(295) and 242(234)cm$H_2$0mins, p<0.18; sigmoid colon - 152(107) to 194(111)cm$H_2$0mins, p<0.19. Review of the results presented in table 29 demonstrates that there was no significant difference in either the resting or post meal motility in either the transverse, descending or sigmoid colon in patients when compared to controls.

Discussion

The pattern of response to meal stimulation was different from that obtained with neostigmine (see figs 30,31,44,45). The motility gradient which is present in controls following the administration of neostigmine could not be demonstrated using a 1000 calorie meal. The probable reason for this is that the colonic response to a meal is mediated, in part, through the vagus nerve and has its greatest effects on the transverse colon. If we consider the number of contractions >5mmHg , then the greatest activity is seen in the transverse colon in both patients and controls with a lesser, though still significant response in the sigmoid colon. This pattern of activity is not reflected in the overall motility where there appears to be

no statistical difference between motility (area under the motility curve) in the transverse, descending and sigmoid colon following the administration of a 1000 calorie meal.

For the purposes of these two studies (neostigmine 0.01mg/kg s.c. and 1000cal meal), two separate resting periods were selected for analysis. Traditionally these studies are carried out by comparing the parameters of motility for the hour prior to and following the stimulus. A difference in the basal motility in the resting phase may explain the failure to achieve statistical significance in overall motility. However, as can be seen from tables 30,31 there was no statistical difference between the basal motility patterns in the transverse, descending and sigmoid colons for both studies in patients and controls.

Although the overall motility recorded in response to both a 1000 calorie meal and neostigmine 0.1 mg/Kg S.C. was similar in both groups, analysis of individual parameters suggests that the way in which the colon responds to these stimuli is different in patients and controls. Both low pressure and high pressure waves were recorded in patients and controls, but it is not sufficient just to record the frequency of such events, due consideration must be given to the nature of the motility observed.

Colonic Mass Contractions in Patients & Controls

Colonic mass actions are infrequent events in the colon. Their aetiology is unclear, but colonic distension, intraluminal content and the colonic response to a meal are three factors which are thought to be involved in the initiation of mass actions. In this study, mass actions were seen in 6 of 10 patients and 6 of 8 controls. Recording times in the patients who failed to demonstrate mass actions were 21 hours 25 minutes, 18 hours 30 minutes, 20 hours and 5 minutes, and 20 hours and 21 minutes, respectively. In the 2 controls where mass actions were not seen, recording times were 4 hours and 5 hours, respectively. Therefore, failure to see a mass action in 2 of the controls might have been due to an inadequate observation period. The same criticism cannot be upheld in the case of the patients with PC/PH constipation where at least one migrating motor complex in the observation period might have been anticipated.

The results for the following section are summarised in tables 32,33, figs 46-49.

Mass actions in controls, were accompanied by a pressure rise of 160(47)cmH_2O. In contrast, mass actions in patients with PC/PH constipation produced a pressure rise of 81(34)cmH_2O, $p<0.001$ The distance travelled by mass actions was similar in patients and controls, 28(15)cm vs 31(12) cm,

respectively, $p<0.33$. In controls, mass actions travelled at the speed of 1.0(0.8)cm/s. In contrast, mass actions in patients travelled at 1.7(1.0)cm/s, $p<0.002$.

As mass actions are infrequent, objective comments on the numbers seen in this study cannot be made . However, subjectively, they are present in a similar proportion of patients and controls.

Mass actions progressed over similar distances in patients and controls. However, the strength of contraction in patients was less than in controls. This observation may partly explain the delayed transit seen in patients. The reason for the low pressure propagating waves in patients is unclear. From the previous experiments, the colon, in both patients and controls, responded to neostigmine to the same extent. This suggests that the efferent limb of the peristaltic reflex is intact. Failure to recruit fibres for the contractile effort in the mass action may be due to insufficient afferent input from the sensory side of the peristaltic reflex. As can be seen, (figs 50, 51) the mass action in controls represents a powerful contractile force, which frequently occludes the catheter to the maximum pressure of 223 mmHg. By contrast the propagating wave in constipation patients (fig 52) is a low pressure peristaltic wave. The observation that mass actions in patients travel faster than control is surprising. As the start of the mass

action in the constipation group is less well defined than in the control group, measurement of the wave speed may have been subject to observer error. However, this alone is unlikely to account for the high degree of statistical significance seen between patients and controls. A second possibility is that part of the pressure wave in the constipation group represents a progressive pressure rise within a segment of colon and not genuine peristalsis. Whatever the explanation, there is clearly a difference in the nature of the colonic mass action between patients and controls.

The simple interpretation of the results presented suggests that MMC's in patients with PC/PH constipation run the same distance, but are not carrying out the work along the way.

4.5 Anorectal Manometry Studies

The results for anorectal manometry are summarised in tables 34-36, figs 53-57.

The resting anal canal pressure was 59.1(16.8)mmHg in patients and 56.9(16.4)mmHg in controls, P<0.88. Maximum squeeze pressure in the anal canal was not statistically different in patients, 111.5(38.6)mmHg compared with controls, 89.6(24.7)mmHg, P<0.19. Anal canal length was similar in patients when compared to controls, 39.8 (5.6)mm and 37.7(7.6)mm respectively P<0.14. There was no difference in first sensation of rectal filling which occurred at 52.3(43.4)mls in patients compared with 42.4(26.4)mls in controls, P<0.8. The volume required to produce an urge to defaecate was similar in patients, (159.2(87.5)mls) and controls, (119(51.2)mls), P<0.38. Examination of the rectal anal inhibitory reflex demonstrated a percentage relaxation of the internal sphincter of 67.5(22.2)% in patients and 53.1 (22.1)% in controls, P<0.15. Compliance during rectal filling was not significantly different in patients, (0.22 (0.25)mmHg per ml) compared with controls (0.20(0.22)mmHg per ml), P<0.8, fig 57.

The physiological variation for each parameter can be readily seen from the graphs (figs 53-57). Resting pressure, maximum squeeze pressure and anal canal length were each

normal in patients with postchildbirth/post hysterectomy constipation. The non-significant trend towards decreased rectal sensitivity may be explained by the recordings made in two of the patients. At subsequent laparotomy, both patients were found to have a large redundant rectum and colon and may have been idiopathic slow transit patients who attributed their symptoms to childbirth in one case and hysterectomy in another. The remaining eight patients fall within the physiologically normal range for rectal sensation. A considerable variation was seen in the degree of relaxation produced by rectal distension in both patients and controls. However, the demonstration of a minimum 30% reduction in sphincter tone in 9 of 10 patients excludes the possibility of short segment Hirschsprungs disease in this group.

In summary, patients with PC/PH constipation have normal anorectal physiology and therefore preservation of at least the lower rectum should be considered in patients selected for surgery.

SUMMARY

The observations in the *in vivo* studies presented in this section can be summarised as follows.

1. Gastric emptying is normal in patients with PC/PH constipation.

2. Radio-opaque marker studies define a segmental delay in the left colon.

3. Dynamic radioisotope colonic transit studies indicate that delay is localised to the sigmoid colon.

4. (i) The colonic response to a meal and neostigmine is normal in patients with PC/PH constipation.

   (ii) Basal motility is raised in patients with PC/PH constipation.

   (iii) Significantly, mass actions of reduced amplitude are seen in PC/PH constipation.

5. Anorectal manometry is normal in patients with PC/PH constipation.

PART II

ELECTRICAL AND MECHANICAL PROPERTIES *IN VITRO*

OF

COLONIC SMOOTH MUSCLE RESECTED FOR CONSTIPATION

CHAPTER 5

IN VITRO METHODOLOGY

5.1 Introduction.

5.2 Receptor mediated responses in human taenia coli.

- Tissue preparation

5.3 Myo-electrical activity in human taenia coli

- Tissue preparation

5.4 Peristalsis in human sigmoid colon

- Tissue preparation

5.5 Drugs and solutions used in study

5.6 Analysis of data

5.1 INTRODUCTION

Having identified, *in vivo*, a segmental abnormality of transit in the rectosigmoid colon of patients suffering from post childbirth/hysterectomy constipation, it was important to ascertain the nature of the dysmotility. This section of a thesis examines aspects of colonic smooth muscle function; its basal myoelectrical activity, receptors involved, the effects of afferent and efferent nerve stimulation and the role of the peristaltic reflex in colonic motility. Four basic questions were addressed.

1. Is myo-electrical activity present in both constipated and control smooth muscle and can it be influenced by both agonists and antagonists?

2. What receptors mediate the nerve induced response?

3. What is the neuronal basis for contraction and relaxation in human colonic smooth muscle?

4. Is the peristaltic reflex present in patients with PC/PH constipation?

DEMONSTRATION OF RECEPTOR MEDIATED RESPONSE IN HUMAN SIGMOID TAENIA COLI

5.2 Preparation of Tissues

Tissues for this experiment was taken from the distal sigmoid colon in constipated patients and controls, the latter from macroscopically normal colon adjacent to sigmoid carcinomas. Tissue was placed immediately into oxygenated Krebs solution at $4^{o}C$ following its removal at laparotomy.

The longitudinal muscle, mucosa and submucosa were separated by sharp dissection from the adjacent circular muscle and pinned out, mucosa upwards, on a Sylgarded petri dish avoiding undue stretch and bathed continuously in oxygenated Kreb's solution. Following removal of the mucosa and submucosa by sharp dissection, the circular muscle was seen lying transversely and superimposed on the underlying taenia coli. Strips of taenia coli (1x0.2cm) were cut in the line of the longitudinal fibres. Adhering circular smooth muscle was not dissected free so as to avoid injury to the myenteric plexus, which lies between the two muscle layers. Tissues were then mounted in a 10 ml, water-jacketed organ bath in oxygenated (O_2 95% CO_2 5%) Krebs solution at $37\pm0.5^{o}C$ in an electric assembly of 2 concentric silver wire electrodes 5mm apart for the application of electric field stimulation. The distal end of the muscle strip was

tied to a silver hook by means of a silk thread (4/O) while the proximal end was attached, similarly to an isometric strain gauge transducer to measure tension and the signal amplified for display on a chart recorder (Grass SD9), (fig 58). Square wave stimulation, frequency 0.5 - 64Hz, duration 0.1ms, 40 volts was supplied from the Grass stimulater. Tissues were allowed to equilibrate for at least one hour prior to experimentation. Unused tissue was transferred to oxygenated Krebs solution and kept at $4^{o}C$ for use the following morning. During equilibration, the tissues were washed through several times with Krebs solution. Following equilibration, 1Gm tension was applied. Drugs were added directly to the organ bath. A 5 minute time cycle was used between drug administrations until the first mechanical response was observed. Thereafter, a 15 minute period elapsed between drug administrations. The 10 ml organ bath was washed through four times between drug administrations.

5.3 Myo-electrical Activity from constipated and control taenia coli

Extra cellular myo-electrical activity was measured in human taenia coli using Golenhoffen/von Low apparatus (figure 59) (164). Strips of taenia coli (2x0.2cm) were passed through the capillary of the Golenhoffen apparatus to lie across four platinum wire electrodes. Two of these

electrodes were used for recording electrical activity and the other two for electric field stimulation of the tissue. The capillary of the Golenhoffen was continually perfused with oxygenated Krebs solution (O_2 95% CO_2 5%) at 37 ± 0.5°C. One end of the tissue was fixed to a metal hock and the opposite end was attached with silk thread (4/0) to an isometric strain gauge transducer (Grass 7T03C). A resting tension of 1g was applied to each tissue. The recording electrodes were connected to an A.C. preamplifier (Neurolog NL103, low frequency cut off 0.1Hz) and the signals filtered (Neurolog NL115). Both electrical and mechanical signals were amplified via a cathode ray oscilloscope (Tektronix, 5103N) before being recorded on, and further amplified by, an ultraviolet oscillograph (EMI, SE 6150 Mk II) Recordings of electrical and associated mechanical activity were amplified and displayed on an UV recorder. Electrical signals were calibrated by passing 0.1 mV from an A.C. preamplifier (Neurolog NL103)

The effects of increasing tension and agonist/antagonist stimulation were recorded. Aliquots of drugs were added to the perfusate by injection into the main channel.

5.4 Recording of Peristalsis from Human Sigmoid Colon

This experiment was performed using a modification of the Trendelenberg Apparatus (165). Segments of intact sigmoid colon (10cm) were mounted in a 500 ml water-jacketed organ bath containing oxygenated 95% O_2 5%CO_2 Krebs solution, at 37 $\pm$ 0.5^{o}C (fig 60). The proximal end which had been marked at the time of resection in theatre was fixed to a perspex tube (8cm) using plastic retention clips, forming a perfect seal. A manometry catheter was passed through the perspex tube to measure intraluminal pressure. Its proximal end was sealed against the perspex tube using a rubber bung, and the tissue was then suspended from an isotonic transducer and counter balanced with a 30 gm weight. The opposite end of the bowel was fixed similarly over a U-tube using plastic retention clips, again forming a perfect seal. The U-tube was connected to a reservoir containing 500 mls of Krebs solution. The reservoir was attached to a movable ratchet, thus allowing it to be raised and lowered. A second transducer in the reservoir measured changes in pressure caused by the outflow of fluid. Changes of volume within the sigmoid colon were therefore seen as a fall or rise in the fluid level within the reservoir. As the reservoir was raised, fluid flowed from the reservoir to the sigmoid colon, which was distended to the point where reflex

contraction occurred. At this point, fluid was then pushed from the sigmoid colon back into the reservoir, which was recorded as an increase in volume by the transducer.

Tissues equilibrated for three hours in oxygenated Krebs solution, during which time the tissue was washed through on several occasions. A second water-jacketed reservoir of oxygenated Krebs solution prevented cooling of the tissue during these experiments. Using this apparatus the longitudinal contraction of the taenia coli, the intraluminal pressure and the volume of fluid expelled was measured for both constipated and control sigmoid colons.

For each experiment the reservoir was elevated for a period of 10 minutes to induce peristalsis, then lowered to allow a 15 minute rest period.

The experimental models, tissue preparations and recording apparatus are standard to laboratory based pharmacology (87,89,91,93-97,109,110,164,165).

5.5 Drugs and solutions.

The following drugs were used ($1x10^{-8}$ - $1x10^{-4}$M)

Carbachol

Atropine

Diltiazem

Dimethylphenylpiperazimium (DMPP)

Hexamethonium

Neostigmine

Eserine

Nicotine

Sodium Nitroprusside (SNP)

Adenosine Monophosphate (AMP)

Adenosine Triphosphate (ATP)

Cyclic AMP

Phenylephrine

A description of the pharmacology of the drugs used in the in-vitro experiments is given in Appendix 4.

All experiments were performed at 37± 0.5°C, Ph 7.4, in freshly made up Kreb's solution of the following composition (mM):

NaCl	118.4
$NaHCO_3$	25
$NaH_2 PO_4$	1.1
KCl	4.7
$MgCl_2$	1.3
$CaCl_2$	2.7
Glucose	11

5.6 Analysis of Data

Receptor mediated excitatory responses were expressed as grammes tension (T max) achieved. Nerve mediated relaxations were expressed as a % reduction in muscle tension against maximum relaxation produced by sodium nitroprusside ($1x10^{-5}M$). Data obtained for patients and controls was compared using Students' t test. Dose response curves were derived in a similar fashion to the previous experiment by expressing individual drug-induced contractions as a percentage of the maximum tension produced in that tissue. Dose-response curves for patients and controls were compared using regression analysis. Results were considered significant at $P<0.05$

Insufficient tissues were examined in the Golenhoffen apparatus due to the requirements of other contemporaneous experiments. For this reason it was not possible to apply strict statistical analysis to this experiment. However, changes in amplitude and frequency of electrical and mechanical activity in response to agonist and antagonist stimulation are reported for constipated and control tissues.

The frequency amplitude and pressure of peristaltic contractions were recorded for both constipated and control tissue. The threshold volume and pressure required to initiate reflex contraction was also recorded. The effect of local anaesthetic agents, ganglion stimulants, ganglion blocking agents, cholinergic agonists and antagonists were observed and the effect on the aforementioned parameters recorded. Data was compared using the Students t-test and results were considered significant at $P<0.05$.

CHAPTER 6

IN VITRO PROPERTIES OF HUMAN SIGMOID COLON

6.1 Nerve mediated responses in sigmoid taenia coli

6.2 Drug-mediated responses in sigmoid taenia coli

6.3 Examination of the peristaltic reflex in human sigmoid colon

6.4 Electromechanical properties of sigmoid taenia coli

Results

Considerable patience was required when dealing with colonic smooth muscle. Some tissues initially considered inactive after a period of equilibration, subsequently responded to both electrical and receptor-mediated stimulation. Tissues which were spontaneously active at the beginning of an experiment usually retained the ability to respond to nerve stimulation throughout the experiment. In general, tissues did not seem to fatigue during the course of any experiment, nor did nerve/agonist responses diminish. Strips of taenia coli regularly developed spontaneous activity, which was taken as a sign that equilibration had been achieved. This spontaneous activity was not nerve-mediated (fig 61) as it was not abolished by either atropine (1x10-6M) or tetrodotoxin (1x10-5 M). These contractions were due to repeated depolarisation and repolarisation of the cell membrane and is an inherent

property of pacemaker activity. It is however mediated via calcium channels in the smooth muscle cell and was abolished by diltiazem (1x10-5M).

On occasion, basal tone would increase during an experiment. As the effects of nerve stimulation are dependent on the inherent tone of the preparation for their demonstration, individual tissues could exhibit both excitatory and inhibitory responses depending on the tone present. Thus nerve stimulation could give rise initially to excitation, but later elicit an inhibitory response (fig 62). Some tissues also exhibited pacemaker activity in that they showed cyclical increases in basal tone. This property appeared to be myogenic in origin, as it was unaffected by tetrodotoxin (fig 63).

When recording myo-electrical activity from human colon using the Golenhoffen/von Low apparatus, a similar picture to that observed with organ bath techniques emerged. After an initial quiescent period, electrical activity and a rise in tone developed spontaneously. The frequency of the electrical activity increased over the next 2 hours and a regular electrical discharge pattern emerged. This electrical and mechanical activity persisted without diminution throughout the experiment.

In the peristalsis experiments, if the 10 minute stimulation period and 15 minute resting periods were observed, peristalsis was reproducible, over a period of several hours.

Nerve mediated responses from fresh tissue and that stored overnight at $4^{o}C$ were similar. However, if kept for a 2nd day at $4^{o}C$ tissue responses to electrical stimulation and drugs declined. Carbachol induced contractions in both fresh and stored tissue were the same, although the time taken to reach the maximum response was longer in one day old tissue. The ED50 which determines the sensitivity of the tissue was the same for fresh and day old tissue. For these reasons only fresh and one day old tissue was used in these experiments.

6.1 Nerve Mediated Responses in Sigmoid Taenia Coli

As in laboratory animal experiments, stimulation parameters of 1-64Hz, 0.5-1 ms and supramaximal voltage 30 to 70 volts were employed (93-100). The mechanical responses produced by these parameters were found to be insensitive to atropine ($1x10^{-6}M$) suggesting that they were not mediated through cholinergic nerve stimulation. The persistence of these responses in the presence of tetrodotoxin ($1x10^{-5}M$) indicated that the stimulation parameters used in animal models produced a direct stimulation of smooth muscle when

applied to the human situation. Both atropine ($1x10^{-6}M$) and tetrodotoxin ($1x10^{-5}M$) completely abolished the excitatory component when stimulation parameters were reduced to 0.15ms and 40 volts.

Figure 64 shows the effect of increasing voltage and pulse width producing a direct stimulation of human colon. Thereafter, nerve-mediated responses were always performed within these stimulation parameters and confirmation of the effectiveness of atropine and tetrodotoxin was repeated with each experiment.

In the presence of low resting tone, nerve stimulation (1-64Hz, 0.1ms, 30-40 volts) produced excitatory responses in both constipated (7 of 20 strips) and control (12 of 25 strips) taenia coli (optimum frequency 64 Hz),fig 65, table 35. Contractions (fig 66) were abolished in the presence of atropine ($1x10^{-6}$) confirming their cholinergic nature (fig 67).

The abolition of excitatory responses by atropine occasionally unmasked a contraction following the end of nerve stimulation which was not cholinergic. This rebound contraction was abolished by tetrodotoxin ($1x10^{-5}M$) confirming its neural origin (fig 68). In constipated colon, although fewer tissues contracted to nerve

stimulation (n=7), the magnitude of response was similar to that of control colon (n=12)(fig 65). The reason for the paucity of excitatory responces in constipated colon could have arisen from from a reduced cholinergic supply or from a simultaneous stimulation of the inhibitory nerves within the tissue.

In contrast to the results from tissues in low tone, raised basal tone (spontaneous or carbachol induced), nerve stimulation always produced relaxation of the taenia coli in both tissues (optimum frequency 1Hz), fig 69, table 36. On cessation of nerve stimulation, a rebound contraction was seen in control (n=25) and constipated (n=20) taenia coli (figs 70,71). Statistical analysis of the percentage relaxation demonstrated a similar degree of relaxation in both control and constipated sigmoid taenia coli, except at a frequency of 2 Hz when tissues from constipated colon were more responsive (table 36). The significance of this observation is unknown. Perhaps this is a chance observation, in view of the fact that at no other frequency is statistical significance achieved.

The nature of the rebound contraction has not been studied in depth in this thesis, but there is evidence from animal work that this rebound phenomenon is, at least in part, mediated through prostaglandins (166). In two tissues

we were able to examine this possibility. Although tissue numbers prevent us from making any definitive statements it is interesting to see that indomethicin (a prostaglandin synthetase inhibitor) abolished the rebound response demonstrated in (fig 72). This is an interesting observation which requires further investigation.

The results of these experiments demonstrate that, depending upon experimental conditions control sigmoid taenia coli responds to nerve stimulation by producing both excitation and inhibition. Nature has devised a system whereby high frequency stimulation preferentially excites the taenia coli, whereas low frequency stimulation produces relaxation. Constipated colon also relaxes to nerve stimulation, indicating the patency of non-adrenergic non-cholinergic myenteric nerves. The infrequency of excitatory responses in tissues from constipated patients suggests but does not prove that the cholinergic nerves maybe compromised.

6.2 Drug-mediated Responses in Sigmoid Taenia Coli

Dose related drug-mediated responses were elicited in both control (n=25) and constipated (n=16) sigmoid taenia coli, using the muscarinic agonist carbachol (fig 73,74). The Log-dose response curve is sigmoid in shape and

contractions occur over a concentration range 5×10^{-8}M to 2×10^{-5}M. Regression analysis of the dose response curves (fig74) demonstrates that sigmoid taenia coli from constipated patients is more sensitive to cholinergic stimulation than control tissue, $p<0.01$

Following Canon's law of denervation supersensitivity (26,27), this observation might suggest that increased cholinergic sensitivity is present in the constipated sigmoid colon. A second possibility is that tissue from controls came from an older age group with malignant disease and that the differences observed might be a feature of receptor loss with age, ie colonic smooth muscle from elderly patients is comparatively less sensitive than that of younger people.

6.3 Examination of the Peristaltic Reflex in Human Sigmoid Taenia Coli

Following fluid distension of a segment of normal human sigmoid taenia coli, two patterns of motility could be observed. Segmentation contraction was observed in some tissues, occurring at a frequency of 0.7 per minute produced by contraction of the longitudinal muscle element alone with no obvious contribution from the circular muscle (fig 75). This activity was insensitive to atropine or tetrodotoxin, indicating that it was myogenic and not neurogenic in origin. Segmentation was, however, abolished by diltiazem, which blocks voltage-sensitive Ca^{+} channels (fig 76).

The second motility pattern was peristalsis, itself, occurring at a higher frequency of 1.3 per minute (fig 77). The neural origin of the peristaltic reflex was confirmed by the action of hexamethonium ($1x10^{-6}M$), which either abolished all activity (fig 78, 79) or converted the pattern from peristalsis to segmentation (fig 80).

Peristalsis appeared to be initiated by both stretch and pressure receptors located within the colon. Raising the fluid reservoir caused an immediate rise in intraluminal pressure (fig 81). There followed a receptive relaxation of the tissue during which period the intraluminal pressure fell. Once the critical volume was reached, the peristaltic reflex was initiated. Notably, peristalsis was induced

under these conditions only when the pressure had declined to just above resting values. When the fluid reservoir was then lowered, the segment of colon emptied and peristalsis ceased.

The peristaltic reflex was both temperature and oxygen dependent. Initially, Krebs solution was replaced using solution maintained at room temperature. This resulted in tissue insensitivity to distension which was restored when the temperature returned to 37°C. To overcome this, a second reservoir was employed, which kept a fresh supply of oxygenated (CO_2 5% O_2) Krebs solution maintained at 37°C. This allowed peristalsis to be induced whenever necessary.

The sensitivity to oxygen is clearly demonstrated in (fig 82) as removal of the oxygen supply quickly resulted in diminution of activity. Re-introduction of the oxygen supply was promptly followed by the return of spontaneous activity.

Peristalsis was induced in control tissues which were initially slow in their response to fluid distension by neostigmine ($1x10M^{-6}M$) (fig 83) or eserine ($1x10^{-6}M$) (fig 84). Peristalsis was abolished by addition of the local anaesthetic agent, procaine (fig 85)

Under physiological conditions, peristalsis in 11 control sigmoid colons was induced at an intraluminal pressure of 5.6 (1.2) mmHg and intraluminal volume of

44(6)mls. In contrast, none of the tissues from 8 sigmoid colons resected for constipation, demonstrated spontaneous peristalsis at this pressure/volume (fig 86). Segmentation contractions were seen in 2 of the specimens from constipated patients. Neither raising the intraluminal pressure to 7.6 (1.3) mmHg (intraluminal volume = 47(5)mls) nor adding neostigmine ($1x10^{-4}M$) induced regular peristalsis. However, neostigmine ($1x10^{-4}M$) induced a single contraction, the magnitude of which was increased by the ganglion stimulating agent, nicotine (fig 87). These results demonstrate that specimens of sigmoid colon from constipated patients are less sensitive to physiological stimuli than control sigmoid colon, indicating an abnormality of the afferent sensory pathway. A second possibility is that sufficient cholonergic denervation has occurred to prevent organised peristaltic contraction of the sigmoid colon taking place. However, in the presence of neostigmine ($1x10^{-4}M$) and nicotine ($1x10^{-5}M$) a contraction was induced, indicating that the efferent pathway will act, if given a sufficient stimulus.

6.4 Electromechanical Properties of Sigmoid Taenia Coli

Simultaneous extracellular (electrical) and mechanical activity were recorded in 8 segments of taenia coli (control - 4, PC/PH - 4) using the Golenhoffen/von Low

apparatus. Insufficient tissues were studied for statistical analysis to be undertaken. Therefore the effect of the various agonists on both tissue groups is described, but not compared. Each agonist/antagonist was added to each muscle strip from both tissue groups.

Fig 88 gives a clear demonstration of spontaneous activity recorded from control sigmoid colon. In this example a single burst of electrical activity gives rise to a single contraction. In the second example, (fig 89) two electrical depolarisations occur in rapid succession, which results in the associated mechanical contractions summating. It is this electrical activity which gives rise to the spontaneous mechanical activity seen in both control and constipated tissues (fig 90, 91). The myo-electrical activity persisted in the presence of both atropine ($1x10^{-6}M$) and Phentolamine ($1x10P^{-6}M$) (fig 92), indicating that the activity was myogenic in origin and was not under direct neuronal control. Myo-electrical activity was either induced or increased in frequency by the addition of the cholinergic agonist carbachol ($1 x10^{-6}M$), (fig 93) indicating that while spontaneous activity is myogenic in origin, its frequency could be modified by neurotransmitters released from adjacent nerve endings. Similarly it could be induced by electric field stimulation (fig 94).

In the presence of low resting tone, the ganglion stimulating agent DMPP increased the frequency of myo-electrical activity, and accompanying contractions (fig 95). In contrast, when tone was elevated, DMPP relaxed sigmoid taenia coli, and reduced myo-electrical activity (fig 96). This observation is in keeping with previous studies in which electrical field stimulation produced excitation in low tone and relaxation in the presence of elevated tone.

ATP produced the dose dependent relaxations in both constipated and control tissues (fig 97). The frequency of myo-electrical activity also decreased and eventually ceased at which time the taenia had relaxed. The duration of the complete relaxation was further prolonged by increasing the concentration of ATP (fig 98). In the same way, SNP, which is a source of nitric oxide, produced dose dependent relaxations in both constipated and control taenia coli. The duration of this relaxation could be prolonged by increasing the concentration of the SNP. Phenylephrine decreased the frequency of myoelectrical activity and relaxed human sigmoid taenia coli (fig 101). CAMP also reduced the frequency of myoelectrical activity and relaxed both constipated and control tissue.

As all tissues, both constipated and control, demonstrated spontaneous electrical activity a primary myopathy is unlikely to be the cause of the dysmotility observed in PC/PH constipation patients. In addition, modification of this activity by both excitatory and inhibitory neurotransmitters suggests that the coupling mechanism between the efferent transmitter and receptor is functioning adequately. Further studies are required to to confirm statistically the impression that there is no difference in the myo-electrical activity, both at rest and under pharmacological stimulation between each group of tissues.

The results of this series of experiments can be summarised as follows:-

1. Spontaneous myogenic activity, is present in both control and constipated sigmoid taenia coli, indicating that a primary myopathy is not present in the latter.

2. Excitatory (cholinergic) responses are less frequently elicited in constipated sigmoid taenia coli, than control tissue. This implies a change

either in receptor number, the characteristics of the coupling mechanism or in the amount of the transmitter released.

3. Inhibitory (non-adrenergic, non-cholenergic) relaxations are the main response to electric field stimulation in both constipated and control taenia coli, indicating that the intrinsic nerves in these tissues are intact.

4. Sigmoid taenia coli from constipated patients is more sensitive to cholinergic agonists than control tissue. This implies a change either in receptor number or the efficiency of the associated coupling mechanism.

5. Significantly, in contrast to control tissue, organised peristalsis neither occurs spontaneously nor can be induced in sigmoid colon resected for PC/PH constipation.

SUMMARY AND CONCLUSION

The purpose of this section is to draw together the results of all the experiments to date.

It was initially thought that PC/PH constipation developed following trauma to the pelvic nerves. These patients, it was believed, would have normal transit through the proximal gastro-intestinal tract. To test this view, gastric emptying studies were performed. This simple test, well tolerated by both patients and controls (healthy volunteers) established that PC/PH constipation was a distinct sub-group of idiopathic constipation in which the rate of gastric emptying was normal. However, variation among controls was such that delayed gastric emptying may be present in healthy adults who are asymptomatic. In the idiopathic group of patients studied, there were some whose rate of gastric emptying fell within the expected range of normal. This suggests that some patients with idiopathic constipation may also have normal proximal function.

Having identified a trend towards normal proximal gastrointestinal motility in patients with PC/PH constipation, the next step was to isolate that segment of the colon in which the dysmotility was most apparent. The usefulness of radio-opaque marker studies has previously been described (57-59) and discussed earlier in this thesis. The more elaborate methodology of segmental transit studies

adopted here, while identifying an area of delayed transit in the left colon, appeared to be of little clinical benefit. Again, the physiological range of normal was such that although statistical significance was achieved for transit through the left colon, a clinical decision to perform segmental resection could only have been made in 2 patients on the basis of this study alone. Although, only 13 patients were studied, this particular test has been disappointing. Colonic mass actions are infrequent but result in the movement of colonic content over a large distance in a few seconds. Two x-rays, taken 10 minutes apart might give completely different results. Despite this being a straightforward study, performed with the benefits of a non-invasive technique, there are obvious limitations to its use, especially as the sole method of transit and therefore it cannot be used in isolation.

To reduce the observation period required to identify isolated regions of colonic dysmotility, dynamic radio-isotope transit was performed. The results gave the first clear evidence that PC/PH dysmotility was maximised in the region of the hindgut. However, unlike the 2 previous studies (gastric emptying and radio-opaque marker studies) which were performed under physiological conditions, dynamic radio-isotope scanning was performed under pharmacological conditions. Bisacodyl is a sufficiently powerful

non-specific stimulant of the myenteric plexus and as such it may be possible to force radio-isotope along the proximal colon of PC/PH patients at apparently normal speeds. Therefore, lesser degrees of proximal dysmotility in such patients may be overlooked by the use of this drug. Notwithstanding, dynamic radio-isotope transit times through the transverse and descending colon was exactly the same in all cases. These results contrast with those published previously (68) using the same technique in which a heterogeneous group of patients gave a heterogeneous response.

Colonic manometry although difficult to perform, presented some of the most interesting results. The response to a meal stimulus suggests that in the proximal colon, contractions >5cmH_2O, >50cmH_2O, mean pressure and motility index (area under the motility curve) are normal in patients with PC/PH constipation. In contrast, failure of the sigmoid colon in these patients to generate high pressure waves in response to a 1000 cal meal is interesting. However, it should be borne in mind that the rate of gastric emptying varied between individuals and presumably the stimulus to the sensory pathways mediating the 'gastrocolic reflex' may not have been the same in all

patients and controls. Therefore, in any one patient in whom the colonic response to a meal was being studied, it would be important to know the rate of gastric emptying.

In an effort to overcome some of the inherent problems in studying the response of the human colon to physiological stimuli, neostigmine provocation testing was employed. Neostigmine raised colonic motility as measured by colonic manometry, in all segments of the colon in patients and controls to the same level. This suggested that post-ganglionic cholinergic fibres and their receptors were intact and functional. This study, like the dynamic radio-isotope study is open to the criticism that the stimulus stresses the colon in both patients and controls to non-physiological levels. The rate of absorption of neostigmine (0.1mg/kg s.c.) may also have varied among individuals. In retrospect, a slow intravenous infusion of neostigmine might have been a more appropriate route of administration. In contrast to the meal stimulus, neostigmine (0.1mg/kg s.c.) induced high pressure waves in the sigmoid colon in the patients and clearly represents a much more powerful stimulus to the hindgut than a 1000 calorie meal. Neostigmine acts throughout the human gastrointestinal tract and its effect will be greatest where the number of nerves and receptors are most prevalent, ie in the sigmoid colon (56). The nature of the response of the

sigmoid colon to the stimulus of a 1000 calorie meal is less clear. The 'gastrocolic reflex' is mediated by afferent fibres from the proximal jejunum through to efferent fibres in the vagus nerve. For this reason it is most accurately recorded in the transverse colon. The nature of the pathways which elicit a response in the sigmoid colon is not clearly defined and therefore the response in the sigmoid colon may be a secondary and not a primary event, reflecting increased colonic motility in the adjacent transverse and descending colon.

The interpretation of colonic motility in normal subjects is clearly difficult. The low pressure waves, which are seen repeatedly during recordings are difficult to interpret, but probably represent segmentation contractions within the human colon. The motility pattern which is singularly open to objective interpretation is the colonic mass action. While units of motility, eg number of contractions greater than 5cmH_2O, contractions greater than 50cmH_2O, mean colonic pressure and motility index (area under the curve) convey an impression of changes in motility, they are in fact no more than numbers. In contrast, the mass action is the propagative power house of colonic motility and conveys to the researcher the only true image of movement within the human colon. Failure to generate high pressure waves in the colon of PC/PH patients

is, the most obvious difference in motility patterns between constipated patients and controls. This finding warrants further investigation.

The *in vitro* studies performed in this project demonstrate that the elements which produce both contraction and relaxation of the human colon in both constipated and control tissue are present. In a pharmacological setting, there was effectively no difference in the pattern of nerve stimulated contraction and relaxation between patient and control groups. The degree of relaxation in strips of constipated sigmoid taenia coli at 2 Hz exceeded that of control tissue. This may be important in the pathogenesis of PC/PH constipation implying as it does, a compromise of neuronal or effector control. Further studies are required to demonstrate the neurotransmitter which acts most effectively at a frequency of 2 Hz. Both constipated and control tissue responded equally well to drug induced stimulation, indicating that the receptor mechanisms which mediate the efferent responses are intact. Constipated colon appeared to be more sensitive to carbachol than control colon and this may indicate a difference in the transmitter/receptor coupling mechanism. As in the *in vivo* experiments, interpretation of *in vitro* responses is difficult given the limitations of this experimental approach: (A) non-physiological methods of stimulation,

especially the choice of frequency and voltage, constitute an artificial efferent stimulus; (B) strips of taenia were studied in isolation, *in vitro*, when in the normal situation they function in concert with the adjacent circular muscle.

For this reason, the peristalsis experiments are particularly relevant. Here, a physiological stimulus (a bolus of fluid) was used to induce peristalsis in the human sigmoid colon. This served as a useful model and demonstrated that a physiological stimulus was unable to induce regular peristalsis in the constipated colon. A similar finding followed the manometry experiment *in vivo* in which a 1000 calorie meal failed to induce high pressure waves in the sigmoid colon of PC/PH patients.

The results obtained in the *in vivo* and *in vitro* studies should be correlated with an in depth review of the neurohistology of the human colon. A problem which arises when studying the neurohistology of any organ is whether the features seen represent the primary pathology or are secondary to the initial insult. However, it is important to correlate histology, pharmacology and physiology in an attempt to clearly define the nature of the disease process. Identifying a relative lack of acetylcholinesterase in constipated colon would help to clarify the observations, which have been made in the *in vitro* studies. The staining of the neurofilaments would also determine whether injury

has occurred at the preganglionic or postganglionic level. Abnormalities in the ganglia which have been previously demonstrated (50-55) may also be present in the tissues we have studied. The addition of electron micrography might help to elucidate the nature and extent of ganglion destruction. This work in itself could form the basis of a separate thesis.

Given the observations presented in this thesis there is circumstantial evidence to implicate the sigmoid colon in the pathogenesis of hindgut dysmotility. To attribute this exclusively to cholinergic denervation is not justified on the basis of the work presented. If cholinergic denervation was the sole cause of the problem, then one would expect the area of dysmotility to extend to the region of the splenic flexure (that being the distribution of the pelvic parasympathetic outflow). The sigmoid colon does contain the greatest number of cholinergic nerves and proportionately greater number of cholinergic receptors (56). In addition, there is a high concentration of 5-HT receptors in the sigmoid colon. These 2 facts suggest that the sigmoid colon is an organ unto itself. Unlike the rest of the colon, which is concerned with mixing, dehydration and storage of faecal matter, the sigmoid colon is concerned with evacuation. Partial denervation of the pelvic parasympathetic outflow may well manifest itself most

effectively in the sigmoid colon. It is perhaps therefore not unreasonable to postulate an isolated hindgut neuropathy confined to the region of the rectosigmoid.

What is clear from this work is that idiopathic slow transit constipation is not a single condition and perhaps 3 or 4 differing pathologies exist. In order to clarify further the relationship between difficult childbirth/hysterectomy and constipation it would be necessary to study the colonic motility parameters of patients with idiopathic constipation and make comparisons. Indeed it would be interesting in the future to compare posthysterectomy patients with those attributing their symptoms to a traumatic childbirth as the mechanisms of injury in each case are by no means clear.

If we refuse to acknowledge the possibility that some cases of constipation are due to regional dysmotility then our surgical management will not have progressed beyond the case reports first presented by Arbuthnot Lane. Colectomy and iliorectal anastomosis gives a satisfactory result in the majority of patients but may over treat a small group of patients in whom limited resection is all that is required. To continue with total colonic resection for all patients with severe constipation is unacceptable for the smaller subgroup of patients in whom we may be getting the diagnosis wrong.

References

(1) Lane W Arbuthnot. The results of operative treatment of chronic constipation. Br Med J 1908;i:126-30.

(2) Lane W Arbuthnot. In: The operative treatment of chronic constipation. Nisbet: London 1909.

(3) Lane W Arbuthnot. Chronic intestinal stasis. Br Med J 1909;i:1408-11.

(4) Lane W Arbuthnot. Chronic intestinal stasis. Br Med J 1913;ii:1125-8.

(5) Walsh PV, Peebles-Brown DA, Watkinson G. Colectomy for slow transit constipation. Ann R Coll Surg Eng 1987;69:71-75.

(6) Preston DM, Hawley PR, Lennard-Jones et al. Results of colectomy for severe idiopathic constipation in women (Arbuthnot Lanes disease) Br J Surg 1984;71:547-552.

(7) Moore-Gillon V. Constipation: what does the patient mean? J R Soc Med 1984;77:108-110.

(8) Hinton JM, Lennard-Jones JE. Constipation: definition and classification. Postgrad Med J 1968;44:720-3.

(9) Connell AM, Hilton C, Irvine G et al. Variation of bowel habit in two population samples. Br Med J 1965;2:1095-9.

(10) Wrick KL, Robertson JB, Van oest et al. The influence of dietry fibre source on human intestinal transit and stool output. J Nutr 1983;113:1464-79.

(11) Passaretti S, Tittobello A, Capozzi C et al. Cisapride accelerates total intestinal transit in patients with irritable bowel syndrome - associated constipation. Progr Med 1987;ii:121-129.

(12) Preston DM, Lennard-Jones JE. Severe chronic constipation of young women: "idiopathic slow transit constipation". Gut 1986;27:41-8.

(13) Waiter A, Devroede G, Duranceau A et al. Constipation with colonic inertia - a manifestation of systemic disease? Dig Dis Sci 1983;28:1025-33.

(14) Preston DM, Lennard-Jones JE. Colonic motility and response to intraluminal bisacodyl in slow-transit constipation. Gut 1983;23:A891.

(15) Lennard-Jones (1985) Constipation: pathophysiology, clinical features and treatment. In coloproctology and the Pelvic Floor (edited by Henry, MM, Swash, M). Butterworths, London. pp 350-375.

(16) Bannister JJ, Timms JM, Barfield LJ et al. Physiological studies in young women with chronic constipation. Int J of Colorect Dis 1986;1:175-182.

(17) Preston DM, Butler MG, Smith B et al. Neuropathology of slow transit constipation. Gut 1983;24:A997

(18) Preston DM, Lennard-Jones JE, Thomas BM. The balloon proctogram. Br J Surg. 1984;71:29-32.

(19) Read NW, Timms JM, Barfield LJ et al. Impairment of defaecation in young women with severe constipation. Gastroenterology 1986a;90:53-60.

(20) Barnes PRH, Lennard-Jones. Balloon expulsion from the rectum in constipation of different types. Gut 1988;29:17-20.

(21) Turnbull GK, Lennard-Jones JE, Bartram CI. Failure of rectal expulsion as a cause of constipation: why fibre and laxatives sometimes fail. Lancet 1986;i:767-769.

(22) Preston DM, Lennard-Jones JE. Is there a pelvic floor disorder slow transit constipation? Gut 1981;22:A809.

(23) Kuipers HC, Bleijenberg. The spastic pelvic floor syndrome. A cause of constipation. Dis Col Rectum 1985;28:669-72.

(24) Bannister JJ, Timms JM, Barfield LJ et al. Physiological studies in young women with chronic constipation. Int J of Colorect Dis. 1986;1:175-182.

(25) Bannister JJ, Lawrence WT, Smith A et al. Urological abnormalities in young women severe constipation. Gut 1988;29:17-20.

(26) Cannon WB. A Law of denervation. Am J Med Sci 1939;198:737.

(27) Cannon WB, and Rosenblueth A. The Supersensitivity of Denervated Structures; a Law of Denervation, New York The Macmillan Company.

(28) Abel-Raham M, Toppercer A, Duguay C et al. Urorectodynamics in patients with colonic enertia. Urology 1981;18:4 428-432.

(29) MacDonald A, Shearer M, Paterson PJ et al. Relationship between outlet obstruction constipation and obstructed urinary flow. Br J Surg 1991;78:693-695.

(30) Snooks SJ, Swash M, Mathers SE et al. Effect of vaginal delivery on the pelvic floor: a 5-year follow-up. Br J Surg 1990;77:1358-1360.

(31) Smith AN, Varma JS, Binnie NR et al. Disordered colorectal motility in intractable constipation following hysterectomy. Br J Surg 1990;77:1361-1366.

(32) Roe AM, Bartolo DCC, Mortensen NJMcC. Slow transit constipation. Comparison between patients with and without previous hysterectomy. Dig Dis Sci 1988;33:1159-63.

(33) Parys BT, Haylen BT, Hutton JL et al. The effects of simple hysterectomy on vesicourethral function. Br J Urol 1989;64:594-599.

(34) Christenson J, Schulze-Delrieu K. Nerves in the colon: discovery and rediscovery. Gasteroenterology 1985;89:222-3.

(35) Fukai K, Fakuda H. The intramural pelvic nerves in the colon of dogs. J Physiol 1984;354:89-98.

(36) De Groat WC, Kawatani M. Reorganisation of sympathic preganglionic connections in cat bladder ganglia following parasympathic denervation. J Physiol 1989;409:431-49.

(37) Ekstrom J, Motmberg L. Functional evidence for sprouting of decentralised parasympathetic neurons in rat urinary bladder. Acta Physiol Scand 1984;112:7-15.

(38) Mitchell GAG. Anatomy of the autonomic nervous system. Edinburgh and London: E&S Livingstone,1953:190.

(39) Lannon J, Weller E. The parasympathetic supply of the distal colon. Br J Surg 1947;34:373-8.

(40) Catchpole BN. Motor pattern of the left colon before and after surgery for rectal cancer; possible implications in other disorders. Gut 1988;29:624-630.

(41) Kumar D. In vitro inhiibitory effect of progesterone on extra-uterine human smooth muscle. Am J Obstet Gynaec 1962;84:1300-4.

(42) Gill RC, Bowes KL, Kingma YJ. Effect of progesterone on canine colonic smooth muscle. Gastroenterology 1985;88:1941-7.

(43) Scott LD, De Flora E. Cholinergic responsiveness of intestinal muscle in the pregnant guinea pig. Life Sci 1989;44:7,503-8.

(44) Bruce LA, Behsudi FM. Progesterone effects on three regional gastrointestinal tissues. Life Sci 1979;25:729-34.

(45) Hinds JP, Stoney B, Wald A. Does gender or menstrual cycle affect colonic transit. Am J Gastroenterology 1989;84:123-126.

(46) Dyer NH, Dawson AM, Smith B et al. Obstruction of the bowel due to lesion in the myenteric plexus. Br Med J 1969;1:686-9.

(47) Krishnamurthy S, Schuffler MD, Rohrman CA et al. Severe idiopathic constipation is associated with a distinctive abnormality of the colonic myenteric plexus. Gastroenterology 1985;88:26-34.

(48) Schuffler MD, Bird TD, Sumi SM et al. A familial neuronal disease presenting as intestinal pseudo-obstruction. Gastroenterology 1978;75:889-98.

(49) Schuffler MD, Jonak Z. Chronic intestinal pseudo-obstruction caused by a degenerative disorder of the myenteric plexus:the use of Smith's to define the neuropathology. Gastroenterology 1982;82:476-86.

(50) Schuffler MD, Baird HW, Fleming C.R et al. Intestinal pseudo-obstruction as the presenting manifestation of small cell carcinoma of the lung; a paraneoplastic neuropathy of the gastrointestinal tract. Ann Intern Med 1983;98:129-34.

(51) Smith B. The neuropathology of intestinal pseudo-obstruction. In Chey W.Y, ed. Functional disorders of the digestive tract. New York: Raven 1983:231-6.

(52) Kune GA. Megacolon in adults. Br J Surg 1966;53:199-205.

(53) Smith B, Grace RH, Todd IP. Organic constipation in adults. Br J Surg 1977;64:313-4.

(54) Kluck P, Kate FJW, Ruud Schouten W et al. Efficacy of antibody NF2F11 staining in the investigation of severe long-standing constipation. A preliminary report. Gastroenterology 1987;93:872-5.

(55) Koch TR, Carney JA, Go L et al. Idiopathic chronic constipation is associated with decreased colonic vasoactive intestinal peptide. Gastroenterology 1988;94:300-10.

(56) Burleigh DE. Evidence for a functional cholinergic deficit in human tissue resected for constipation. J Pharm Pharmacol 1988;40:55-57.

(57) Hinton JM, Lennard-Jones JE, Young AC. A new method for studying gut transit times using radio-opaque markers. Gut 1969;10:842-7.

(58) Arhan P, Devroede G, Jehannin B et al. Segmental colonic transit time. Dis Col & Rect 1981 24;8:625-29.

(59) Metcalf AM, Philips SF, Zinsmeister AR et al. Simplified assessment of segmental colonic transit. Gastroenterology 1987;92:40-47.

(60) Miller R, Duthie GS, Bartolo DCC et al. Anismus in patients with normal and slow transit constipation. Br J Surg 1991;78:690-692.

(61) Bassotti G, Gaburri M, Imbimbo BP et al. Colonic mass movements in idiopathic constipation. Gut 1988;29:1173-1179.

(62) Waldron D, Bowes KL, Kingma YJ et al. Colonic and anorectal motility in young women with severe idiopathic constipation. Gastroenterology 1988;95:1388-94.

(63) Krevsky B, Maurer AH, Fisher RS. Patterns of colonic transit in chronic idiopathic constipation. Am J Gastroenterology 1989;84:2,127-132.

(64) Ritchie JA, Ardran GM, Truelove SC. Motor activity of the sigmoid colon in humans. A combined study by intraluminal pressure recordings and cineradiography. Gastroenterology 1962;43:642-668.

(65) Dinoso V,P Murthy SNS, Goldstein J, Rosner B. Basal motor activity of the distal colon. A reappraisal. Gastroenterology 1983;85:637-642.

(66) Reynolds JR. The application of radio-telemetry techniques in the investigation of gastrointestinal function in health and disease. MD Thesis University of Nottingham 1988.

(67) Connell AM, McCall J, Misiewicz J, Rowlands EN. Observations on the clinical use of radio pills. Br. Med. J. 1963;ii:771-774.

(68) Kamm MA, Lennard-Jones JE, Thompson DG et al. Dynamic scanning defines a colonic defect in severe idiopathic constipation. Gut 1988;29:1085-1092.

(69) Kamm MA, Hawley PR, Lennard-Jones JE. Outcome of colectomy for severe idiopathic constipation. Gut 1988;29:1085-1092.

(70) Hosie KB, Kmiot WA, Keighley MRB. Constipation: another indication for restorative proctocolectomy. Br J Surg 1990;77:801-802.

(71) Kamm MA, van der Sijp JRM, Hawley PR, Philips RKS, Lennard-Jones JE. Left hemicolectomy with rectal excision for severe idiopathic constipation. Int J Colorect Dis 1991;6:49-51.

(72) Barnes P.R.H, Hawley P.R, Preston D.M et al. Experience of posterior division of the puborectalis muscle in the management of chronic constipation. Br J Surg. 1985;72:475-7.

(73) Kamm MA, Hawley PR, Lennard-Jones JE. Lateral division of the puborectalis muscle in the management of severe constipation. Br J Surg 1988;75:661-3.

(74) Keighley MRB, Shouler P. Outlet syndrome: is there a surgical option? J R. Soc. Med 1984;77:559-64.

(75) Pinho M, Yoshioka K, Keighley MRB. Long term results of anorectal myectomy for chronic constipation. Br J Surg 1989;76:1163-64.

(76) Hallan RI, Williams NS, Melling J et al. Treatment of anismus in intractable constipation with Botulinum A toxin. Lancet 1988;ii:714-16.

(77) Bleijenberg G, Kuypers AC. Treatment of spastic pelvic floor syndrome with biofeedback. Dis Col Rectum 1987;30:108-11.

(78) Lestar B, Penninckx F, Kerrenams R. Biofeedback defaecation training for anismus. Int J Colorect Dis 1991:6;202-207.

(79) Loening-Baucke V. Modulation of abnormal defaecation dynamics by biofeedback treatment in chronically constipate children with encopresis. J Paediatr 1990:116;214-22.

(80) Binnie NR, Kawimbe BM, Papachrysostomou M et al. EMG biofeedback as a domicillary treatment of anismus. Br J Surg 1989:76;1339A.

(81) Gray H. Anatomy of the Human Body (29th American ed), Lea and Febiger. Philadelphia, 1973.

(82) Romanes GJ. Cunningham's Textbook of Anatomy, (11 ed). Oxford University Press, London, 1972.

(83) Eastwood GL. pp 1-16 in Bustos-Fernandez L, (ed). Colon Structure and Function, Plenum, New York 1983.

(84) Gardner E, Gray DJ, O'Rahilly R. Anatomy. A Regional Study of Human Structure (3rd ed.). W.B. Saunders, Philadelphia 1969.

(85) Lundgren O, Jodal M. pp. 211-232 in Bustos-Fernandez L, (ed). Colon Structure and Function Plenum, New York, 1983.

(86) Jodal M, Lundgren O. pp. 187-209 in Bustos-Fernandez L, (ed). Colon Structure and Function Plenum, New York, 1983.

(87) Stockley HL, Bennet A. The intrinsic innervation of human sigmoid colonic muscle. Procedings of the fourth International Symposium on Gastrointestinal motility, 1974. Edited by EE. Daniel, Mitchel, Vancouver. pp 163-176.

(88) Bucknell A. 1966 Ph.D. thesis (Lond).

(89) Crema A, Del Tacca M, Frigo GM, Lechini S. Presence of an non-adrenergic inhibitory system in the human colon. Gut 1968;9:633-637.

(90) Jacobowitz D, Koelle GB. Histochemical correlations of acetylcholinesterase and catecholamines in post ganglionic autonomic nerves of the cat, rabit and guinea pig. J Pharmacol Exp Ther 1965;148:225-37.

(91) Furness JB, Costa M. The nervous release and the action of substances which affect intestinal muscle through neither adrenoreceptors nor cholinoreceptors. Philos Trans R Soc Lond (Biol Sci) 1973;265:123-33.

(92) Baumgarten HG, Ehinger B, Falck B. Microspectrofluorimetric the differentiation between primary and secondary catacholamines in tissues. Life Sci 1967;6:2465-8.

(93) Bennett A, Garrett JR and Howard ER. Adronergic myenteric nerves in Hirschsprung's disease. Br Med J 1968;1:487-9.

(94) Fishlock TG and Parks AG. A study of human colonic muscle in vitro. Br Med J 1963;5358:666-7

(95) Bucknell A, Whitney B. A preliminary investigation of the pharmacology of the human isolated taenia coli. Brit J Pharmacol 1964;23:164-75.

(96) Del Tacca, Soldani G, Selli M et al. Action of catecholamines on release of acetylcholamine from human taenia coli. Europ J Pharmacol 1970;9:80-4.

(97) Middleton SJ, Cuthbert AW, Shorthouse M, Hunter JO. Nitric oxide affects mammalian distal colonic smooth muscle by tonic neural inhibition. Br. J. Pharmacol. 1993;108:974-979.

(98) Bult H, Boeckxstaens GE, Pelkmans PA et al. Nitric oxide as an inhibitory non-adrenergic, non-cholinergic neurotransmitter. Nature 1990;345:346-347.

(99) Palmer RMJ, Rees DD, Ashton DS, Moncada S. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 1988;153:1251-1256.

(100) Knowles RG, Palacios M, Palmer RMJ, Moncada S. Kinetic characteristics of nitric oxide synthase from rat brain. J. Biochem 1990;269:207-210.

(101) Nakatsu K, Diamond J. Role of cGMP in relaxation of vascular and other smooth muscle. Can. J. Physiol. Pharmacol. 1987;67:251-262.

(102) Rees DD, Palmer RMJ, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. Br. J. Pharmacol. 1989;96:418-424.

(103) Moore PK, Al-Swayeh OH, Chong NWS et al. L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vaso-dilatation *in vitro*. Br. J. Pharmacol 1990;99:408-412.

(104) Boeckxstaens GE, Pelkmans PA, Bult H et al. Non-adrenergic, non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. Eur. J. Pharmacol 1990;190:239-246.

(105) Dalziel H, Thornbury K, Ward S, Sanders K. Involvement of nitric oxide synthetic pathway in inhibitory junction potentials in canine proximal colon. Am. J. Physiol 1991;260:G789-G792.

(106) Desai KM, Sessa WC, Vane JR. Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. Nature 1991;351:447-479.

(107) Tottrup A, Birgitte G, Svane D. Involvement of the L-arginine-nitric oxide pathway in internal anal sphincter relaxation. Gastroenterology. 1992;102:409-415.

(108) Burliegh D. N^G-nitro-L-arginine reduces non-adrenergic, noncholinergic relaxations of human gut. Gastroenterology 1992;102:679-683.

(109) O'Kelly T, Brading A, Mortensen N. Nerve mediated relaxation of the internal anal sphincter: the role of nitric oxide. Gut. 1993;34:689-693.

(110) Bennet A, Whitney B. A pharmacological study of the motility of the human gastrointestinal tract. Gut 1966(a);7:307-316.

(111) Gagnon DJ, Devroede G, Belisle S. Excitatory effects of adrenaline upon isolated preparations of human colon. Gut 1972;7:307-316.

(112) Boeckxstaens GE, Pelckmens PA, Rampart M et al. Pharmacological characterisation of 5-Hydroxytryptamine receptors in the canin terminal ileum and ileocolonic junction. J Pharmacol Exp Ther 1990;254(2):652-658.

(113) Talley NJ, Philips SF, Haddad A et al. GR 380 32F (Ondanfetron), a selective 5-HT_3 receptor antagonist slows colonic transit in healthy man. Dig Dis Sci 1990;35(4):477-480

(114) King FD and Sanger GJ. Gastrointestinal motility enhancing agents. Annal Rep Med Chem 1988;23:201-210.

(115) Craig DA and Clark DE. Peristalsis evoked by 5-HT and Renzapride: evidence for putative 5-HT_4 receptor activation. Br J Surg Pharmacol 1981;102:563-564.

(116) Craig DA and Clark DE. Pharmacological characterization of a neuromal receptor for 5-Hydroxytryptamine in guinea pig ileum with properties similar to the 5-Hydroxytryptamine$_4$ receptor. J Pharmacol Exp Ther 1990;252:1378-1386.

(117) Yang YZ, Cooke HJ, Sue HC and Fertel R. Histamine augments colonic secretion in guinea pig distal colon. Am J Physio 1990;258(3 Pt 1):P G 432-g.

(118) Hishingmuna S and Uchida MK. Short-term desensitisation of phosphatidylinositol turnover via muscarinic acetyl choline receptors and histamine H1 receptors in smooth muscle. Biochem Biophys Res Comun 1989;1962(2):733-9.

(119) Bennett A, Eley KG, and Scholes GB. Effect of prostaglandins E1 and E2 on human, guinea-pig and rat isolated small intestine. Br J Pharmacol 1968;34:630-8.

(120) Fleshler and Bennett A. Action of prostogladin E on a longituodenal muscle on the guinea pig isolated colon. Br J Pharmacol 1969;35:351-352.

(121) Bennett A and Fleshler B. Prostoglandins in the gastrointestinal tract. Gastroenterology 1970;59:790-800.

(122) Barrowman JA, Bennet A, Hillenbrand P et al. Diarrhoea in thyroid medullary carcinoma: role of prostaglandins and therapeutic effect of nutmeg. Br Med J 1975;3(5974):11-12

(123) Ferri G-L, Adrian TE, Ghatei MA et al. Tissue localization and relative distribution of regulatory peptides in separate layers from the human bowel. Gastroenterology 1983;84:777-786.

(124) Wattchow DA, Furness JB and Costa M. Distribution and co-existence of peptids in nerve fibres of the external muscle of the human gastro-intestinal tract. Gastroenterology 1988; 95:32-41.

(125) Burleigh DE and Furness JB. Distribution and actions of galanin and vasoactive intestinal peptide in the human colon. Neuropeptides 1990;16(2):77-82

(126) Ishizawa M. Effect of vaso-active intestinal peptid on the motility of guinea pig colon in vitro. Nippon Heikatsukin Gakkai Zasshi 1988;24(3);185-92.

(127) Milner P, Crowe R, Calm MA et al. Vaso-active intestinal peptic levels in sigmoid colon from idiopathic constipation and diverticular disease. Gastroent 1990;99(3):666-75.

(128) Daniel EE. Sympossium on Colonic Function[1] (Electrophysiology of the Colon) Gut 1975;16:298-329.

(129) Furness JB, Lloyd KC, Sternini C and Walsh JH. Projections of substance P, vaso-active intestinal peptide and tyrozine hydroxalase immunoreactive nerve fibres in the canin intestine, with special reference to the innervation of the circular muscle. Arch Histol Cytol 1990;(53)2:129-40.

(130) Katsoulis S and Conlin JM. Effects of guinea pig neurotensin (<SER7> neurotensin) on gastrointestinal smooth muscle. Eur J Pharmacol 1987;140(3):353-6.

(131) Hellstrom PM, Rosell S. Effects of neurotensin, substance P and methionine-enkephalin on colonic motility. Acta Physiol Scand 1981;113:147-54.

(132) Ischizawa M. Effects of GABA and homotaurine on the colonic motility of the guinea pig. Nippon Heikatsukin Gakkai Zasshi 1987;23(6):441-7.

(133) Schang JC, Dapoigny M and Devroede G. Stimulation of colonic peristalsis by vasopressin colon electromyographic study in normal subjects and patients with chronic idiopathic constipation. Can J Physiol Pharmacol 1987;65(10):2137-41.

(134) Wilay J, Owyang C. Dopaminergic modulation of rectosigmoid motility; action of Domperidone. J Pharmacol Exp Ther 1987;242(2):548-51.

(135) Hellstorm PM. Mechanisms involved in colonic vaso-constriction and inhibition of motility induced by neuropeptide Y. Acta Physiol Scand 1987;129(4):549-56.

(136) Sun EA, Snape WJ Jr, Cohen S et al. The role of opiate and cholinergic neurons in the gastrocolonic response. Gastroenterology 1982;82:689-93.

(137) Snape WJ Jr, Matarazzo SA, Cohen S. Effect of eating and gastrointestinal hormones on human colonic myoelectrical and motor activity. Gastroenterology 1978;75:373-8.

(138) Egberts EH, Johnson AG. The effect of cholecystokinin on human taenia coli. Digestion 1977;15:217-22.

(139) Polak JM, Bloom SR, Sullivan SN. Enkephalin-like immunoreactivity in the human gastrointestinal tract. Lancet 1977;i:972-4.

(140) Schiller PW, Lipton A, Horrobin DF et al. Unsulphated C-terminal 7-peptide of cholecystokinin: a new ligand of opiate receptor. Biochem Biophys Res Commun 1978;85:1332-8.

(141) Hoyle CH, Knight GE, Burnstock G. Suranim antagonises responses to P2 - purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. Br J Pharmacol 1990;99(3):617-21

(142) Holdgate AM, Read NW. Relationship between small bowel transit time and absorbtion of a solid meal. Influence of metoclopromide, magnesium sulphate and lactulose. Dig Dis Sci 1983;28:812-819.

(143) Wood JD. Enteric neurophysiology. Am J Physiol 1984;247:G585-8.

(144) Brown GL, Pascoe JE. Conduction through the inferior mesenteric ganglion of the rabbit. J Physiol (Lond) 1951;118:113-23.

(145) Job C, Lundberg A. Reflex excitation of cells in the inferior mesenteric ganglion by stimulation of the hypogastric nerve. Acta Physiol Scand 1952;26:366-82.

(146) Coweroft PJ, Holman ME, Szurzewski JH. Excitatory input from the distal colon to the inferior mesenterric ganglion in the guinea pig. J Physiol (Lond) 1971;219:443-61.

(147) Weems WA, Szurewski JH. Modulation of colonic motility by periferal neural inputs to neurons of the inferior mesenteric ganglia. Gastroenterology 1977;73:273-8.

(148) Spiller RC, Brown ML, Philips SF. Decreased fluid tolerance, accelerated transit, and abnormal motility of the human colon induced by oleic acid. Gastroenterology 1986;91:100-7.

(149) Kamath PS, Philips SF, O'Connor MK et al. Colonic capacitance and transit in man: modulation by luminal contents and drugs. Gut 1990;31:443-449.

(150) Spiller RC, Brown ML, Philips SF. Emptying of the terminal ileum in intact man: influence of meal residue and ileal motility. Gastroenterology 1987;92:724-9.

(151) Spiller RC, Brown ML, Philips SF, Azpiroz F. Scintigraphic measurements of canine ileocolonic transit: Direct and indirect effects of eating. Gastroenterology 1986;91:1213-20.

(152) Snape WJ Jr, Wright SH, Battle WM et al. The gastrocolonic response: evidence for a neural mechanism. Gastroenterology 1979;77:1235-40

(153) Snape WJ Jr, Carlson GM, Cohen S. Human colonic myoelectric activity in responce to prostigmine and the gastrintestinal hormones. Am J Dig Dis 1977;54:1005-11.

(154) Dinoso VP, Meshkinpour H, Lorber SH et al. Motor responces of the sigmoid colon and rectum to exogenous cholecystokinin and secretin. Gastroenterology 1973;65:438-444.

(155) Misiewicz JJ, Holdstock DJ, Waller SL. Motor responses of the human alimentary tract to near-maximal infusion of pentagastrin. Gut 1967;8:463-469.

(156) Bennet A, Misiweicz JJ, Waller SL. Analysis of the motor effects of gastrin and pentagastrin on the human alimentary tract in vitro. Gut 1967;8:470-474.

(157) Logan CJH, Connell AM. The effect of a synthetic gastrin-like pentapeptide (ICI 50,123) on intestinal motility in man. Lancet 1966;1:996-999.

(158) Dockray GJ, Taylor IL. Heptadecapeptide gastrin: measurement in blood by specific radioimmunoassay. Gastroenterology 1976;71:971-77.

(159) Narducci F, Bassotti G, Gaburri M et al. Distention stimulated motor activity of the human transverse, descending and sigmoid colon. Gastroenterology 1985;88:1515

(160) Duthie HL and Gairns FW. Sensory nerve endings and sensation in the anal region of man. Br J Surg 1960;47:585-595.

(161) Lane RHS and Parks AG. Function of the anal sphincter following colo-anal anastomosis. Br J Surg 1977;64:596-599.

(162) Finlay IG, Carter K, McLeod I. A comparison of intrarectal infusion of mass and gas on anorectal angle and anal canal pressure. Br J Surg 1986;73:1025.

(163) MacDonald A, Paterson PJ, Baxter JN, Finlay IG. Relationship between intra-abdominal and intrarectal pressure in the proctmetrogram. Br J Surg 1993;80:1070-71.

(164) Gollinhoffen K, von Low D. Elektrophysiologische untersuchungen zur normalen spontanaktivitat der isolinerten taeni coli des meerschweinchens. Pflugers Arch 1970;314:312:328

(165) Trendelenberg D Arch. Exp. Path. Pharmak. 1917;81:55.

(166) Botella A, Delvaux M, Fioramonti J, Bueno L. Stimulatory (EP1 and EP3) and inhibitory (EP2) prostaglandin E2 receptors in isolated ileal smooth muscle cells. European Journal of Pharmacology 1993;273(1):131-7.

(167) Bowman WC and Rand MJ. Textbook of Pharmacology. Second edition Blackwell Scientific Publications. London 1980.

Appendix 1.

POSITION OF MANOMETRY CATHETER PERFUSION POINTS

A= ascending colon

T= transverse colon

D= descending colon

S= sigmoid colon

R= rectum

Controls

	1	2	3	4	5	6	7
1	A	T	T	T	D	S	S
2	A	T	T	T	D	S	S
3	T	T	T	T	D	S	S
4	T	T	D	D	S	S	S
5	T	T	T	D	S	S	S
6	D	D	D	D	S	S	S
7	S	S	S	S	R	R	
8	A	T	T	T	D	D	S
9	A	T	T	D	D	S	S

Patients							
1	T	T	T	D	D	S	S
2	A	T	T	T	D	S	S
3	T	T	T	D	D	S	S
4	A	T	T	D	D	S	S
5	A	A	T	T	T	D	D
6	D	D	D	S	S	S	S
7	D	D	D	S	S	S	S
8	A	T	T	T	T	A	S
9	T	T	T	D	D	S	S
10	T	T	T	D	D	S	S

Appendix 2.

Parameters for wave classification

(1) Time constant for baseline changes — 4 seconds.

(2) Minimum level at which wave is registered as started — 5 cmH_20.

(3) Minimum level at which wave is registered as ended — 5 cmH_20

(4) Amplitute of oscillation within wave to be registered as a peak — 2 cmH_20.

(5) Minimum duration for wave to be included in summary — 0.8 seconds

(6) Minimum height for wave to be registered as high amplitude — 50 cmH_20.

(7) Minimum duration for wave to be registered as long — 60 seconds.

The baseline for any segment of recording is taken as the minimum pressure and the average wave gradient is recorded. This means that if the patient's position varies, resulting in an increase in baseline pressure, the magnitude of any waves recorded during that period is calculated from the new baseline pressure. The area under the motility curve is calculated by approximating each wave to an isosceles triangle.

$$\text{Area under curve} = \frac{\text{base x height}}{2}$$

Where the gradient of a wave varied with time, the computer calculated the average gradient.

Appendix 3

COMPONENTS OF THE 1000KCAL MEAL

FOOD	Wt (g)	Kcal	Fat (g)	Protein (g)
white bread	46	107	0.8	3.6
roast beef	132	297	16.6	36.8
mayonaise	22	158	17.4	0.4
ice cream	132	218	10.8	4.4
Fortisip	150	225	9.8	7.5
TOTAL	482	1005	55.4	52.7

COMPOSITION OF FORTISIP Cow & Gate Nutricia Ltd.

Average contents per 100ml

Energy	150Kcal
Protein	5.0g
Fat	6.5g
Carbohydrates	17.9g
Lactose	<0.025g
Sodium	80mg
Potassium	150mg
Calcium	50mg
Phosphorus	50mg
Magnesium	22mg
Chloride	80mg
Iron	1.5mg
Zinc	1.05mg
Manganese	0.6mg
Copper	0.15mg
Iodine	9mcg

Vitamins	
Vit A	105mcg
Vit D	0.75mcg
Vit E	4.8mg
Thiamin	0.11mg
Riboflavin	0.15mg
Niacin	1.5mg
Pantoth. acid	0.75mg
Vit B_6	0.15mg
Folic acid	37.5mcg
Vit B_{12}	0.3mcg
Biotin	22.5mcg
Vit. C	7.5mg
Inositol	34.5mg
Choline	67.5mg
Water	78.6g
Osmolarity	385mOsm/l

Appendix 4

DESCRIPTION OF DRUGS USED IN IN-VITRO EXPERIMENTS.

CARBACHOL

Used principally as a muscarinic agonist but which also has some affinity for nicotinic receptors. It is related to naturally occurring acetylcholine and is of similar potency. Substitution of the acetyl group by a carbamyl unit makes the substance less readily hydrolysed by cholinesterase. Its muscarinic activity results in contraction of colonic smooth muscle.

ATROPINE

A naturally occurring alkaloid (Atropa Belladonna) is a competitive antagonist of acetylcholine at muscarinic receptors. It has no effect on the resting tone of colonic smooth muscle but will completely block nerve-mediated excitatory contractions.

NEOSTIGMINE

A synthetic reversible anticholinesterase. This drug potentiates the effect of acetylcholine at neuromuscular junctions. Its effect would be to initiate or increase the rate of peristalsis in normal colon.

ESERINE

This drug is also a synthetic anticholinesterase and therefore promotes peristalsis.

HEXAMETHONIUM

A tetra-ammonium compound which is a non-depolarising ganglion blocking agent. It binds to a receptor and blocks the associated channel. It produces a progressive reduction in peristalsis in the colon.

NICOTINE

A naturally occurring alkaloid. Low concentrations of nicotine stimulate nicotinic ganglia and facilitate the transmission of impulses. When larger doses are applied, the initial stimulation is followed by desensitisation and blockade of transmission. It increases the frequency of peristalsis in human colon and its effect is blocked by the ganglion blocking agent, Hexamethonium.

DIMETHYL-4-PHENYLPIPERAZINIUM (DMPP)

This drug is a ganglion stimulating agent which differs from nicotine in that the initial stimulation is not followed by a dominant blockade. Its stimulatory action mimics the initial excitatory post synaptic potential and is blocked by hexamethonium. DMPP is about three times more potent than nicotine. It increases the frequency of peristalsis in normal colon.

SODIUM NITROPRUSSIDE (SNP)

This drug produces profound relaxation of colonic smooth muscle due to the production of nitric oxide.

ATP, ADP, AMP, CYCLIC AMP

Recognised neurotransmittors in purinergic nerves. Adenyl compounds produce relaxation of colonic smooth muscle. ATP and ADP are the most active and are equipotent.

PHENTOLAMINE

A potent competitive a receptor blocker. It prevents sympathetic stimulation of the human colon. It has no effect on the intrinsic activity of the colon.

PHENYLEPHRINE

A powerful post-synaptic *a* receptor stimulater with little effect on *b* receptors. Its direct action accounts for the greater part of its effect. It relaxes colonic smooth muscle.

The description of these drugs is taken from a standard textbook of pharmacology (167).