SMALL BOWEL MOTILITY IN ULCERATIVE COLITICS UNDERGOING ILEAL POUCH-ANAL ANASTOMOSIS

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by
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"Those early weeks, when Campbell had spent hours waiting about in what Hadden had called the kebab squad, hanging about ghoulishly for operations that rarely delivered the goods, were not to be repeated. "

Colin Douglas, "Ethics Made Easy"

SUMMARY

1. Although much clinical research has been carried out in the last 14 years to examine the postoperative function of patients undergoing ileal pouch-anal anastomosis (IPAA), little is known about the small bowel which is used to create the reservoir in this procedure. This thesis has therefore examined the preoperative small bowel motility characteristics of patients with ulcerative colitis (UC) undergoing IPAA using *in vivo* and *in vitro* techniques. The *in vitro* studies were compared against control subjects of whom most were undergoing elective right hemicolectomy for non-obstructing neoplasms. During part of the research project access to ileal tissue from patients with idiopathic slow transit constipation (ISTC) undergoing subtotal colectomy and ileorectal anastomosis (SC+IRA) enabled a study of this interesting subgroup of patients to be made. A review of the IPAA operation performed at Glasgow Royal Infirmary between 1988 and 1995 i.e. totally stapled restorative proctocolectomy (TSRP), was also carried out.

2. An ambulatory manometry catheter assembly with 6 solid-state transducers, straddling the small bowel from duodenojejunal flexure to ileocaecal junction, was used to assess fasting patterns of small bowel motility in 9 UC patients and 2 patients with familial adenomatous polyposis (FAP) awaiting IPAA. In the 9 UC patients (7 intact; 2 staged i.e. previous subtotal colectomies) nocturnal recordings were possible in 8. In 1 staged UC patient, catheter advancement to terminal ileum was impossible and with hindsight, following IPAA, this was thought to have been caused by previous postoperative adhesions. The second staged UC patient had catheter migration into the stoma bag following insertion and recordings were therefore invalid. The 7 intact UC patients and 1 FAP patient were studied for a

total of 81 hours in the nocturnal period. The 7 UC patients (6 males) had trace recordings analysed for migrating motor complex (MMC) characteristics including total number, cycle length, mean duration and mean velocity. Non-propagating MMCs, discrete clustered contractions (DCCs) and prolonged propagating contractions (PPCs) were also noted. The median recording period was 8.7hours (h), (range 6.5-9.5h) and the nocturnal stool frequency 2 (range 0-7). Other propagating contractions per subject included 1 PPC (0.9-2) and 2.1 DCCs (0-6). Calculation of Spearman's rank correlation coefficient failed to show any statistically significant associations between these motility parameters and nocturnal stool frequency. In particular the MMC pattern failed to show any relationship to nocturnal stool frequency. Recordings made in 5 UC patients during the diurnal period were considered "too noisy" to show any meaningful relationship between stool frequency and motility parameters because of intense postprandial motor activity. On the basis of this pilot study, ambulatory small bowel manometry was considered to be uncomfortable for the patient, the data obtained time-consuming to analyse, unsuitable for assessment of patients who had previously undergone subtotal colectomy and limited by expensive equipment which was too easily damaged. While possible, the technique did not seem to be a practical way of assessing small bowel motility routinely in prospective IPAA patients. Albeit on the basis of 7 nocturnal recordings, the technique also failed to show any relationship between gross patterns of small bowel motility and stool frequency in intact UC patients.

3. When isolated mucosal-free strips of human ileum were suspended in conventional jacketed organ baths, bursts of rhythmic oscillations in tone (spontaneous activity) were noted in 59 control strips (60%) and 73 UC strips

(49%). Analysis of this activity, in horizontally orientated strips, in a Golenhofen apparatus to enable synchronous measurement of both extracellular electrical and mechanical activity showed marked differences in the results from the 2 techniques. In 3 groups of patients i.e. control subjects, UC and ISTC patients, the Golenhofen measurements showed that spontaneous mechanical contractions were faster, stronger and of shorter duration than those measured in conventional organ baths. These differences reached statistical significance in 6 of the 9 comparative measurements among the recording techniques in the 3 patient categories. When the electrical and mechanical characteristics of UC (n=7) and ISTC (n=7) patients were compared with controls (n=10), significant differences were noted in terms of the number of electrical spike bursts and the duration of electrical spike bursts between ISTC patients and controls: 3.86$\pm$0.14 versus 9.71$\pm$1.78 electrical spikes per spike burst, P=0.03; 0.66$\pm$0.12 versus 2.18$\pm$0.62s duration of electrical spike bursts, P=0.04. Although trends emerged in the results from UC strips, suggesting the opposite in terms of greater numbers of electrical spikes per spike burst and longer duration of electrical spike bursts these differences did not reach statistical significance. These results suggest that, although the intestinal pacemaker is functional in the small bowel of ISTC patients, it generates spontaneous mechanical activity as a result of less intense, shorter duration electrical activity than controls. This supports the concept of ISTC being a panenteric disorder which is not solely confined to the colon. In UC patients undergoing IPAA no statistically significant differences were observed in spontaneous electrical and mechanical parameters when compared with control subjects.

4. The effects of cooling/ rewarming and certain drugs (potassium chloride, carbachol, atropine, diltiazem, sodium nitroprusside and metronidazole) were

examined using the Golenhofen apparatus in control, UC and ISTC samples of ileum (3 patients, 3 muscle strips per category). Stepwise cooling from 37.5°C to 27.5°C reduced the frequency and amplitude of slow wave activity and the preceding spike bursts of electrical activity. Neither the resting tone nor the maximum amplitude of isometric contraction was affected by these temperature changes in the 3 patient groups. Rewarming produced reversal of these effects with visible recovery of electrical activity.

5. The presence of voltage-operated and receptor-operated channels were demonstrated in ileal tissue from the 3 patient groups using potassium chloride, carbachol, atropine, diltiazem and sodium nitroprusside (SNP). No differences were observed amongst the 3 patient groups in terms of the pattern of tissue reponse to each of these spasmogens and relaxants. Metronidazole, an antibiotic commonly used to treat pouchitis, an idiopathic inflammatory condition which can affect up to 31% of IPAA patients with UC, had no effect on extracellular electrical or mechanical activity.

6. In carbachol-induced tone, both control and UC samples of ileum responded to electrical field stimulation (EFS, 1-64Hz, 0.05ms, supramaximal voltage) with a relaxation response followed by a rebound contraction. There were no statistically significant differences between control and UC tissues in terms of frequency/ response curves. The nitric oxide synthase (NOS) antagonist N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4}M) inhibited nerve-mediated relaxations in control tissues (10 patients, 17 muscle strips) by 60-90%. A more heterogeneous group of responses were noted in 18 UC patients (58 muscle strips, 11 synchronous IPAA procedures, 7 staged IPAA procedures). Thus, only 5 (10 muscle strips)

behaved in a similar fashion to control ileum i.e. L-NAME effectively inhibited nerve-mediated relaxations by 60-90%. L-NAME was only weakly effective in a further 3 patients (11 muscle strips) and ineffective in 5 patients (12 muscle strips). A further 5 UC patients (25 muscle strips) undergoing IPAA showed no evidence of functioning enteric neurons with EFS failing to elicit relaxation responses. NO would therefore seem to be the main inhibitory transmitter in control ileum but, in patients with UC, whilst some may behave in a comparable fashion to control tissue others have relaxation responses which suggest a possible alteration in the relative potencies of other inhibitory neurotransmitters. This may be due to the systemic effects of UC, to the medications used to treat this condition or both. Those patients, with *in vitro* absence of neuronal relaxations to EFS may exhibit features of a more aggressive form of UC. Alternatively, these tissues may have been traumatised by resection and therefore less likely to have responded to stimuli in the organ bath.

7. The NO-donor SNP in strips of control human ileum (n=30, 6 patients) produced a TTX-insensitive and therefore not neuronally mediated relaxation. The SNP-induced relaxation was independent on K^+-channel opening as demonstrated by the ineffectiveness of K^+-depolarising solution or apamin to modify its effect. The compound oxyhaemoglobin (HbO) which has a high affinity for NO and scavenges it from extracellular media did not appreciably prevent or reverse the relaxant responses to SNP in control human ileum. SNP-relaxation in human small bowel did not appear to be mediated by nerves or affected by K^+-channel opening. The ineffectiveness of HbO on SNP-induced relaxation suggests that, contrary to belief, this NO-donor relaxes smooth muscle in human ileum by mechanisms which are independent of extracellular NO generation.

8. Histochemical staining for reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase which colocalizes with NOS was carried out in 4 control and 11 UC patients. In the control group, 2 individuals were undergoing reverse ileal onlay grafts for postvagotomy diarrhoea. One of the UC patients had fulminant disease whilst one had pouch dysfunction requiring formation of a defunctioning ileostomy. Strong uptake of the stain was noted in the intestinal mucosa and myenteric plexus of all tissues studied. Nerve fibres showing strong NADPH-diaphorase activity were evenly distributed throughout the inner circular and outer longitudinal layers. There were no visual differences amongst the 2 patient groups in terms of intensity or distribution of staining.

9. A retrospective audit of 103 attempted TSRP procedures at Glasgow Royal Infirmary between 1988 and 1995 (87 UC; 9 FAP; 6 ISTC; 1 hereditary non-polyposis colorectal cancer, HNPCC), revealed no operative mortality, minimal operative morbidity and a pouch excision rate of 8%. In 60 patients with a maximum of 12 months established function, mean stool frequencies were x5 by day and x1 by night. Fifty-three patients (88.3%) were satisfied with overall long-term function. Of the 7 FAP pouches and 39 UC pouches undergoing detailed functional assessment the FAP subgroup had superior day and night stool frequencies as well as continence scores. The long-term functioning UC pouches showed a wider spectrum of function. The clinical impression of a difference in function of the small bowel in the UC and FAP patients undergoing IPAA, as reported in other pouch series in the surgical literature, was borne out by this audit. Moreover, the contribution of small bowel motility problems to pouch dysfunction

was clinically apparent with some individuals showing pouch dysfunction in the absence of pouchitis, pouch-anal outlet problems or anal sphincter defects.

10. These preliminary studies show that perioperative assessment of small bowel motility in prospective IPAA patients with a diagnosis of UC is possible using ambulatory small bowel manometry, although the technique itself is restricted by the difficulties associated with catheter insertion and data analysis. No association between small bowel motility and nocturnal stool frequency was evident in 7 intact UC patients. *In vitro* studies of small cuffs of ileum obtained at the time of IPAA were easier to perform and yielded more data than the small bowel manometry studies. Examination of spontaneous activty in control and UC tissues was influenced by the orientation of the muscle strip and the apparatus used to assess pacemaker activity. Golenhofen apparatus measurements, compared with conventional jacketed organ bath recordings gave data which showed spontaneous mechanical activity in control and UC strips to be faster, stronger and of shorter duration. No statistically significant differences in spontaneous activity parameters were noted between UC and controls. In contrast, the chance to study similar muscle strips from patients with ISTC revealed the unexpected finding that the latter group have differences in their spontaneous extracellular electrical activity characteristics. These suggest that in ISTC, the disease process is not confined exclusively to the colon and that differences in pacemaker activity may suggest differences in the number and/ or function of the interstitial cells of Cajal (ICC), which are believed to act as pacemakers for most of the gastrointestinal tract. Differences exist in the inhibitory nerve-mediated responses of UC and control tissues. In UC, alternative neuronal mechanisms appear to be utilised in relaxation in some patients whilst in others there are no demonstrable nerve-mediated

responses. Some UC patients do, however, have responses which are affected by L-NAME in the same way as control tissues, implying that NO is the main inhibitory neurotransmitter in these UC individuals. The classic NO-donor SNP produced its relaxant effects in human ileum by mechanisms which were not nerve-mediated, not dependent on K^+ channel opening and not prevented or reversed by HbO. The latter observation challenges the traditional concept that SNP produces its smooth muscle relaxation by liberating extracellular NO. NADPH-diaphorase staining showed that the facility to generate NO is present in the ileal smooth muscle of control and UC tissues. The heterogeneous reponses to L-NAME seen in UC patients implies that whilst NO can be generated in inhibitory ileal neurons, the cascade of events bringing about the relaxation response seen in control ileal smooth muscle is either modified, with other inhibitory transmitters playing a greater role in the process or alternatively abolished in some UC patients. These changes are presumably a direct legacy of the systemic effects of chronic UC and/or long-term drug treatments used to treat this condition. Collectively, these data support the concept that the ileum in UC patients undergoing IPAA is different from that of control counterparts, or indeed the ileum of FAP patients undergoing IPAA. The significance of these differences in terms of long-term pouch function will require further investigation.

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In 1992 my remit was to "do an MD on pouches". Numerous individuals have helped me over the ensuing period in an attempt to achieve this goal and I would therefore like to express my thanks to the following:

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Finally, this MD Thesis would never have been completed without the continuous support and encouragement of my family. Above all, I should like to thank my wife Jane and my son, James Robert for their tolerance of this major intrusion into family life. I dedicate the thesis to them with love and gratitude.

PRESENTATIONS AND PUBLICATIONS

The work described in this thesis has been presented at the following meetings of scientific societies.

Presentations:

1. "Mechanical activity of the ileum in chronic ulcerative colitis".
JS McCourtney, IG Finlay, JN Baxter, JW Craig, TC Muir.
Caledonian Society of Gastroenterology Autumn Meeting,
Ninewells Hospital, Dundee, 12th November 1993. (Oral presentation)

2. "Nitroprusside relaxant properties in human intestinal smooth muscle : is NO the answer?"
JS McCourtney, IG Finlay, JN Baxter, JW Craig, TC Muir.
Scottish Society of Experimental Medicine Annual Research Meeting, Ninewells Hospital, Dundee, 3rd December 1993. (Poster presentation)
Awarded prize for Best Poster Presentation

3. "Spontaneous activity of the ileum in ulcerative colitis".
JS McCourtney, IG Finlay, JN Baxter, JW Craig, TC Muir.
Glasgow Gut Club, Southern General Hospital, Glasgow, 28th January 1994. (Oral presentation)

4. "The role of nitric oxide in ileal enteric nerve-mediated relaxation in patients with ulcerative colitis undergoing pelvic pouch surgery".

JS McCourtney, JN Baxter, IG Finlay, JW Craig, TC Muir

The 15th International Symposium on Gastrointestinal Motility, Rome, Italy, 8th November 1995. (Poster presentation) + (Oral presentation, plenary session).

Also presented at:

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5. "The ileum used to make the pouch in ulcerative colitis may be pharmacologically abnormal".

JS McCourtney, JN Baxter, IG Finlay, TC Muir.

Tripartite Meeting (The Association of Coloproctology of Great Britain and Ireland; The Section of Coloproctology of the Royal Society of Medicine; American Society of Colon and Rectal Surgeons; Section of Colon and Rectal Surgery of the Royal Australasian College of Surgeons; Colorectal Surgical Society of Australia), London 10th July, 1996. (Oral presentation)

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Also presented at:

Falk UK Workshop: The Pelvic Ileal Reservoir in Ulcerative Colitis, Oxford, 19th April, 1997. (Oral Presentation)

6. "Small bowel manometry patterns and nocturnal stool frequency in prospective ileoanal pouch patients with ulcerative colitis"

JS McCourtney, JN Baxter, KB Carter, IG Finlay

Falk UK Workshop: The Pelvic Ileal Reservoir in Ulcerative Colitis, Oxford, 19th April, 1997. (Oral Presentation)

7. "Is there NO relaxation in ulcerative colitis pouch ileum?"

JS McCourtney, JN Baxter, IG Finlay, TC Muir

Royal Society of Medicine Overseas Meeting, Coimbra, Portugal 7th May 1997. (Poster presentation)

8. "Clinical and functional outcome of totally stapled restorative proctocolectomy"

JS McCourtney, IG Finlay

Royal Society of Medicine Overseas Meeting, Coimbra, Portugal 7th May 1997. (Oral presentation)

9. "Totally stapled restorative proctocolectomy"

JS McCourtney, IG Finlay

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10. "Small bowel motility and nocturnal stool frequency in ulcerative colitis"

JS McCourtney, JN Baxter, KB Carter, IG Finlay

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11. JS McCourtney, DM Hemingway, IG Finlay, TC Muir. Abnormal spontaneous pacemaker activity in the small bowel of patients with idiopathic slow transit

constipation. Association of Coloproctology of Great Britain and Ireland Annual Scientific Meeting, Jersey, 29th June- 1st July 1998. (Oral presentation- submitted)

Publications

1. JS McCourtney, IG Finlay, JN Baxter, JW Craig, TC Muir. Nitroprusside relaxant properties in human intestinal smooth muscle: is NO the answer? *Scottish Med J* 1994; **39**: 155. (abstract)

2. JS McCourtney, JN Baxter, IG Finlay, JW Craig, TC Muir. The role of nerve-mediated relaxation in patients with ulcerative colitis undergoing pelvic pouch surgery. *Neurogastroenterol Mot* 1995; **7**: 274. (abstract)

3. JS McCourtney, JN Baxter, IG Finlay, TC Muir. The ileum used to make the pouch in ulcerative colitis may be pharmacologically abnormal. *Int J Colorect Dis* 1996; **11**:144. (abstract)

4. JS McCourtney, IG Finlay. Totally stapled restorative proctocolectomy. *Br J Surg* 1997 **84**: 808-12. (paper)
Int J Colorect Dis 1997 **12**; 154. (abstract)

5. JS McCourtney, JN Baxter, KB Carter, IG Finlay. Small bowel motility and nocturnal stool frequency in ulcerative colitis. *Int J Colorect Dis* 1997 **12**; 155. (abstract)

6. JS McCourtney, IG Finlay, JN Baxter, JW Craig, TC Muir. Altered inhibitory transmission in ileal smooth muscle of patients with ulcerative colitis undergoing ileoanal pouch surgery. *Dis Colon Rectum* (paper: submitted)

7. JS McCourtney, DM Hemingway, IG Finlay, TC Muir. Abnormal spontaneous pacemaker activity in the small bowel of patients with idiopathic slow transit constipation. *Dis Colon Rectum* (paper: submitted)

DECLARATION

I declare that I was solely responsible for both the concept and the experimental design of this thesis. The work reported in this thesis has not been submitted previously for another degree.

The studies described in the experimental section were carried out between October 1992 and July 1994 while I worked in the University Department of Surgery at Glasgow Royal Infirmary. I had sole reponsibility for the design, execution and statistical analysis of the ambulatory small bowel manometry study and *in vitro* smooth muscle pharmacology studies, along with the processing of the tissue samples for histochemistry and subsequent photomicrography.

For a three month period (January-March 1993) I collaborated with Ms Alison J Campbell, a Senior Honours BSc student at the Department of Pharmacology, Glasgow University in a pilot study to examine some of the basic pharmacological properties of human terminal ileum. This study formed the basis of her thesis presented in partial fulfilment for the Degree of Bachelor of Science (with Honours) in Pharmacology (see references). Some of these control data are referred to in the thesis overleaf.

The clinical studies described in this thesis were performed with the approval of the Ethics Committee, Glasgow Royal Infirmary. Radiological screening of the patients in the small bowel manometry section met with Radiation Protection guidelines.

Between January 1992 and January 1996 it was my sole responsibility to collect the data for the review of IPAA at Glasgow Royal Infirmary.

ABBREVIATIONS

ATP	Adenosine triphosphate
ATZ	Anal transitional zone
β	Beta
BRP	Bovine retractor penis
Ca^{++}	Calcium ion
CD	Crohn's disease
cGMP	Cyclic guanosine 3′, 5′- monophosphate
CGRP	Calcitonin gene regulating peptide
cm	Centimetre(s)
cNOS	Nitric oxide synthase (constitutive isoenzyme)
DCCs	Discrete clustered contractions
D-NAME	N^{ω}-nitro-D-arginine methyl ester
EDHF	Endothelium-derived hyperpolarizing factor
EDRF	Endothelium-derived relaxing factor
EFS	Electrical field stimulation
EGG	Electrogastrography
FAP	Familial adenomatous polyposis
GRCs	Giant retrograde contractions
h	Hour(s)
HbO	Oxyhaemoglobin
HNPCC	Hereditary non-polyposis colorectal cancer
5HT	5-Hydroxytryptamine
IPAA	Ileal pouch-anal anastomosis
IAS	Internal anal sphincter

IBS	Irritable bowel syndrome
ICJ	Ileocaecal junction
IDMEC	Interdigestive myoelectrical complex
IL-4	Interleukin-4
IL-10	Interleukin-10
iNOS	Nitric oxide synthase (inducible isoenzyme)
ISTC	Idiopathic slow transit constipation
"J"	Double-limbed
K^+	Potassium ion
L-ARG	L-Arginine
L-NAME	N^{ω}-nitro-L-arginine methyl ester
L-NMMA	N^{ω}-monomethyl-L-arginine
L-NNA	N^{ω}-nitro-L-arginine
LOS	Lower oesophageal sphincter
m	milli (10^{-3})
MAF	Mini-activity fronts
min	Minute(s)
MMC	Migrating motor complex
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NADPHd	Reduced nicotinamide adenine dinucleotide phosphate-diaphorase
NANC	Non-adrenergic, non-cholinergic
NKA	Neurokinin A
NKB	Neurokinin B
NO	Nitric oxide
NOS	Nitric oxide synthase

P	Probability
PBS	Phosphate buffer solution
PPCs	Prolonged propagating contractions
RAIR	Rectoanal inhibitory reflex
r_S	Spearman's rank correlation
"S"	Triple-limbed
s	Second(s)
SEM	Standard error of mean
SNP	Sodium nitroprusside
TTX	Tetrodotoxin
TSRP	Totally stapled restorative proctocolectomy
μ	Micro (10^{-6})
UC	Ulcerative colitis
VIP	Vasoactive intestinal peptide
"W"	Quadruple-limbed

CHAPTER 1

WHY STUDY SMALL BOWEL MOTILITY IN PATIENTS WITH ULCERATIVE COLITIS UNDERGOING RESTORATIVE PROCTOCOLECTOMY WITH ILEAL POUCH-ANAL ANASTOMOSIS?

1.1 INTRODUCTION

Ulcerative colitis (UC) is an inflammatory disorder of the colonic mucosa which spontaneously relapses and remits. The pathogenesis of UC is multifactorial and probably results from a complex interaction among genetic, endogenous and exogenous modifying factors (Lowes & Jewell, 1990). Since predisposing and trigger factors have not been clearly identified, current therapeutic strategies for UC are targetted to the immunopathogenic mechanisms mediating the inflammatory process which produce tissue injury in this condition. A significant proportion of patients with chronic UC will, however, require surgical intervention, particularly if the inflammation involves the entire colon and rectum.

Traditionally, the surgical procedure of choice for patients with chronic UC was panproctocolectomy (removal of the entire colon and rectum). This operation entails a permanent abdominal end ileostomy and patients are totally incontinent of faeces and require to wear continuously an external stoma appliance. Although most patients with an ileostomy adjust to having a stoma and return to a relatively normal lifestyle, approximately 50% of patients, despite a well constructed ileostomy, suffer chronic appliance-related problems (Becker, 1993). There are also significant psychological and social implications for such patients. Not surprisingly, therefore, surgeons have, for many years, sought alternative strategies, the most recent of which has been the modern restorative proctocolectomy with ileal pouch-anal anastomosis (Parks & Nicholls, 1978). This operation is now the preferred surgical treatment for UC (Williams, 1989a). Restorative proctocolectomy involves excising the diseased colon and rectum with the creation of a pouch or reservoir from the remaining terminal ileum which is then either sutured or stapled to the anal sphincter mechanism (see Fig 1.1).

In spite of the potential advantages of IPAA, with over 90% of patients having satisfactory preservation of faecal continence (Pemberton *et al*, 1987)

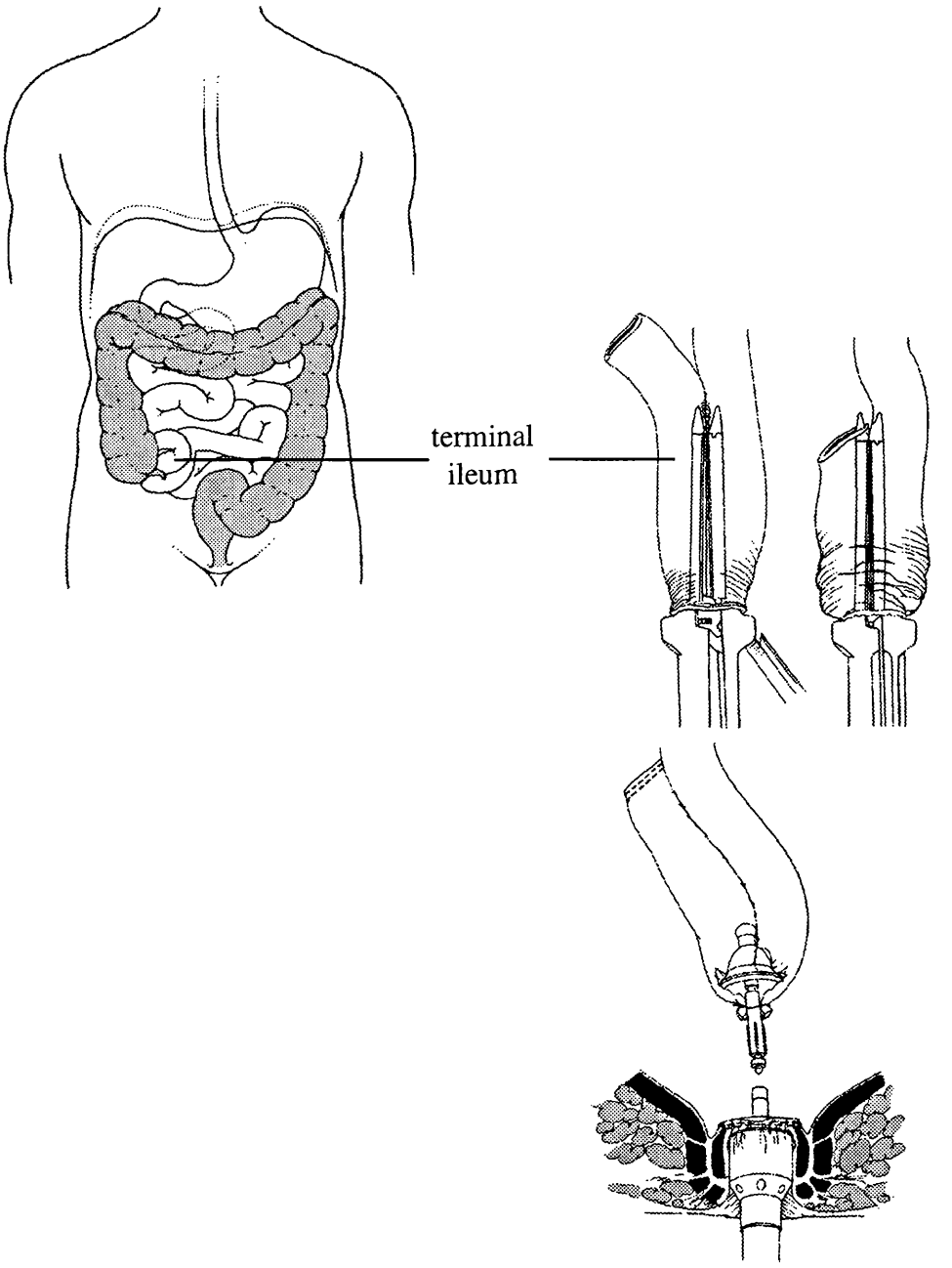


Fig 1.1 Totally stapled restorative proctocolectomy.
Shaded area represents excised colon and rectum.

some individuals develop persistent and excessive stool frequency following restorative proctocolectomy. In the absence of sphincter impairment this suggests that factors, already present in the ileum used to construct the pouch, may be involved in these continence difficulties (dyscontinence) (Dozois *et al*, 1989). Measurement of small bowel motility in established IPAA patients with a spectrum of pouch activity has shown that small bowel motility may indeed be a determinant of pouch function (Groom *et al*, 1994).

Although the human terminal ileum is one of the major anatomical components used in IPAA to treat chronic UC, its *in vitro* and *in vivo* motility characteristics are unknown. Indeed, little is known about the motility of the human terminal ileum in health. Work in laboratory animals has, however, shown that enteric neural and smooth muscle functions are altered in non-inflamed areas of the gastrointestinal tract as a consequence of distal inflammation (Marzio *et al*, 1990; Jacobson *et al*, 1993a-b). Whether the same holds true for patients with chronic UC undergoing IPAA is, as yet, unestablished.

In an attempt to clarify why small bowel motility should be investigated in UC patients undergoing IPAA this chapter reviews the development of modern restorative proctocolectomy with IPAA; discusses the factors used to assess pouch functional outcome; and looks at current practice in terms of preoperative clinical and physiological assessment of prospective UC patients.

1.2 RESTORATIVE PROCTOCOLECTOMY WITH ILEAL-POUCH ANAL ANASTOMOSIS (IPAA) IN THE MANAGEMENT OF ULCERATIVE COLITIS

1.2.1 HISTORICAL BACKGROUND

The role of surgery in the management of UC has evolved over the last 100 years in tandem with developments in surgical practice, anaesthesia and resuscitation. Surgical treatment of UC has developed from the early, life-saving technique of colonic irrigation, through diversion of the faecal stream to enable complete physiological rest of the inflamed colon, to surgical excision of diseased colon and rectum either with or without restoration of intestinal continuity. These important developments in the surgical management of UC are summarised below.

The first case report of successful surgical intervention in UC described irrigation of the inflamed mucosa via a left iliac sigmoid colostomy to treat severe UC (Mayo Robson, 1893). Thereafter, the technique evolved from irrigation via a colostomy to irrigation with various fluids through the appendix stump (appendicostomy), which minimised the leakage of excoriating ileal contents onto the abdominal wall. This remained the surgical treatment of choice in UC until the 1940's (Corbett, 1945). However, one major limitation with colonic irrigation was that the colon was defunctioned inadequately.

Failure to improve symptoms by colonic irrigation alone in some patients with severe UC led to the introduction of the concept of diverting the faecal stream to rest completely the inflamed colonic mucosa (Brown, 1913). This was achieved by dividing the ileum and bringing the end out on to the abdominal wall as a flush ileostomy. A tube caecostomy was then used to irrigate the colon. While diversion by ileostomy with colonic irrigation remained central to the treatment of

severe UC for the first half of this century, it was regarded as a salvage procedure designed for the treatment of almost moribund patients with UC.

By the Second World War, general improvements in resuscitation and anaesthetic techniques enabled proctocolectomy with formation of a permanent end ileostomy to become the recognised operative procedure for the management of severe UC (Strauss & Strauss, 1944). However, major morbidity associated with ileostomy formation such as stomal stenosis and excessive effluent output persisted until techniques were introduced to create everted ileostomies (Brooke, 1952; Turnbull, 1953). By everting the stoma, large losses of nutrients and fluid were avoided and serositis, which leads to stricture formation, effectively abolished. Further advances in adhesives and lightweight stoma appliances minimised problems with skin excoriation and enabled patients to lead active lifestyles after colectomy.

Because of problems relating to the perineal dissection in proctocolectomy such as sexual dysfunction (Stahlgren & Ferguson, 1959) and perineal sinus formation (Watts *et al*, 1966), methods were devised to excise the diseased rectum whilst retaining some or all of the anus and the sphincter mechanism. Of these, conservative proctocolectomy with retention of both the internal and external anal sphincters along with the mucosa of the lower anal canal avoided a perineal wound and preserved the perineum with its important supportive function. Doubts about the efficacy of this procedure, in terms of providing uncomplicated perineal healing (Talbot *et al*, 1989) resulted in it failing to gain widespread acceptance. Intersphincteric proctectomy, on the other hand, preserved both an intact external anal sphincter with levator ani muscles (Lyttle & Parks, 1977). This form of rectal excision resulted in improved perineal wound healing and allowed easier eradication of any perineal sepsis which might develop.

Despite these improved techniques of rectal excision and ileostomy formation, considerable interest had focussed on preserving intestinal continuity following colonic excision in UC. Ileorectal anastomosis following colectomy

was the initial alternative sphincter-sparing procedure to attract attention (Devine & Devine, 1957). This procedure, still used by some surgeons to treat UC and FAP, represents a compromise between preserving anorectal and sexual function on one hand, with the putative risk of leaving diseased rectal mucosa on the other. Over 90% of patients undergoing this procedure return to full health with an operative mortality as low as 5% in some series (Aylett, 1960). In a review of 17 studies on ileorectal anastomosis for the treatment of UC, 1206 patients were found to average 4-5 bowel motions per day and 99% of these individuals had normal continence (Parc *et al*, 1985). Fifteen percent of these patients, however, eventually underwent bypass procedures or completion proctectomy. However, neoplastic transformation in the retained rectal mucosa is a real risk, occurring in 2-20% of reported series. Colectomy with ileorectal anastomosis is certainly an attractive option in patients with minimal rectal involvement but close surveillance is mandatory to minimise the possibility of developing rectal cancer and to monitor disease progression or exacerbation in the remaining rectal segment, which often requires further medical or surgical intervention.

In an attempt to overcome the total incontinence associated with a permanent ileostomy, the concept of an intra-abdominal reservoir was introduced (Kock, 1969). This created an abdominal ileostomy which required no stoma appliance, with the added advantage for the patient of being able to evacuate the stoma when socially convenient. Further modification of the continent ileostomy occurred with the introduction of a nipple valve to create a leak-proof one-way valve system (Faren *et al*, 1973), but failure required valve revision in over 50% of patients. Subsequent technical improvements in valve construction reduced this revision rate to a reported 10% of patients (Dozois *et al*, 1980; Gerber *et al*, 1983). The Kock continent ileostomy procedure is both technically demanding and associated with high complication rates. While still carried out at specialised institutions, modern restorative proctocolectomy with IPAA has completed the evolution of the continent ileostomy procedure to the end of its evolution.

1.2.2 EVOLUTION OF THE MODERN OPERATION OF ILEAL POUCH-ANAL ANASTOMOSIS

The risks associated with the retention of a potentially diseased rectum following total colectomy and ileorectal anastomosis, together with the technical aspects of constructing a continent permanent ileostomy led to the evolution of the procedure of modern restorative proctocolectomy with IPAA (Parks & Nicholls, 1978). This operation evolved from the fusion of two concepts: the ileoanal pull-through, which consisted of total colectomy, rectal mucosectomy and pull-through of the distal ileum with anastomosis to the anus; and the ileal reservoir.

Ileoanal anastomosis was first attempted at the beginning of this century (Hochenegg, 1900), but poor functional outcome and high morbidity prevented its widespread acceptance as a standard surgical technique. Similar problems were observed when ileoanal anastomosis was performed in Germany on a 10 year-old boy with FAP (Nissen, 1933), and some 10 years later in Minnesota, USA when Wangensteen attempted to duplicate this operation in a young adult male with chronic UC (Wangensteen, 1943). Disappointing results were also noted in studies of mucosectomy prior to ileoanal anastomosis both in dogs (Ravitch & Sabiston, 1947) and in a limited number of humans with severe UC (Ravitch, 1948). Coloanal anastomosis, following mucosal stripping of the distal rectum and a colonic pull-through procedure was first described in the treatment of megacolon (Yancey *et al*, 1952) and later applied to the management of Hirschsprung's disease with good results (Soave, 1962). However, increased stool frequency and erratic continence limited the use of the Soave procedure in adult patients. Distal rectal mucosal stripping combined with a total colectomy and a straight ileoanal anastomosis was first applied in the treatment of FAP (Safaie-Shirazi & Soper, 1973) and later in young patients with UC (Martin *et al*, 1977). However, urgency, nocturnal incontinence and excessive stool frequency dogged initial efforts at performing straight ileoanal anastomosis in adults.

The concept of constructing a reservoir to increase neorectal capacity, in an effort to address the problem of excessive stool frequency following ileoanal anastomosis was first explored in animal studies. A triple limb ileal pouch combined with an ileoanal anastomosis was studied in dogs (Valiente & Bacon, 1955), as was the effectiveness of a double-barrelled isoperistaltic pouch (Karlan *et al*, 1959). In humans, the first acceptable results following restorative proctocolectomy were achieved by combining a triplicated ("S") ileal pouch with distal rectal mucosectomy and handsewn pouch-anal anastomosis (Parks & Nicholls, 1978).

Since 1978, several reservoir designs have been advocated in restorative proctocolectomy procedures. These include the "J" pouch (Utsunomiya *et al*, 1980), the lateral side-to-side ileal pouch (Fonkalsrud & Ament, 1978) and the "W" reservoir (Nicholls & Lubowski, 1987). Each of these reservoir configurations appear to have similar functional results provided that they are of sufficient capacity, are positioned in the true pelvis with a short outflow tract and that the constructed pouch-anal anastomosis is tension-free.

As the modern IPAA procedure has evolved, the length of the remaining rectal muscular cuff has been shortened (Grant *et al*, 1986) or completely excised (Chaussade *et al*, 1989; Slors *et al*, 1989) by many surgeons. The role of mucosectomy has also generated considerable debate (O'Connell & Williams, 1991). While some surgeons begin proximal stripping of the mucosa at the dentate line (Parks & Nicholls, 1978; Parks *et al*, 1980; Nicholls, 1987) arguing that this removes all the diseased mucosa and prevents any chance of recurrent UC, FAP or neoplastic transformation, others strip above the anal transitional zone (ATZ) (Martin *et al*, 1985). Advocates of the latter technique claim superior functional results in terms of continence (Miller *et al*, 1990; Lavery *et al*, 1990).

Recently, surgeons have avoided mucosectomy by constructing stapled pouch-anal anastomoses (Heald & Allen, 1986; Johnston *et al*, 1987; Williams, 1989; Kmiot & Keighley, 1989; Sugerman *et al*, 1991), 1-2cm above the dentate

line. Those workers have argued that the excellent functional results obtained with these techniques outweigh the putative risk of leaving a small cuff of potentially diseased mucosa. Nevertheless, if a stapled pouch-anal anastomosis is performed without mucosectomy then regular surveillance of the retained cuff is mandatory (Schmitt *et al*, 1992; Seow-Choen *et al*, 1991).

Controversy persists over which operative technique of restorative proctocolectomy with IPAA is superior. In an important prospective randomised study from St Mark's (Seow-Choen *et al*, 1991), anal function was compared after handsewn ileoanal anastomosis with mucosectomy versus stapled ileoanal anastomosis without mucosectomy. No functional advantage was conferred by use of the latter. Subsequent studies have also supported these findings (Gozzetti *et al*, 1994; Becker & Raymond, 1986). At present, surgeons adopt their own technique of choice: at our Institution we perform a totally stapled "J" IPAA procedure (see Appendix and Fig 1.1). From the literature to date there seem to be few differences in functional results between a well-sewn IPAA and a well-stapled one.

IPAA now represents an acceptable alternative to conventional proctocolectomy and ileostomy, in selected patients with UC and FAP. Indeed, some consider that IPAA should be the elective operation of choice for young UC patients requiring surgical intervention (Williams, 1989a). In most large series of patients undergoing IPAA, the overall functional results have been encouraging (Pemberton *et al*, 1987; Wexner *et al*, 1989; Keighley *et al*, 1993; Mortensen, 1988; Stryker *et al*, 1986). Improvements in technique have lowered operative morbidity and improved overall functional outcome so that a patient undergoing IPAA can expect a stool frequency of 4 to 6 motions per 24 hour period, complete continence, and spontaneous defaecation with the ability to defer defaecation for more than 30 min (Mortensen, 1988). There is, however, considerable variation in clinical results and 10% of pouch patients are estimated to have a poor outcome with respect to their preoperative condition (Stryker *et al*, 1986). The factors

which have been established as contributing to the determination of long-term pouch function are reviewed below.

1.2.3. FUNCTIONAL DETERMINANTS

For an ileoanal pouch to function well as a substitute rectum (neorectum), the ileum which is used to create the reservoir must adapt to its new role and environment. In clinical practice, IPAA performance is assessed by monitoring four variables (Levitt & Lewis, 1991). These are summarised in Table 1.1.

SPONTANEITY OF DEFAECATION

The majority of IPAA patients can empty their reservoirs spontaneously. However, failure of spontaneous defaecation, with patients requiring to self-catheterise the pouch to achieve defaecation was formerly associated with a defective "S" pouch design. This has since been eliminated, largely by modification of the "S" pouch to shorten its efferent limb (Hallgren *et al*, 1989).

ABILITY TO DEFER DEFAECATION

Most ileoanal pouch patients can postpone defaecation voluntarily for at least fifteen minutes (Stryker *et al*, 1985). The minority who cannot are defined as having pouch *urgency*. The factors causing this unusual phenomenon are poorly understood. Although some workers have identified patients with poor external anal sphincter function who experience symptoms of urgency (Nasmyth *et al*, 1986a), this has not been encountered by others (Scott *et al*, 1989). While exclusion of patients with poor preoperative sphincter function reduces the incidence of urgency, other contributing factors, as yet undetermined, must exist.

CONTROL OF FAECAL CONTINENCE

Poor postoperative continence following IPAA has been most commonly associated with impaired internal anal sphincter (IAS) function, which itself is

FUNCTIONAL PARAMETER
Spontaneous defaecation
Ability to defer defaecation for at least 30 min
Faecal continence
Stool frequency (day and night)

Table 1.1: Clinical determinants of function in established IPAA patients. (after Levitt & Lewis, 1991)

reflected in a reduction in resting anal canal pressure (Chaussade *et al*, 1989; Johnston *et al*, 1987; Nasmyth *et al*, 1986a; Scott *et al*, 1989; Primrose *et al*, 1987; O'Connell *et al*, 1988). Other factors such as the effectiveness of pouch evacuation (Nasmyth *et al*, 1986a) and stool consistency (Beart *et al*, 1985) have also been implicated.

Irrespective of the surgical technique used to construct the ileoanal pouch, a reduction in anal pressure is a common sequel of IPAA. Patients, however, generally report satisfactory continence in the early postoperative period which would imply considerable functional sphincter reserve (Stryker *et al*, 1986; Pescatori & Parks, 1984; Williams *et al*, 1989). In addition, both continence and IAS function improve postoperatively and gradually return to preoperative levels (Becker *et al*, 1985; Kmiot *et al*, 1989).

Poor continence following IPAA seems more likely to reflect poor patient selection rather than sphincter injury related to operative technique. Individuals with limited functional sphincter reserve because of advanced age or previous sphincter injury are most likely to experience incontinence problems following IPAA (Chaussade *et al*, 1989; Kmiot *et al*, 1989; Nicholls & Pezim, 1985; McHugh *et al*, 1987).

STOOL FREQUENCY

Pouch volume appears to be the single most important influence on stool frequency. Thus, a larger pouch is often associated with a lower stool frequency (Nasmyth *et al*, 1986b; Becker *et al*, 1985; O'Connell *et al*, 1987; Nicholls & Pezim, 1985; Neal *et al*, 1982). Moreover, pouch capacity tends to increase with time and this is mirrored by a reciprocal fall in stool frequency (Becker *et al*, 1985; Williams, 1986). It is unclear as to how pouch capacity influences the pattern of defaecation. Work which has questionned the view that the pouch acts purely as a reservoir includes scintigraphic analysis of IPAA patients. This technique showed that the actual reservoir area included ileum immediately above

the pouch (O'Connell *et al*, 1986). Comparative studies of patients with straight ileoanal anastomoses and IPAA patients showed no significant difference in the respective capacity of each type of neorectum (Dozois, 1986; Taylor *et al*, 1983).

Pouch capacity is also related to compliance with the larger pouch proving the more compliant. In turn, compliance is linked with stool frequency (Nasmyth *et al*, 1986a; Scott *et al*, 1989). Indeed, large pouches have less active motility profiles and have greater threshold volumes (O'Connell *et al*, 1987). The latter term refers to the volume of pouch material which triggers propulsive wave activity associated with the desire to defaecate. How variations in pouch capacity influence pouch compliance and motility are unknown (Levitt & Lewis, 1991). Intrapouch pressure measurements, during continuous water distension, have been used to characterise compliance and pouch motor activity in patients with good and poor functional outcomes (Levitt *et al*, 1992). Using this provocative test of pouch performance, those individuals with poor function had a reduced pouch compliance and primary disorders of pouch motility.

Other factors which influence stool frequency are total daily stool volume (O'Connell *et al*, 1987) and the degree of pouch emptying (Stryker *et al*, 1986; O'Connell *et al*, 1987; Dozois, 1986). Higher stool volumes are associated with a greater frequency of defaecation following IPAA (O'Connell *et al*, 1987). The former are directly related to dietary intake. The factors which result in poor pouch emptying are not fully understood, but outlet obstruction is one of the most important (Pescatori & Parks, 1984; O'Connell *et al*, 1986).

Two other factors which have an adverse effect on overall pouch function are postoperative sepsis (Beart *et al*, 1985; Keighley *et al*, 1988) and pouchitis (Madden *et al*, 1990; Hulten, 1989). Postoperative sepsis may result in a smaller, less compliant pouch but this is unproven (Keighley *et al*, 1988). Pouchitis results in increased stool frequency and increased urgency; pouchmetrography has been used to characterise the pouch motility profile in patients with this condition (Levitt *et al*, 1992).

1.3 PREOPERATIVE ASSESSMENT OF THE ULCERATIVE COLITIC PATIENT UNDERGOING ILEAL POUCH-ANAL ANASTOMOSIS-CURRENT SURGICAL PRACTICE

1.3.1 PATIENT SELECTION

While IPAA may seem to be, at least in theory, the ideal operation for the elective management of UC, each case has, nevertheless, to be assessed individually. The implications of IPAA on the patient's lifestyle must be considered along with technical factors and the advantages of this operation weighed against alternative surgical strategies for UC.

The relative advantages and disadvantages of IPAA (see Table 1.2) have to be carefully discussed with each patient and a joint decision reached on whether IPAA or an alternative operation, most commonly proctocolectomy and end ileostomy, is most appropriate for that particular individual.

Technical aspects often determine whether or not IPAA is appropriate. For example, the length of the small bowel mesentery is very important in determining the ease with which a terminal ileal reservoir may be led into the pelvis to enable pouch-anal anastomosis. Both the short obese patient and the very tall patient can have restricted lengths of small bowel mesentery. Likewise, previous surgery may restrict the length of available small bowel for pouch construction. Dense adhesions after previous surgery for acute UC may also cause technical difficulties in the pelvic dissection prior to pouch-anal anastomosis. Careful consideration of these factors is mandatory before attempting ileoanal pouch surgery. The potentially difficult patient has to be informed preoperatively of the possibility of technical failurewhich would necessitate permanent end ileostomy formation.

The main indication for ileoanal pouch surgery in chronic UC is the case which is refractory to medical therapy or complicated by frequent attacks of severe acute colitis. Patients with longstanding disease which interferes with physical, social or employment factors are also strong candidates for elective

ADVANTAGES	DISADVANTAGES
Improved body image **Improved social life** **Less interference with occupation** **No occupational barriers** **Psychosocial advantages** **Less odour during/between emptying** **Less time to manage**	**More extensive operation** **Longer convalescence** **Requires greater motivation** **Perianal leakage** **Perianal irritation** **Pouchitis** **More frequent visits to toilet** **Long-term surveillance necessary** **10% failure rate requiring pouch excision**

Table 1.2: Advantages and disadvantages of IPAA. The above quality of life factors should be considered for each UC individual requiring elective surgery. IPAA is considered against the traditional operation of panproctocolectomy and permanent ileostomy formation (see Pezim & Nicholls, 1985).

pouch surgery. Another large subgroup of patients who undergo IPAA at Glasgow Royal Infirmary are those who have been treated at other institutions by initial subtotal colectomy with rectal stump preservation for toxic megacolon or complications of acute UC. Individuals with severe or high-grade dysplastic lesions biopsied on colonoscopic surveillance for chronic total UC, or low-grade dysplastic changes on raised lesions are also advised to undergo ileoanal pouch surgery as prophylaxis against developing colorectal carcinoma. In certain patients with toxic UC refractory to medical therapy, urgent IPAA within 48 hours of admission is performed in selected cases at Glasgow Royal Infirmary, providing the histological diagnosis is established, perforation has not occurred and the patient does not require intensive preoperative parenteral nutrition. This last indication for ileoanal pouch surgery is a local policy and although encouraging results have been obtained at Glasgow Royal Infirmary and at other institutions (McCourtney & Finlay, 1993; Mowschenson *et al*, 1993), one must acknowledge that in this clinical situation it is traditional colorectal practice to perform subtotal colectomy, rectal stump preservation and end ileostomy (O'Kelly & Mortensen, 1992).

Table 1.3 lists the absolute and relative containdications to ileoanal pouch surgery respectively. The relative contraindications must be assessed on an individual basis. Clearly, elderly patients must be considered for pouch surgery only after a thorough clinical and physiological evaluation to identify latent anal sphincter weakness has been made. Paediatric patients and their parents require careful counselling, but excellent long-term functional results in carefully selected, well-motivated children (McCourtney *et al*, 1996; Romanos *et al*, 1997) have been obtained. Patients with major psychiatric disorders should probably not be offered the procedure. Likewise, those individuals who are educationally subnormal should also be carefully evaluated before embarking on ileoanal pouch construction, which even if technically succesful, may fail because the impact of the surgery and subsequent period of pouch adaptation is too great and the patient

RELATIVE	ABSOLUTE
Age **Psychological factors** **Poor nutritional status** **General inanition** **Indeterminate colitis**	**Crohn's disease** **Distal rectal/anal carcinoma** **Anal sphincter damage** **Faecal incontinence** **Obstetric trauma** **Immobility/infirmity**

Table 1.3: Contraindications to IPAA.

cannot cope (Mortensen, 1993). The diagnosis of "indeterminate colitis" accounts for 10-15% of all cases of idiopathic inflammatory bowel disease (Pezim *et al*, 1989). This entity reflects the absence of characteristic features which enable either UC or CD to be diagnosed. Problems can ensue, when faced with this diagnosis, in trying to decide the most appropriate surgical procedure. Further clinical and radiological assessment can often lead to a firm decision being made regarding the question of ileoanal pouch surgery. The majority of cases of indeterminate colitis can undergo pouch formation with satisfactory functional results (Pezim *et al*, 1989). Nevertheless, where serious concern over occult CD exists a preliminary subtotal colectomy with rectal stump preservation should be performed. Thereafter the rectum should be biopsied, on a serial basis if necessary, to confirm or refute the diagnosis of CD, though in the defunctioned state, rectal histology can be difficult to interpret and may have features which are hard to distinguish from CD (Shepherd, 1991).

1.3.2. PREOPERATIVE PREPARATION- GENERAL ASPECTS

Several clinical aspects must be assessed before a patient with UC undergoes ileoanal pouch surgery. These are broadly similar to those evaluated in the preoperative preparation of patients undergoing other major elective colorectal procedures and include a complete examination of the colon, if still present, by barium enema or colonoscopic examination, histology to exclude CD and identify UC cases with dysplastic changes, preoperative asessment of the patient's cardiorespiratory function and general fitness, correction of anaemia and electrolyte disturbances and evaluation by a stomatherapist. At Glasgow Royal Infirmary the patient is generally admitted 48h prior to operation to enable these tests to be completed and a full bowel preparation commenced the day before surgery. This preoperative "work-up" is also carried out at other pouch centres, both in the United Kingdom (Thomas & Taylor, 1991; O'Kelly & Mortensen, 1992) and in North America (Pemberton, 1993). The preoperative assessment of

the two functional components of the ileoanal pouch *viz.* the anal sphincter mechanism and the small bowel are discussed below.

1.3.3 ANORECTAL PHYSIOLOGY

Most centres perform anorectal physiology tests on prospective IPAA patients routinely. Although used largely as a research tool, these provide important objective information about basic characteristics such as resting and squeeze pressure, anal canal length and sensitivity and the presence or absence of a rectoanal inhibitory reflex (RAIR). Many papers (see review by Bartolo & Duthie, 1993) have reported the pre- and postoperative changes in anorectal measurements with various IPAA techniques. In conjunction with the more recent introduction of endoanal ultrasonography, these tests yield useful data on the pre-pouch functional and structural characteristics of the anal sphincter mechanism. Table 1.4 summarises the most common anorectal physiology tests which are routinely performed in pouch centres.

In practical terms, however, anorectal physiological tests are useful only as clinical research tools. Few, if any, patients are rejected from IPAA on the basis of an abnormal anal canal pressure profile (O'Kelly & Mortensen, 1992). Furthermore, an experienced surgeon can obtain just as much information regarding anal sphincter function from digital examination (Hallgren *et al*, 1989).

1.3.4 SMALL BOWEL ASSESSMENT

In the clinical situation, where doubt exists over a diagnosis of UC, Crohn's disease (CD) may be excluded, to some extent, by small bowel radiological investigations i.e. small bowel enema or follow-through studies. Occasionally, radiolabelled white cell scans may be performed to identify inflammatory changes in the terminal ileum which are suggestive of CD. Currently, however, UC patients who undergo IPAA do not have preoperative small bowel functional assessment, either at Glasgow Royal Infirmary or at other

ANORECTAL ASSESSMENT
***Digital examination** ***Anal canal manometry** Resting pressure (internal anal sphincter) Squeeze pressure (external anal sphincter) RAIR testing Anal canal motility ***Anal canal length** **Anal canal sensation** **External anal sphincter electromyography** **Pudendal nerve terminal motor latency**

Table 1.4: Preoperative anorectal assessment of prospective IPAA patients.
RAIR= rectoanal inhibitory reflex.
*= tests commonly used in practice at Glasgow Royal Infirmary.
(see Thomas & Taylor, 1991).

centres specialising in this operation. Indeed, the motility characteristics of the small bowel in patients with UC have never been assessed. This is a surprising statistic when one considers that several thousand patients with UC have undergone IPAA since the modern operation was first described (Parks & Nicholls, 1978).

1.4 AIMS OF THESIS

Currently the best "chance" of a successful result following IPAA lies in the creation of a "large volume reservoir" in patients who have good preoperative anal sphincter function (Levitt & Lewis, 1991). However, poor clinical outcome with excessive stool frequency is seen in some patients following ileoanal pouch surgery despite having normal anal sphincter function, a good capacity reservoir and no pouchitis (Stryker *et al*, 1986). Differences in small bowel motility characteristics in patients with high and low stool frequencies following IPAA may explain this phenomenon (Groom *et al*, 1994). Further, the functionally superior results reported in some series of FAP patients undergoing IPAA, as compared with UC counterparts, has led to the suggestion that the remaining small bowel is functionally different in these two conditions (Dozois *et al*, 1989). Clearly, pre- and postoperative studies of small bowel motility characteristics in patients with UC undergoing IPAA would be necessary to resolve these questions.

Although some workers have examined the relationship of small bowel motility to IPAA function *after* reservoir construction (see section 2.5.3), surprisingly there has yet to be a study of small bowel motility *prior* to pouch surgery. This deficiency is compounded by the fact that the *in vitro* motility characteristics of the terminal ileum used to construct the pouch have never been assessed in UC and only examined briefly in control subjects (see section 4.2).

Two basic questions regarding small bowel motility therefore need to be answered in IPAA for UC:

1. Do inter-individual differences in patterns of small bowel motility correlate with individual preoperative stool frequencies in patients with UC before IPAA is performed?

2. What are the *in vitro* characteristics of human terminal ileum in health and UC?

This thesis attempted to answer these questions by studying, *in vivo* and *in vitro*, the small bowel motility characteristics of UC patients undergoing IPAA. Solid state ambulatory manometry was used to assess, *in vivo*, the preoperative patterns of human small bowel motility in patients undergoing IPAA. In resected specimens from control and IPAA patients with UC, specific interest was focussed on two areas of smooth muscle function. These areas comprised the characteristics of and effects of drugs on spontaneous myogenic activity in the human terminal ileum and the role of nitric oxide (NO) as a mediator of inhibitory neurotransmission in this region of the gut in control and UC patients.

The background literature to these two areas of research and the *in vivo* and *in vitro* experimental work which was carried out are reported in the following chapters.

CHAPTER 2

SMALL BOWEL MOTILITY- *IN VIVO* ASPECTS

2.1 INTRODUCTION

A study of small bowel motility in preoperative patients with UC would help to establish whether inter-individual differences in small bowel motility patterns correlated with respective preoperative stool frequencies before IPAA is performed. However, the term "motility" covers a diverse range of smooth muscle functions matched only by the wide variety of techniques used to investigate them. Clinical techniques used to assess small bowel motility are listed in Table 2.1.

Clearly, studies of small bowel motility can focus at different functional levels such as the single smooth muscle cell, whole tissue preparation or intact animal. This chapter examines the properties of small bowel which determine its motility characteristics, assesses the recording techniques which can be used to investigate human small bowel motility and reviews the data obtained from small bowel manometry studies in healthy individuals, in patients with motility disorders and in those who have undergone IPAA.

2.2 BACKGROUND

2.2.1 FUNCTIONS SERVED BY SMALL BOWEL MOTILITY

Contractions of the small bowel are complex. They carry out several important functions in both the fed state, when nutrient assimilation takes place and in the fasting state, when nutrients are absent (different contraction patterns in both fed and fasting states are discussed in section 2.4.1). During the fed state, small bowel contractions may assist in the control of gastric emptying, mix intraluminal contents with pancreatic, biliary and enteric secretions, bring intraluminal contents into contact with the absorptive surface of the mucosa and bring about an overall net caudad (downstream) movement. In the fasting state, muscle contractions may prevent disuse atrophy of the smooth muscle cells in the wall of the small bowel. Contractions are also coupled to intestinal secretory

SMALL BOWEL MOTILITY INVESTIGATION TECHNIQUES
SMALL BOWEL MOTOR/ MYOELECTRIC ACTIVITY **Intraluminal manometry** **Serosal electromyography** **Transluminal electromyography** **SMALL BOWEL TRANSIT** **Breath hydrogen analysis** **Scintigraphy**

Table 2.1: Clinical techniques used to investigate small bowel motility.

events and occur periodically, propelling the secretions caudad. Along with digestive secretions, small bowel contractions in the interdigestive phase maintain a low microbial count in the proximal gut. Furthermore, these contractile patterns may also be altered or be absent in certain disease states where clinical signs and symptoms suggest disrupted gut motility. To date two studies have shown that alterations in fasting motility patterns can lead to small bowel bacterial overgrowth (Vantrappen *et al*, 1977; Scott & Cahall, 1982).

2.2 PHYSIOLOGICAL ASPECTS OF SMALL BOWEL MOTILITY

Several basic physiological phenomena underlie contractile activity in the small bowel. These include electrical "slow waves", action potentials, coordination or propagation of motility, the migrating motor complex (MMC) and postprandial motility. Appreciation of these aspects of intestinal smooth muscle activity is important when attempts are made to interpret motor events in the small bowel.

ELECTRICAL "SLOW WAVES"

Slow waves (also referred to as pacemaker potential, basic electrical rhythm or electrical control activity) are rhythmic oscillations of membrane potential of smooth muscle cells. They can be detected by several *in vitro* and *in vivo* techniques. The electrical signals can be recorded by intracellular microelectrodes in single smooth muscle cells *in vitro*, or from groups of smooth muscle cells using extracellular electrodes *in vitro* (see section 4.4) or *in vivo* (see section 2.3). In the small bowel, the intrinsic frequency of the slow wave decreases in a caudad direction. This gradient is thought to enable propulsion of gut contents in all mammals (Phillips, 1988). In humans, the gradient was first documented using electrical recording techniques (Christensen, 1966). The mechanism by which a faster proximal pacemaker in the small bowel captures and drives the more distal small bowel is thought to involve a series of relaxation

oscillators (Sarna *et al*, 1971; Sarna, 1984). However, the cellular mechanisms which regulate this gradient of spontaneous myogenic activty remain unknown.

ACTION POTENTIALS AND SLOW WAVES

The maximum rate at which contractions in different areas of the small bowel occur is governed by the slow wave. When smooth muscle membrane depolarisation occurs, in association with a slow wave, an action potential is recorded. Furthermore, when depolarisation takes place in association with all slow waves in a particular region of the small bowel, this area will contract at its maximum frequency. Indeed, this frequency gradient of small bowel contractions shows the same frequency gradient as the slow wave. However, the small bowel may also contract in response to only some or none of the slow waves. This capacity for the membrane potential (slow wave) to either trigger an action potential or not, enables intestinal muscular contractions to vary in intensity and underlies the subtle motility patterns which exist in small bowel. These aspects are discussed more fully in Chapter 4, Section 4.1. The varying patterns of contractile activity or inactivity in the small bowel are determined by the balance of local factors such as gut contents, extrinsic and intrinsic innervation, as well as neurotransmittters.

COORDINATION OR PROPAGATION OF MOTILITY

As well as a gradient for slow waves along the length of the small bowel there is also a "phase-lag" which results in the slow waves appearing to move in a caudad direction. Slow waves not only determine the pace of contractile events at sites in the small bowel as they pass downstream but also enable mechanical events to be propagated. Stationary contractions in the small bowel move gut contents in both directions simultaneously, whereas propagated mechanical activity causes propulsion of contents. The mechanisms which regulate the degrees of coordination in intestinal smooth muscle are unknown, but

undoubtedly contribute to the wide range of transit rates seen in small bowel. The variations in degrees of coordination and propagation of small bowel motility are most notable in the digestive (postprandial) state.

THE MIGRATING MOTOR COMPLEX

Periodic activity in the stomach and small bowel was first described by Boldyreff in Pavlov's laboratory in 1905 (Wingate, 1981). However, it was not until Szurszewski identified a regular motility cycle in fasting small bowel, using electromyography in conscious dogs (Szurszewski, 1969) that this phenomenon was clearly identified. In the latter study a migrating burst of intense spike activity lasting 90-120 min was detected by a series of electrodes positioned along the gut from duodenum to ileum. A phase lag between each sensor corresponded to the time required for motor pattern propagation and the intense spike activity was noted to alternate with phases of inactivity (quiescence). This phenomenon is now recognised as a universal feature of mammalian small bowel. In electrical terms the interdigestive myoelectrical complex (IDMEC) is a short burst of spiking activity over several minutes during which every slow wave leads to an action potential. The IDMEC migrates along the small bowel with a velocity and periodicity such that when one burst reaches the ileum, another starts in the duodenum. This cycle of 90-120 min has been divided into four phases in the dog small bowel (Code & Marlett, 1975). Human interdigestive motility was classified into four phases in a similar manner to that of Code and Marlett, by Vantrappen and colleagues in 1977 (Vantrappen *et al*, 1977); in recent years Phase IV designation has largely been abandoned since it is often poorly developed. These phases of mechanical activity are summarised in Table 2.2. Phase III of interdigestive motility is known as the migrating motor complex (MMC). For examples see Chapter 3.

FASTING (INTERDIGESTIVE) MOTOR ACTIVITY
PHASE I **Motor quiescence** **PHASE II** **Persistent but irregular motor activity** **PHASE III** **Regular contractions superimposed on every electric depolarisation**

Table 2.2: Normal fasting (interdigestive) motor activity. Phase III (migrating motor complex, MMC) lasts about 10min and migrates slowly down the small intestine before recurring after a period of about 90min.

POSTPRANDIAL MOTILITY

In the postprandial (fed) pattern of small bowel contractions replacement of the MMC occurs with an almost continuous low level of contractile activity (for example see Chapter 3). This makes detailed analysis difficult, and in many cases, impossible. The random patterns of spiking and contractility can persist for several hours and are influenced by both the calorific value of the meal and the nutrient constituents. After a meal the human MMC usually first reappears in the mid-jejunum. At present, little else is known about the organisation of human postprandial motor activity. Unfortunately, many patients with clinical signs of disturbed small bowel motility experience their symptoms *after* eating.

2.3 METHODS OF RECORDING HUMAN SMALL BOWEL MOTILITY

Human gastrointestinal motor activity can be evaluated using one or more of three basic techniques. These comprise cutaneous recordings of myoelectric activity, postoperative serosal myoelectric recordings and intraluminal monitoring.

Electrodes placed on the skin surface to obtain recordings of myoelectric activity are the least invasive of these recording methods. However, in reality their application is limited to the measurement of gastric antral myoelectric activity (electrogastrography, EGG) (Abell & Malagelada, 1988). This technique has no useful role in assessing small bowel motility.

Implantation of serosal electrodes at the time of open surgery enables accurate postoperative recordings of myoelectric activity but is largely restricted in humans because of its invasive nature (Richter & Kelly, 1986; Miedema *et al*, 1992).

Recordings of intraluminal myoelectric and/or contractile activity represent a semi-invasive compromise between the two previously described techniques. Indeed, this is the most widely used technique to evaluate motor

function along the entire length of the human gut. In the upper gastrointestinal tract the subject can be intubated either orally or nasally with a flexible recording catheter; in the colon the recording device is usually positioned with the aid of a colonoscope, while intraluminal measurements in the anorectum are easily obtained from catheters inserted under direct vision. Transluminal electromyography with bipolar electrodes has been used to investigate human small bowel (Fleckenstein, 1978). However, concerns over the safety and reliability of suction, wick and mucosal clip electrodes have limited the application of this technique. Moreover, the myoelectric tracings obtained from intraluminal bipolar electrodes are difficult to interpret and are used less commonly than recordings of contractile activity obtained either with manometric tips or solid state transducers.

The principles underlying manometric assessment of the human small bowel and the manometry techniques which are available to study this region of the gut are discussed below.

2.4 MANOMETRIC TECHNIQUES

2.4.1 PRINCIPLES OF MANOMETRY

The technique of manometry records gastrointestinal tract mechanical activity by detecting and measuring changes in intraluminal pressure caused by gut wall contractions. In basic physics terms, however, a manometer is a device which measures the pressure of fluid or gas in an enclosed chamber. The instrument consists of a U-shaped tube filled with liquid. The unknown pressure of the fluid or gas in the chamber is applied to one limb of this tube and the height of the column of liquid in the other limb above the equilibrium level equals this unknown pressure. To record gut contractility this method has been modified by the use of low compliance open-tip perfused-tube (pneumohydraulic) systems. An alternative and more modern manometry recording technique involves

intraluminal pressure measurements using tube-mounted electronic transducer strain gauges (solid state).

2.4.2 PNEUMOHYDRAULIC MANOMETRY

Pneumohydraulic manometry relies on the perfusion of distilled water at a constant pressure through one or more side openings in an intraluminal recording catheter. When gastrointestinal smooth muscle contracts sufficiently to occlude the lumen at the level of the opening, the flow of water is restricted. Such resistance to perfusate outflow causes an increase in pressure inside the catheter tubing which is in turn measured by a resistive transducer mounted on a diaphragm. The pressure amplitude which is generated in this way is proportional to the resistance to perfusate outflow. This, in turn, depends on the force with which the smooth muscle contracts and on the length of gut segment affected by this contraction. Pneumohydraulic manometry therefore records the force with which the contraction occurs. Contractions which fail to occlude the manometric tip are not recorded with this technique. Similarly, a contraction at one site will produce little or no alteration in intraluminal pressure at adjacent non-contracting sites.

Pneumohydraulic manometry catheters for small bowel recording are multilumen enabling recordings to be made at several different levels. The tubing itself is made of polyvinylchloride which permits bonding of several tubes and also retains flexibility for passage along the gut. The tube dimensions range from inner diameters of 0.75mm to outer diameters of 2.0mm. As a result, perfusion of distilled water through these small lumens is very restricted, with typical infusion rates of 0.5-3ml/min. Such a hydraulic capillary system achieves high fidelity recording of intraluminal pressure at a low infusion rate. A reservoir of water is maintained at a high constant pressure (100mmHg), and is reduced to atmospheric presure by the narrow calibre capillary tubing which has a high resistance to flow. The flow rate is largely unaffected by gut contractions since the pressure in the

manometry tube lumen is always low in comparison with the reservoir pressure. As the infusion system compliance is therefore minimised, the manometer itself becomes the main source of compliance in the pneumohydraulic system. Water-perfused manometry catheters have proved to be reliable and accurate but the accompanying cumbersome apparatus limits this technique to bedside studies. A recent advance, which has overcome this drawback, is the ability to record contractile activity over 24 hour periods with ambulatory solid state manometry systems.

2.4.3 SOLID STATE MANOMETRY

Solid state manometry catheters comprise a fine tube (2.0mm outer diameter) on which are mounted pressure-sensitive electronic strain gauge sensors. Measurements of pressure are relayed from the sensors to a portable electronic recorder. Data from the latter can be downloaded at the end of the study period to a computer for storage, replay and analysis.

Since many motility abnormalities become more apparent during routine activities or over several hours, ambulatory techniques enable important measurements of gut contractile activity to be made in subjects who are under "as near normal" environmental conditions. However, these solid state ambulatory catheters are more expensive than their water-perfused counterparts and are less robust. In recent years they have, nevertheless, been used with increasing frequency in motility studies.

2.4.4 MULTICHANNEL MANOMETRIC RECORDINGS

The small bowel motor functions of mixing and propulsion depend on both contractions at a given location and their spatial relationship with contractions at adjacent sites. Pneumohydraulic and solid state manometry catheters are therefore generally equipped with multiple recording channels in order to obtain meaningful recordings over several sites in the gut. With

multichannel manometry the recording tip/sensors can be spaced far apart to measure motor activity over one or more distinct areas, or instead spaced close together to measure contractions from a short gut segment. Although these two strategies give different types of information on gut motiltity they each record the temporal and spatial organisation of contractions. Temporal organisation refers to the characteristics of contractions at a given anatomical site; these vary with time and include frequency, amplitude and duration. Spatial organisation, on the other hand, refers to the relationship between contractions which occur at adjacent gut sites. The specific characteristics of spatial organisation which are commonly noted in manometry studies include propagation (or non-propagation) of contractions, as well as their direction, velocity and length of propagation.

The temporal and spatial organisation characteristics which have been established in certain patterns of human small bowel motility are reviewed below. These include studies of asymptomatic individuals of patients with certain disease states associated with abnormal small bowel motility and of patients who have undergone IPAA.

2.5 HUMAN SMALL BOWEL MANOMETRY PATTERNS

2.5.1 HEALTH

The spatial and temporal organisation of small bowel contractions may change in response to different ingested nutrients and different motility requirements. For the majority of time, the small bowel mixes ingested food with enteric and exocrine secretions, moving gut contents in a caudad direction. However, occasionally, under certain circumstances, the small bowel has to move luminal contents rapidly in either orad or caudad directions, such as vomiting and mass movements respectively. In addition, the small bowel has to keep its lumen free of residual debris between meals. Each of these functions is carried out by a

different type of contraction. Three categories of small bowel contraction are recognised (see Table 2.3).

INDIVIDUAL PHASIC CONTRACTIONS

The basic unit of contractile activity in the fasted and fed state is the individual phasic contraction. In the human jejunum, individual phasic contractions are spatially and temporally discoordinated when measured from recording sites just 2cm apart (Sarna, 1989). The term "discoordination" refers to the fact that most of the individual phasic contractions in the jejunum vary both in amplitude and frequency and that few, if any, propagate distances of 2cm or more. Although these contractions cause very slow distal propulsion, they enable mixing of the luminal contents to occur with exposure of nutrients to the absorptive surface area of the jejunal mucosa. Occasionally, larger amplitude and longer duration single contractions which sweep over several centimetres are detected in the jejunum. This "individual migrating contraction" may have a role in propelling jejunal contents rapidly over short distances in a caudad direction.

The spatial and temporal organisation of individual phasic contractions in healthy and diseased human small bowel have not been fully characterised. However, this type of contraction may dictate small bowel mixing characteristics and transit rates. Any organisational changes to these contractions may therefore produce absorptive and transit disorders.

ORGANISED GROUPS OF CONTRACTIONS

Two organised groups of contractions are recognised in the human small bowel. These are the migrating motor complex (MMC) and discrete clustered contractions (DCCs)

CATEGORIES OF SMALL BOWEL CONTRACTION
INDIVIDUAL PHASIC CONTRACTIONS **ORGANISED GROUPS OF CONTRACTIONS** **Migrating motor complex** **Discrete clustered contractions** **SPECIAL PROPULSIVE CONTRACTIONS** **Prolonged propagating contractions** **Giant retrograde contractions** **Mini-activity front**

Table 2.3: Categories of small bowel contraction.

Migrating motor complex

As discussed in section 2.2.2, the MMC is the mechanical activity front in phase III of fasting motility. In the human small bowel the MMC is a periodically occurring band of contractile activity which normally originates in the proximal duodenum and migrates caudad to the ileocaecal junction (ICJ). It normally occurs only in the fasting state with a cycle length of 150 min. Cycle length is defined as the time interval between two successive MMCs passing the most proximal manometry port/sensor. At any given site the MMC lasts for a period of 6-10 min and migrates caudad with a velocity of 1-4 cm/min. The effects of eating on the MMC cycle are discussed in section 2.2.2.

Discrete clustered contraction

Discrete clustered contractions (DCCs) are groups of contractions lasting 1-2min which migrate over short distances in a caudad direction. Unlike MMCs, DCCs may occur in both the fasting and fed states, and are characterised by their irregularity and occur in an unpredictable sequence. For this reason DCCs have been difficult to investigate in health and disease states.

SPECIAL PROPULSIVE CONTRACTIONS

In certain physiological circumstances the small bowel is required to propel gut contents in orad or caudad directions. This is achieved by prolonged propagating contractions (PPCs); giant retrograde contractions (GRCs); and mini-activity fronts (MAFs).

Prolonged propagating contractions

The prolonged propagating contraction (PPC) is a large amplitude, sustained contraction which migrates rapidly down the small bowel (>60cm/min). It is about 1.5-2 times larger in amplitude and 4-6 times longer in duration than the phasic contractions seen in the MMC (Sarna, 1987). PPCs are caused by

profound smooth muscle depolarisation which removes it from the control of the slow wave. Once initiated, PPCs migrate uninterruptedly into the terminal ileum and often across the ICJ into the proximal colon. The PPC is highly propulsive as a result of its characteristic large amplitude, long duration and unimpeded caudad propagation. In health, these contractions can occur normally in the terminal ileum where they propel semi-solid material into the colon. PPCs can also be induced by the presence of short-chain fatty acids (SCFAs) in the small intestine. This may act as a reflex control mechanism to prevent reflux of colonic contents into the lower small bowel, since SCFAs are normally found in the colon. At present, the precise function of PPCs in health is unknown. However, they may have a pathophysiological role in abdominal pain and diarrhoea (Kellow & Phillips, 1987).

Giant retrograde contractions

The gaint retrograde contraction (GRC) is one of the motor correlates of vomiting (Sarna, 1989). It originates, just prior to vomiting, in the upper small bowel and is 1.5-2 times larger in amplitude and 2-4 times longer in duration than the phasic contractions of the MMC. The GRC migrates orad at a velocity of 8-10cm/s. In this fashion it evacuates the contents of the upper small bowel into the stomach in preparation for vomiting.

Mini-activity front

A third type of special propulsive contraction can be associated with nausea and vomiting. The mini-activity front (MAF) consists of regular contractions lasting for short periods of 2-3min. Unlike the MMC, the MAF may, instead of migrating caudad, either remain stationary or propagate orad.

2.5.2 MOTILITY DISORDERS

Small bowel motility in humans is affected by a spectrum of conditions. Alterations to the spatial and temporal organisation of specialised small bowel contractions as a result of disease are reviewed below.

MMC patterns in motility disorders

Manometry has been used to correlate MMC characteristics with such motility disorders as diarrhoea or constipation but has failed to define a consistent relationship. The MMC is present in diabetes mellitus (Malagelada *et al*, 1980), Chaga's disease (Oliveira *et al*, 1983), peptic ulcer disease (Miranda *et al*, 1985; Thompson *et al*, 1982), myotonic dystrophy (Nowak *et al*, 1984) and irritable bowel syndrome (Kellow & Phillips, 1987). During prolonged small bowel manometry measurements in IBS, abnormalities in the MMC cycle length and abnormal motor patterns have been noted which correlate with symptoms (Kellow *et al*, 1990; Kellow *et al*, 1992). More recently, in patients with diarrhoea-predominant IBS, manometry has failed to show any abnormalities of fasting small bowel motility (Gorard *et al*, 1994).

In some studies the MMC is always present while in others it is absent in some patients. These studies included investigation of idiopathic intestinal pseudo-obstruction (Sarna *et al*, 1978; Summers *et al*, 1983), scleroderma (Rees *et al*, 1982), and small bowel overgrowth (Code & Marlett, 1975). Attempts to correlate specific MMC characteristics, such as cycle length, duration, direction of migration, velocity and distance of propagation, with motility disorders in certain disease states have been unsuccessful. It seems likely that alterations in these MMC characteristics would have a significant impact on small bowel motility. Indeed, in contrast to animal studies, MMC characteristics are highly variable in normal human subjects (Kellow *et al*, 1986; Kerlin & Phillips, 1982). Further, since MMC are only present in the fasting period, extrapolation of changes in MMC characteristics to small bowel motility in the fed state would be difficult.

Nevertheless, motility disorders of neural or chemical origin may manifest manometrically as failure to disrupt the MMC following a meal or early return of MMC in the postprandial period. In addition, MMCs appearing in the digestive state could alter small bowel transit times.

Discrete clustered contractions in motility disorders

DCCs have been associated with symptoms of abdominal discomfort in patients with IBS (Kellow & Phillips, 1987). These patients were also found to have DCCs which occurred in the fasting period for a greater percentage of time and at a greater amplitude than DCCs in control subjects. DCCs have also been observed in cases of pseudoobstruction and can be induced by administration of both mannitol and cisapride (Read, 1986).

Prolonged propagating contractions in motility disorders

The occurrence of PPCs in proximal small bowel is abnormal and can be associated with abdominal pain (Sarna, 1989; Kellow & Phillips, 1987) while the incidence of PPCs and their site of origin relative to the ileocaecal junction are both increased significantly during nematode infection (*Trichinella spiralis*), (Cowles & Sarna, 1988), and abdominal irradiation (Otterson *et al*, 1988). Both of these factors may contribute to the diarrhoea experienced by patients in such circumstances (Sarna & Otterson, 1989).

2.5.3 ILEAL POUCH- ANAL ANASTOMOSIS

To date three groups of workers have studied small bowel motility in patients *following* IPAA (see Table 2.4). There were, however, considerable differences in both the configurations and types of manometry catheters used in each of these studies, as well as in study design. Nevertheless, interesting data have emerged concerning the motility of the small bowel after IPAA. The findings are described below.

YEAR	AUTHOR	NUMBER OF PATIENTS	DIAGNOSES	POUCH DESIGN	MANOMETRY SYSTEM
1985	Stryker *et al,*	8	UC: 8	8 "J"	Water-perfused
1989	Chaussade *et al,*	11	UC: 10 FAP: 1	4 "S" 7 "J"	Water-perfused
1994	Groom *et al,*	12	UC: 10 FAP: 2	5 "W" 6 "J" 1: Ileoanal Kock	Solid state

Table 2.4: Published studies of small bowel motility in established IPAA patients. For references see Bibliography.

The Mayo Clinic study

Stryker and colleagues used small bowel manometry to assess 8 IPAA patients with a preoperative diagnosis of UC (Stryker *et al*, 1985). The patients had a variable clinical outcome ranging from 2-17 stools per 24 hours, and were compared with 6 healthy volunteers. Follow-up occurred at 4-24 months after pouch surgery. Using a pneumohydraulic manometry system, both small bowel and pouch contractile activity were recorded simultaneously. Small bowel motility was measured with a nine-channel catheter with side-ports at 10,20,30,40,50,75,100 and 125cm from the tip while a second separate three-lumen assembly was placed transanally into the pouch to monitor propagation of proximal small bowel motility. Fasting and fed recordings were made over periods of 20 hours and 6 hours respectively and analysed for the presence of MMCs, PPCs and DCCs.

There were no differences between the patient groups in terms of MMC frequency, duration or velocity of propagation. PPCs had similar durations and velocities in each group. However, PPC distribution differed; in controls, PPCs occurred only in the terminal ileum, but in 3 IPAA patients they were noted at the most proximal recording port at 125cm proximal to the pouch. DCCs occurred in all controls and in over half of the IPAA patients, with similar frequency, duration and velocity values. The Mayo Clinic group failed to show any correlation between motility patterns and frequency of pouch evacuation. Further, no temporal association was found between motility patterns and individual defaecation events.

The French study

Motility of the jejunum was examined in 11 patients and 6 control subjects to investigate whether small bowel motility was altered after IPAA because of mechanical obstruction and/or bacterial overgrowth (Chaussade *et al*, 1989). Pneumohydraulic manometry and D-glucose breath tests were performed in 6

patients with 6 months of pouch function and in 5 patients with 12 months of pouch function. The manometry catheter consisted of four side-holes located at 0,30,60 and 90cm from the tip, with the most proximal port positioned under radiological control at the duodenojejunal flexure. Recordings were made in 11 patients during fasting (4 hours) and in 7 patients after a liquid meal (1 hour). Using D-glucose breath testing, only 2 patients had bacterial overgrowth. MMC frequencies in the fasting period were unchanged between the 2 groups; PPCs were infrequent in the 6 control subjects and were never recorded in the fed period of the study. Four IPAA patients however, had numerous PPCs and 2 of these had evidence of jejunal bacterial overgrowth. Three of these 4 patients had triplicated "S" pouches and 2 required to catheterise the reservoir to achieve defaecation. In the fasting state, DCCs appeared with equal frequency between patients and controls. However, postprandially DCCs were recorded in all 7 IPAA patients (6 of whom had no evidence of bacterial overgrowth), but were not seen in the control group.

The authors concluded that the presence of DCCs could have arisen from a functional obstruction as a result of small bowel anastomosis due to the high pressure zone of the anal sphincters since the same motility pattern was noted in the jejunum of patients with proximal or distal partial small bowel obstruction (Summers *et al*, 1983).

The St Mark's study

The St Mark's group used a solid state ambulatory technique to determine whether small bowel motility was important in determining the frequency of defaecation following IPAA and to examine whether small bowel motility was propagated into the pouch (Groom *et al*, 1994). Both small bowel and pouch motility were compared in 2 groups of patients prospectively selected as having low or high stool frequencies. Twelve patients were studied with a range of 9-108 months pouch function (5 with good functional outcome, 7 with poor functional

outcome). Functional outcome in these patients was inversely related to their 24 hour stool frequencies. The ambulatory small bowel manometry catheter comprised 5 solid state pressure transducers at 20,40,60,80 and 100cm from the distal tip, which was screened fluoroscopically to a position 20-30cm orad from the pouch. Once positioned, a second manometry catheter, with a solid state pressure transducer 1cm from the tip was inserted transanally into the pouch. Patients were studied whilst fully ambulant on an unrestricted diet. At the end of the ambulatory study, anal manometry, pouch-pressure volume and video evacuation pouchography studies were performed to assess anal sphincter function, pouch compliance and reservoir size respectively.

The major finding was that IPAA patients with poor pouch function had a significantly greater number of MMCs than those with good pouch function, during the fasting nocturnal period. Only 2 out of a total of 120 MMCs in the 12 patients studied were propagated from the small bowel into the pouch. DCCs did not propagate into the pouch but in 1 patient PPCs were found to pass into the reservoir. Whilst stool volume over a 24 hour period and radiological pouch size were not significantly different between the patient groups, those with poor pouch function had a reduced maximum reservoir capacity. It was concluded that the differences in the maximum tolerated volume may have been related to changes in pouch sensitivity rather than to reservoir size. Such an alteration in pouch sensitivity in patients with poor functional outcome may have coexisted with the disturbances in nocturnal small bowel motility. Thus, although propagation of coordinated small bowel motility into the reservoir was rare, the results from this study suggested that small bowel motility *orad* to the pouch was related to pouch functional outcome and 24 hour stool frequency.

Although each of the above studies examined *postoperative* patterns of small bowel motility, *preoperative* manometry has never been performed to study small bowel motility in patients awaiting IPAA. The following chapter describes a

pilot study which examined small bowel manometry patterns in prospective IPAA patients who suffered from UC.

CHAPTER 3

SMALL BOWEL MANOMETRY PATTERNS IN ULCERATIVE COLITICS UNDERGOING ILEAL POUCH-ANAL ANASTOMOSIS

3.1 INTRODUCTION

Given that IPAA has been used in the surgical management of UC for some fifteen years, it is surprising that only three small bowel manometry studies of IPAA patients have been reported. The most recent study on this subject and arguably the one which produced the most clinically relevant data was carried out by the St Marks' group who used ambulatory solid state manometry to measure small bowel motility in IPAA patients (Groom *et al*, 1994). This study demonstrated that small bowel motility parameters *orad* to the pouch were related to the pouch functional outcome and 24h stool frequency, but concluded that further studies were required to determine whether these existed *before* reservoir creation.

The main aim of the present study was therefore to assess differences between individuals in terms of small bowel motility patterns in prospective UC patients awaiting IPAA using ambulatory solid state manometry and to establish whether individual small bowel motility profiles correlated with defaecation patterns prior to removal of the inflamed colon and formation of IPAA. A second aim was to evaluate ambulatory small bowel manometry as a potential tool for preoperative investigation of IPAA patients.

3.2 PATIENTS AND METHODS

Patients

The patients investigated by small bowel manometry fell into two categories which are detailed below.

1. Preliminary study

Since small bowel manometry had never been previously used at Glasgow Royal Infirmary a preliminary series of small bowel intubations and manometry

recordings were carried out in three patients with known gastrointestinal motility problems. Their clinical details are listed in Table 3.1.

2. Prospective IPAA patients

Eleven of 12 consecutive IPAA patients consented to undergo ambulatory small bowel manometry over a 10 month period. The preoperative diagnosis was UC in 9 and FAP in 2 patients. Clinical details for these patients are listed in Table 3.2. Two patients with UC had undergone previous subtotal colectomy and preservation of rectal stump for toxic megacolon. Access to age- and sex-matched control subjects was not possible.

Equipment

A 3mm diameter pliable catheter 250cm in length was used to record small bowel motility (Gaeltec Limited, Dunvegan, Isle of Skye, Scotland). The catheter contained six solid state pressure transducers located at 5, 25, 45, 65, 95 and 125cm from the distal tip (Fig 3.1). A central lumen allowed inflation/ deflation of a 7ml balloon tied on to the tip.

Subjects were intubated via the nasointestinal route following an overnight fast. Radiological screening was used to confirm the passage of the catheter through the pylorus. At this point, the balloon was inflated to facilitate caudad migration of the catheter. Regular radiological screening was used to monitor the progress of the catheter and the time taken for it to reach its final tip position in the terminal ileum noted (Fig 3.2). Once in the terminal ileum, the balloon was deflated and the catheter taped to the nostril to maintain its final position (Fig 3.3). The manometry catheter was then connected to a 7-channel portable recorder with 512kBite digital memory (7-MPR recorder, Gaeltec Ltd, Isle of Skye, UK). Pressures were sampled in each channel at a frequency of 8Hz. The recorder was carried in a shoulder holster and had a patient-initiated event marker.

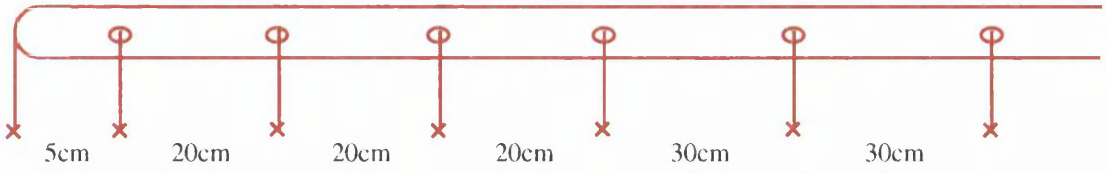
Note: The displays of transducer recordings from these small bowel manometry studies (see Figs 3.5-9) are orientated in the opposite direction to

STUDY	PATIENT	SEX	AGE	DIAGNOSIS	PREVIOUS SURGERY
1	JMcA	F	76	Slow transit constipation	No
2	EH	F	49	Systemic lupus erythematosis	No
3	LG	F	36	Irritable bowel syndrome	No

Table 3.1: Clinical details of patients in preliminary small bowel manometry study.

STUDY	PATIENT	SEX	AGE	DIAGNOSIS	PREVIOUS SURGERY
4	CM	F	29	UC-Chronic	Yes-subtotal colectomy
5	ZS	M	30	UC-Chronic	No
6	JB	M	48	UC-Chronic	No
7	AR	M	24	UC-Chronic	No
8	WF	M	35	UC-Chronic	No
9	RK	M	29	UC-Chronic	No
10	YS	F	15	FAP	No
11	DK	M	29	UC-Chronic	No
12	KH	M	39	FAP	No
13	SB	M	30	UC-Chronic	Yes-subtotal colectomy
14	JB	F	36	UC-Chronic	No

Table 3.2: Clinical details of prospective IPAA patients undergoing small bowel manometry. UC, ulcerative colitis; FAP, familial adenomatous polyposis.



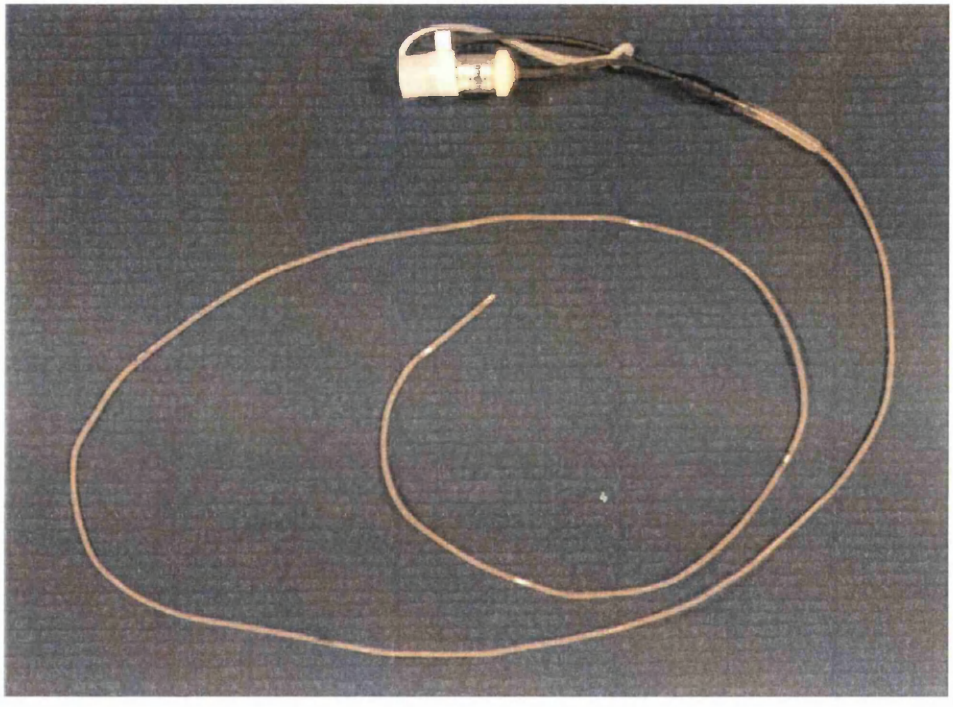


Fig 3.1 Small bowel manometry catheter specifications: diagram shows positioning of six pressure transducers.

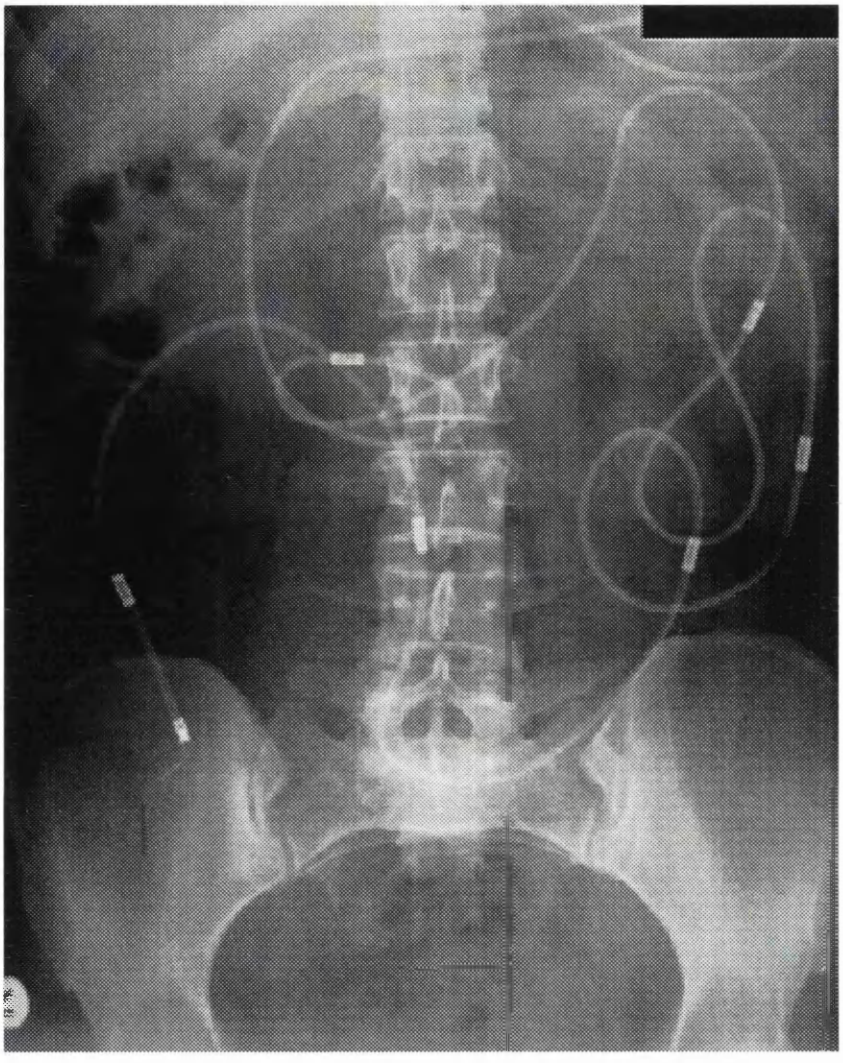


Fig 3.2 Abdominal x-ray showing satisfactory final position with catheter tip in terminal ileum.

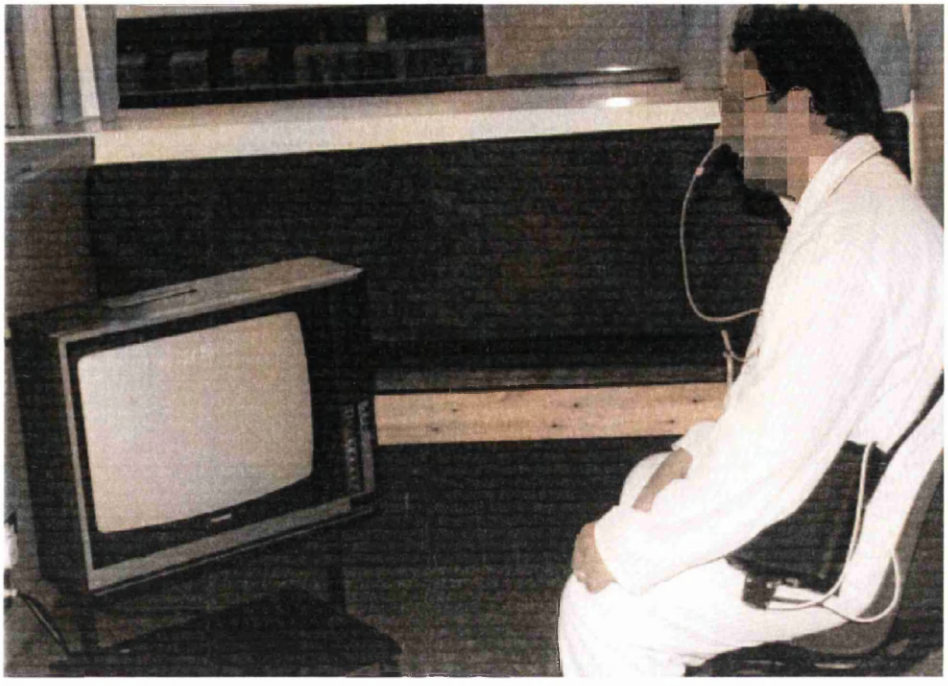


Fig 3.3 Patient undergoing ambulatory small bowel manometry study.

conventional displays of such recordings, due to problems with the initial set-up of the system. The same trace orientation was, however, maintained for the duration of the study period. Data storage and trace analysis were unaffected by this display problem. Fig 3.5 illustrates the orientation of the small bowel manometry traces obtained in this pilot study.

Study conditions

Recording started once the tip was in its final position. Patients were fully ambulant during the study and on an unrestricted diet. Diaries of activities were kept by each individual. The patients were also asked to record times of eating and drinking, defaecation, passage of flatus and micturition. Topical pharyngeal anaesthesia was applied in a few situations where the patient complained of throat discomfort.

Data analysis

The data were downloaded to a Viglen IV/25 personal computer for analysis which was performed manually by reviewing all records in their entirety on the monitor screen. For analysis, each study was divided into diurnal and nocturnal study periods. The diurnal period was defined as the interval between waking and going to bed (usually 2200h). The nocturnal period was taken from 2200h to 0700h, and during this period the subjects fasted.

Small bowel manometric activity was analysed to define the following periods:

1. phase I- quiescence (absence of motor activity),
2. phase II- motor activity characterised by irregular contractions,
3. phase III- regular contractile activity at a frequency of 11-13/min, lasting at least three min. Phase III activity represents the migrating motor complex (MMC). Propagation of motor activity was defined as caudad progression as measured in at least 3 transducers. Non-propagating motor activity was defined as

contractile events recorded at only 1 transducer (acknowledging, however, that these may have propagated over a short distance).

During phase II activity, the traces were analysed for the presence of DCC (rhythmic bursts of phasic contractions of duration shorter than three minutes and occurring with a much greater periodicity than phase III contractions. The presence of PPCs were also noted. These were defined as single pressure waves, propagated over at least three sites, and lasting 20-30s or more with pressures up to 100mmHg.

In the nocturnal study, the durations of phases I, II and III were measured, in addition to the MMC cycle length. The latter was defined as the interval between 2 successive phase III activity fronts passing the most proximal transducer. The duration and extent of caudad propagation of each MMC activity front was also measured.

3.3 RESULTS

1. Preliminary study

Several technical problems were encountered during the learning phase of small bowel manometry and these are summarised in Table 3.3.

A total of 29h of recording (14h nocturnal, 15h diurnal) were obtained from patients 2 and 3. Because of the position of the catheter tip in patient 1 recording was abandoned. Although a satisfactory terminal position of the small bowel manometry catheter was achieved in patient 2, her disease process (systemic lupus erythematosis, SLE) made identification of MMC activity impossible. This in itself was an interesting feature which would merit further investigation. Conclusive evidence of MMC activity and caudad progression was seen in patient 3. However, because this individual required intravenous metoclopramide, itself a prokinetic agent which facilitates gastric emptying, to enable passage of the catheter through the pylorus after 24 hours of unsuccessful intubation, the traces were declared unsuitable for meaningful analysis. A further problem with this recording was the

STUDY	PATIENT	INTUBATION PROBLEMS
1	JMcA	Double loop through pylorus prevented caudad migration
2	EH	48 hours to negotiate catheter tip to terminal ileum
3	LG	Intravenous metoclopramide required to pass catheter tip through pylorus

Table 3.3: Intubation problems encountered in preliminary small bowel manometry study.

failure of the sixth transducer to detect pressure changes (see trace in Fig 3.5). Subsequent testing by the manufacturer confirmed the presence of water in the catheter assembly.

2. Prospective IPAA patients

One technical problem arose during small bowel intubation in the prospective ileoanal pouch patients. This occurred because of catheter impaction at the duodenojejunal flexure in study 4 which precluded further advancement. Interestingly, at subsequent laparotomy and IPAA, dense adhesions were noted around this area which were related, presumably, to this patient's previous emergency subtotal colectomy for fulminant UC.

The median length of time required to negotiate the catheter to its final resting position in the terminal ileum in the remaining 10 patients was 7.5h (range 6-48h). Unfortunately, patient 11 demanded the catheter to be withdrawn shortly after successful intubation. Successful ambulatory small bowel manometry recordings were made in the remaining nine patients (8 UC, 1 FAP). The total recording period for these patients was 140h (81h nocturnal); in four cases (patients 10,11,13,14), diurnal recording was not carried out at the request of the individuals, who were uncomfortable and felt unable to continue with the study following the nocturnal recording period. Diurnal small bowel manometry recordings were made, however, in the other five subjects (patients 5-9) for a total of 59h.

The small bowel manometry pilot study is summarised in Fig 3.4. Trace orientation and examples of motility patterns are shown in Figs 3.5- 3.9.

Fasting nocturnal small bowel motility (2200h-0700h)

The data for the nocturnal study are summarised in Tables 3.4 and 3.5. Clearly, a spectrum of small bowel activity was noted during the fasting period in the nine patients studied.

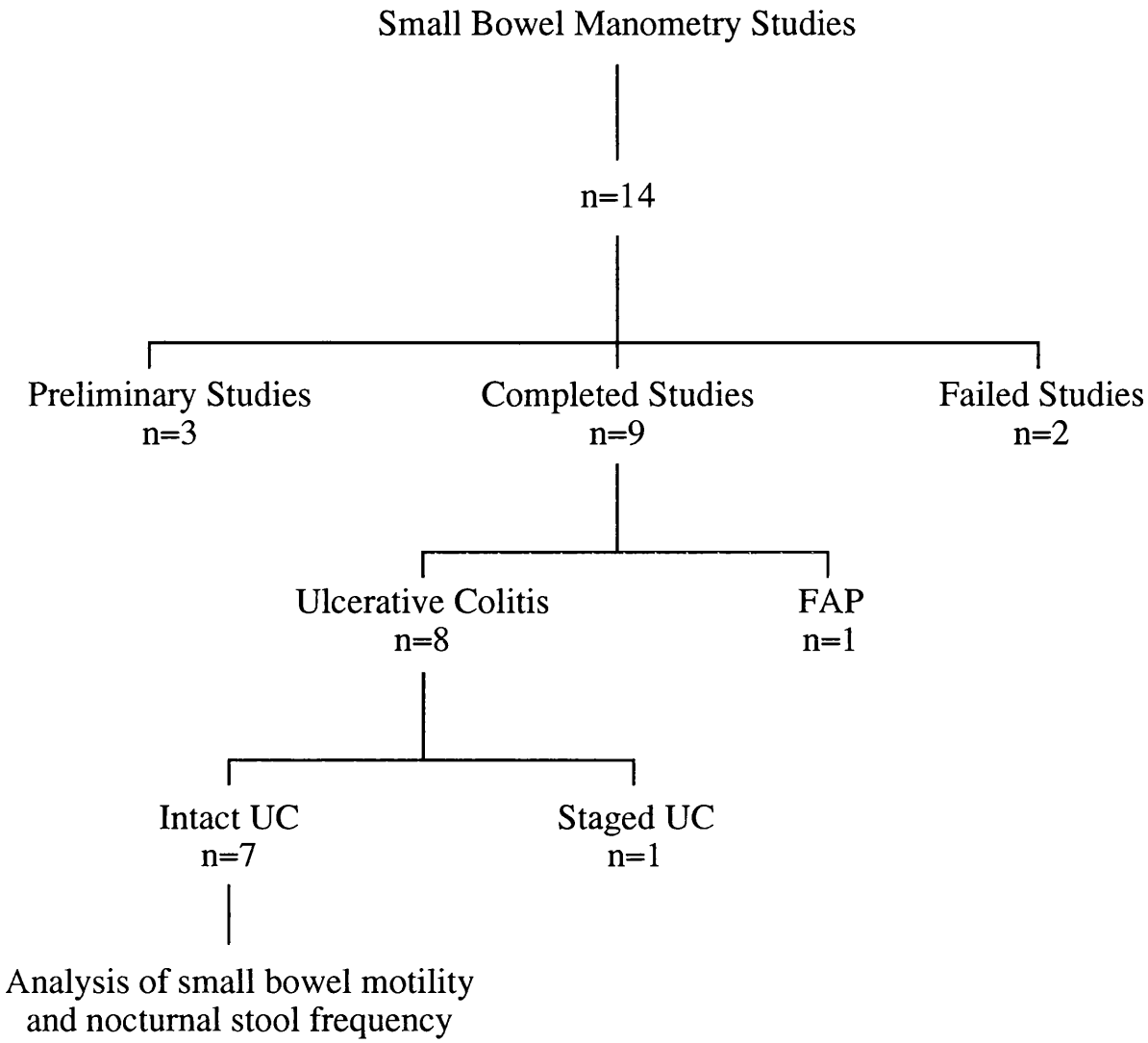
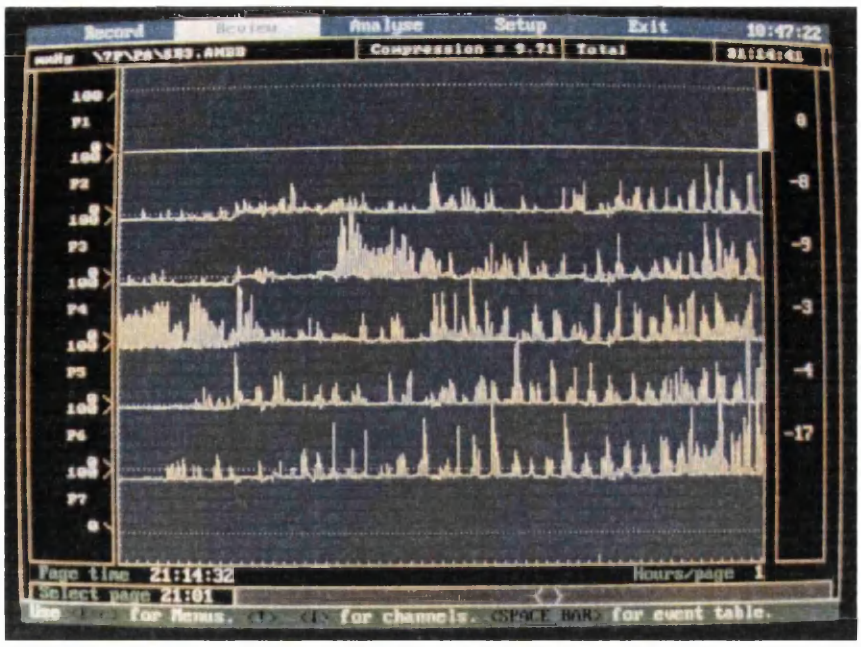
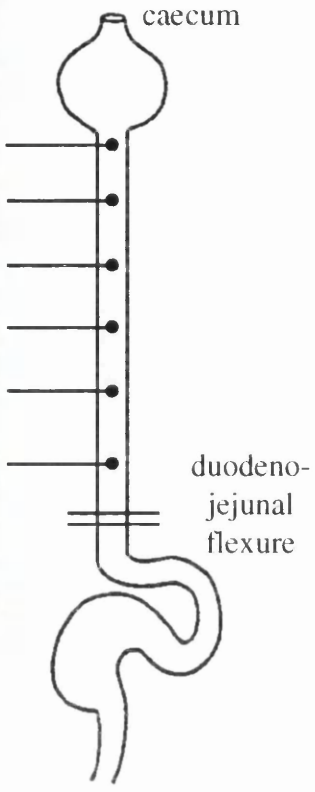


Fig 3.4 Flow chart of ambulatory small bowel manometry studies in 14 patients.
FAP, familial adenomatous polyposis; UC, ulcerative colitis.





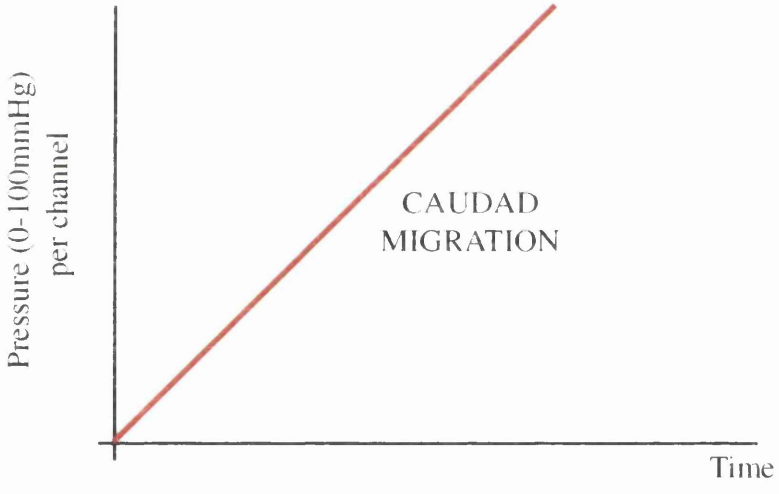


Fig 3.5 Anatomical orientation and interpretation of manometry recording. Note absence of signal in top channel because of transducer failure.

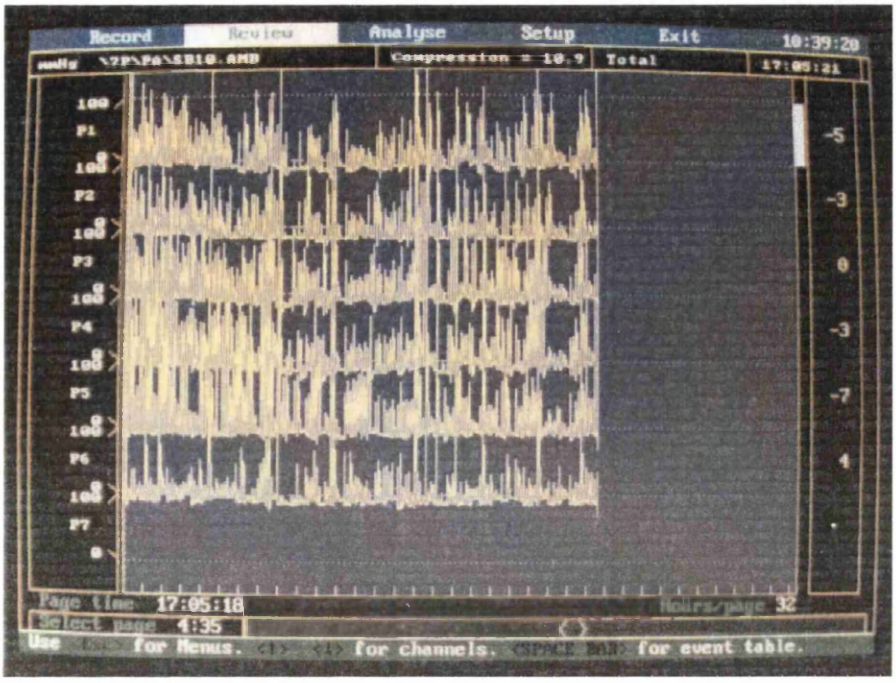


Fig 3.6 Compressed 24 hour small bowel manometry recording.

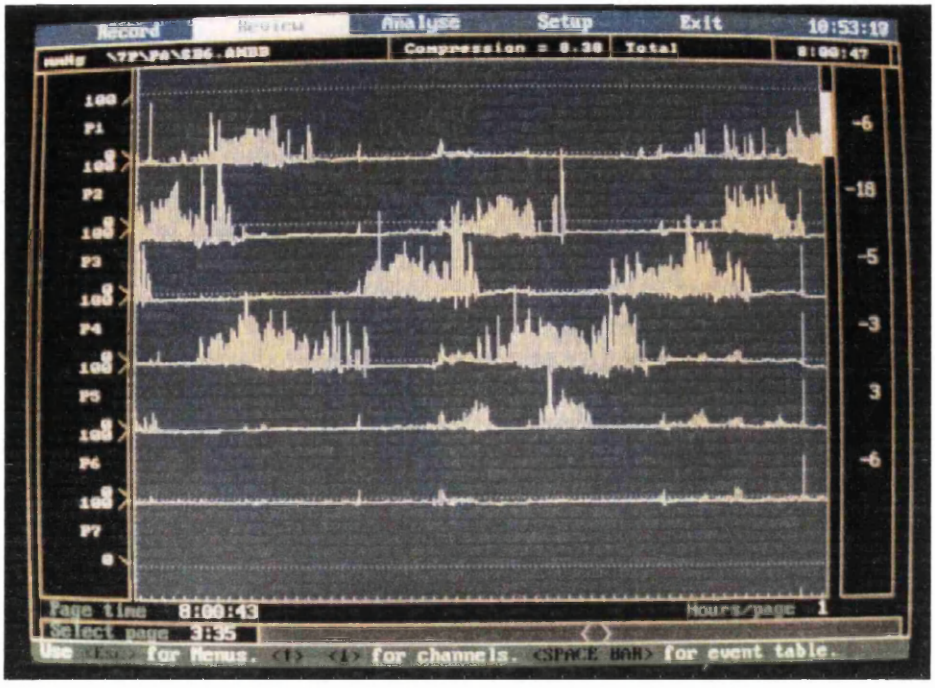


Fig 3.7 Migrating motor complexes: screen width 60 min.

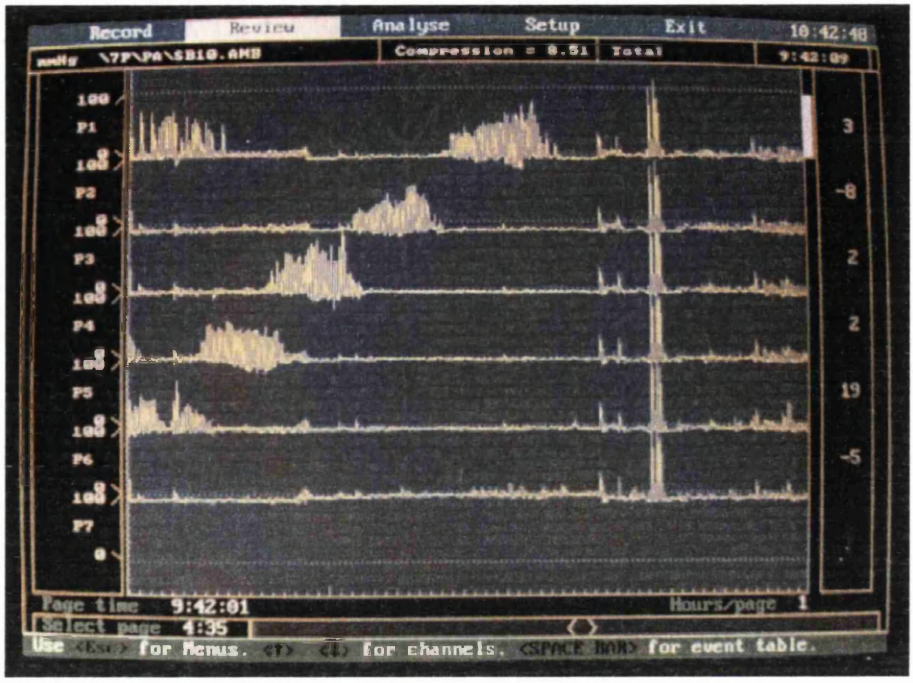


Fig 3.8 Defaecation following migrating motor complex. Note synchronous pressure effects detected in all 6 channels.

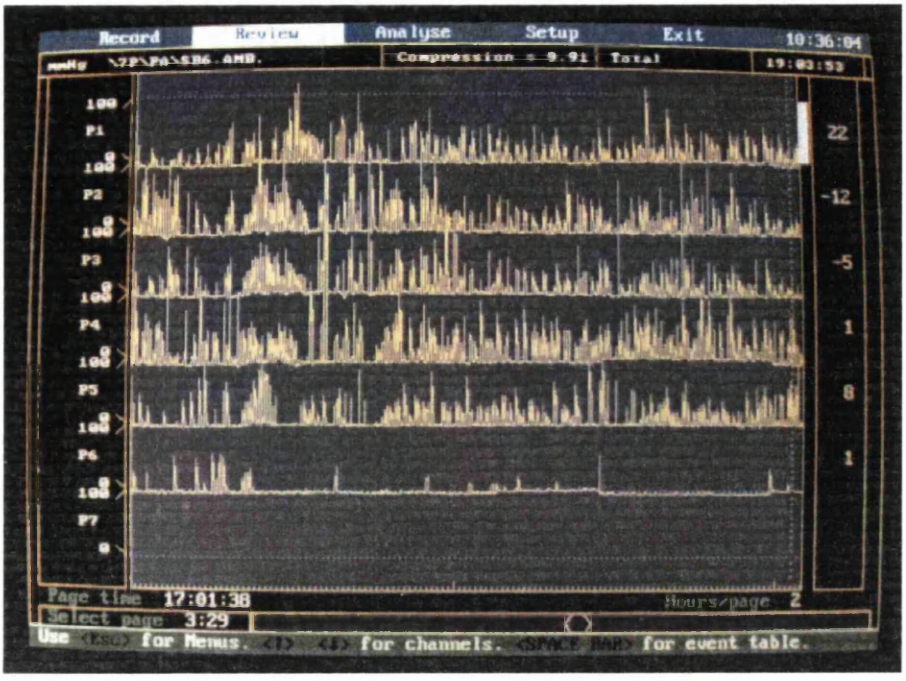


Fig 3.9 Postprandial small bowel manometry trace (diurnal study).

NOCTURNAL SMALL BOWEL MOTILITY PARAMETERS	UC n=8	FAP n=1
MMCs		
Propagating MMCs	**54**	**5**
Non-propagating MMCs	**18**	**0**
Total number of MMCs	**72**	**5**
Number of MMCs per subject	**8 (4-15)**	**5**
Duration of MMC cycle (min)	**62 (18-190)**	**27**
Velocity of MMC cycle (cm/min)	**3 (1-5)**	**3**
INTERDIGESTIVE PHASE DURATION		
Phase I (min)	**80 (26-139)**	**33**
Phase II (min)	**19 (7-33)**	**10**
Phase III (min)	**10 (6-15)**	**9**

Table 3.4: Nocturnal small bowel motility in prospective IPAA patients. UC, ulcerative colitis; FAP, familial adenomatous polyposis; MMC, migrating motor complex. Values expressed as median (range).

NOCTURNAL MMC FEATURES	UC n=8	FAP n=1
Propagating MMCs	54	5
Number of MMCs reaching distal transducer	50	0
Most proximal sensor at which MMC was first detected:		
P1- Terminal ileum	0	0
P2	0	0
P3	8	0
P4	11	0
P5	18	2
P6- Duodenojejunal flexure	17	3
Total number of non-propagating MMCs	17	
Location and number of non-propagating MMCs recorded		
P1- Terminal ileum	4	
P2	3	
P3	2	
P4	5	
P5	1	
P6- Duodenojejunal flexure	2	

Table 3.5: Features of nocturnal migrating motor complexes (MMCs) and other propagating contractions in prospective IPAA patients. UC, ulcerative colitis; FAP, familial adenomatous polyposis; PPC, prolongred propagating contraction; DCC, discrete clustered contraction.

There was no evidence of non-propagating MMCs in study 10 (FAP, female, age 15yrs). Otherwise, the motility parameters measured for this patient fell within the ranges encountered in the UC patients.

Interestingly, the highest total number of MMCs were in study 13 (UC-previous subtotal colectomy, age 30yrs), which had the only patient in the series with an end ileostomy.

Diurnal small bowel motility (0700h- 2200h)

The diurnal motility recordings for the five UC patients are summarised in Table 3.6.

Much of the recording time was spent in the postprandial state (see Fig 3.9). Consequently, trace analysis to determine MMC cycle length was impossible.

Relationship of small bowel motility with nocturnal defaecation patterns

Evaluation of the relationship between nocturnal defaecation patterns and motility was possible in the seven intact UC patients. The patient in study 3 was excluded from these calculations since it was not possible to document accurately the defaecation events from his end ileostomy. Data for these patients are summarised in Table 3.7.

There were no statistically significant relationships amongst the nocturnal defaecation patterns with respect to the total number of MMCs, propagating MMCs, non-propagating MMCs, MMC cycle length, MMC mean duration , MMC mean velocity, number of PPCs, or number of DCCs and nocturnal stool frequency. Individual relationships between motility parameters and nocturnal stool frequency were calculated using Spearman's rank correlation, r_S (Kirkwood, 1988). The respective correlation coefficients for each motility parameter are listed in Table 3.8. The phase III motility parameters of the 7 intact UC patients studied nocturnally and the distribution of stool frequency are shown in Table 3.9.

DIURNAL MMC CHARACTERISTICS	
Total diurnal recording period (h)	**59**
Hours per patient	**12 (10-13)**
Total number of MMCs	**19**
Number per patient	**3.8 (1-7)**
Velocity of propagation (cm/min)	**2.64 (1.09- 3.65)**
Time to establishment of MMC post-cibal (min)	**314 (212-370)**
Other propagating contractions	
PPC	**2 (0.7-3.5)**
DCC	**7 (5-11)**

Table 3.6 Migrating motor complex (MMC) and other propagating contraction characteristics of 5 patients in the diurnal study. PPC, prolonged propagating contraction; DCC, discrete clustered contraction. Values expressed as median (range).

NOCTURNAL MOTILITY PARAMETERS	
Nocturnal stool frequency	**2 (0-7)**
Total MMCs	**7 (4-12)**
Propagating MMCs	**6 (3-9)**
Non-propagating MMCs	**3 (0-3)**
MMC cycle length (min)	**41 (9.5-190.5)**
MMC duration (min)	**8.8 (5.9-15.3)**
MMC mean velocity (cm/min)	**2.64 (0.63-4.94)**
Nocturnal PPCs	**1 (0.86-2)**
Nocturnal DCCs	**5 (4-6)**

Table 3.7 Nocturnal motility parameters in 7 intact patients with ulcerative colitis. MMC, migrating motor complex; PPC, prolonged propagating contraction; DCC, discrete clustered contraction. Values expressed as median (range).

SMALL BOWEL MOTILITY PARAMETER	CORRELATION COEFFICIENT, r_S, WITH NOCTURNAL STOOL FREQUENCY	P
Total number of MMCs	**0.26**	**n.s**
Propagating MMCs	**0.22**	**n.s.**
Non-propagating MMCs	**0.36**	**n.s**
MMC cycle length	**-0.54**	**n.s.**
MMC mean duration	**-0.50**	**n.s**
MMC mean velocity	**0.43**	**n.s.**
Mean number of PPCs	**-0.62**	**n.s**
Mean number of DCCs	**-0.36**	**n.s.**

Table 3.8 Correlation coefficients for motility parameters and nocturnal stool frequency in 7 intact patients with ulcerative colitis. r_S, Spearman's rank correlation coefficient; P, probability; n.s., not significant.

NOCTURNAL INTERDIGESTIVE MOTILITY PARAMETERS AND STOOL FREQUENCY	
Interdigestive Cycle	
Phase I duration (min)	**81.7 (26.4-139)**
Phase II duration (min)	**13 (6.8-52.5)**
Phase III duration (min)	**8.78 (5.9-15.3)**
Total number of stools	**14**
Number of defaecations during each motility phase (% total)	
Phase I	**12 (86%)**
Phase II	**1 (7%)**
Phase III	**1 (7%)**

Table 3.9 Interdigestive motility parameters of the 7 intact ulcerative colitics studied nocturnally and the distribution of stool frequency during the study period. Results expressed as median (range).

The nocturnal small bowel motility parameters recorded for the 2 other patients (studies 10 and 13), are summarised in Table 3.10.

3.4 DISCUSSION

Ambulatory small bowel manometry is not easy to perform; several factors contribute to the difficulty of this technique. Firstly, intubation of the entire length of the small bowel, relying on gravity and peristalsis to negotiate the catheter into position, is a tedious and unpleasant process which may be affected by catheter-related or anatomical problems. In the preliminary study, looping of the catheter through the pylorus prevented further caudad migration and intubation of the small bowel had to be abandoned (study 1). Similarly, in the study of prospective IPAA patients 1 individual (study 4) had a failed intubation because the catheter would not pass the duodenojejunal flexure. This was later discovered to be due to dense adhesions.

Another problem with the technique is the fragility of the catheter. The two most common types of damage caused during intubation are crushing of the transducers by the patient's teeth if looping occurs in the mouth during insertion; and lacerations, again by inadvertent biting during catheter insertion, to the silicone sleeving of the assembly which enables water to seep into both the tubing and transducers (as experienced in study 3). Necessary catheter repairs are also expensive.

In addition to the costs of the catheter (in this sudy one catheter at discounted 1993 prices cost £3000), further expense is incurred with the need for radiological screening. An average of 8 sessions of screening (range 3-12) were required for the 11 prospective IPAA patients. This also carries a small amount of radiation exposure to both the patient and clinical staff. While ambulatory small bowel manometry could be performed on an out-patient basis, in practice patients

NOCTURNAL MOTILITY PARAMETERS	Study 10: FAP	Study 13: Staged UC
Total MMCs	5	15
Propagating MMCs	5	12
Non-propagating MMCs	0	3
MMC cycle length (min)	26.8	21
MMC mean duration (min)	8.8	8.83
MMC mean velocity (cm/min)	2.82	4.34
Nocturnal PPCs	1	6
Nocturnal DCCs	2	5

Table 3.10 Motility parameters of other patients studied during nocturnal period. FAP, familial adenomatous polyposis coli; UC, ulcerative colitis. MMC, migrating motor complex; PPC, prolonged propagating contraction; DCC, discrete clustered contraction.

prefer to remain in hospital during the recording period. The cost of hospitalisation for periods of up to 3 days must also be taken into account when assessing this technique.

Interpretation of the data from small bowel manometry studies can be time-consuming. Analysis of 10 traces in this study took 60 hours. Although computer programmes have been devised to assist with the analysis, the traces in the present study and in all the other published series of small bowel manometry in IPAA patients were analysed manually.

Because of the associated difficulties with small bowel manometry large numbers of patients have not been recruited to this or previous IPAA patient studies. The lack of patient numbers compromises the validity of statistical tests applied to study data.

In the present study, a control group of patients, or more prospective FAP patients awaiting IPAA would have been helpful to permit comparisons with the UC recordings. Access to control (volunteer) subjects, which would have entailed remuneration, was not an available option. Furthermore, the number of FAP patients awaiting IPAA, who were studied, represent the total number of FAP pouches which were constructed in a 12 month period at a tertiary referral centre. Thus, in practical terms, recruitment of either control subjects or more FAP patients was not possible during the study period.

The main area of investigation in the present study was the nocturnal period. This was useful for two reasons. Firstly, it has been validated as a reliable time period to assess fasting motility patterns (Kumar *et al*, 1990). Secondly, the overnight stool pattern is a major determinant of long-term functional outcome (see Appendix). It was therefore important to investigate whether a relationship existed between patterns of fasting motility and nocturnal stool frequency in prospective IPAA patients. What conclusions can therefore be drawn from the data obtained? Clearly, the measured motility parameters showed no correlation with stool frequency in the 7 UC subjects studied. This answers in part, the main question

raised by the St Mark's study (Groom *et al*, 1994), i.e. do small bowel motility profiles which correlate with postoperative stool patterns in IPAA patients with good and poor functional outcomes exist before pouch surgery?

The differences in the number of MMCs generated between individuals as well as cycle duration and MMC velocity are well documented (Groom *et al*, 1994). However, in the present study the lack of correlation between motility parameters and nocturnal stool frequency may be due to the fact that the colon was *in situ*. Thus, any variation in small bowel activity may be dampened by the absorptive and reservoir capacity of the colon. When the colon is excised and IPAA performed, this may reveal the effect of inter-individual variation in small bowel behaviour with respect to stool frequency.

Prior to the St Mark's study (Groom *et al*, 1994) small bowel motility patterns had been implicated with different patterns of defaecation. In 1990, Kellow and colleagues performed 24 hour small bowel ambulatory manometry studies in 12 patients with irritable bowel syndrome (IBS) and compared these results with those from 8 healthy controls (Kellow *et al*, 1990). Diarrhoea-predominant IBS patients had significantly more frequent small bowel MMCs than control subjects.

Since, in the present study, no statistically significant correlations have been demonstrated to exist between small bowel motility parameters and stool frequency in preperative IPAA patients, the motility changes noted in patients with good and poor functional outcomes may have been related to functional differences in the actual pouches. These differences in frequency of pouch evacuation could in turn feed back to the small bowel proximal to the pouch via intrinsic neural pathways to modify its behaviour. Unfortunately, in the St Mark's patient group (Groom *et al*, 1994), reservoir design varied. Although the total volume and radiological size of the reservoirs in the good and poor functional groups were similar on performing pouch pressure-volume studies and video evacuation pouchography, differences were noted in the respective maximum tolerated pouch

volumes. Those authors concluded that such differences were possibly related to altered pouch sensitivity rather than pouch size. A postoperative investigation of the 7 intact UC patients used in the present study, with further small bowel manometry combined with pouch-pressure volume measurements, stool volume and pouch sensitivity recordings would be helpful in establishing whether this postulated modification of small bowel motility by the pouch actually takes place. The 7 patients in this study would have the added advantages of having been standardised in terms of diagnosis, pouch design and absence of the effects of previous abdominal surgery. In the St Mark's study (Groom *et al*, 1994), however, the reservoirs of the 12 patients (10 UC, 2 FAP) comprised five "W" and six "J" pouches, with one ileoanal Kock reservoir.

While the two other studies of small bowel motility in patients following IPAA failed to show any difference in MMC frequency, neither categorised the pouch subjects in terms of clinical function. In the French study (Chaussade *et al*, 1989) only jejunal motility was considered and the study period lasted only 4 hours. This was an inadequate time period in which to detect differences in small bowel motility (Groom *et al*, 1994). This study also looked at a heterogeneous group of pouch patients comprising 11 patients (10 UC, 1 FAP) with a mixture of 4 "S" and 7 "J" reservoirs. Stryker *et al* recorded both jejunal and ileal motility for a longer time period, although non-ambulant water-perfused manometry was used (Stryker *et al*, 1985). In this study the periodicity and other parameters of the MMC were not significantly different between patients and control healthy sunjects. The Mayo Clinic study (Stryker *et al*, 1985), was also superior to the French study (Chaussade *et al*, 1989), since it examined patients with the same original disease (8 UC), who underwent a standardised pouch operation (Beart *et al*, 1982).

If one assumes that IPAA formation modifies proximal small bowel motility, it would be helpful to investigate the motility characteristics of the terminal ileum used to fashion the pouch. This could be achieved by looking at the *in vitro* characteristics of a small portion of ileum at the time of pouch

construction. Alternatively, pouch motility could be investigated in an animal model. However, this has the disadvantage that there is no satisfactory animal model which reproduces the local and systemic effects of chronic UC.

While manometry has been used to investigate the motility characteristics of the terminal ileum (Hammer *et al*, 1993), the costs of manufacturing special catheters and problems intubating the most inaccessible area of the gastrointestinal tract made this an unattractive option, within the financial and practical constraints of this thesis. From the outset, *in vitro* analysis of ileum obtained at the time of pouch construction seemed to be a more useful and cheaper way of studying small bowel motility in UC patients undergoing IPAA.

The second section of this thesis deals with a series of experiments which were carried out in an attempt to characterise certain aspects of ileal motility. As a prelude, the following chapter reviews the literature relating to the aspects of motility which were investigated *in vitro* in samples of ileum obtained at the time of IPAA formation.

CHAPTER 4

SMALL BOWEL MOTILITY - *IN VITRO* ASPECTS

4.1 INTRODUCTION

From the concluding remarks of the preceding chapter *in vitro* motility studies may represent a more practical and cheaper way of studying human small bowel motility characteristics in UC patients undergoing IPAA. However, unlike the vast amount of literature which exists on the *in vitro* small bowel motility characteristics of various laboratory animals (see Furness & Costa, 1987) there is very little information available on the *in vitro* motility characteristics of human small bowel. This is, in part, due to ethical constraints regarding human tissue research. Any work which is undertaken on human small bowel *in vitro* must therefore target specific areas which are both suitable and practical to investigate. With these factors in mind, preliminary *in vitro* studies were undertaken in UC patients undergoing IPAA (see Chapter 5).

As a precursor, this chapter therefore reviews the relevant background areas which underpin the *in vitro* motility experiments described in Chapter 5. These areas include: the properties of spontaneous gastrointestinal pacemaker activity, the pharmacological characteristics of the human small bowel in health and inflammatory bowel disease, the nonadrenergic, noncholinergic (NANC) inhibitory control of gastrointestinal motility and the role of the L-arginine-nitric oxide pathway in NANC transmission in gastrointestinal smooth muscle.

4.2 SPONTANEOUS PACEMAKER ACTIVITY IN SMALL BOWEL

Small bowel manometry can determine gross patterns of fasting motility (see Chapter 3), but cannot accurately document segmental or peristaltic activity. For practical and ethical reasons it would be impossible to assess *in vitro* the motility characteristics of the human small bowel. Thus, the only segment of small bowel which is potentially available for study in patients with UC undergoing IPAA is the most distal edge of terminal ileum. One way to examine motility, in such a limited quantity of tissue would be to use either extra- or intracellular recording techniques to measure both these electrical and mechanical events which

characterise the oscillatory contractions seen in mammalian intestinal smooth muscle (see Bolton, 1989). Indeed, this would be an obvious starting point in examining the basic *in vitro* motility characteristics of any intestinal smooth muscle preparation before proceeding to studies of nerve-evoked and receptor-evoked responses, The following is a brief overview of the electrophysiology, origin and function of spontaneous activity in the small bowel of several species, including human.

The two transient electrical phenomena which are seen in gastrointestinal smooth muscle are the action potential and the slow wave (see Fig 4.1). While the action potential (spike burst) generally lasts for, at most, several milliseconds the slow wave can persist for several seconds and does not carry the membrane potential to such positive values as the action potential does. Spike bursts of action potentials are generally superimposed on the crests of slow waves and are not usually discharged in the intervals between slow waves when the membrane potential is at its most negative. The action potential consisits of an upstroke (depolarizing phase) followed by repolarization to a potential that may be more negative than the level at which it originally arose (afterhyperpolarization). The nature of the ionic species responsible for the upstroke and downstroke of the spike have been investigated in several species (see Bolton, 1989). In general, the upstroke of the action potential is caused by potential-sensitive voltage dependent Ca^{++} channels operating i.e conducting Ca^{++}. The downstroke (repolarization) is probably due to a vigorous increase in K^{+} conductance. The ionic basis of slow waves, on the other hand, is less clear and various hypotheses suggesting both voltage-dependent and metabolic components have been proposed. Regular spontaneous contractions observed in intestinal smooth muscle are presumed to indicate action potentials and/or slow wave discharge, but slow wave discharge alone, or in the absence of action potential discharge may be associated with weak or no contractions (Bolton, 1989).

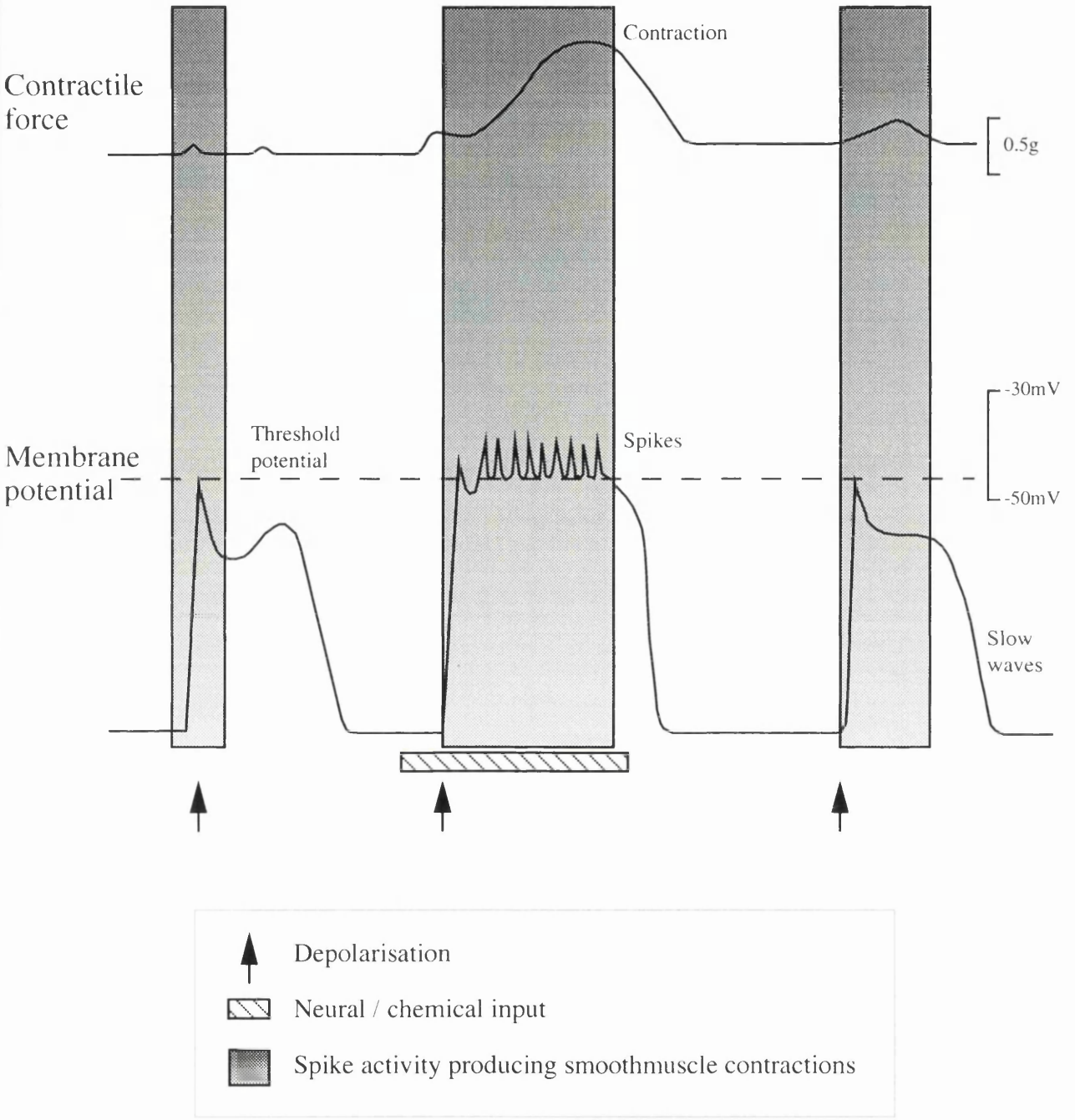


Fig 4.1 Transient electrical phenomena seen in gastrointestinal smooth muscle.

Anatomically, the upper duodenum has the highest intrinsic slow wave discharge frequency and this appears to decline caudad (Diamant & Bortoff, 1969). In this way a gradient of slow wave frequencies exist in the small intestine from duodenum to ileum *in vivo*. The origin of the slow wave has in recent years been re-examined. Originally slow waves were thought to originate in the longitudinal muscle (Bortoff *et al*, 1981) and to spread passively to the circular muscle where they either decayed electrotonically (Bortoff *et al*, 1981), or propagated by regenerative amplification (Connor *et al*, 1977). However, intracellular recordings in the longitudinal, inner and outer circular muscle layers of several species, including human, showed that spontaneous slow waves were generated in non-neural cells located between the longitudinal and outer circular muscle layer and by non-neural cells located between the outer and inner circular muscle layers (Hara *et al*, 1986).

Recently, the interstitial cells of Cajal (ICCs), a group of cells in the gastrointestinal tract redescribed in 1893 by the Spanish neuroanatomist Santiago Ramon y Cajal (Cajal, 1893), have been proposed as the cells from which slow waves originate and therefore may act as the pacemakers of gut motor activity (see reviews by Hagger *et al*, 1997; Thuneberg, 1982). They have also been proposed as the intermediaries of the neural control of gut muscular activity. Extensive studies of human small bowel have enabled the locations of ICC to be mapped in the gut wall using a variety of histochemical staining techniques (see sections 5.4.1 and 5.4.3 for discussion). It is interesting to compare the work which located slow wave generation in the muscle layers of human jejunum (Hara *et al,* 1986) with more recent work which has mapped human small bowel ICC location (Hagger *et al*, 1997), (see Fig 4.2). Although the work of Hara and colleagues did not provide direct confirmation, it did at least support the hypothesis that the ICCs generate slow waves in human small bowel.

The slow waves, thought to be generated by ICCs, are thought to determine the segmental contraction pattern of the gut and contribute to peristalsis. Excitatory

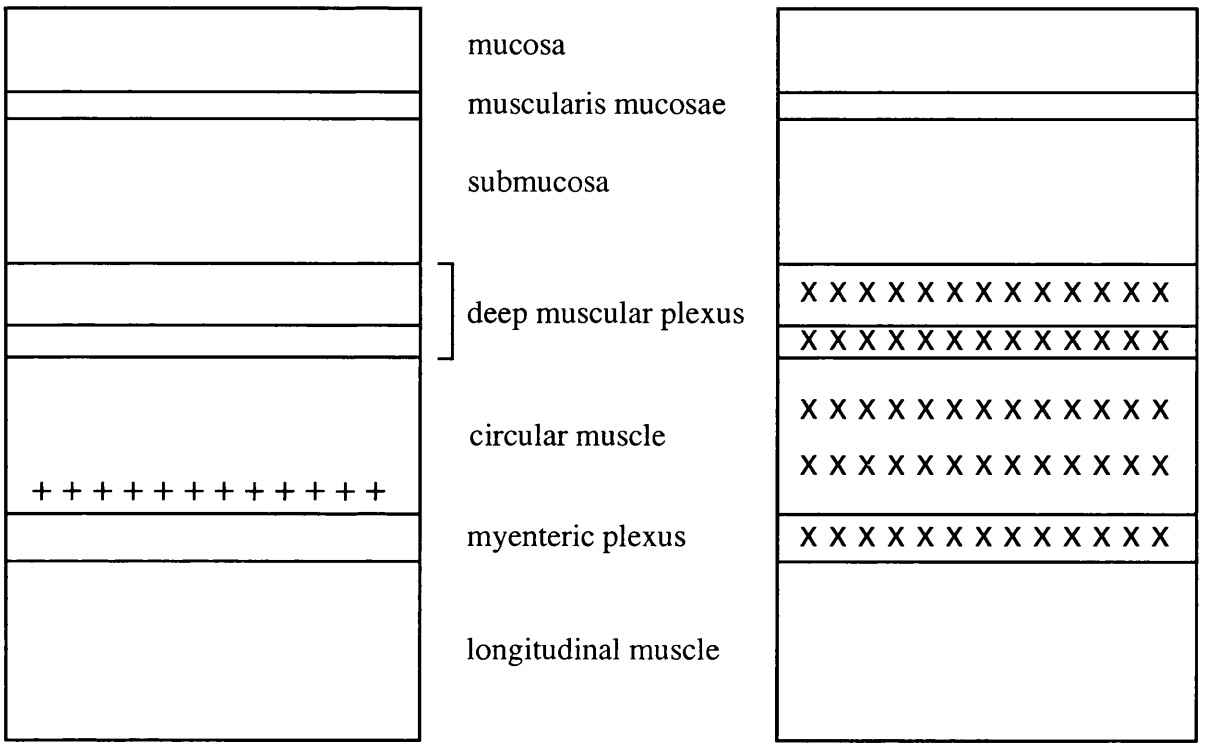


Fig 4.2. Comparative diagrams showing location of slow wave activity in human jejunum muscle layers with immunohistochemical localisation of human small bowel interstitial cells of Cajal (ICC).
Adapted from Hara *et al*, 1986 and Hagger *et al*, 1997.

and inhibitory transmitters and hormones appear to regulate whether slow waves carry spike bursts, and if so, how many. Generally the spike rather than the slow wave is associated with muscle contraction. In some way ascending and descending neural pathways must control whether contraction associated with spike bursts on the crest of a slow wave remains localised (segmental) or is propagated (peristaltic). Although the small bowel of most species shows regular and continuous slow wave activity, peristaltic activity is less evident than segmental contractions (Bolton, 1989). As yet, the involvement of nervous activity in determining this pattern remains unestablished. Whilst slow wave activity in human small bowel has been studied by Hara and colleagues (Hara *et al,* 1986), the spontaneous activity characteristics of ileum in patients with UC remains unknown.

4.3 PHARMACOLOGY OF HUMAN ILEUM

A careful review of the literature has revealed 2 abstracts, a brief report and 5 papers which have been published on the pharmacological characterisitics of human ileum in the last 30 years (1964-1994), see Table 4.1.

Fishlock reported the contractile effect of 5-hydroxytryptamine (5HT) on the circular muscle of the human ileum which was abolished by lysergic and diethylamide (LSD) and partially blocked by hexamethonium and morphine (Fishlock, 1960). One year later, Bennett demonstrated that ACh elicited contraction of both the longitudinal and circular muscle layers of the human ileum via muscarinic receptors, while the sympathomimetic amines (phenylephrine, noradrenaline, adrenaline and isoprenaline) relaxed both muscle layers and inhibited spontaneous contractions (Bennett, 1965). The contractile effects of exogenously applied histamine were also noted.

In 1980 Shirazi and colleagues published an abstract reporting the *in vitro* mechanical activity of transverse and longitudinal muscle strips from human terminal ileum (Shirazi *et al*, 1980). They found no differences in mechanical activity between the two muscle layers in terms of amplitude, frequency or resting

YEAR	AUTHOR(S)	TOPIC	PATIENT STATUS
1964	Fishlock (abstract)	Effects of 5HT on circular muscle	Control
1965	Bennett (paper)	Basic characteristics of circular/ longitudinal muscle layers	-
1980	Shirazi *et al,* (abstract)	Mechanical activity of circular/ longitudinal strips	Control
1986	Hara *et al,* (paper)	Slow wave properties	Control
1989	Maggi *et al,* (paper)	Effects of EFS and exogenous neuropeptides on longitudinal muscle	Control
1990	Maggi *et al,* (paper)	Effects of EFS and exogenous neuropeptides on circular muscle	Control
1991	Maggi *et al,* (paper)	Effects of NOS inhibitors on NANC relaxation in circular muscle	Control
1992	Maggi *et al,* (paper)	Inhibition of nerve-mediated contractions in circular muscle by tachykinin antagonists	Control

Table 4.1: Published work on the smooth muscle pharmacology of human ileum. 5HT, 5 hydroxytryptamine; EFS, electrical field stimulation; NOS, nitric oxide synthase; NANC, non-adrenergic, non-cholinergic. For references see Bibliography.

tension. However, the mechanical responses of each muscle layer to electrical field stimulation (EFS) were generally opposite to one another, with the circular muscle tending to relax and the longitudinal layer contracting to EFS.

The work by Hara and colleagues on slow wave activity in human small bowel has been reviewed in the previous section (Hara *et al*, 1986).

Undoubtedly the greatest contribution to the study of human ileal smooth muscle pharmacology has been made by Maggi and co-workers (see Table 4.1) who focussed on the contractile effects of tachykinins- a family of peptides which include substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). Maggi and colleagues showed that in the longitudinal muscle of the human ileum both cholinergic excitatory and non-adrenergic, non-cholinergic (NANC) nerves affected motility and that several neuropeptides (NKA and SP) produced potent motor effects mediated by activation of NK-2 receptors (Maggi *et al*, 1989). VIP and calcitonin gene-related peptide (CGRP) each inhibited motility analogous to NANC stimulation. In circular muscle of human ileum cholinergic excitatory and non-cholinergic inhibitory nerves each affected motility while tachykinins exerted a potent contractile effect, independently of cholinergic nerves, via NK-1 and NK-2 receptors (Maggi *et al*, 1990). Subsequent work with newly developed tachykinin antagonists showed that the circular muscle of human ileum had NK-2 receptors as well as NK-1 receptors (Maggi *et al*, 1992). When activated, the former were largely responsible for the non-cholinergic rebound contraction to nerve stimulation.

The effects of nitric oxide synthase (NOS) inhibitors on NANC relaxation elicited by EFS in human ileal circular muscle were also studied (Maggi *et al*, 1991). NOS inhibitors abolished the NANC-mediated relaxation in this tissue implicating NO as the inhibitory transmitter of these nerves.

Clearly only a handful of papers have been published on human ileal smooth muscle pharmacology. The data described above refer only to specimens obtained from patients undergoing colorectal and urological resections for

neoplasm i.e. control subjects. There are no previous studies on ileum from patients with UC. While a few other workers have performed *in vitro* research on human jejunum (Bauer *et al*, 1991; Stark *et al*, 1993), their findings cannot be extended to another anatomically distinct region of the gut, nor indeed applied to the ileum of UC patients undergoing IPAA.

An investigation of the smooth muscle pharmacological characteristics of UC pouch ileum must therefore rely on using the limited cuff of ileum discarded at the time of reservoir construction. For obvious reasons this places immediate restraints on any attempts at a systematic study to characterize all the aspects of spontaneous and nerve-mediated motility mechanisms.

In recent years there has been increased interest in both the role of NO as a NANC inhibitory transmitter in the mammalian gut and in its possible pathogenic role in UC and CD (see Chapter 6). A study of this aspect of inhibitory control in ileal motility would be interesting in UC patients undergoing IPAA. The background to NANC inhibitory transmission in the gut, as well as the literature concerning NO in gastrointestinal function and disease are reviewed in the following section.

4.4 NITRIC OXIDE (NO) AND NONADRENERGIC, NONCHOLINERGIC (NANC) INHIBITORY TRANSMISSION IN GASTROINTESTINAL SMOOTH MUSCLE

4.4.1 INTRODUCTION

The quest to identify the neurotransmitter from NANC nerves has stemmed from observations made over the last 100 years. Whilst sympathetic nerves were regarded as releasing only noradrenaline while parasympathetic nerves released only acetyl choline, several independent pieces of research suggested that this was an oversimplification of the peripheral autonomic nervous system. Electrical stimulation of the sacral parasympathetic outflow via the pelvic nerves led to penile

erection that was unaffected by atropine (Langley & Anderson, 1895). Shortly thereafter, electrical stimulation of the vagus was shown to elicit both motor and inhibitory responses from the stomach (Langley, 1898). Several workers also reported that atropine only partially blocked motor responses of mammalian urinary bladder preparations following parasympathetic nerve stimulation (Langley & Anderson, 1895; Henderson & Roepke, 1934; Ambache, 1955). Of these workers, only Henderson and Roepke concluded that the nerves were releasing a transmitter other than acetyl choline. With the introduction of selective adrenergic neurone blocking drugs, some smooth muscles were shown to be innervated by nerves which were neither adrenergic nor cholinergic. Electrical evidence for the distinct existence of non-adrenergic inhibitory nerves was first provided by Burnstock and colleagues using guinea-pig taenia coli (Burnstock *et al*, 1964). The following year vagal stimulation in the cat elicited relaxations which were resistant to both atropine and guanethidine (Martinson, 1965).

Over the last three decades NANC nerves have been shown to supply the smooth muscle of the upper, middle and lower regions of the alimentary tract of all mammals investigated (For reviews see Furness & Costa, 1987; Burnstock, 1981; Gillespie, 1982; Gillespie, 1990). NANC inhibitory neurones reflexly regulate smooth muscle relaxation during stomach filling and the subsequent movement of food and debris along the length of the gut. NANC nerves are also involved in sphincteric smooth muscle relaxation at the lower oesophageal sphincter (LOS), (Code & Schlegel, 1968), pylorus (Vanderwinden *et al*, 1993), ICJ (Boeckxstaens *et al*, 1990) and in the IAS (Rayner, 1974). They are responsible for the receptive relaxation and accommodation of both the stomach (Abrahamson, 1973) and gall bladder (Al-Hassani & Davidson, 1979; McKirdy & Johnson, 1991), as well as the descending inhibition of peristalsis (Hirst & McKirdy, 1974; Julé, 1980).

NANC nerves do not, however, comprise a homogeneous population with a single neurotransmitter. Several putative transmitters have been implicated in NANC neurotransmission or in cotransmission with "classical" transmitters in the

gut. These other neurotransmitters have included ATP (Burnstock *et al*, 1970, Burnstock *et al*, 1972, Burnstock, 1981), 5-hydroxytryptamine (5HT), (Gershon, 1979), vasoactive intestinal peptide (VIP), substance P and neuropeptide Y (Cuello, 1982; Lundberg & Hokfeldt, 1983; Bartfai *et al*, 1988). However, smooth muscles such as the bovine retractor penis muscle or rat anococcygeus the putative NANC inhibitory transmitter did not resemble any of the above substances. A novel smooth muscle inhibitory factor was isolated from both of these tisssues in the 1970s (Ambache *et al*, 1975; Gillespie & Martin, 1978). Further studies of this smooth muscle inhibitory factor over the last 15 years culminated in providing the first evidence that nitric oxide (NO) was released from NANC nerves (see Section 4.4.3).

In recent years NO has been the topic of much research. Its role in the central nervous system, the cardiovascular system, in immunology and haematology, as well as smooth muscle has been investigated extensively. The following section reviews the role of the L-arginine-nitric oxide pathway in gastrointestinal function and disease.

4.4.2 THE L-ARGININE- NITRIC OXIDE PATHWAY: BACKGROUND

In the gastrointestinal tract NO has important physiological and pathological roles (see reviews by Moncada, 1992; Rand, 1992; Sanders & Ward, 1992; Stark & Szurszewski, 1992; Moncada & Higgs, 1993). The following discussion reviews the background to the discovery of NO and considers its roles in NANC neurotransmission in gastrointestinal smooth muscle.

The gas NO is a labile molecule, synthesised from the semiessential amino acid L-arginine (L-ARG) by a family of enzymes, the NO synthases, through the L-arginine-nitric oxide pathway (Moncada, 1992). NO is endogenous and stimulates soluble guanylate cyclase in a number of tissues, including the brain, the adrenal gland and platelets (see Fig 4.3). NO is also cytotoxic being released by activated macrophages following immunological challenge; it may function as an effector

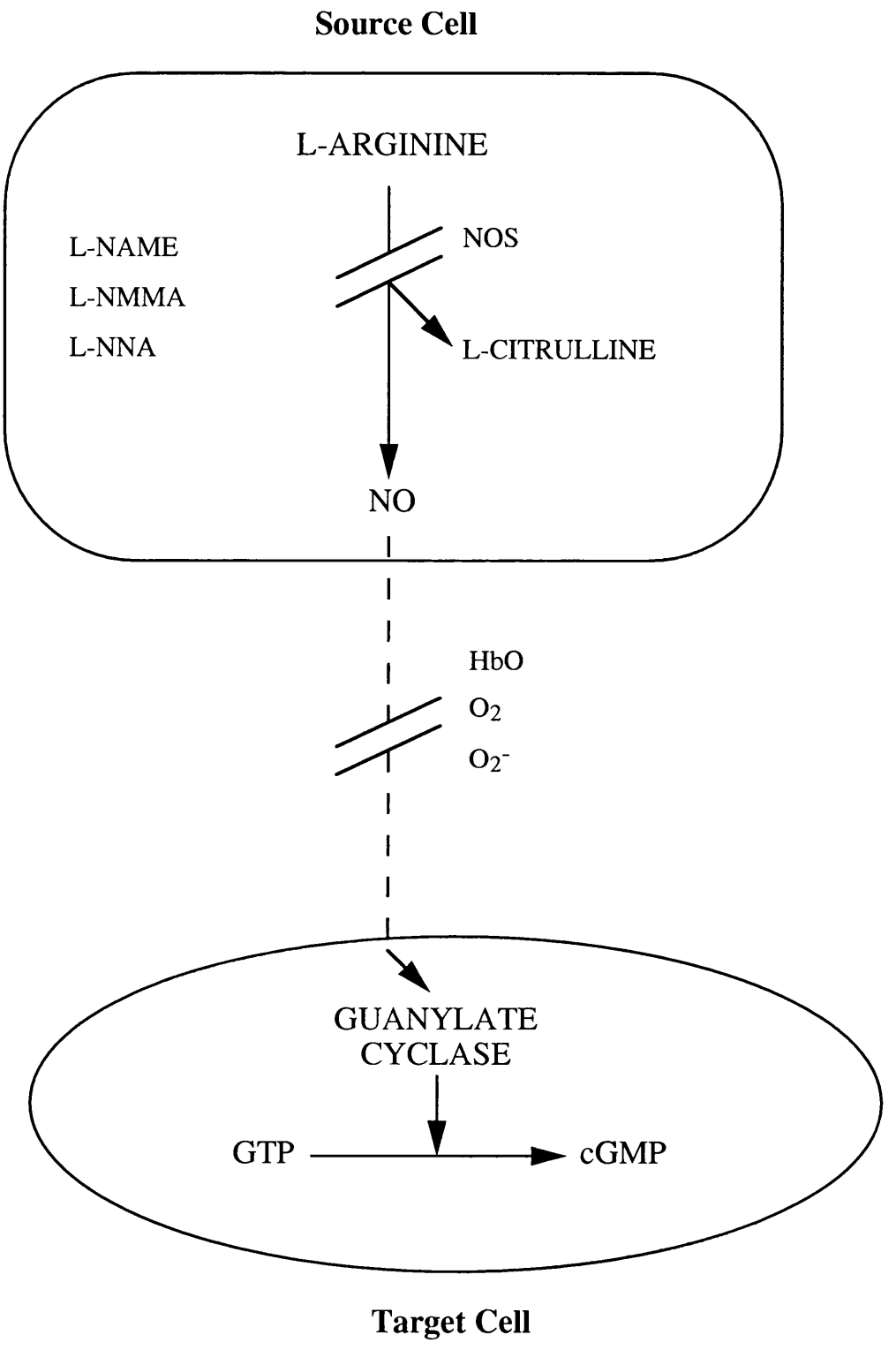


Fig 4.3 Schematic diagram of nitric oxide (NO) synthesis and action. Nitric oxide synthase (NOS) is shown to be inhibited in the source cell by the stereospecific antagonists L-NAME, L-NMMA and L-NNA (see text). Extracellular NO is inhibited by oxyhaemoglobin (HbO), superoxide anion (O^-_2) and oxygen (O_2). On entering the target cell NO activates guanylate cyclase. NO may also have effects in source cell.
(diagram modified from Stark & Szurszewski, 1992)

molecule mediating cytotoxicity in immune reactions against tumour cells and intracellular parasites (Hibbs *et al*, 1988; Stuehr *et al*, 1989; Salvemini *et al*, 1989; Liew *et al*, 1990). NO therefore has two diverse biological properties i.e. intercellular communication and cytotoxicity. Underlying these properties are two different classes of NO synthase (NOS). One is constitutive, cytosolic, Ca^{++} calmodulin-dependent (cNOS) and generates picomoles (10^{-12}M) of NO in response to receptor activation. Constitutive means that it is present and active without exposure to inducing agents. The other class of NO is cytosolic Ca^{++} calmodulin-independent (iNOS) and produces nanomoles (10^{-9}M) of NO over long periods following induction by immunological stimuli. Importantly, iNOS induction is inhibited by glucocorticoids. Both cNOS and iNOS enzymes are NADPH- and tetrahydrobiopterin-dependent. Induction of cNOS by bacteria or lipopolysaccharide (LPS) in gut tissue, including smooth muscle has also been reported; this may have pathophysiological significance (Oguchi *et al*, 1992). Endothelial NOS may differ from the neuronal forms, thus distinguishing three forms of the enzyme (Schmidt *et al*, 1992).

NO has unique properties making it quite unlike any other biological mediator (Ignarro, 1989). NO is a colourless gas which is slightly water-soluble. In dilute solution, NO has a half-life of<10 seconds because it is rapidly oxidised to inorganic nitrite and nitrate. NO is also destroyed by superoxide anion. Superoxide dismutase protects NO from breakdown by the latter (Rubanyi, 1986; Gryglewski, 1986). NO binds to oxyhaemoglobin (HbO) which rapdily terminates its biological actions (Palmer *et al*, 1987; Ignarro *et al*, 1987; Martin *et al*, 1986), and in biological systems NO has a half-life of <5 seconds (Palmer *et al*, 1987). The enzymatic synthesis of NO is competitively antagonised by stereospecific analogues of L-arginine such as N^{ω}-monomethyl-L-arginine (L-NMMA), N^{ω}-nitro-L-arginine (L-NNA), and N^{ω}-nitro-L-arginine methyl ester (L-NAME). When NO is produced by vascular endothelial cells it diffuses into the cytosol of the adjacent vascular smooth muscle cells where it binds to soluble guanylate

cyclase, a cytosolic haem-containing protein (Wolin *et al*, 1982). When NO binds to this enzyme production of 5'-cyclic guanosine monophosphate (cGMP) is activated with subsequent relaxation of smooth muscle (Gruetter *et al*, 1981; Katsuki *et al*, 1977).

In the human, endothelium-dependent relaxation has been demonstrated in rings of vascular tissue (Thom *et al*, 1985; Luscher *et al*, 1988), and is more pronounced in arteries than in veins. This phenomenon suggests that arteries generate more NO than veins. The NO produced in the cardiovascular system contributes to the regulation of basal and stimulated regional blood flow (Vallance *et al*, 1989a; Vallance *et al*, 1989b). Endothelium-derived NO can also diffuse into nearby adhering platelets in the blood vessel lumen to inhibit platelet aggregation by a cGMP-dependent mechanism and acts in synergy with prostacyclin to inhibit the aggregation of platelets by increasing their levels of cyclic adenosine monophosphate (cAMP), (Radomski *et al*, 1987). NO inhibits platelet adhesion and platelets themselves generate NO which acts as a negative-feedback mechanism to inhibit platelet activation (Radomski *et al*, 1990; Radomski & Moncada, 1991). NO derived from vascular endothelium may therefore have an important role in hypertension, shock and atherosclerosis.

Endogenous NO is also produced in nonvascular tissues including neutrophils, macrophages, brain, adrenal gland, renal epithelial cells, mast cells and peripheral neurones in gut, lung and penis. In the gastrointestinal system NO mediates relaxation of the muscularis externa and may play an important role in gastrointestinal mucosal blood flow, mucosal protection, haemodynamic response to liver disease, mediation of liver toxicity and regulation of hepatocyte function (Stark & Szurszewski, 1992).

The discovery that NO was an important messenger molecule came about more as a product of gradual evolution rather than through instant realisation. Review of the literature shows that several unrelated areas of research each moving

towards the common conclusion that NO plays an important physiological role in gastrointestinal function.

The identification of nitrosamines as potent carcinogens and their biosynthesis from nitrates led concerned workers to establish that nitrates could be synthesised by mammalian tissues. In addition, work revealed that the level of production of nitrates was increased under conditions of infection or insult by inflammatory agents (Green *et al*, 1981; Wagner *et al*, 1983).

Research into macrophage activity showed that when macrophages are activated by bacterial toxins, cytokines or lipopolysaccharides, they produce nitrite and nitrate from the precursor L-arginine (Stuehr & Marletta, 1985; Iyengar *et al*, 1987). NO is an intermediate compound in the process of macrophage oxidation of L-arginine (L-ARG) to nitrite and nitrate (Marletta *et al*, 1988). More recently, NO has been identified as an important cytotoxic activated macrophage effector molecule (Hibbs *et al*, 1988; Nathan & Hibbs, 1991).

Workers studying the mechanisms of control of cardiovascular smooth muscle showed in 1980 that blood vessel endothelium was essential for the vasorelaxant effects of acetylcholine, and also demonstrated that this effect was mediated by the release of an endothelial substance that relaxed vascular smooth muscle (Furchgott & Zawadzki, 1980). The latter was termed endothelium-derived relaxant factor (EDRF). Over the next five years many studies were devoted to the characterisation of the pharmacological and chemical properties of EDRF. The well-known effects of the nitrovasodilators (i.e. nitroglycerin, amyl nitrite and sodium nitroprusside, SNP) to relax cardiovascular smooth muscle led to the suggestion that NO may indeed be EDRF. In 1987, this was confirmed by two groups working independently who using chemical assay and bioassay proved that NO was released from vascular endothelium and that the effects of NO and EDRF were indistinguishable (Palmer *et al*, 1987; Ignarro *et al*, 1987). NO is, however, not the sole agent responsible for causing hyperpolarization in vascular smooth muscle. More recently, other types of agonists in addition to EDRF have been

found to hyperpolarize the vascular smooth muscle membrane in an endothelium-dependent manner. These include endothelium-derived hyperpolarizing factor (EDHF, Chen *et al*, 1988) and prostanoids (Parkington *et al*, 1993)

Having discovered that NO was EDRF, it was logical to study its role on other smooth muscles. By 1989, three independent groups had reported that NO was the major NANC inhibitory neurotransmitter in the anococcygeus muscle, an accessory gut muscle in the rat and mouse (Gillespie *et al*, 1989; Li & Rand, 1989; Ramagopal & Leighton, 1989) and subsequently in gut preparations (Bult *et al*, 1990; Hata *et al*, 1990; Li & Rand, 1990; Toda *et al*, 1990; Will *et al*, 1990; see review by Rand & Li, 1995). The research findings over the last 7 years which implicated NO as a mediator of inhibitory NANC transmission are reviewed below.

4.4.3 EVIDENCE SUPPORTING THE L-ARGININE-NO PATHWAY AS A MEDIATOR OF INHIBITORY NANC TRANSMISSION

Unlike striated muscle, gastrointestinal smooth muscle is under both inhibitory and excitatory neuronal control. Excitation of smooth muscle results in contraction; in smooth muscle aligned in a circumferential manner this produces constriction and movement of gut contents. Inhibition, on the other hand, causes relaxation and a reduced resistance to movement of contents. The "law of the intestine", viz. physiological stimulation of the intestinal wall causes contraction orally and relaxation anally has been evident since the work of Bayliss and Starling at the end of the last century (Bayliss & Starling, 1899). Indeed, those workers reported that in dog small intestine, vagal stimulation caused relaxation followed by a powerful contraction and that neither component was abolished by atropine. The orientation of these reflexes are essential to normal gut motility. Trendelenburg reported in 1917 that peristalsis could be recorded in isolated whole gut lengths of animal intestine, thus demonstrating that the enteric nervous system was the primary control mechanism of motility (Trendelenburg, 1917). Not surprisingly the polarity of the reflexes evoked by local stimulation is mirrored by

the orientation of excitatory and inhibitory motor neurones. Thus excitatory motor neurones project orad and inhibitory motor neurones project caudad (Brookes *et al*, 1991). Using atropine and a variety of selective adrenergic antagonists it became evident in the early 1960's that inhibitory neurotransmission to enteric smooth muscle was mediated by transmitters that were nonadrenergic, noncholinergic (NANC) in nature. Although strong electrical stimulation of mesenteric nerves activated postganglionic sympathetic nerve fibres and evoked adrenergic inhibition (Finkleman, 1930), it was shown that in the taenia coli intrinsic inhibitory motor neurones appeared to use a NANC transmitter (Burnstock *et al*, 1963) which was resistant to adrenergic blocking drugs and therfore non-sympathetic. By 1972, adenosine triphosphate (ATP) or a related compound was implicated as the NANC transmitter released by inhibitory motor neurones in the taenia coli of the guinea-pig and in other regions of the gut, since exogenous application of ATP mimicked the relaxation elicited by electrical stimulation of motor nerve fibres (Burnstock, 1972). Almost in tandem with the proposal of ATP as a putative NANC neurotransmitter, came the discovery of vasoactive intestinal polypeptide (VIP) and the demonstration that it caused a powerful relaxation of enteric smooth muscle (Said & Mutt, 1970). Further support came when it was shown that this peptide could be released by nerve stimulation (Fahrenkrug *et al*, 1978). Over the intervening years considerable controversy existed over whether the inhibitory neurotransmitter to gastrointestinal smooth muscle was ATP (purinergic theory) or VIP (peptidergic theory), (see review by Stark & Szurszewski, 1992). In both cases support was incomplete while in some gastrointestinal smooth muscles neither seemed to be involved (Manzini *et al*, 1986; Goyal *et al*, 1980; Biancani *et al*, 1984; Behar *et al*, 1989; Furness & Costa, 1987; Niel *et al*, 1983; Costa *et al*, 1986). In retrospect, considerable data showed that ATP and VIP, neither separately nor together, could account for all the types of NANC inhibitory transmission to gut smooth muscle. NO, a potent relaxant of vascular smooth muscle and the anococcygeus muscle was therefore an obvious choice as the third

candidiate. Recent work has indicated that a nonpurinergic, nonpeptidergic system involving NO may mediate NANC neural inhibition of gastrointestinal smooth muscle (see review by Stark & Szurszewski, 1992).

Early work carried out in Glasgow by Gillespie and colleagues on the innervation of rodent and bovine genitalia-associated smooth muscle (well before the identification of EDRF as NO), indicated that a nonpurinergic, nonpeptidergic neurotransmitter was involved in NANC neurotransmission (Gillespie, 1972; Bowman & Drummond, 1984; Bowman *et al*, 1982). In these preparations, NANC inhibition was apparently independent of any established neurotransmitter, cGMP appeared to be the second messenger mediating relaxation and the effect of NANC nerve stimulation was antagonised by oxyhaemoglobin (HbO) which binds NO. The mediator of NANC inhibition in these tissues had chemical properties and actions similar to those of EDRF. Later work revealed that NO is the mediator of NANC neurotransmission in these muscles (Gillespie *et al*, 1989; Gibson *et al*, 1990; Gillespie & Sheng, 1990). In 1990, Bult and colleagues reported that NO was released on stimulation of enteric NANC nerves (Bult *et al*, 1990). Since then an increasing body of evidence has accumulated to support the concept that NO acts as an inhibitory NANC neurotransmitter in the gut.

Before a substance can be classed as a neurotransmitter it must meet a number of criteria. Release of a putative neurotransmitter from neurones must be detectable and exogenous application of the substance should have identical effects on postjunctional receptors to those produced when it is released by nerve stimulation. Antagonists should also have the same effect on the response to exogenous application of the neurotransmitter and on nerve stimulation. A mechanism for transmitter inactivation must exist and the neurones must be shown to have mechanisms for neurotransmitter synthesis and storage. The evidence to date which shows that most of these criteria have been met for NO, supporting a role for NO as a neurotransmitter in the gastrointestinal tract, is summarised below. A role for NO in NANC transmission has been demonstrated in several animal

preparations. For comprehensive lists of these experiments see reviews by Brookes, 1993, and Stark & Szurszewski, 1992.

Release of NO

In vitro animal experiments have shown that electrical field stimulation (EFS) of NANC nerves released a chemical from both the canine ileocolonic junction and rat stomach which caused relaxation when superfused over vascular smooth muscle preparations (Bult *et al*, 1990; Furness & Costa, 1987). The chemical in question was NO, or a related compound since it was inactivated by superoxide anion and HbO and its release was prevented by a NOS inhibitor. The origin of its release appeared to be neuronal since it was dependent on the frequency of EFS and sensitive to tetrodotoxin (TTX), a nerve toxin which blocks neuronal action potential conduction. These early experiments did not, however, exclude the possibility that the EFS was releasing a neurotransmitter which in turn liberated NO from non-neuronal tissues, such as gastrointestinal smooth muscle.

Identity of action

The effects of exogenously applied NO and the effects of NANC nerve stimulation have been found to be similar in numerous animal and human gastrointestinal smooth muscle preparations. Gross similarities exist in the relaxation produced by application of exogenous NO with that evolved by EFS of NANC nerves in tissue strips from the LOS, stomach, small intestine and IAS from various animal species (Boeckxstaens *et al*, 1990; Boeckxstaens *et al*, 1991a; Toda *et al*, 1990; De Man *et al*, 1991; Stark *et al*, 1991; Murray *et al*, 1991; Rattan & Chakder, 1992). To mimic the effects of endogenous NO in tissue preparations, exogenous NO can be applied in a variety of forms. NO in gaseous form, dissolved in degassed boiled water has been used by Boeckxstaens and colleagues

(Boeckxstaens et al, 1991b). However, there are technical problems with this method such as the instability of pure NO gas and difficulty in assessing absolute concentrations. Other agents which release NO in solution, on or in target smooth muscle cells, such as SNP, nitroglycerin and amyl nitrite have been used to apply NO to tissues under experimental conditions.

Work in isolated circular muscle from segments of rat colon showed that relaxation evoked by exogenous NO produced the same effect i.e. descending relaxation, that is seen in response to intraluminal balloon distension (Hata *et al*, 1990). Intracellular recording techniques also revealed that exogenously applied NO evoked a membrane hyperpolarization that is similar to inhibitory junction potentials (IJP) evoked by NANC nerve stimulation, in studies of smooth muscle cells from canine jejunum, ICJ and colon (Stark *et al*, 1991; Ward *et al*, 1992; Thornbury *et al*, 1991; Ward *et al*, 1992).

Preparations of human tissue have also been studied. Circular muscle from non-diseased human jejunum was used to study the mechanisms by which NO mediates NANC inhibition (Stark *et al*, 1993). In the human tissue exogenous NO evoked membrane hyperpolarization and inhibition of mechanical activity. Although this mimicked the general response to NANC nerve stimulation, analysis of the electrical responses revealed marked differences between the exogenous and endogenous NO-induced mechanisms. The workers concluded that of the two hyperpolarization components seen in the human jejunal NANC inhibitory junction potential (IJP), NO may mediate the later sustained smaller amplitude hyperpolarization but not the initial rapid hyperpolarization.

Effect of antagonists

As yet no competitive antagonists exist for NO. This may be because NO does not appear to produce its effects through a typical membrane-associated receptor. The role of NO in NANC inhibitory neurotransmission has however, been investigated by using agents such as HbO which inactivates NO (Palmer *et al*,

1987; Ignarro *et al*, 1987; Martin *et al*, 1986) and L-ARG analogues which can be used to inhibit NO synthesis. When either NO synthesis is inhibited or exogenous HbO applied *in vitro*, the mechanical relaxation obtained by EFS of NANC nerves is attenuated in a variety of smooth muscle preparations including human jejunum (Stark *et al*, 1993), colon (Burleigh, 1992) and IAS (Burleigh, 1992). Intracellular recordings *in vitro* of circular muscle from human jejunum have also shown that HbO and NOS inhibitors attenuate or block IJP evoked by NANC nerve stimulation (Stark *et al*, 1993).

Mechanism of inactivation

A specific mechanism for the breakdown or removal of NO in gastrointestinal tissue has not been identified. Two factors may underlie this. Firstly, NO is extremely unstable and spontaneously oxidises to nitrite and nitrate, with a half-life of only a few seconds, thus eliminating the need for a specific degradation pathway. Secondly, substances that inactivate NO, including oxygen and HbO are present in gastrointestinal tissue and would be expected to limit the action of NO released from NANC nerves to a localised area for a limited time period.

Neural synthesis and storage of NO

While the evidence above strongly suggests that NO plays an important role in mediating NANC inhibitory transmission in gastrointestinal smooth muscle, there is no definitive proof that NO is a neurotransmitter. Moreover, NO could be acting alternatively in a second messenger role within smooth muscle cells. If this was the case, then release of an unspecified neurotransmitter from NANC nerves would stimulate the synthesis of NO within gastrointestinal smooth muscle cells (Ignarro *et al*, 1990). Two areas of research have supported the concept that NO is in fact acting as a neurotransmitter. Immunohistochemical staining in cells in the myenteric plexus and in neuronal processes in the circular muscle of the rat

duodenum localised, for the first time, NOS (Bredt *et al*, 1990). Further work with HbO which showed that the effects of stimulating NANC nerves could be inhibited by this large cell-impermeable molecule, suggested that NO had to exist extracellularly at some point after its neuronal generation (Boeckxstaens *et al*, 1990; Toda *et al*, 1990; De Man *et al*, 1991; Stark *et al*, 1991; Thornbury *et al*, 1991). Other studies have shown that NO can be generated in some gastrointestinal smooth muscle cells in response to VIP (Grider *et al*, 1992). Furthermore, the possibility that release of a putative NANC neurotransmitter initiates NO production in a nonneural cell, which in turn acts on neighbouring smooth muscle cells to produce relaxation cannot be ruled out.

Thus a mechanism for storing NO in enteric nerves has not been demonstrated. Free NO stored in vesicles seems unlikely since it easily permeates lipid membranes. Storage may in fact be unnecessary if synthesis of NO matches neuronal release, simply balancing supply with demand. Ignarro has suggested that an alternative storage mechanism may involve an acid-stabilised S-nitrosothiol intermediate which could be packaged and stored in vesicles, with nerve stimulation leading to exocytosis and spontaneous decompression of the intermediate to form NO on exposure to the nonacidic extracellular space. This would certainly be comparable with storage and release of classical neurotransmitters. Work in vascular endothelium has shown that NO is stored in thiol-bound intermediates (Mülsch *et al*, 1991).

In addition to the release of NO following NANC nerve stimulation workers have investigated whether there is a continual background synthesis and release of NO affecting tone in gastrointetsinal smooth muscle. Data suggest that ongoing release of NO may occur in sphincters or smooth muscles that show predominantly active tone or slow changes in tone like the gastric fundus. However, in smooth muscles that show predominantly phasic activity and little active tonic activity such as the ileum or jejunum, ongoing release of NO does not seem to occur. There is no evidence to date which clearly identifies the source of

NO in smooth muscle in which there is ongoing release. Both neuronal and nonneuronal cells may synthesise NO. Isolated gastric muscle cells can themselves generate NO (Grider *et al*, 1992), as can white blood cells (Hibbs *et al*, 1988; Stuehr *et al*, 1989; Salvemini *et al*, 1989) and blood vessels of the gastrointestinal tract (Ozaki *et al*, 1991; Tøttrup *et al*, 1991; Whittle *et al*, 1990). Work has also shown that NOS activity exists in rat gastric mucosa (Brown *et al*, 1992). The possibility that mucosa-generated NO may modulate the function of underlying gastrointestinal smooth muscle may be significant in inflammatory conditions such as UC and CD (see Chapter 6).

Mechanism of NO action

1. Role of cGMP

The relaxation of gastrointestinal smooth muscle produced by NO may be mediated by increased levels of cGMP. cGMP-dependent protein kinase has been found in the gastrointestinal smooth muscle of a number of species including humans (Miller *et al*, 1986). Relaxation of sphincter preparations including human LOS, opossum LOS and canine IAS, in response to NANC nerve stimulation is associated with increases in cGMP in smooth muscle (Grous *et al*, 1991; Torphy *et al*, 1986). Relaxation evoked in guinea-pig taenia coli is also associated with an increase in cGMP (Shikano *et al*, 1988). The mechanism through which increases in cGMP levels, brought about by NO, result in smooth muscle relaxation is unknown. Modulation of levels of intracellular Ca^{++} may be implicated (see Stark & Szurszewski, 1992). Activation of cGMP-dependent protein kinases may lead to phosphorylation of proteins in the contractile apparatus, calcium-sequestering mechanisms or ion channels. Ramagopal and Leighton reported that EFS in the rat anococcygeus muscle led to a reduction in intracellular Ca^{++} that could be blocked by L-NMMA (Ramagopal & Leighton, 1989). In canine gastric antrum, SNP decreases the amplitude of the plateau potential of gastric action potentials and inhibits the increase in cytosolic Ca^{++} and tension associated with the plateau

potential (Ozaki *et al*, 1991). These results imply that NO inhibits voltage-dependent Ca^{++} channels reponsible for the plateau potential. SNP had no significant effect on either upstroke potential of the gastric slow wave or levels of free cytosolic Ca^{++}. There was, however, a significant decrease in the force of contraction triggered by the upstroke potential, suggesting that NO may reduce in addition the Ca^{++} sensitivity of the contractile elements. This effect may be mediated by cGMP, as cGMP inhibits Ca^{++}-induced contraction in this tissue.

2. Role of hyperpolarisation evoked by NO

Exogenously applied NO causes a membrane hyperpolarisation in gastrointestinal smooth muscle which resembles the IJP seen following NANC nerve stimulation (Stark *et al*, 1991; Thornbury *et al*, 1991; Ward *et al*, 1992; Stark *et al*, 1993). Whether this hyperpolarisation is caused by a direct effect of NO on membrane channels, or mediated by a NO-induced increase in cGMP is unknown. Methylene blue, which blocks the action of NO through the inhibition of guanylate cyclase, attenuates NO-induced IJP in the circular smooth muscle of opossum oesophagus and canine small intestine, demonstrating that increased levels of cGMP are important in NO-evoked hyperpolarisation (Christinck *et al*, 1991). Of course NO may act through cGMP-independent mechanisms, as has been demonstrated in NO-induced osteoclast inhibition (MacIntyre *et al*, 1991) and in fibroblast cell lines (Garg & Hassid, 1991). However, the effectiveness of methylene blue in numerous gastrointestinal smooth muscle preparations suggests this is unlikely. Work in canine intestine has shown that the membrane potential during NO-evoked hyperpolarisation approaches the expected potassium equilibrium potential suggesting that an increase in K^+ conductance mediates the hyperpolarisation (Stark *et al*, 1991; Thornbury *et al*, 1991). In patch-clamp recordings from canine colonic myocytes in the cell-attached configuration NO enhanced the open probability of Ca^{++}-activated K^+ channels (Thornbury *et al*, 1991; Sanders & Ward, 1992). This evidence suggests that this type of K^+ channel mediates hyperpolarization in response to NANC neurotransmission and

exogenous application of NO. However, IJP in many gastrointestinal smooth muscles are unlikely to be mediated by such channels (Sanders & Ward, 1992). Apamin, a compound isolated from bee venom, has been shown to block a component of the IJP in many preparations, but not canine colon. This agent has been shown to block a small-conductance Ca^{++}-dependent K^+ channel but the expression of this channel, using single-channel patch-clamp studies has yet to be demonstrated in gastrointestinal smooth muscles. The level of expression of these channels is very low in some preparations. Indeed, it is possible that they are expressed in a very small quantity in gastrointestinal smooth muscle (Sanders & Ward, 1992). Very few K^+ channels need to be activated to cause hyperpolarisation because the input resistance of gastrointestinal smooth muscles is very high at the resting membrane potential (Sanders, 1989). In many sites in the gastrointestinal tract apamin blocks only a portion of the inhibitory response (Costa *et al*, 1986; Vogalis & Sanders, 1990; He & Goyal, 1993; Rae & Muir, 1996), suggesting that two or more conductances may mediate these reponses. Humphreys and colleagues (Humphreys *et al*, 1991) recently suggested that only the apamin-insensitive component of IJP is mediated by another transmitter. Recent data from Sanders and colleagues have raised the possibility that both apamin-sensitive and insensitive IJP exist in the canine pylorus (Sanders & Ward, 1992) and canine ileocolonic sphincter (Ward *et al*, 1992).

Physiological role of NO in NANC inhibition

1. Relationship of NO to other putative NANC neurotransmitters

Several different NANC neurotransmitters may be involved in initiating the same inhibitory response and similar responses in different gastrointestinal smooth muscles and species may be due to different transmitters. The evidence reviewed above provides compelling evidence that NO functions as a NANC transmitter in several gastrointestinal smooth muscles. However, a role for other transmitters such as VIP and ATP cannot be excluded. Indeed, there is a large amount of

evidence which supports a neurotransmitter role for these substances in NANC neurotransmission (Furness & Costa, 1987). Some of the work which has raised the possibility that more than one NANC neurotransmitter is present in gastrointestinal smooth muscle and that more than one NANC neurotransmitter is released on NANC nerve stimulation is summarised below.

In guinea-pig ileum NANC elicited IJP have two components: a rapid short latency hyperpolarisation that is apamin-sensitive and a slower long-lasting hyperpolarisation that is apamin-resistant (Niel *et al*, 1983). This implies that either more than one transmitter mediates the IJP or that a single inhibitory neurotransmitter may mediate two different receptors linked to K^+ channels with different kinetics or modes of activation. Further work in the guinea-pig has shown that the relative contributions of apamin-sensitive and apamin-resistant mechanisms vary between regions of the gut (Costa *et al*, 1986). Further evidence for multiple mediators of inhibitory neurotransmisssion came from work in the rat intestine which showed that ATP antagonists and ATP desensitisation had differing effects in the duodenum and ileum (Manzini *et al*, 1986). Similarly, in several preparations, VIP antiserum blocks the response to exogenous VIP but partially reduces the effects of NANC nerve stimulation, supporting the hypothesis that a neurotransmitter in addition to VIP may be released from inhibitory NANC neurons (Goyal *et al*, 1980; Biancani *et al*, 1984; Behar *et al*, 1989).

Several NANC neurotransmitters may be released on NANC nerve stimulation. In experiments on the role of NO in NANC neurotransmission, incomplete attenuation of the inhibitory effects of NANC nerve stimulation by NOS inhibitors and HbO have been demonstrated in canine LOS (De Man *et al*, 1991), canine jejunum (Stark *et al*, 1991), canine ICJ and ileum (Boeckxstaens *et al*, 1990), canine colon (Dalziel *et al*, 1991) and rat gastric fundus (Boeckxstaens *et al*, 1991; Li & Rand, 1990).

NO may act as the final mediator of relaxation, induced by other putative NANC transmitters such as VIP and ATP. ATP-induced relaxations in canine

ileum and ICJ are reduced by both HbO and NOS inhibitors, suggesting that NO is the final mediator for relaxation (Boeckxstaens *et al*, 1991). VIP-induced relaxations in rat gastric fundus (Li & Rand, 1990) and opossum IAS (Rattan & Chadker, 1992) were also observed to be reduced by NOS inhibitors. The mechanism by which NO acts as a final mediator for relaxation initiated by other putative NANC transmitters such as ATP or VIP could occur in one of several ways. Firstly, as a neurotransmitter released from a final effector neuron in response to ATP or VIP; secondly, as a mediator released from a non-neuronal cell in response to ATP or VIP, or as a second messenger produced in smooth muscle in response to ATP or VIP. In guinea-pig gastric fundus VIP release stimulates NO production in target smooth muscle cells (Grider *et al*, 1992). However, in other preparations such as canine LOS (De Man *et al*, 1991), opossum LOS (Tøttrup et al, 1992) and rat duodenum (Irie *et al*, 1991), ATP- and VIP-induced relaxations were not affected by NOS inhibitors, indicating that ATP and VIP relaxed smooth muscle by NO-independent mechanisms. These findings support the theory that multiple inhibitory neurotransmitters can be released to act through parallel pathways during NANC nerve stimulation.

As yet it is unknown whether putative NANC neurotransmitters, including NO, are colocalized in the same neurones and if they are colocalized whether they are released simultaneously on NANC nerve stimulation. *In vitro* work, showing co-release of multiple NANC transmitters may simply be an artefact resulting from EFS of muscle strips. Electrical stimulation could activate all enteric neurones simultaneously. In physiological terms the relative roles of various NANC transmitters may be related to their half-lives. Thus, NO would be expected to cause rapid transient relaxation, whereas other NANC neurotransmitters with longer half-lives would be responsible for prolonged relaxations. The interplay between neurones releasing one or more NANC neurotransmitters would determine the type and duration of relaxation.

Physiological role of NO in gastrointestinal motility

An important component of the intestinal peristaltic reflex is a descending inhibition mediated by NANC enteric inhibitory neurons. Experiments in isolated segments of rat colon have shown that the descending relaxation evoked by balloon distension is mimicked by exogenous NO and is prevented by inhibitors of NOS. This suggests that NO mediates this reflex (Hata *et al*, 1990). Likewise, receptive relaxation in the isolated guinea-pig stomach is prevented by NOS inhibitors (Desai *et al*, 1991). Inhibitory modulation of the phasic contractions in canine gastric antrum appears to result from the continuous spontaneous release of NO (Ozaki *et al*, 1991). From *in vitro* animal experiments, NO-mediated inhibition of gastrointestinal smooth muscle appears important for a variety of physiological functions. More recently, *in vivo* research has supported this concept by demonstrating that vagally-mediated relaxation of the opossum LOS (Tøttrup *et al*, 1991), canine stomach (Allescher *et al*, 1992) and rat stomach (Lefebvre *et al*, 1992) is mediated by NO. Thus, from animal work NO appears to have a physiological role in NANC inhibition. Much less is known however, about the role of NO in human gastrointestinal motility.

McKirdy and colleagues reported that the involvement of NO in NANC relaxation of human LOS strips (McKirdy *et al*, 1992) and NO has been implicated in NANC relaxation of the circular muscle of human ileum (Maggi *et al*, 1991). More recently, Burleigh demonstrated that NO was involved in NANC relaxations of the human IAS (Burleigh, 1992). However, unlike the IAS, relaxations in the circular muscle from human sigmoid colon were only partially mediated by NO.

While a large amount of evidence supports the concept of a role for NO in NANC-induced relaxations of gastrointestinal smooth muscle, there are still unanswered questions and discrepancies over the possible mechanisms of inhibitory neurotransmisssion in the gut. Complex multiple mechanisms exist which interact to elicit NANC-mediated relaxation. No single transmitter underlies inhibition of tone in gastrointestinal smooth muscle. In addition, the importance of

one transmitter relative to others varies according to anatomical location, the species and experimental techniques used to study the transmitter in question.

As yet the level of action and interaction between putative NANC neurotransmitters has not been settled. Current information suggests that NO, ATP and VIP are probably released from the same varicosities of inhibitory motor neurones. NO increases intracellular cGMP levels leading to hyperpolarisation and relaxation. VIP increases intracellular cAMP levels causing relaxation and possibly causing small hyperpolarisations in some preparations. ATP acts apparently through ion channels, hyperpolarising the cell membrane thus reducing Ca^{++} permeability and intracellular Ca^{++} concentrations leading to relaxation. This gives limitless possibilities for interactions between different transmitter systems. More work is required to establish the targets for phosphorylation by the relevant protein kinases and to examine the specific effects of cyclic nucleotides.

Caution is required when interpreting the effects of an agonist or antagonist on the activity of a putative NANC neurotransmitter *in vitro*. Transmitter interactions and the possibility that several neurotransmitters may be simultaneously released from the same nerve ending on stimulation, mean that even if a specific NOS antagonist completely blocks inhibition this does not preclude a major role for other neurotransmitters at this site. Such an observation simply means that NO release is required for muscle relaxation. Transmitters may not simply have additive effects. Indeed, the effects of two transmitters may multiply with one being permissive and synergistic with the effects of the others.

More work is also needed to investigate the causal relationship between hyperpolarisation and smooth muscle relaxation. At least theoretically it is possible for changes in coupling between intracellular Ca^{++} and the contractile apparatus to cause relaxation without altering membrane potential (Bolton & Large, 1986). Hyperpolarization of the muscle membrane will tend to close voltage-activated Ca^{++} channels in the muscle cell membrane and thus decrease intracellular levels of Ca^{++}. However, the hyperpolarization may also serve other roles including

"resetting" of ion channels that are rapidly inactivated at normal potentials. The actions of NO or VIP, mediated through second messengers (viz. cGMP and cAMP respectively) may cause a reduction in intracellular Ca^{++} concentrations through phosphorylation of ion channels, changes in Ca^{++} storage mechanisms or to alterations in the sensitivity of the contractile apparatus to intracellular Ca^{++} (for a detailed review of Ca^{++} homeostasis in smooth muscle see Tsien & Tsien, 1990).

At present, very little information exists about neurotransmission in human gastrointestinal smooth muscle. To investigate NO as a NANC neurotransmitter in the human gut will require much work to study different anatomical sites and the relative contributions which NO makes to relaxation and indeed to IJP. The identity of the transmitter(s) that mediates the NO-independent IJP in human and nonhuman muscles will also be an important area for future research. Finally, once control human tissue has been assessed work can be extended to disease states to establish whether changes in NO and other neurotransmitter functions are present and if they are a cause or effect of the disease process in question.

CHAPTER 5

SMALL BOWEL MOTILITY - *IN VITRO* STUDIES OF ISOLATED HUMAN ILEUM

5.1 INTRODUCTION

A few studies have examined, *in vitro*, the pharmacological properties of the isolated human terminal ileum from control human subjects. However, to date there have been no related studies on specimens from patients with UC. This is surprising since the terminal ileum is used to construct the reservoir in the procedure of IPAA. This chapter reports my *in vitro* investigations of the spontaneous myogenic activity in isolated human terminal ileum from control and UC patients. The role of NO in inhibitory nerve-mediated transmission and in SNP-induced relaxation in human ileum were also examined.

5.2 METHODS

5.2.1 PATIENTS

Between October 1992 and July 1994, 171 *in vitro* experiments were performed on human ileal smooth muscle. Five hundred and thirty-four strips from 61 patients (25 UC, 21 control, 15 other conditions) were studied in organ bath and Golenhofen apparatus (see sections 5.2.3 and 5.2.4). The details for each of the three patient categories are shown in Tables 5.1-5.3.

Twenty-five patients with UC were investigated (17 males) with a mean age of 34.5years. Two patients underwent subtotal colectomies followed by staged TSRP and had ileal samples taken at both procedures for *in vitro* experiments (studies 1-9; studies 20-23). Four cases of fulminant UC who underwent emergency subtotal colectomy were included in the study. IPAA was performed in 19 of the patients in this group, of whom 8 had been treated previously with subtotal colectomy (staged). One patient with quiescent chronic UC had a colonic carcinioma treated by right hemicolectomy.

Controls were chosen on the basis of non-inflammatory pre- or neoplastic conditions who were undergoing surgical procedures involving terminal ileal

STUDY	SEX	DATE	PATIENT	AGE	DIAGNOSIS	OPERATION	STRIPS
1	M	28/10/92	WM	57	Colonic neoplasm	Right hemicolectomy	3
2	F	16/02/93	MMcK	80	Colonic neoplasm	Right hemicolectomy	9
3	M	16/02/93	JB	78	Colonic neoplasm	Revision of ileostomy	9
4	M	17/02/93	AL	61	Colonic neoplasm	Right hemicolectomy	9
5	F	22/02/93	MH	66	Colonic neoplasm	Right hemicolectomy	9
6	M	24/08/93	JH	73	Colonic neoplasm	Right hemicolectomy	10
7	M	07/09/93	JW	60	Colonic neoplasm	Right hemicolectomy	15
8	M	13/10/93	DMcL	56	Colonic neoplasm	Right hemicolectomy	10
9	M	20/11/93	JP	39	Colonic neoplasm	Right hemicolectomy	7
10	F	23/11/93	MK	62	Colonic neoplasm	Right hemicolectomy	11
11	F	14/12/93	WM	66	Colonic neoplasm	Right hemicolectomy	6
12	M	12/01/94	IS	51	FAP	Totally stapled restorative proctocolectomy-staged	4
13	F	01/02/94	AR	77	Colonic neoplasm	Right hemicolectomy	8
14	F	04/02/94	MMACN	57	Colonic neoplasm	Excision coloanal anastomosis;ileostomy formation	6
15	F	08/02/94	ML	47	Colonic neoplasm	Right hemicolectomy	8
16	F	09/02/94	MMcL	78	Colonic neoplasm	Right hemicolectomy	6
17	F	01/03/94	HMcG	73	Colonic neoplasm	Right hemicolectomy	6
18	M	08/04/94	PT	72	Colonic neoplasm	Right hemicolectomy	6
19	F	29/04/94	EL	46	Colonic neoplasm	Right hemicolectomy	4
20	M	02/06/94	DMcK	31	HNPCC	Totally stapled restorative proctocolectomy	4
21	M	08/06/94	KH	39	FAP	Totally stapled restorative proctocolectomy	5

Table 5.1: *In vitro* studies on ileal tissue from control subjects. FAP, familial adenomatous polyposis; HNPCC, Hereditary non-polyposis colorectal cancer.

STUDY	SEX	DATE	PATIENT	AGE	DIAGNOSIS	OPERATION	STRIPS
1	F	04/12/92	CMACF	28	UC-Fulminant	Subtotal colectomy	2
2	F	28/01/93	DL	22	UC-Chronic	Revision of ileostomy	7
3	F	02/02/93	PM	22	UC-Fulminant	Subtotal colectomy	7
4	F	24/02/93	JY	34	UC-Chronic	Totally stapled restorative proctocolectomy	7
5	M	31/03/93	JMcG	52	UC-Chronic	Totally stapled restorative proctocolectomy-staged	10
6	F	04/08/93	PW	35	UC-Chronic	Totally stapled restorative proctocolectomy	9
7	M	11/08/93	KMcG	27	UC-Chronic	Loop ileostomy formation for pouch dysfunction	16
8	M	18/08/93	ZS	30	UC-Chronic	Totally stapled restorative proctocolectomy	6
9	F	19/08/93	CMACF	29	UC-Chronic	Totally stapled restorative proctocolectomy-staged	6
10	M	25/08/93	JS	26	UC-Chronic	Totally stapled restorative proctocolectomy	13
11	M	01/09/93	JB	48	UC-Chronic	Totally stapled restorative proctocolectomy	14
12	M	15/09/93	AMcK	37	UC-Chronic	Totally stapled restorative proctocolectomy-staged	14
13	M	29/09/93	AG	35	UC-Chronic	Totally stapled restorative proctocolectomy	20
14	M	14/10/93	AR	24	UC-Chronic	Totally stapled restorative proctocolectomy	5
15	F	03/11/93	PMcK	41	UC-Chronic	Totally stapled restorative proctocolectomy	5
16	F	10/11/93	GM	33	UC-Chronic	Totally stapled restorative proctocolectomy-staged	10
17	M	17/11/93	WF	34	UC-Chronic	Totally stapled restorative proctocolectomy	15
18	M	25/11/93	AT	45	UC-Chronic	Totally stapled restorative proctocolectomy	6
19	M	29/12/93	JH	37	UC -Fulminant	Subtotal colectomy	11
20	M	27/01/94	RK	30	UC-Chronic	Subtotal colectomy	8
21	M	16/02/94	WMcG	78	UC-Chronic+Carcinoma	Right hemicolectomy	11
22	F	16/03/94	SMcV	44	UC-Chronic	Totally stapled restorative proctocolectomy-staged	12
23	M	13/04/94	RK	31	UC-Chronic	Totally stapled restorative proctocolectomy-staged	1
24	M	05/05/94	TD	40	UC-Fulminant	Subtotal colectomy	4
25	M	25/05/94	SMcN	16	UC-Chronic	Totally stapled restorative proctocolectomy-staged	6
26	M	26/05/94	DK	29	UC-Chronic	Totally stapled restorative proctocolectomy	12
27	M	22/06/94	AMcP	14	UC-Chronic	Totally stapled restorative proctocolectomy-staged	5

Table 5.2: *In vitro* studies on ileal tissue from ulcerative colitic (UC) patients.

EXPMT	SEX	DATE	PATIENT	AGE	DIAGNOSIS	OPERATION	STRIPS
1	F	29/01/93	CMcG	37	Hindgut neuropathy	Reversal of ileostomy	9
2	M	03/02/93	DS	34	Crohn's disease	Revision of coloanal anastomosis	9
3	M	26/08/93	EB	46	PVD	Reverse ileal onlay graft	9
4	M	31/08/93	KE	36	PVD	Reverse ileal onlay graft	13
5	F	20/10/93	JC	60	Carcinoma; vagotomy	Right hemicolectomy	20
6	F	02/12/93	RH	59	PVD	Reverse ileal onlay graft	6
7	F	05/01/94	FW	29	ISTC	Subtotal colectomy+ileorectal anastomosis	12
8	F	06/01/94	AB	34	ISTC	Subtotal colectomy+ileorectal anastomosis	7
9	F	13/01/94	CL	34	ISTC	Subtotal colectomy+ileorectal anastomosis	7
10	F	19/01/94	MMcC	32	ISTC	Subtotal colectomy+ileorectal anastomosis	12
11	F	26/01/94	AM	32	ISTC	Subtotal colectomy+ileorectal anastomosis	7
12	F	02/02/94	EMcG	47	ISTC	Subtotal colectomy+ileorectal anastomosis	7
13	F	10/02/94	IMcC	70	Crohn's disease	Right hemicolectomy	6
14	F	23/02/94	JI	48	ISTC	Subtotal colectomy+ileorectal anastomosis	7
15	M	01/06/94	EB	31	Angiodyspasia	Right hemicolectomy	6

Table 5.3: *In vitro* studies on ileal tissue from idiopathic slow transit constipation (ISTC) patients and other miscellaneous conditions. PVD, postvagotomy diarrhoea.

resection. For established carcinoma, which was either located in the caecum or right colon, the lesion had to be non-obstructing. Thus, emergency right hemicolectomy procedures for obstructing colonic neoplasms were excluded from the study. Twenty-one patients were included in this category. Seventeen patients were treated by right hemicolectomy for colonic carcinoma. Of the remaining 4, 3 underwent TSRP for FAP or NHPCC. One patient had formation of a permanent ileostomy following excision of a coloanal anastomosis with completion colectomy for metachronous neoplasm. The mean age of the 21 patients (11 males) in this category was 60 years.

The third group comprised cases which arose during the study period. Although they did not fall into either the UC or control categories they were analysed as interesting adjuncts to the main study. They involved terminal ileal resections and consisted of hindgut neuropathy (n=1), Crohn's disease (n=2), postvagotomy diarrhoea (n=3), vaogotomised caecal neoplasm (n=1), caecal angiodysplasia (n=1) and ISTC (n=7). The mean age of the 15 patients (4 males) in this category was 41.9 years.

5.2.2 DISSECTION OF TISSUES

Samples of terminal ileum were obtained from patients undergoing surgical procedures at Glasgow Royal Infirmary and Hairmyres Hospital, Lanarkshire. Specimens were transported in Hartmann's physiological saline chilled to 4°C to the Department of Pharmacology, Glasgow University and all experiments carried out within 24h of resection. The premedication agents and intra-operative drugs administered were noted at the time of tissue collection and are listed in Table 5.4.

All tissues were macroscopically normal with no gross signs of tumour infiltration or inflammation. In particular, representative biopsies of the ileal specimens obtained from the UC IPAA patients were submitted for histological analysis. There were no instances of backwash ileitis (see discussion).

Premedication agents
Diazepam; metoclopramide hydrochloride (Maxolon, Beecham); ranitidine hydrochloride (Zantac, Glaxo); temazepam
Intravenous anaesthetic agents
Propofol (Diprivan, Zeneca); thiopentone sodium (Intraval sodium, Rhone-Poulenc Rorer)
Inhalational anaesthetics
Enflurane (Abbott); isoflurane (Abbott); nitrous oxide; oxygen
Muscle relaxants
Pancuronium bromide (Pavulon, Organon-Teknika); vecuronium bromide (Norcuron, Organon-Teknika)
Analgesics
Alfentanil (Rapifen, Janssen); diclofenac sodium (Voltarol, Geigy); fentanyl (Sublimaze, Janssen); ketorolac trometamole (Toradol, Syntex); morphine sulphate
Local anaesthetics
Bupivicaine hydrochloride (Marcain, Astra)
Lignocaine hydrochloride
Antiemetics
Droperidol (Droleptan, Janssen); ondansetron hydrochloride (Zofran, Glaxo)
Antibiotics
Cefotaxime (Claforan, Roussel); ciprofloxacin hydrochloride (Ciproxin, Beyer); gentamicin sulphate (Cidomycin, Roussel); metronidazole (Flagyl, Rhone-Poulenc Rorer)
Others
Ephedrine hydrochloride; heparin sodium (Minihep, Leo); methoxamine (Vasoxine, Calmic)

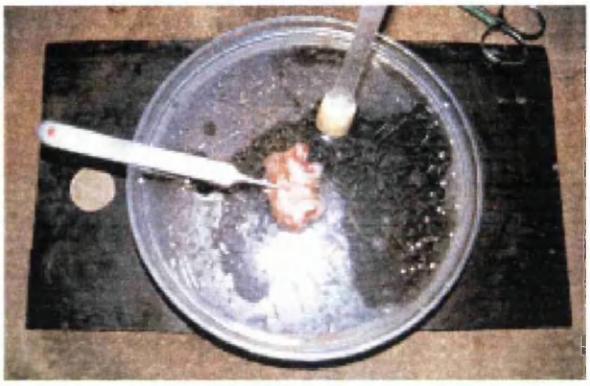
Table 5.4: Drugs administered pre- and intraoperatively prior to resection of specimens for smooth muscle pharmacology experiments.

Surrounding mesentery and fat were removed from the ileal samples and the specimen incised along the antimesenteric border in a longitudinal direction (see Fig 5.1). The bowel segment was pinned out flat with the mucosal surface uppermost. A mucosectomy was then performed with the aid of the dissecting microscope. The circular layer of the muscle wall was preserved and strips measuring 10mm x 2-3mm cut along the longitudinal axis.

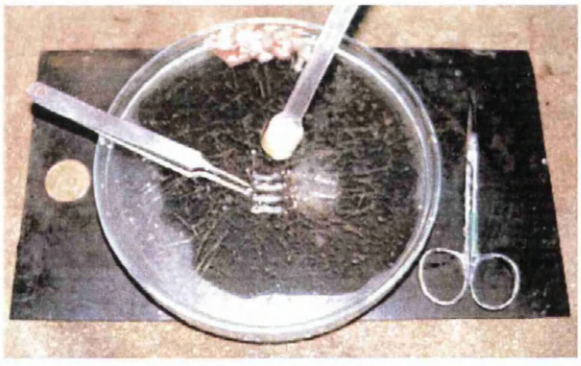
5.2.3 EXTRACELLULAR RECORDING OF SPONTANEOUS ACTIVITY

Simultaneous extracellular recordings of electrical and mechanical activity were made using the Golenhofen apparatus (Golenhofen & Loh, 1970), see Fig 5.2. This apparatus provides an extracellular method of recording changes in the membrane potential of a small number of cells together with simultaneous measurement of the mechanical activity of the whole specimen. The equipment consists of four platinum wire ring electrodes (i.d. ~2.5mm) contained within a narrow, water jacketed (37±0.5°C) glass capillary (i.d. ~2.3mm). These electrodes could be used for recording or stimulating, as required. Electrical signals were amplified (x1000, Neurolog A.C. preamplifier NL104) and filtered (Neurolog NL115, low frequency cut-off 10Hz, high frequency cut-off 10KHz). Mechanical activity was measured using an isometric force-displacement transducer (Grass FT03). Electrical and mechanical activity were displayed on a storage oscilloscope (Hitachi VC-6023) and recorded on an instrumentation tape recorder (Racal, Store 4DS) and U.V. oscillograph (Thorn EMI 6150-Mk II), see Fig 5.3. Longitudinal ileal strips of the same dimensions mentioned above were pulled through the apparatus and left to equilibrate under 1g tension. Drugs were added to the Krebs' solution which continually perfused the tissue chamber. Before entering the Golenhofen apparatus the Krebs' solution flowed through a water jacketed heat exchange coil to maintain it at 37±0.5°C.

1. Whole specimen.



2. Incised over anti-mese nteric length to enable excision of mucosa / submucosa.



3. Preparation of longitudinally orientated muscle strips.

Fig 5.1 Dissection of resected human ileal specimens. Note 1p piece for scale.

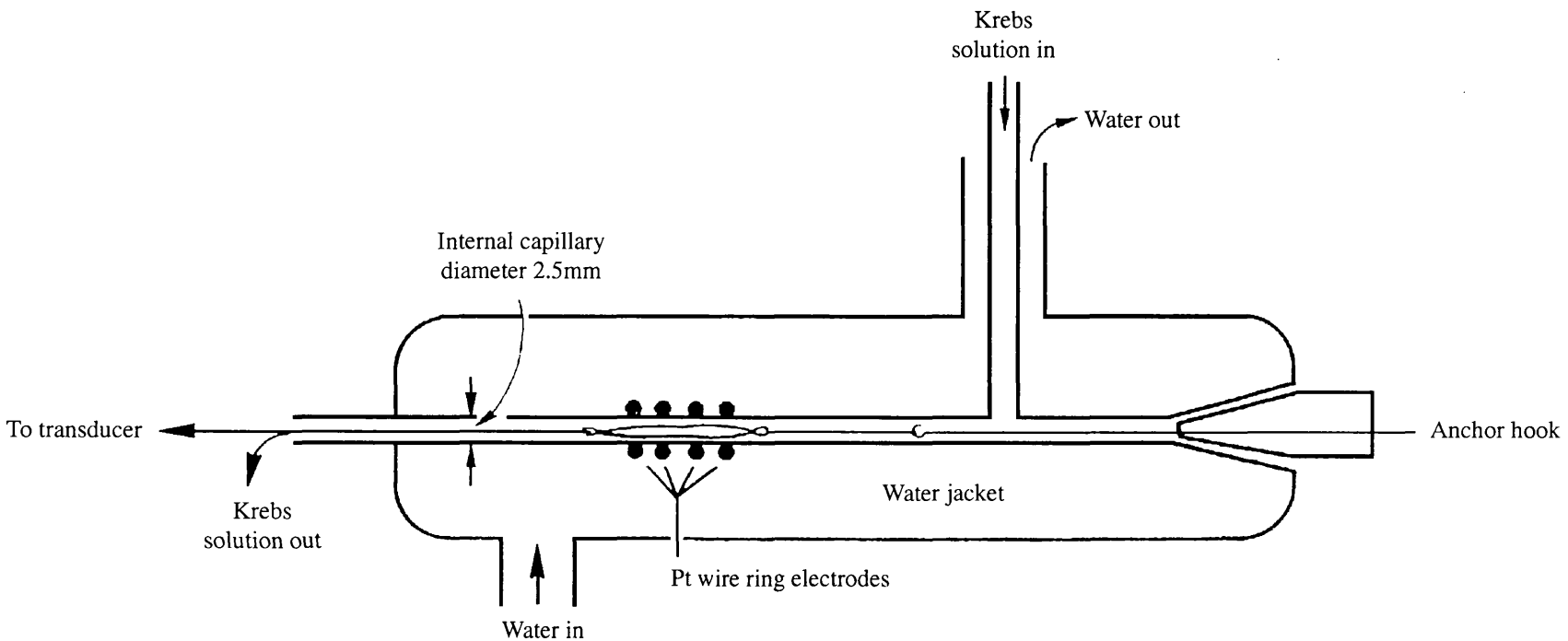


Fig 5.2 The Golenhofen apparatus (Golenhofen & von Loh, 1970).
The tissue to be investigated was passed through a narrow, water-jacketed (37±0.5°C) glass capillary perfused (3ml/min) with oxygenated (95%0_2; 5% CO_2) Krebs solution, as indicated, and mounted between 4 platinum wire ring electrodes for extracellular electrical recording. One end of each tissue was tied, by thread, to an anchor hook, while the other was similarly attached to an isometric force displacement transducer.

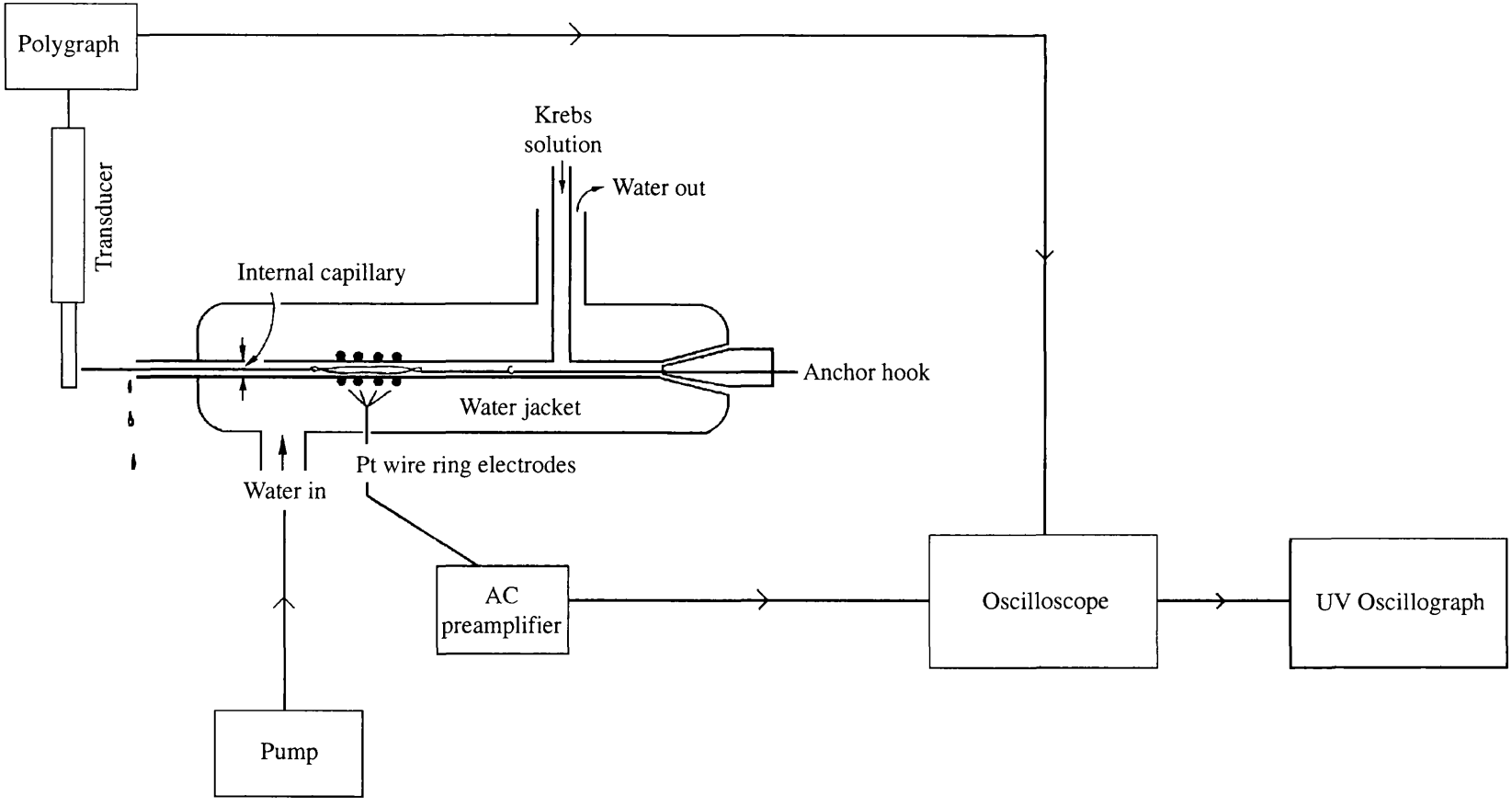


Fig 5.3 Apparatus for simultaneous recording of extracellular electrical and mechanical spontaneous activity in isolated human ileum.

5.2.4 RESPONSES TO NERVE STIMULATION AND DRUGS

Ileal muscle strips were attached to an electrode assembly which comprised two ring electrodes shielded in Araldite as shown in Fig 5.4. The assembly was suspended in conventional, jacketed (10ml) organ bath at 37±0.5°C, placed under 1g of isometric tone and allowed to equilibrate for 60min before experiments were commenced. The organ baths were filled with Krebs' solution (see below). Tissues were gassed continuously with 95% O_2, 5% CO_2 at 37±0.5°C. During equilibration, the organ bath fluid was changed approximately every 15min. To examine inhibitory responses to EFS, tone was raised by the addition of carbachol ($3x10^{-6}$M to 10^{-5}M) before stimulation. The effects of drugs were examined when a reproducible response to stimulation (1-64Hz) was obtained. Inhibitory frequency response curves to EFS were constructed using a fixed number of pulses (64), of width 0.05msec, at supramaximal voltage (60V) and at frequencies ranging between 1 and 64Hz. Trains of stimuli were delivered at 3-5 min intervals.

Antagonists were added to the bath and allowed 15-20min to take effect. All drugs were added by injection to the bath in volumes of 10µl and 30µl.

5.2.5 PHYSIOLOGICAL SOLUTIONS

Hartmann's solution (sodium lactate intravenous infusion) contained the following ions (mM): Na^+ 131, K^+ 5, Ca^{++} 2, HCO_3^-(as lactate) 29, Cl^- 111.

The composition (mM) of Krebs' solution was as follows: NaCl 118.4, $NaHCO_3$ 25, NaH_2PO_4 1.1, KCl 4.7, $MgCl_2$ 1.3, $CaCl_2$ 2.7 and Glucose 11.

5.2.6. DRUGS

The following drugs were used: apamin (Sigma);` L-arginine hydrochloride (L-ARG; Sigma); atropine sulphate (Sigma); carbamylcholine chloride (carbachol, Koch Light); bovine haemoglobin (HbO; Sigma); guanethidine monosulphate (Ciba); metronidazole (Flagyl, Rhone-Poulenc Rorer); N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME; Sigma); nitro blue tetrazolium (NBT, Sigma); β-

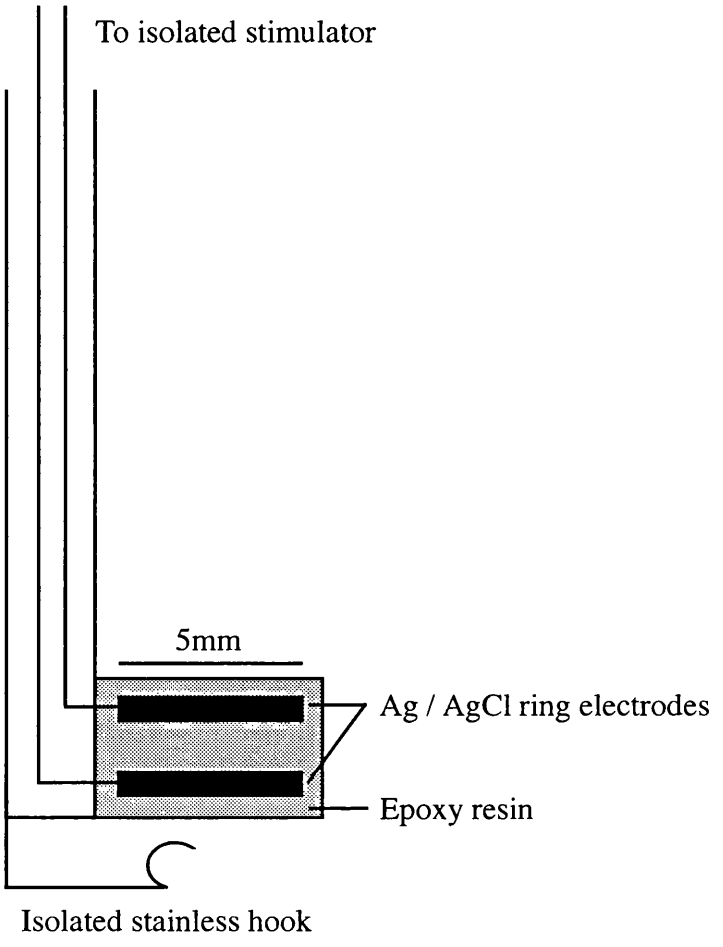


Fig 5.4 Stimulating electrode assembly. The tissue was pulled gently through the ring electrodes; one end had a thread attached to the stainless steel hook at the bottom and the other end was attached by thread to a force displacement transducer (not shown). The entire assembly was held in a 10ml organ bath and attached by wires to a Grass stimulator for electrical stimulation.

nicotinamide adenine dinucleotide phosphate diaphorase (βNADPH, Sigma); N$^{\varpi}$-nitro-D-arginine methyl ester hydrochloride (D-NAME, Sigma); potassium chloride (KCl, Sigma); sodium hydrosulphite (Sigma); sodium nitroprusside (SNP; BDH); sucrose (Sigma); tetrodotoxin (TTX, Sigma). Drug concentrations in the bath refer to the salt, with the exception of apamin, HbO and TTX, which are expressed as concentrations of the base. Stock solutions of the drugs were prepared in distilled water before dilution with Krebs solution prior to their use. HbO was prepared as described by Martin and colleagues (Martin *et al*, 1985).

5.2.7 NADPH-DIAPHORASE HISTOCHEMISTRY

The presence of NO in control and UC ileal specimens was detected microscopically using histochemistry for reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase , which is a NOS (see Chapter 4), using the method described by Valtschanoff (Valtschanoff *et al*, 1992).

Immediately following surgical resection, ileal specimens adjacent to the portion obtained for organ bath experimentation were fixed in a solution containing 4% paraformaldehyde in 0.1M phosphate buffer solution pH 7.3 (PBS). Specimens were immersed in this solution for 24h at 4^{o}C. Cryoprotection was then achieved by immersing the specimens in a solution containing 30% sucrose in 0.1M PBS until they sank to the bottom (~30 min) to prevent cells shattering on subsequent freezing. The tissue specimens were snap frozen in isopentane cooled by liquid nitrogen and placed in a cryostat (Reichert-Jung, Cryocut 1800) for subsequent sectioning. Transverse sections were cut at 20μm and placed on glycerine-coated slides which were set aside at room temperature to permit tissue adhesion. The slides were prepared by coating in a mixture of gelatin 0.5g, chrome alum 0.05g and distilled water made up to a final volume of 100ml. After dipping the slides in this solution they were placed in a vertical slide holder and dried at 60^{o}C in an incubator overnight. Subsequent staining was carried out by immersing the lowest 1-1.5cm of the slide, covered on the outside by aluminium foil, in a solution of

0.1M PBS containing 0.25% Triton X-100 solution and leaving for 10 minutes. Into this solution 0.1M PBS containing 0.2mg/ml nitro blue tetrazolium was added along with 0.5mg/ml β-NADPH. The slides were then incubated at 37°C and examined after 1-2 hours for staining. Thereafter sections were rinsed in 1M PBS to stop the reaction, graded concentrations of ethanol (75%, 95% and 100%) and then to increasing concentrations of Histoclear (50%, 75% and 100%) for approximately 5min each. Because the reaction product was soluble in ethanol, rinses in alcohols were short (1min for each concentration). The slides were allowed to dry before being mounted and were then examined using light microscopy. Permanent records of the NADPH-diaphorase staining were made by photomicrography.

Tissues were obtained from 15 patients for NADPH-diaphorase histochemistry. Their status and disease categories are listed in Table 5.5.

5.2.8 ANALYSIS OF RESULTS

SPONTANEOUS ACTIVITY, THE EFFECTS OF TEMPERATURE AND PERFUSED DRUGS

Following the equilibration period tissues developed spontaneous electrical and mechanical activity. The number of phasic contractions after the onset of spontaneous mechanical activity was determined in a steady-state 5-min period (Fig 5.5). Summation contractions, defined as two or more phasic contractions recorded without a return to baseline tonic contraction, were noted when they occurred. The parameters measured (as illustrated in Fig 5.6) comprised the frequency of contractions (min^{-1}); the mean maximum contraction per burst of mechanical activity (g); and mean duration of each mechanical burst (s). These parameters were measured over a 5min period after the onset of spontaneous mechanical activity. In a preliminary study of spontaneous mechanical activity in strips of terminal ileum from control subjects, UC and ISTC patients, the number

Category	Condition	Surgical procedure	Number of patients
Control	Postvagotomy diarrhoea	Reverse ileal onlay graft	2
	Caecal neoplasm	Right hemicolectomy	2
Ulcerative colitis (UC)	Fulminant UC	Emergency TSRP	1
	Chronic UC; intact	Elective TSRP	5
	Chronic UC; staged	Elective TSRP	3
Others	Previous UC; ileoanal pouch dysfunction	Defunctioning of TSRP with formation of ileostomy	2

Table 5.5: Terminal ileum specimens submitted for NADPH-diaphorase histochemistry- patient categories.

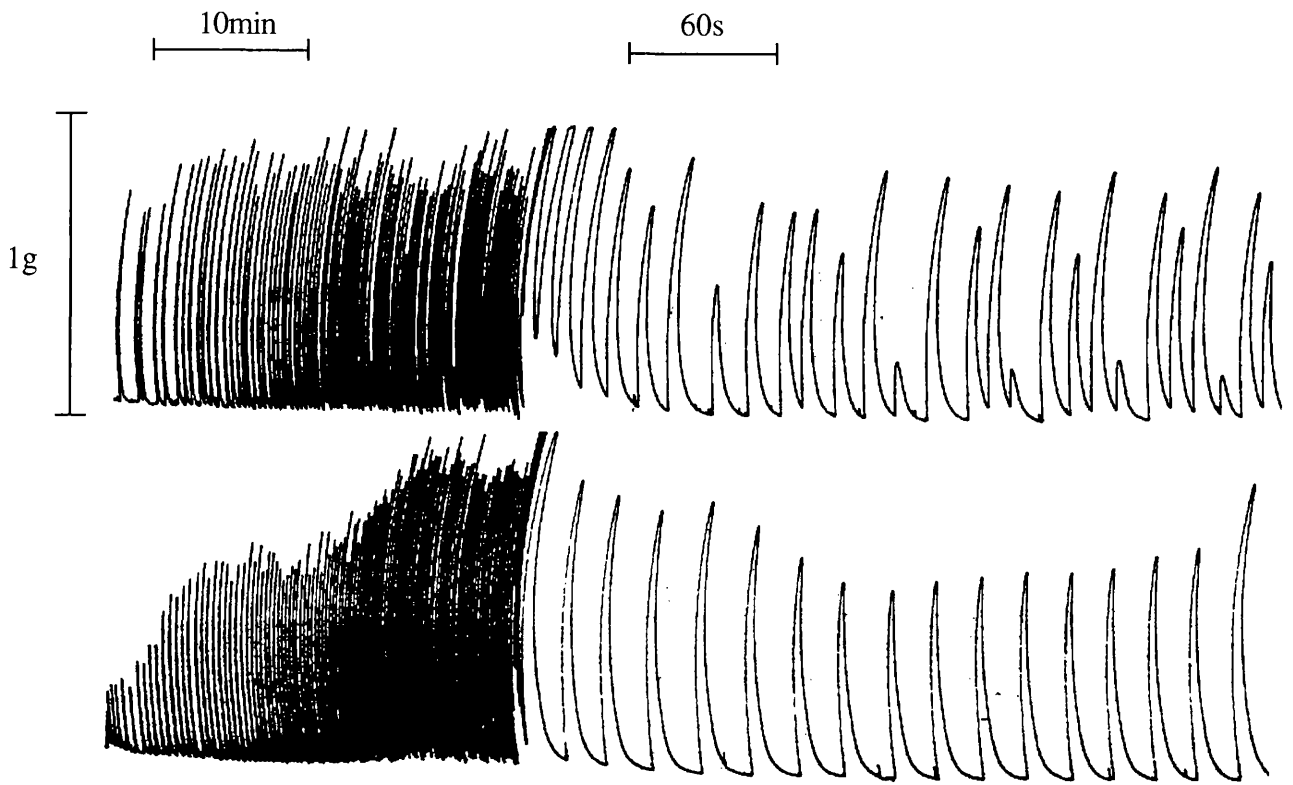


Fig 5.5 Spontaneous mechanical activity in 2 human ileal muscle strips after equilibration under 1g tension in organ bath study. Note the spontaneous mechanical activity in each muscle strip shown at 2 different paper speeds viz. 2.5mm/min and 25mm/min.

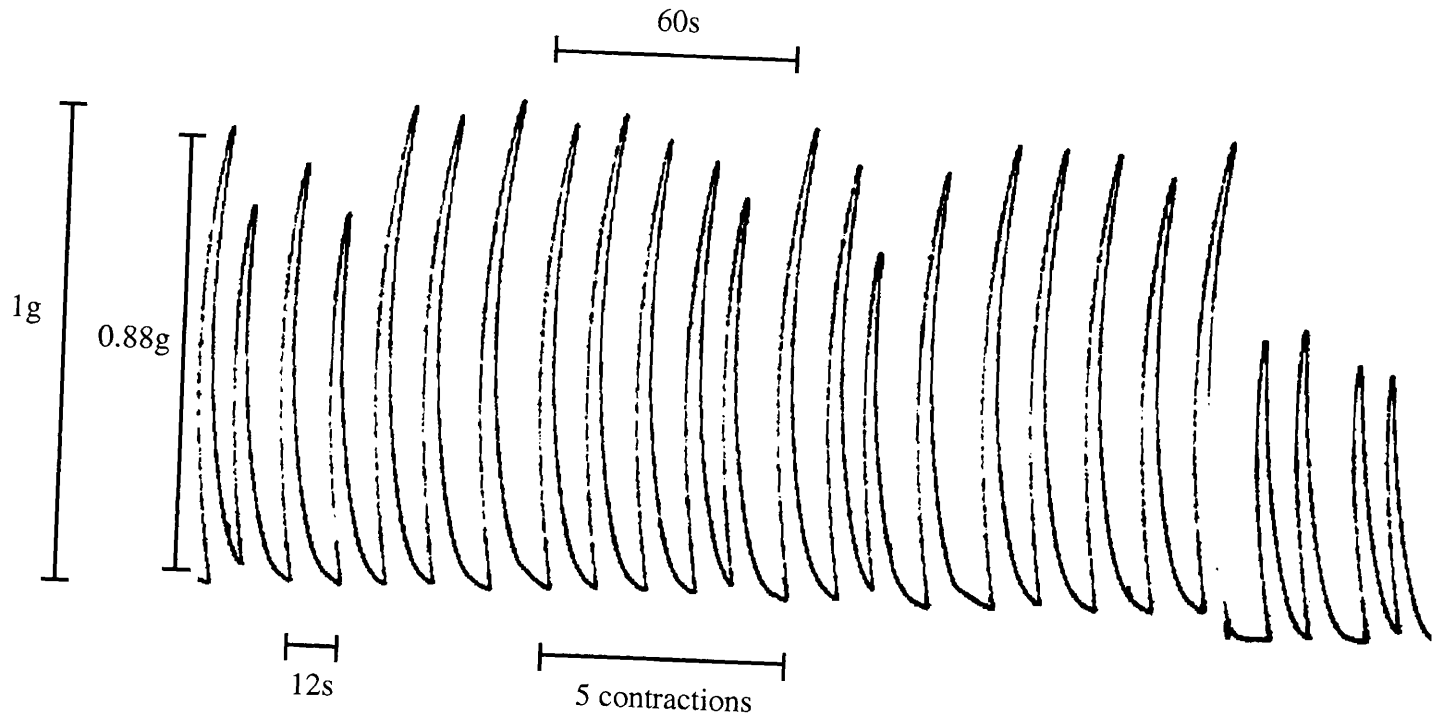


Fig 5.6 Parameters measured in assessment of spontaneous mechanical activity in human ileal muscle strips in organ bath study after equilibration under 1g tension.

of phasic contractions was determined for a 5min period following the onset of spontaneous activity and over a 5min period 30min after the initial measurement. Paired Student's t-test revealed no significant differences between the initial frequency of phasic contractions and the frequency of phasic contractions recorded after 30min for each of the patient categories (see Table 5.6).

In Golenhofen apparatus experiments the same mechanical parameters were measured as above, in addition to the frequency of bursts of spiking of electrical activity (bursts min^{-1}); maximum spike amplitude per burst of electrical activity (mV); number of electrical spikes per burst (spikes $burst^{-1}$); and duration of each electrical burst (s). The spontaneous activity parameters measured in a typical Golenhofen apparatus recording are shown in Fig 5.7.

Comparisons of spontaneous activity parameters among the patient groups were made using Mann-Whitney *U* tests. A probability level of $P<0.05$ was considered statistically significant. ** indicates $P<0.001$ and * $P<0.05$.

INHIBITORY NERVE STIMULATION AND DRUGS

Relaxation (inhibitory) responses to EFS and to drugs were expressed as a percentage of tension developed by the contractile agonist (carbachol 10^{-6}M-$3x10^{-6}$M). The effects of drugs on inhibitory responses to EFS were expressed as a percentage inhibition of nerve mediated relaxation at a given stimulation frequency, pulse width and supramaximal voltage. These results were then expressed as mean $\pm$ S.E.M. A probability level of $P<0.05$ was considered statistically significant. ** indicates $P<0.001$ and * $P<0.05$.

Diagnosis	n	Frequency (min^{-1}) of mechanical spontaneous activity in initial 5min period	Frequency (min^{-1}) of mechanical spontaneous activity in 5min period 30min later	P
Controls	8	3.72 ± 0.69	4.05 ± 0.51	0.71, n.s.
UC	8	2.78 ± 0.22	3.08 ± 0.16	0.29, n.s.
ISTC	6	4.9 ± 1.2	4.97 ± 1.2	0.97, n.s.

Table 5.6: Comparison of mean frequencies of spontaneous phasic contractions in strips of human terminal ileum from controls, ulcerative colitics (UC) and patients with idiopathic slow transit constipation (ISTC), during an initial 5min period of stable spontaneous activity and a second 5min period 30min later in organ bath apparatus.
n= number of patients (one strip analysed per subject).
Results expressed as mean ± SEM; P calculated using paired Student's t-test.
n.s., not significant.

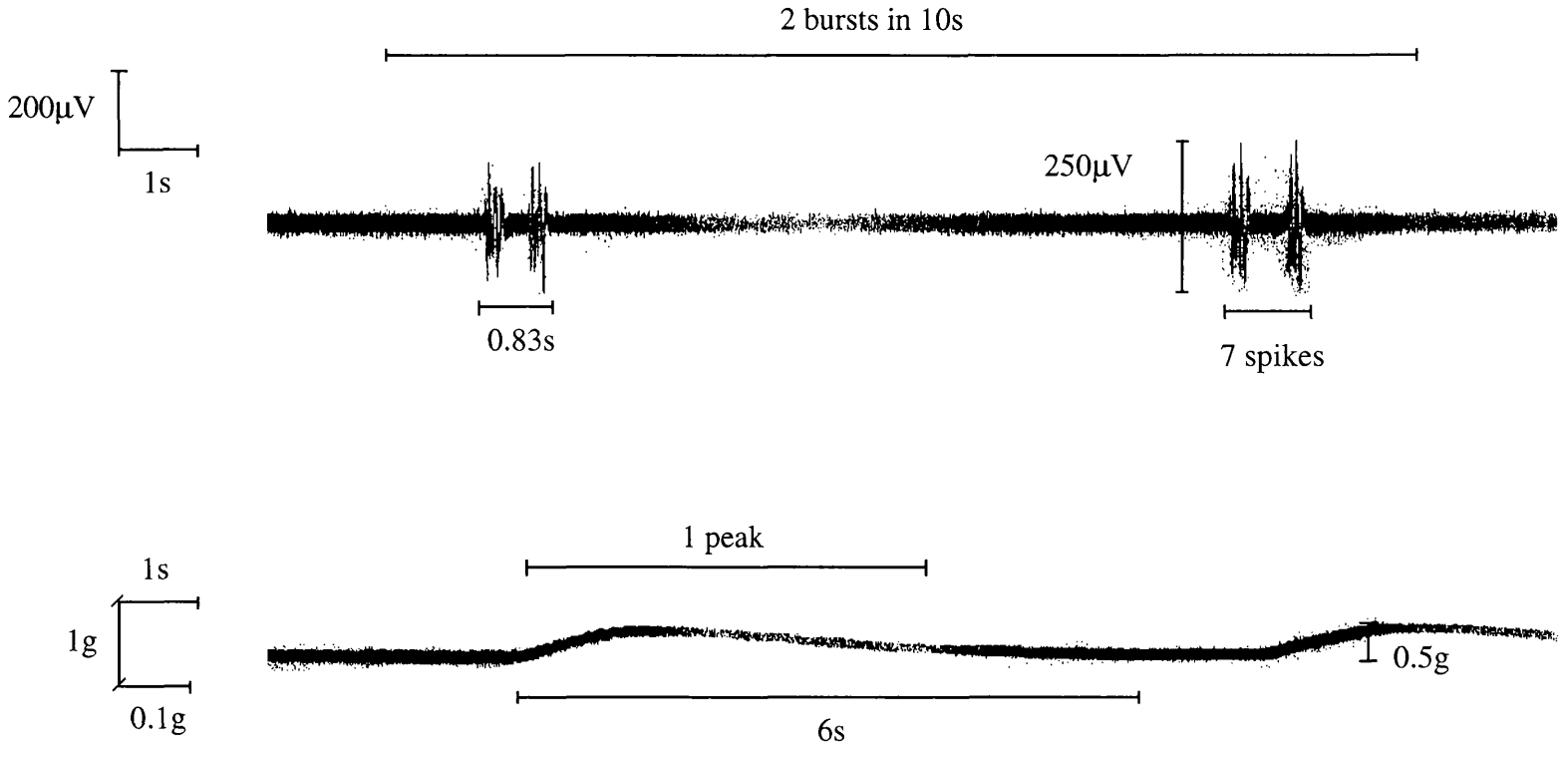


Fig 5.7 Electrical (top trace) and mechanical parameters measured in Golenhofen recording.

5.3 RESULTS

5.3.1 SPONTANEOUS ACTIVITY, THE EFFECTS OF TEMPERATURE AND PERFUSED DRUGS

SPONTANEOUS MECHANICAL ACTIVITY

When strips of ileum from control subjects (16 patients, 99 muscle strips), UC patients (18 patients, 160 muscle strips) and ISTC patients (7 patients, 37 muscle strips) were suspended in jacketed organ baths (Fig 5.4), and allowed to equilibrate under 1g of isometric tension, the presence of spontaneous mechanical activity in strips from each patient group varied (controls, 60%; UC patients, 49%, ISTC patients, 49%). The equilibration period was set at 60min, with 15min washouts. The development of spontaneous mechanical activity occurred at a mean of 56.6min $\pm$ 9.40min in control strips; at a mean of 62.2 $\pm$7.43min in UC strips; and at a mean of 93.3 $\pm$ 16.7min in ISTC strips after organ bath suspension. When compared to control strips there were no significant differences in the times for UC and ISTC strips to develop spontaneous mechanical activity (P=0.43 and P=0.07 respectively, Mann-Whitney *U* test applied). The time taken to develop spontaneous mechanical activity in these preparations may have partly reflected equilibration, rewarming of the tissues after transport and dissection (see effects of temperature below). The development of spontaneous mechanical activity in 2 strips of control ileum from the same patient is shown in Fig 5.5.

The frequency of spontaneous mechanical activity and the mean amplitude of contractions (measured in g of isometric tension) were compared from controls, UC and ISTC patient groups. The data are summarised in Table 5.7. No significant differences were found.

In each of the three patient groups, after 1g of isometric tension had been applied to the suspended ileal strips, further increases in tension to 3g caused an increase in the amplitude of mechanical activity (not shown). This may have been

SPONTANEOUS MECHANICAL ACTIVITY PARAMETER	CONTROL	UC	ISTC
Number of patients (number of muscle strips analysed)	**16 (99)**	**18 (160)**	**7 (37)**
Percentage of strips showing spontaneous activity	**59.69±8.45**	**44.17±6.71 P=0.24**	**49±9.74 P=0.40**
Frequency of spontaneous contractions (min^{-1})	**4.39±0.66**	**3.40±0.61 P=0.23**	**4.54±1.16 P=0.71**
Isometric tension of spontaneous contractions (g)	**0.66±0.14**	**0.84±0.16 P=0.54**	**0.56±0.21 P=0.92**
Duration of spontaneous contractions (s)	**11.7±2.33**	**16.12±2.77 P=0.19**	**18.33±4.34 P=0.20**

Table 5.7: Spontaneous mechanical activity characteristics in each of the three patient groups studied.
Mann-Whitney *u* tests applied to compare UC and ISTC parameters with control subjects.

caused by stretch-induced depolarisation, where more smooth muscle cells in the preparation would be at, or close to their firing threshold. Stretching above 3g, to limits of 5g resulted in a decrease in the frequency of spontaneous mechanical activity. This could have been caused by a stretch-induced form of depolarising block, or simply due to destruction of nexuses and close junctions resulting in diminished syncytial activity. Similar responses to stretch were noted in preparations where simultaneous measurements of spontaneous extracellular electrical and mechanical activity were recorded (see below).

In the organ bath studies, where tissues were suspended vertically rather than horizontally as in the Golenhofen apparatus, no spontaneous activity was seen in 40% of control strips (40 specimens); 51% of UC strips (82 specimens); and 51% of ISTC strips (19 specimens). Although a constant feature in some subjects, the absence of spontaneous activity in patients was not universal. Absence of spontaneous activity, either in one or all of the strips from one patient may have been related to the trauma of transport and dissection. None of the pre- or intraoperative drugs (Table 5.4) was thought likely to have caused abolition of spontaneous mechanical activity. In those cases where there was absence of spontaneous activity in every muscle strip analysed, there were no instances of perioperative administration of Ca^{++} channel blockers such as nifedipine.

SYNCHRONOUS MEASUREMENT OF SPONTANEOUS ELECTRICAL AND MECHANICAL ACTIVITY

GENERAL ASPECTS

Sixty-seven muscle strips from 30 patients (12 controls, 9 UC, 7 ISTC, 1 vagotomised patient, 1 CD) were studied using the Golenhofen apparatus. The 12 control subjects included 1 individual with FAP. Of the 9 UC patients, 3 were intact at the time of TSRP, 1 was intact i.e no previous colonic surgery, at the time of right hemicolectomy for coincidental neoplasm, 3 were staged and 2 had

fulminant UC. Nine strips showed no evidence of spontaneous activity (13.4%). Tables 5.8- 5.10 summarise the details of the 30 patients.

In each of the 3 groups of patients studied with the Golenhofen apparatus, rapid bursts of electrical activity preceded and continued during the mechanical contractions (Fig 5.8). Each spike-burst was accompanied by a burst of contraction with periods of quiescence between individual bursts. Contractions never occurred in the absence of electrical firing, and vice versa, thus the frequency of contractile bursts matched that of electrical firing in all 3 groups.

The activity parameters for control patients with evidence of spontaneously active muscle strips were as follows. The electrical, and mechanical events occurred with a frequency of 9.3 ± 0.96min^{-1} (n=10) and each contained 6.80 ± 1.01 spikes (n=10). The duration of each spike-burst was 1.42 ± 0.31s (n=10) and the amplitude of the largest spike in each was 309.4 ± 59.0 μV (n=10). Each mechanical event contained 1.6 ± 0.6 peaks (n=10) and lasted 8.2 ± 1.99s (n=10). The maximum tension developed during each was 1.88 ± 0.46g (n=10).

The activity parameters for chronic UC patients with evidence of spontaneously active muscle strips were as follows The electrical, and mechanical events occurred with a frequency of 8.6 ± 0.78min^{-1} (n=7) and each contained 9.71 ± 1.78 spikes (n=7). The duration of each spike-burst was 2.18 ± 0.62s (n=7) and the amplitude of the largest spike in each was 352.0 ± 77.9 μV (n=7). Each mechanical event contained 1.14 ± 0.14 peaks (n=7) and lasted 7.9 ± 1.07s (n=7). The maximum tension developed during each was 1.14 ± 0.15g (n=7).

The activity parameters for the ISTC patients studied were as follows. The electrical, and mechanical events occurred with a frequency of 9.1 ± 1.53min^{-1} (n=7) and each contained 3.86 ± 0.14 spikes (n=7). The duration of each spike-burst was 0.66 ± 0.12s (n=7) and the amplitude of the largest spike in each was 304.3 ± 42.4μV (n=7). Each mechanical event contained 1 ± 0 peaks (n=7) and lasted 6.07 ± 0.52s (n=7). The maximum tension developed during each was 1.43 ± 0.13g (n=7).

PATIENT	AGE	SEX	DIAGNOSIS	OPERATION	STRIPS
JP	39	M	Colonic neoplasm	Right hemicolectomy	2
MK	62	F	Colonic neoplasm	Right hemicolectomy	1
WM	66	M	Colonic neoplasm	Right hemicolectomy	1
IS	51	M	FAP	Totally stapled restorative proctocolectomy-previous IRA	1
AR	77	F	Colonic neoplasm	Right hemicolectomy	3
MMcN	57	F	Colonic neoplasm	Coloanal excision + ileostomy formation	1
ML	47	F	Colonic neoplasm	Right hemicolectomy	3
MMcL	78	F	Colonic neoplasm	Right hemicolectomy	1
HMcG	73	F	Colonic neoplasm	Right hemicolectomy	1
PT	72	M	Colonic neoplasm	Right hemicolectomy	1
EL	46	F	Colonic neoplasm	Right hemicolectomy	1
EB	31	M	Angiodysplasia	Right hemicolectomy	1

Table 5.8: Golenhofen studies in control patients. FAP: familial adenomatous polyposis; IRA, ileorectal anastomosis.

PATIENT	AGE	SEX	DIAGNOSIS	OPERATION	STRIPS
WF	34	M	UC-Intact	Totally stapled restorative proctocolectomy	1
AT	45	M	UC-Intact	Totally stapled restorative proctocolectomy	1
JH	37	M	UC -Fulminant	Emergency subtotal colectomy	1
WMcG	78	M	UC-Intact + Ca	Right hemicolectomy	1
SMcV	44	F	UC-Staged	Totally stapled restorative proctocolectomy-staged	2
RK	31	M	UC-Staged	Totally stapled restorative proctocolectomy-staged	4
TD	40	M	UC-Fulminant	Emergency subtotal colectomy	1
SMcN	16	M	UC-Staged	Totally stapled restorative proctocolectomy-staged	1
DK	29	M	UC-Intact	Totally stapled restorative proctocolectomy	2

Table 5.9: Golenhofen studies in ulcerative colitic (UC) patients. Ca, colonic neoplasm.

PATIENT	AGE	SEX	DIAGNOSIS	OPERATION	STRIPS
FW	29	F	ISTC	Subtotal colectomy + ileorectal anastomosis	2
AB	34	F	ISTC	Subtotal colectomy + ileorectal anastomosis	10
CL	34	F	ISTC	Subtotal colectomy + ileorectal anastomosis	2
MMcC	32	F	ISTC	Subtotal colectomy + ileorectal anastomosis	2
AM	32	F	ISTC	Subtotal colectomy + ileorectal anastomosis	2
EMcG	47	F	ISTC	Subtotal colectomy + ileorectal anastomosis	2
JI	48	F	ISTC	Subtotal colectomy + ileorectal anastomosis	10
RH	59	F	PVD	Reverse ileal onlay graft	1
IMcC	70	F	CD	Right hemicolectomy	5

Table 5.10: Golenhofen studies in idiopathic slow transit constipation (ISTC) patients and other miscellaneous conditions. PVD, postvagotomy diarrhoea; CD, Crohn's disease.

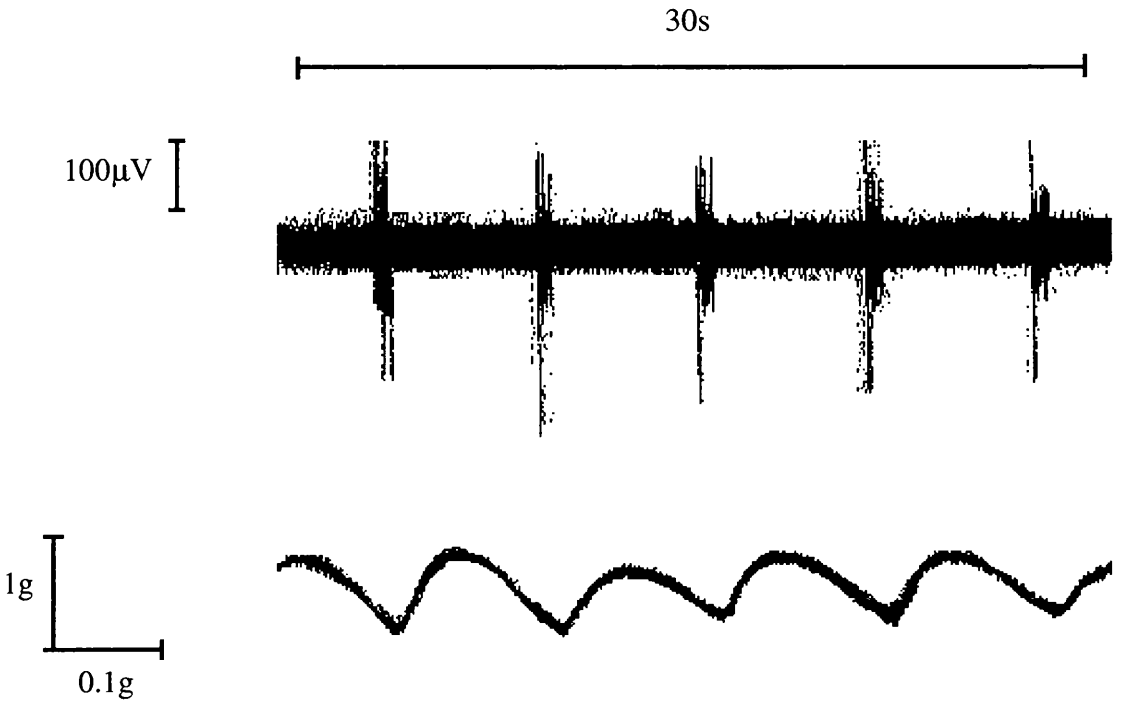


Fig 5.8 The spontaneous electrical (top trace) and mechanical activity recorded in the Golenhofen apparatus from a control strip of human ileal muscle. Each burst of electrical activity was accompanied by contraction, confirming synchronization of electrical and mechanical activity.

Two patients were examined during the course of these experiments who underwent emergency subtotal colectomy for fulminant UC (see Table 5.9). Both individuals had received pre- and intraoperative intravenous doses of hydrocortisone. The first patient (JH) showed some mechanical spontaneous activity in organ bath experiments (4 out of 5 strips) but only weak electrical and mechanical responses in the Golenhofen apparatus which quickly faded and were unsuitable for measurement. The second patient (TD) showed mechanical spontaneous activity in all 3 strips studied in organ bath experiments as well as spontaneous electrical and mechanical activity in the Golenhofen apparatus. The data for TD Golenhofen recordings in 1 tissue strip were as follows. The frequency of spontaneous electrical and mechanical activity was $8min^{-1}$, the number of spikes per burst of electrical activity was 4, the duration of each electrical spike burst was 0.75s and the maximum amplitude of the electrical spike burst was 820 μV. There was 1 peak in each mechanical event and the duration of each was 6.5s. The maximum tension developed during each contraction was 1.4g.

Of the remaining 2 miscellaneous subjects the patient with CD (IMcC) showed no evidence of spontaneous activity in 5 strips analysed. Likewise, the patient with postvagotomy diarrhoea undergoing reverse ileal onlay grafting (RH) showed, rather surprisingly, no spontaneous activity in 1 strip analysed.

No significant differences were detected in spontaneous activity parameters when UC patients were compared with controls (see Table 5.11). However, the ISTC patients were noted to have fewer electrical spike per spike burst and shorter duration electrical spike bursts than control patients. Both of these differences reached statistical significance.

Whilst controls and ISTC patients showed a more regular pattern of spontaneous electrical and mechanical responses, some samples of UC ileum showed irregular patterns in both parameters (Fig 5.9). Such fibrillation-like activity may have been artefactual, caused by subtle changes in the longitudinal orientation of the strip to a more tangential direction. This would have enabled

SPONTANEOUS ELECTRICAL/ MECHANICAL ACTIVITY PARAMETER	CONTROL	UC	ISTC
Number of patients/ strips analysed	10	7	7
Frequency of spontaneous electrical activity/ mechanical contractions (min^{-1})	9.3±0.96	8.6±0.78 P=0.65	9.1±1.53 P=0.46
Number of electrical spikes per spike burst	6.80±1.01	9.71±1.78 P=0.22	3.86±0.14 *P=0.03
Duration of electrical spike burst (s)	1.42±0.31	2.18±0.62 P=0.40	0.66±0.12 *P=0.04
Maximum electrical spike amplitude (μv)	309.4±59.0	352.0±77.9 P=0.93	304.3±42.4 P=0.92
Peaks in mechanical contraction	1.6±0.6	1.14±0.14 P=0.93	1±0
Isometric tension of spontaneous contractions (g)	1.88±0.46	1.14±0.15 P=0.43	1.43±0.13 P=0.92
Duration of spontaneous contractions (s)	8.2±1.99	7.9±1.07 P=0.20	6.07±0.52 P=0.81

Table 5.11: Golenhofen apparatus recordings of spontaneous electrical and mechanical activity in the three patient groups studied.
Mann-Whitney *u* tests applied to compare UC and ISTC parameters with control subjects.

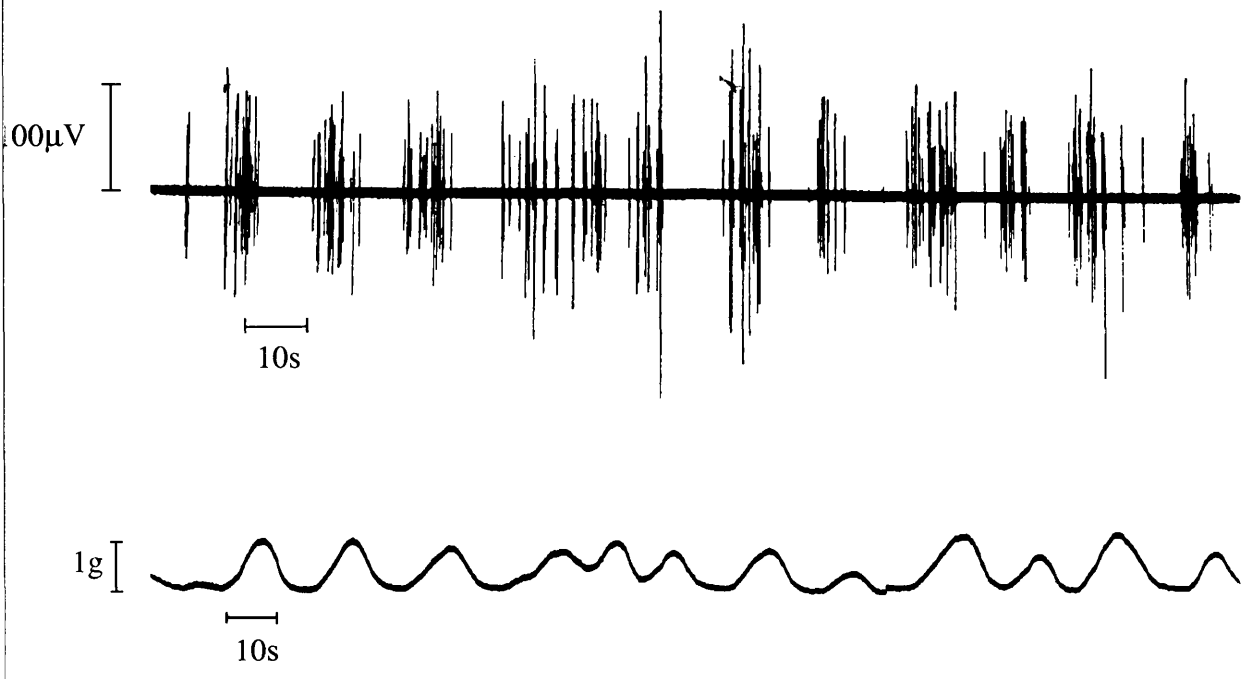


Fig 5.9 The spontaneous electrical (top trace) and mechanical activity recorded in the Golenhofen apparatus in an ileal strip from an ulcerative colitic undergoing IPAA.
Notice the "fibrillation" type pattern of mechanical activity.

greater amounts of inner circular smooth muscle to contribute to the electrical and mechanical events. Alternatively, erratic patterns of spontaneous activity may have reflected disruption of the myogenic pacemaker in terminal ileum by chronic UC.

Both electrical and mechanical events in human ileum were entirely dependent on the presence of extracellular Ca^{++}(not shown). Omission of $CaCl_2$ from the Krebs' solution, which happened accidentally on one occasion, or replacement of it with magnesium chloride abolished both spontaneous spiking and contraction (n=3; results not shown). The effects of diltiazem, a Ca^{++}-channel blocking drug are discussed below. The nerve toxin tetrodotoxin (TTX, 10^{-6}M), which acts by blocking Na^{+} channels, failed to abolish spontaneous pacemaker activity in the human ileum. Thus, in this tissue, the myogenic pacemaker activity relies on the presence of Ca^{++}- rather than Na^{+}-spikes. The TTX results may also imply that the channels for Na^{+} in human ileum are different to those in nerve.

EFFECTS OF COOLING AND REWARMING

Control (n=3), UC (n=2) and ISTC (n=3) tissues were each sensitive to temperature changes in the perfusing Krebs' solution (Fig 5.10). Stepwise cooling from 37.5°C to 27.5°C reduced the frequency and amplitude of slow wave activity and the preceding spike bursts of electrical activity. In general throughout the 3 patient groups there was no accompanying reduction in resting tone as reported in other spontaneously active smooth muscle preparations (Small, 1982). There was also no appreciable change in the maximum amplitude of isometric contraction produced in each temperature range. In 2 control patients, summation of contractions seen initially at 37.5°C disappeared on stepwise cooling but returned when the specimens were rewarmed to 37.5°C. Rewarming in all 3 types of ileal tissue restored mechanical and electrical activity.

No statistically significant differences were detected when UC and ISTC strips were compared to control tissues for changes in spontaneous activity in each temperature range (see Fig 5.11).

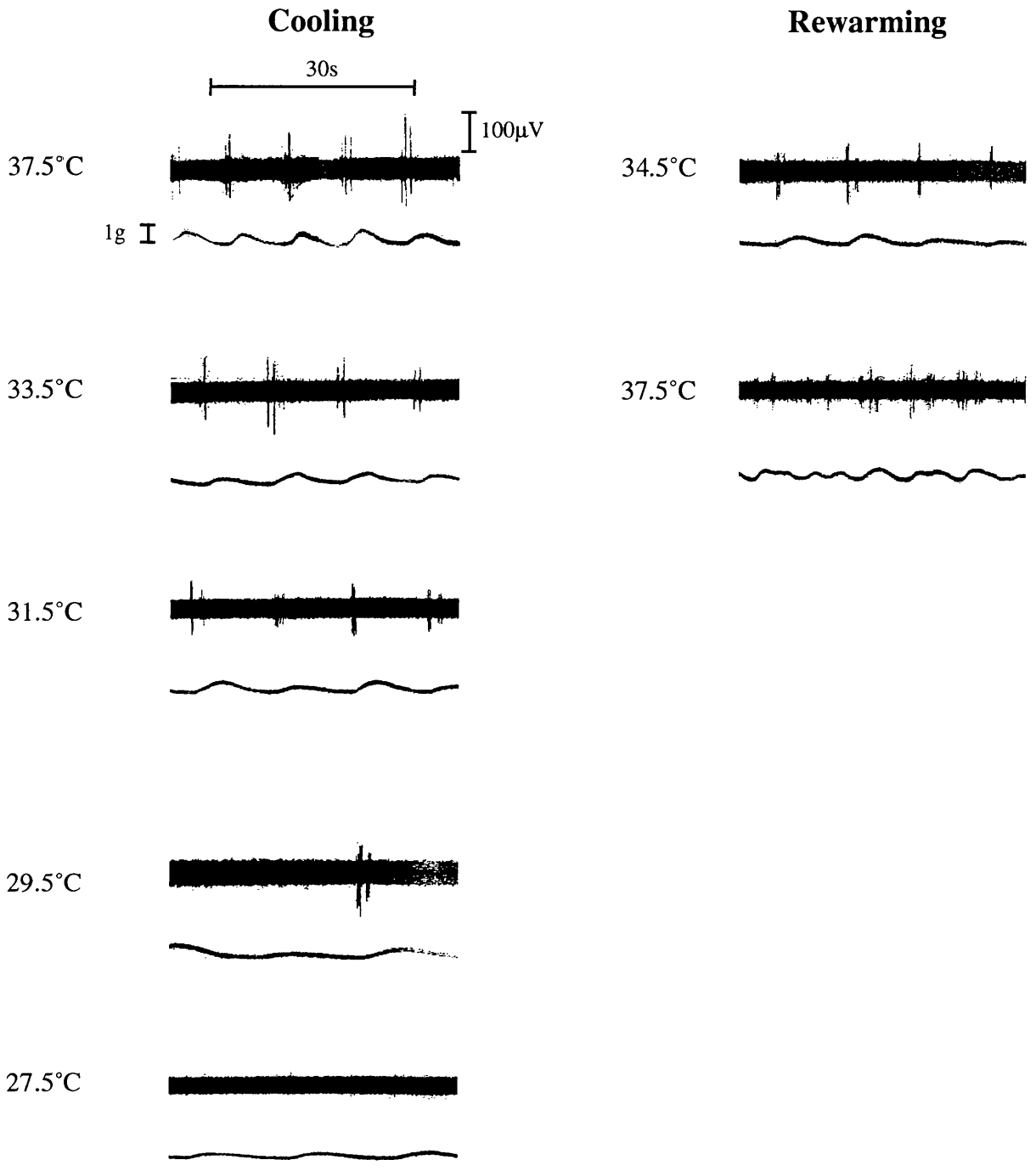


Fig 5.10 Golenhofen recording of the effects of cooling from 37.5°C- 27.5°C and rewarming to 37.5°C in an ileal strip from an ulcerative colitic undergoing IPAA.

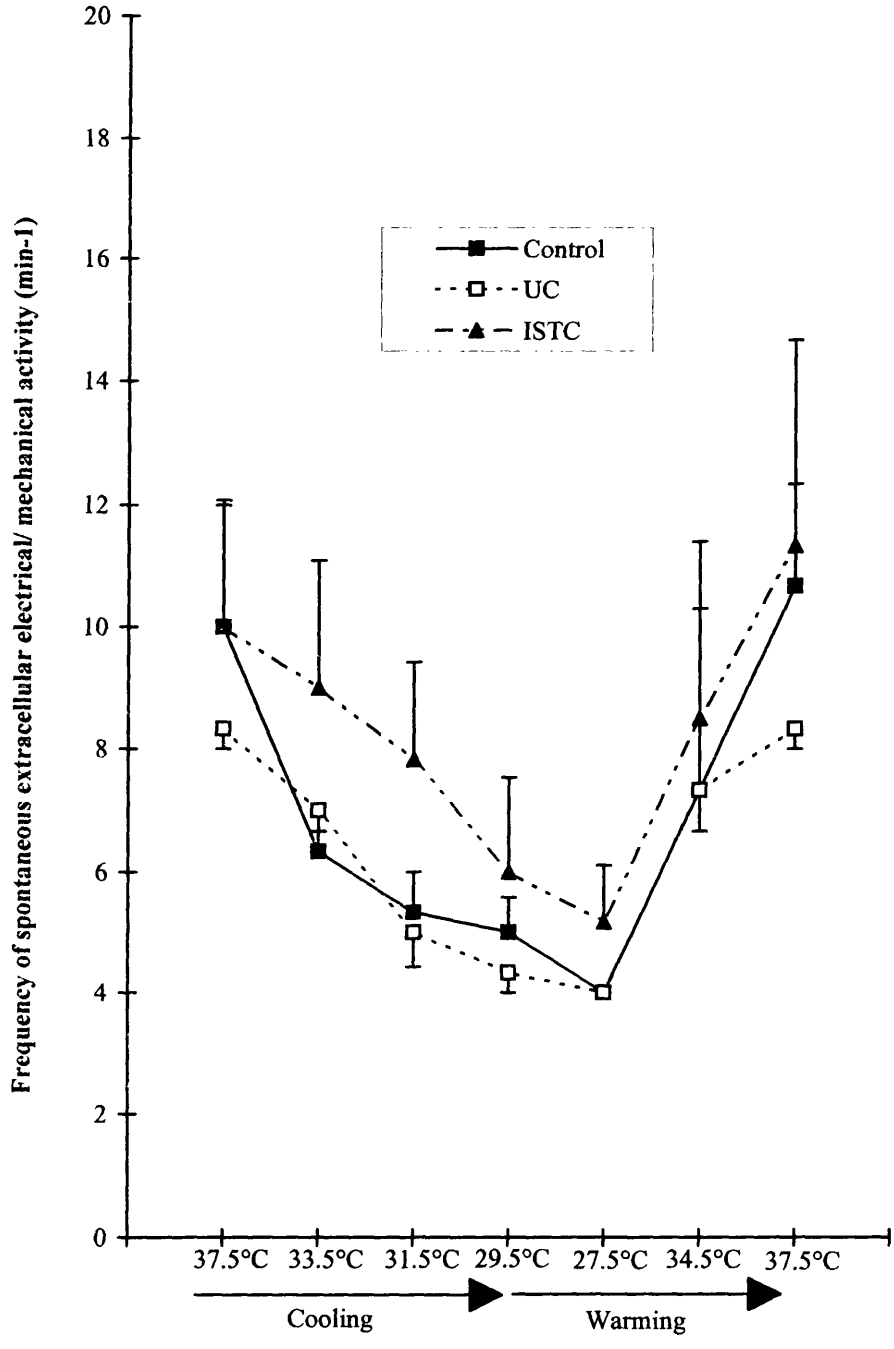


Fig 5.11: Graph of the effects of cooling and rewarming on the frequency (min^{-1}) of spontaneous extracellular electrical (slow wave)/ mechanical activity in ileal muscle strips from control, ulcerative colitic (UC) and idiopathic slow transit constipation (ISTC) patients. n=3 patients, 3 muscle strips, each category. The curves were not significantly different (UC and ISTC compared with controls, Student's t-tests).

EFFECTS OF PERFUSED DRUGS

Potassium chloride

The effects of KCl, used as a spasmogen, were studied in tissues from 9 patients (3 controls, 2 UC, 4 ISTC: 11 strips).

In general, infusion of KCl (0.1M) into the perfusate of the Golenhofen apparatus increased the frequency and amplitude of extracellular electrical spike activity, corresponding with a series of summation contractions which eventually produced a plateau in tone (Fig 5.12; n=5). There were no obvious differences in the patterns of response to KCl among each of the representative strips studied from the 3 patient categories.

Following infusion of KCl (0.1M), (1 UC, 2 ISTC). infusion of carbachol (10^{-6}M) then caused a further rise in mechanical tone with an associated increased extracellular electrical activity (3 strips). Atropine (10^{-6}M) subsequently abolished the rise in tone (1 strip) after 3min, although there was persistent spontaneous extracellular electrical activity.

The spasmogenic effect of KCl (0.1M) was abolished by SNP (10^{-6}M) in 1 strip (control) although, despite baseline tone and no spontaneous oscillations in mechanical tone, some rhythmic electrical activity persisted. The reverse occurred in a second patient (ISTC). Thus, the abolition of spontaneous mechanical and electrical activity after incubation with SNP (10^{-6}M) for 5min was reversed with 6min of incubation with 0.2M KCl. In a third patient (ISTC), pretreated with SNP (10^{-6}M), the relaxant effects wore off and spontaneous activity returned. Incubation with 0.1M KCl failed to have any significant effect, but the spasmogenic effects were demonstrated with the addition of 0.2M KCl.

In one experiment (UC), 0.1M KCl was ineffective in causing depolarisation after the muscle strip had been exposed to diltiazem 10^{-5}M.

Two strips of tissue from a patient with ISTC showed graded responses to two different concentrations of KCl. Incubation with KCl (0.05M) failed to

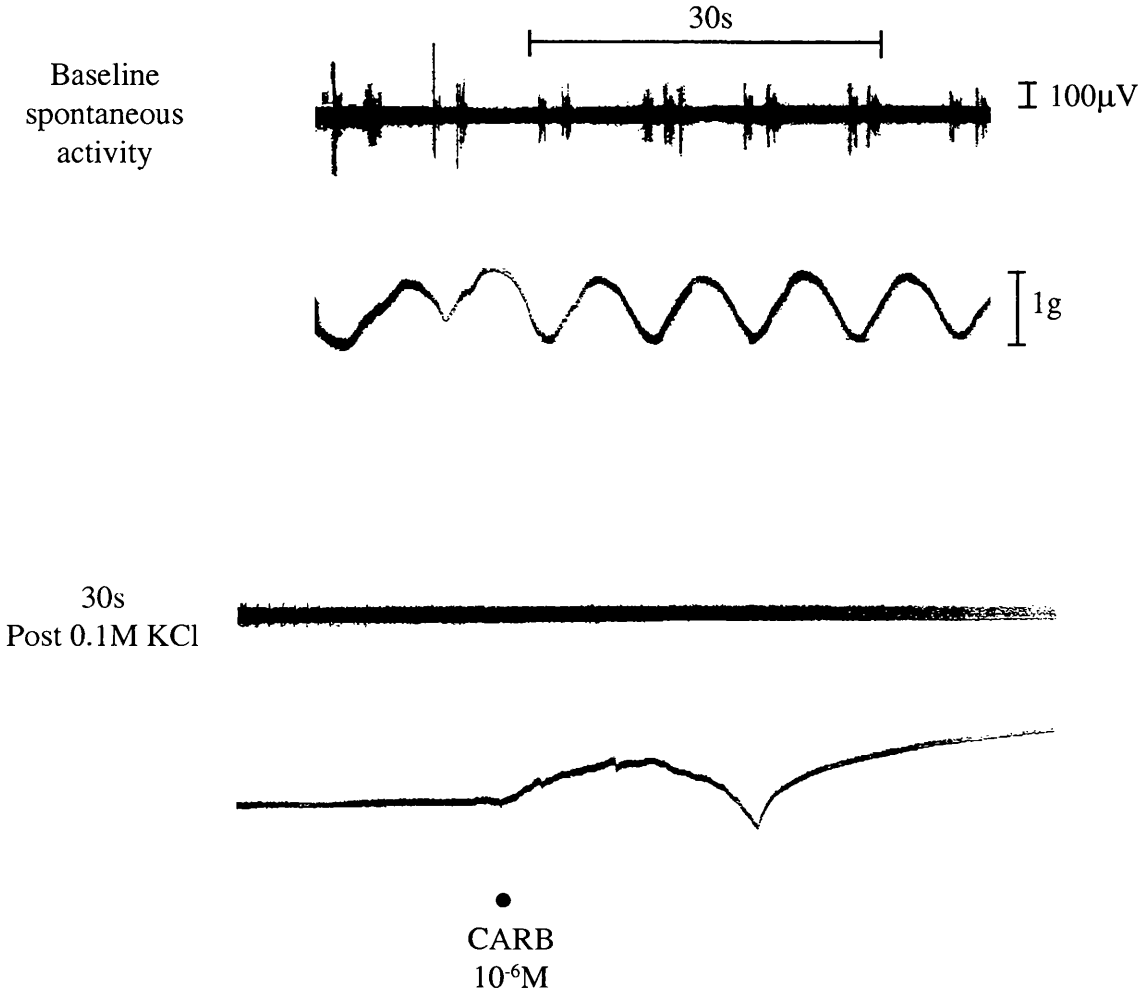


Fig 5.12 The effects of carbachol (10^{-6}M) on the extracellularly recorded (Golenhofen) electrical (top trace) and mechanical responses of a strip of human ileum to infusion of KCl (0.1M). KCl changed the electrical discharge pattern to a continuous one and raised tone. Carbachol (● , 10^{-6}M) further increased tone without visibly altering the electrical activity.

produce any response. KCl (0.1M), on the other hand, produced the characteristic intense extracellular electrical activity accompanied by a series of summation contractions which eventually resulted in a plateau of raised tone.

Carbachol

Carbachol (10^{-6}M) increased the amplitude, frequency and intensity of extracellular electrical activity which corresponded to summation of contractions and a plateau in tone (see Fig 5.13). This occurred in all 6 experiments (2 controls, 1 UC, 3 ISTC; 6 strips).

The median time to produce a plateau in contractions was 4.5min (range 0.5-7min). Interestingly the UC strip produced a maximal response to carbachol (10^{-6}M) after 0.5min; the 2 control strips produced maximal responses at 2 and 4min; and the 3 ISTC strips produced maximal responses at 5, 6.5 and 7min respectively.

As noted above carbachol (10^{-6}M) caused further increases in tone in 3 tissues exposed to the spasmogenic effect of 0.1M KCl (see above).

Atropine

Infusion of atropine (10^{-6}M) reversed the spasmogenic effect of carbachol (10^{-6}M) in 3 experiments (1 UC, 2 ISTC), see Fig 5.13. Spontaneous contractions failed to reappear once baseline tone had been reached although intense extracellular activity was noted in 2 strips. In these strips there were no evidence of discrete clusters of electrical spikes separated by periods of quiescence, as seen in resting spontaneous activity.

Diltiazem

The effects of diltiazem were studied in 4 experiments (1 control, 2 UC, 1 ISTC; 4 strips), see Fig 5.14.

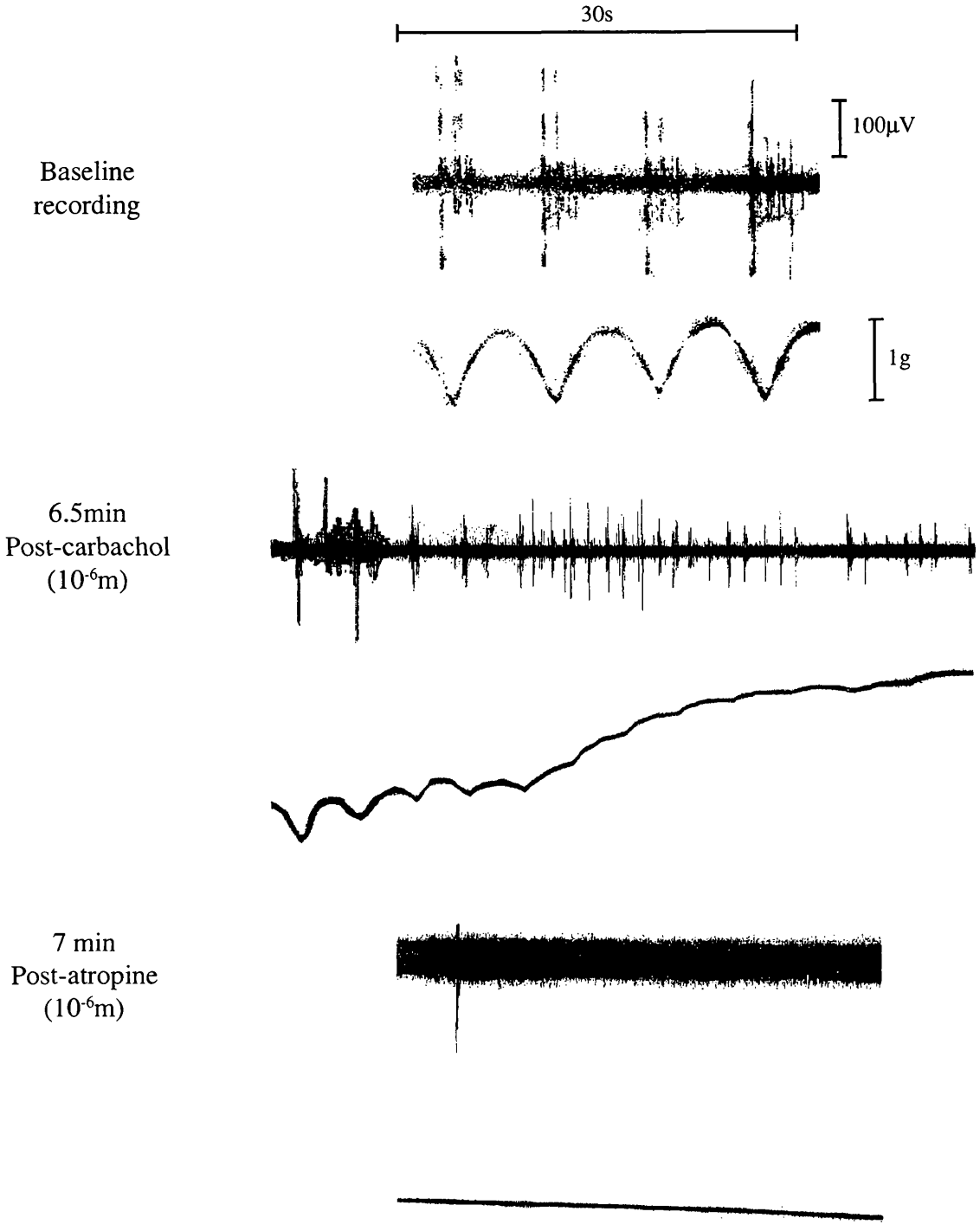


Fig 5.13 The inhibitory effects of atropine (10-6M, lower trace) on the increase in tone and electrical spike change produced by carbachol (10-6M, middle trace) in a strip of control ileum (Golenhofen recording).

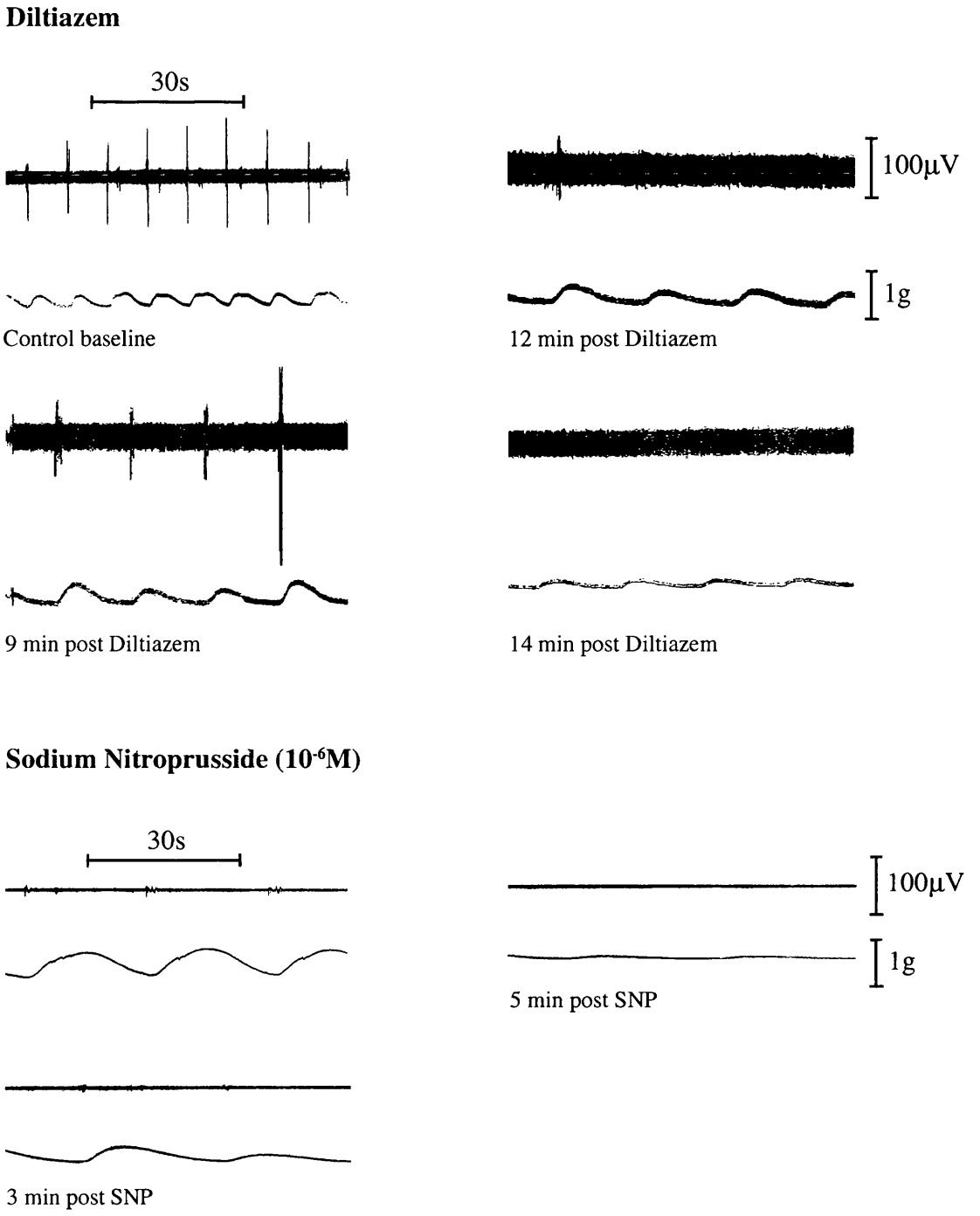


Fig 5.14 The inhibitory effects of diltiazem (10^{-5}M, upper set of recordings) and SNP (10^{-6}M, lower set of recordings) on the electrical (top traces) and mechanical spontaneous activity in samples of control human ileum. Diltiazem and SNP each abolished electrical and mechanical activity.

In the 2 UC strips diltiazem (10^{-5}M) caused abolition of all electrical and mechanical activity by 10min in both strips. Residual tone which was negligible was aslo reduced by diltiazem. In 1 strip some spontaneous activity had returned after 12min.

In 1 ISTC strip diltiazem (10^{-5}M) caused abolition of all electrical and mechanical activity after 30min. Likewise, in a control strip, diltiazem ($3x10^{-5}$M) took 27min to produce a similar abolition of spontaneous activity.

Sodium nitroprusside

SNP (10^{-6}M; n=2), ($3x10^{-6}$M; n=1) and (10^{-5}M; n=1) abolished spontaneous electrical and mechanical activity in all 4 strips studied (Fig5.14).

The median time to abolish spontaneous activity using SNP (10^{-6}M) was 5min (range 2-8min). In 1 UC strip, abolition took 2min, while the 2 ISTC strips studied were relaxed 5min and 8min respectively after exposure to SNP. In one control strip, SNP (10^{-5}M) initially caused a transient increase in both the electrical spike amplitude and the size of mechanical contractions which lasted about 30s before abolition of spontaneous activity occurred after 5min. As with diltiazem, SNP reduced resting tone.

In one experiment, during continuous perfusion of SNP, spontaneous activity re-emerged 10min after its abolition by SNP (10^{-5}M). The mechanical and electrical activity were, however, approximately 70% of their pre-SNP amplitudes, with no change in frequency.

The effects on spontaneous activty of SNP in conjunction with KCl are discussed above.

Metronidazole

Metronidazole, at an organ bath concentration ($5.26x10^{-4}$M) representative of the concentration achieved when a standard 500mg dose is infused intravenously in clinical practice, was studied in 6 experiments (3 controls, 2 UC; 6 strips).

No major effects on spontaneous electrical or mechanical activity were noted.

5.3.2 INHIBITORY NERVE STIMULATION AND THE EFFECTS OF DRUGS

In carbachol-induced tone ($3x10^{-6}M$-$10^{-5}M$), EFS of intramural nerves (1-64Hz, 0.05ms, supramaximal voltage) elicited frequency-related relaxations with an optimum frequency of around 4-8Hz. (see Fig 5.15) There were no differences in tissue responses to inhibitory EFS between control and UC patients (see Fig 5.16). To prevent adrenergic influence, guanethidine ($10^{-6}M$) was present throughout. A non-cholinergic blockade would have been desirable but was not possible since atropine would have abolished the carbachol-induced tone. Indeed, for studies of the effects of drugs on inhibitory nerve stimulation, the experiments often lasted in excess of 120min and carbachol was the only agonist which could sustain tone over this period (unlike substance P; 5-hydroxytryptamine, 5HT and histamine). Thus, the inhibitory nerve responses examined in this series of experiments were non-adrenergic rather than NANC. Interestingly, 2 patients (one control, one UC) provided muscle strips which generated sufficient spontaneous tone to enable the experiments to be conducted in the presence of atropine ($10^{-6}M$) and guanethidine ($10^{-6}M$) i.e. truly NANC conditions. These patients are discussed below.

When tone was raised by carbachol ($3x10^{-6}M$- $10^{-5}M$) to the organ bath, relaxations to EFS with rebound contractions were observed in all the tissues (see Fig 5.15). Frequency-response relationships in control and UC strips were similar, with an optimum frequency of around 4Hz (see Fig 5.16). In control strips relaxations at 4Hz were $70.2 \pm 4.82\%$ of induced tone; in UC tissues $63.1 \pm 3.23\%$. All responses to inhibitory EFS were abolished by TTX ($10^{-6}M$) indicating their neural origin.

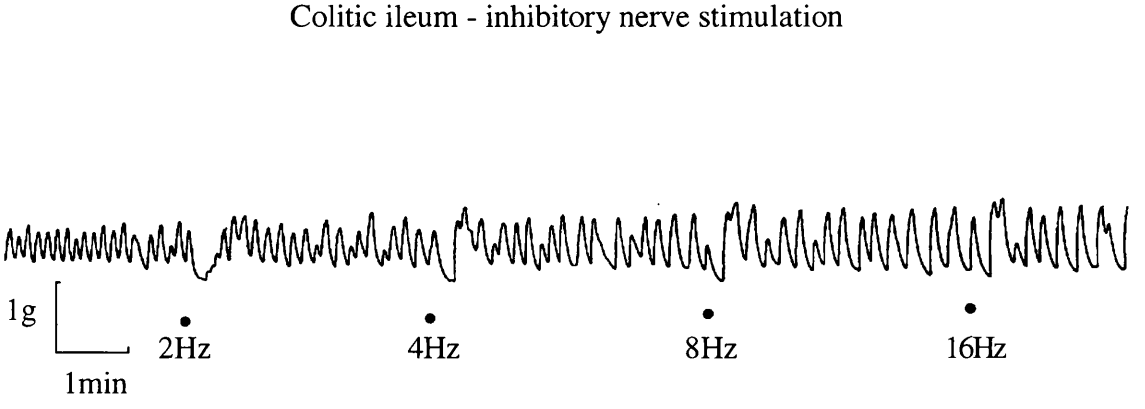


Fig 5.15 The effects of inhibitory EFS, (2-16Hz, 64 pulses, 0.05ms, supramaximal voltage) on the spontaneous mechanical activity of ileum from an ulcerative colitic undergoing IPAA, in the presence of carbachol-induced (10^{-6}M) tone. EFS produced a frequency-dependent inhibition of spontaneous activity.

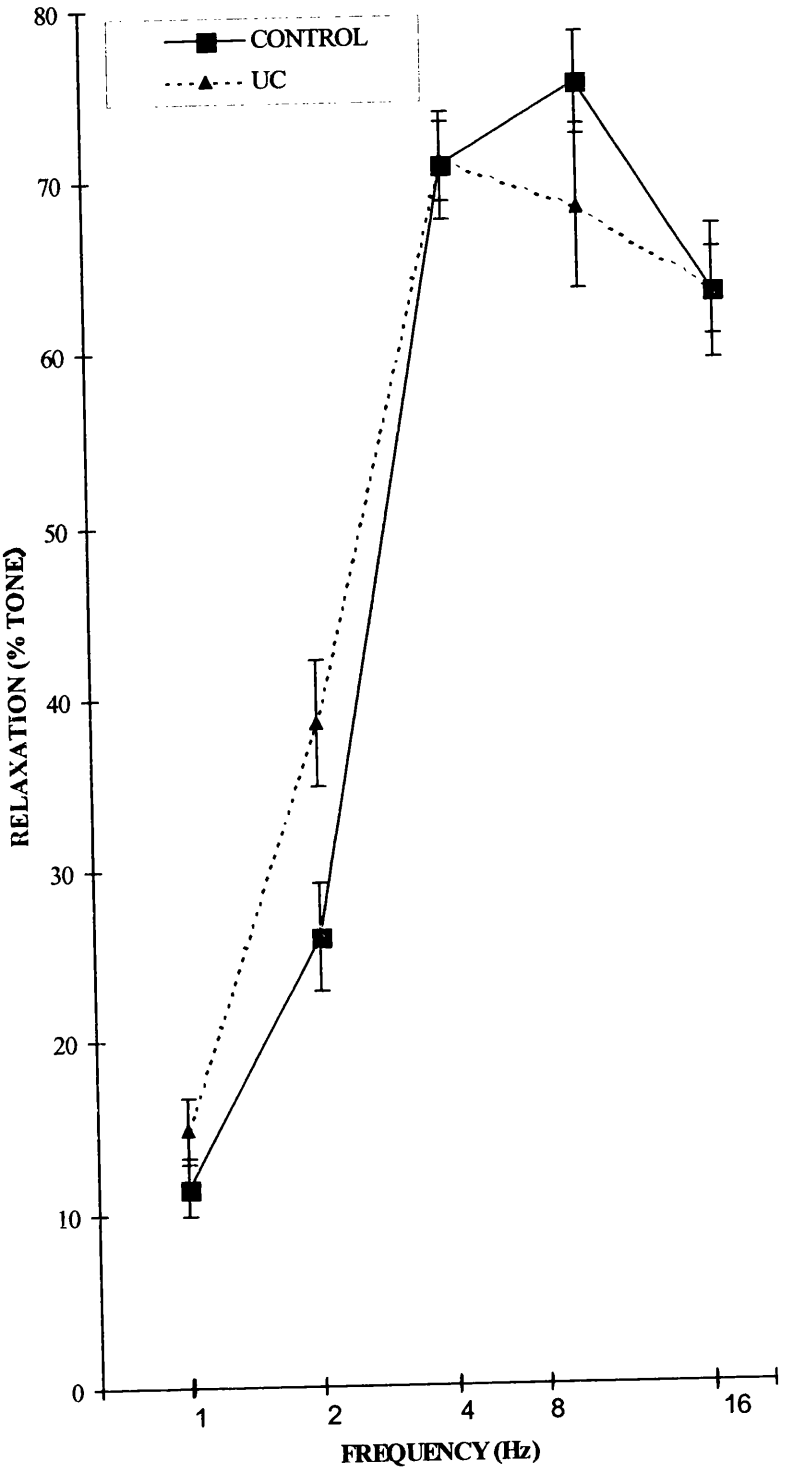


Fig 5.16 Frequency response curves to inhibitory EFS (64 pulses, 0.05ms, supramaximal voltage) in control and ulcerative colitic (UC) ileum. Tissues were pre-contracted with carbachol ($3x10^{-6}M$). Relaxations are expressed as a percentage of carbachol-induced tone. Controls: 4 patients, 11 strips; UC: 4 patients, 9 strips. The curves were not significantly different from each other at any point (Student's t-test applied).

Previous preliminary work had shown that the inhibitory relaxations to EFS were inhibited by L-NAME in a concentration-dependent manner (Campbell, 1993). At 10^{-4}M, inhibition produced by L-NAME was 60-90% in control patients. This dose of L-NAME was therefore selected for study in control and UC strips. In 17 muscle strips from 10 control patients, L-NAME (10^{-4}M) inhibited relaxation by a mean of 80.2 $\pm$ 3.83%. Nerve-mediated responses were present in strips tested from all control patients.

In 2 control subjects (2 muscle strips), and 4 UC patients (6 muscle strips), incubation with the stereospecific enantomer D-NAME (10^{-4}M) had no effect on inhibitory EFS. In 2 UC patients (3 muscle strips), the effects on inhibitory EFS of incubation with 10^{-4}M L-NAME could be stereospecifically reversed by incubating with L-ARG (10^{-3}M), see Fig 5.17.

In 58 muscle strips from 18 UC patients undergoing IPAA a more heterogeneous group of responses was noted on repeated inhibitory EFS after incubation with L-NAME (see Table 5.12). Only 4 of the 11 intact UC patients (36.4%) undergoing IPAA showed responses to L-NAME which were similar to those of the control group (see Fig 5.17), with an inhibition in relaxation response by a mean of 85.6 $\pm$ 6.93% (P=0.62, n.s.; Mann-Whitney *U* test). One of the 7 (14.3%) staged IPAA patients with UC behaved in the same manner with L-NAME incubation. Five of the 18 (27.8%) UC patients showed no response to either excitatory or inhibitory EFS. In a further 3 UC patients (33.3%; 2 synchronous, 1 staged) L-NAME was only weakly effective i.e. 0-60% reduction in response to inhibitory EFS, (see Fig 5.18) whilst 5 patients (27.8%; 1 synchronous, 4 staged) showed no alteration in inhibitory EFS after L-NAME incubation (see Fig 5.19).

In 2 muscle strips from 2 control subjects and 6 muscle strips from 4 UC patients, incubation with the stereospecific enantiomer D-NAME (10^{-4}M) had no effect on inhibitory EFS. In 2 UC patients (3 muscle strips) the effects of incubation with L-NAME (10^{-4}M) on inhibitory EFS could be stereospecifically reversed by incubating with L-ARG (10^{-3}M), see Fig 5.17.

PATIENT	n	L-NAME effective (>60% inhibition)	L-NAME weakly effective (0-60% inhibition)	L-NAME ineffective	Neuronal relaxations absent
Controls	10 (17)	10 (17)			
IPAA-synchronous	11 (37)	4 (7)	2 (7)	1 (3)	4 (20)
IPAA- staged	7 (21)	1 (3)	1 (4)	4 (9)	1 (5)

Table 5.12: The antagonistic effect of incubation with L-NAME (10^{-4}M, 40min) on inhibitory nerve-mediated relaxations (2-16Hz, 64 pulses, 0.05ms, supramaximal voltage) in terminal ileal muscle strips from controls and UC patients undergoing ileal pouch-anal anastomosis (IPAA).
Synchronous, staged- see text.
n= number of patients (number of muscle strips).

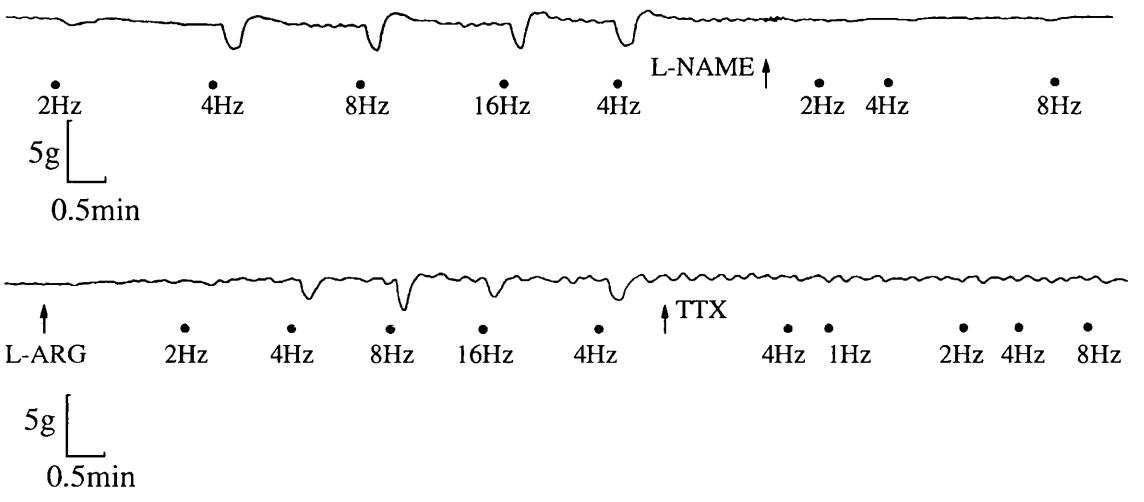


Fig 5.17 The effect of L-NAME (arrow, 10^{-4}M), alone (top trace) and after the addition of L-ARG (arrow, 10^{-4}M, lower trace), on the response to inhibitory EFS (2-16Hz, 64 pulses, 0.05ms, supramaximal voltage) in the presence of carbachol-induced tone (10^{-6}M) in UC ileum. The inhibitory responses to EFS were abolished by L-NAME, an effect reversed by L-ARG (lower trace). The responses to EFS were neuronally mediated; they were abolished by TTX (arrow, 10^{-6}M).

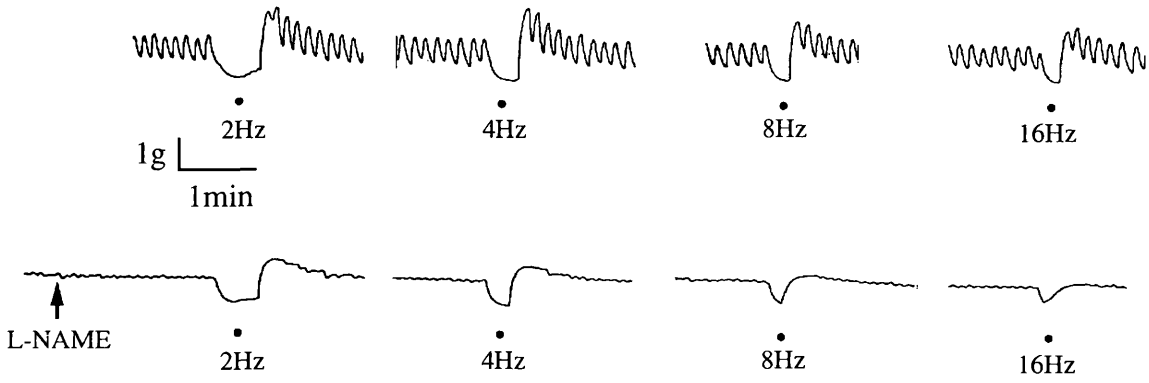


Fig 5.18 The effects of L-NAME (arrow, 10-4M, lower trace) on the inhibitory responses to EFS (2-16Hz, 64 pulses, 0.05ms, supramaximal voltage) in UC ileum. Although present in a similar concentration L-NAME was only weakly effective (Cf. Fig 5.17).

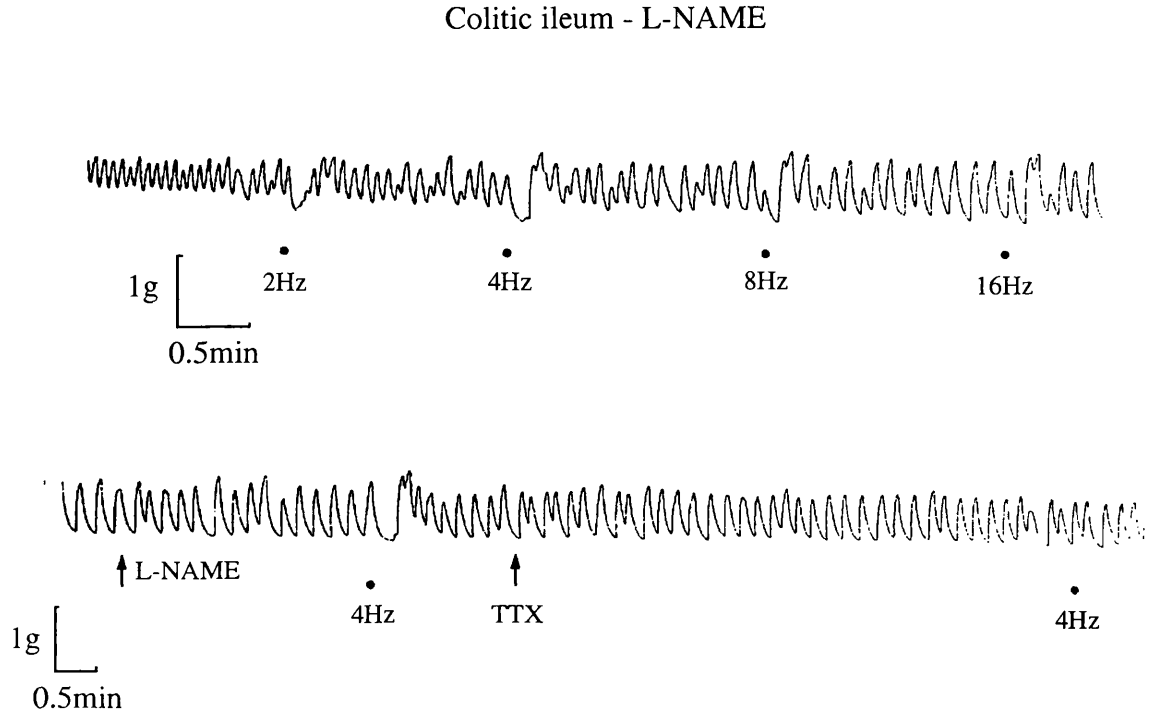


Fig 5.19 The ineffectiveness of L-NAME (arrow, 10^{-4}M, lower trace) on the inhibitory responses to EFS (2-16Hz, 64 pulses, 0.05ms, supramaximal voltage) in UC ileum. The responses to EFS were neuronally mediated; they were abolished by TTX (arrow, 10^{-6}M, lower trace).

The bee venom apamin, which blocks small conductance Ca^{++}-dependent K^+ channels (Maas *et al*, 1980; Castle *et al*, 1989), was used in 3 controls (4 muscle strips) and 3 UC patients (4 muscle strips) to investigate whether a second neurotransmitter may contribute to the process of nerve-mediated relaxation. The results of these studies are shown in Figs 5.20- 5.23.

5.3.3 SODIUM NITROPRUSSIDE (SNP)- INDUCED RELAXATION

The basis of these experiments lay in the apparent contradiction between the classical view (Ignarro *et al*, 1981) that SNP acts by liberating NO or a related substance and the observations that in the sheep urethra the effects of SNP were unaffected by HbO (Garcia-Pascual & Triguero, 1994). With access to human tissue it therefore seemed worthwhile to carry out some preliminary studies to assess the mechanism of SNP-induced relaxation in the ileum of control subjects.

Samples from 6 controls (30 muscle strips) were examined in conventional jacketed organ baths. In the presence of CARB-induced tone ($3x10^{-6}M$), SNP produced dose-related relaxations ($3x10^{-8}M$-$10^{-4}M$), (not shown). As previously demonstrated, SNP remained effective at producing relaxation in the presence of TTX and also after tissue exposure to L-NAME ($10^{-4}M$).

The SNP-induced relaxation of human ileum was unaffected by apamin ($5x10^{-7}M$) or by KCl (0.1M). When HbO ($3x10^{-5}M$) was incubated with tissue strips however, SNP relaxation was neither prevented nor reversed (n=3), see Fig 5.24.

5.3.4 NADPH-DIAPHORASE HISTOCHEMISTRY

NADPH diaphorase in the layers of the human small bowel (see Fig 5.25) was demonstrated in cryostat transverse sections (20μm) of terminal ileum from both control and UC patients. Many NADPH-diaphorase-positive structures were seen in all the sections (Fig 5.26a-d). Strong uptake of the stain was noted in the intestinal mucosa and the myenteric plexus. In the latter, dense blue deposits of the

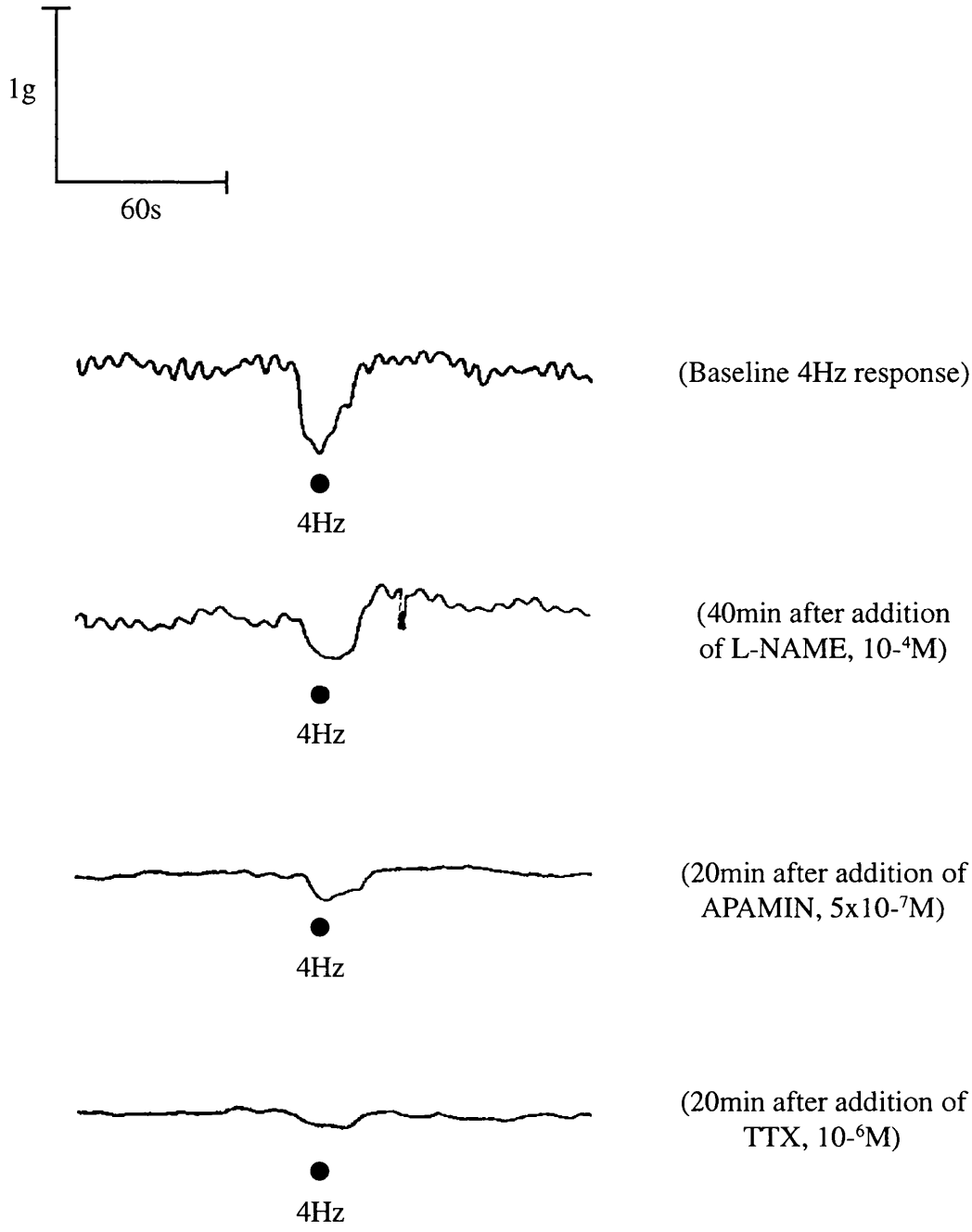


Fig 5.20 The effects of L-NAME (10-4M), alone and after the addition of apamin ($5x10^{-7}M$) on the inhibitory responses of control ileum to EFS (4Hz, 64 pulses, 0.05ms, supramaximal voltage). The 4Hz response was reduced by L-NAME alone, was further diminished by apamin and abolished by the additional presence of TTX($10^{-6}M$). Diagrams in chronological order moving downwards.

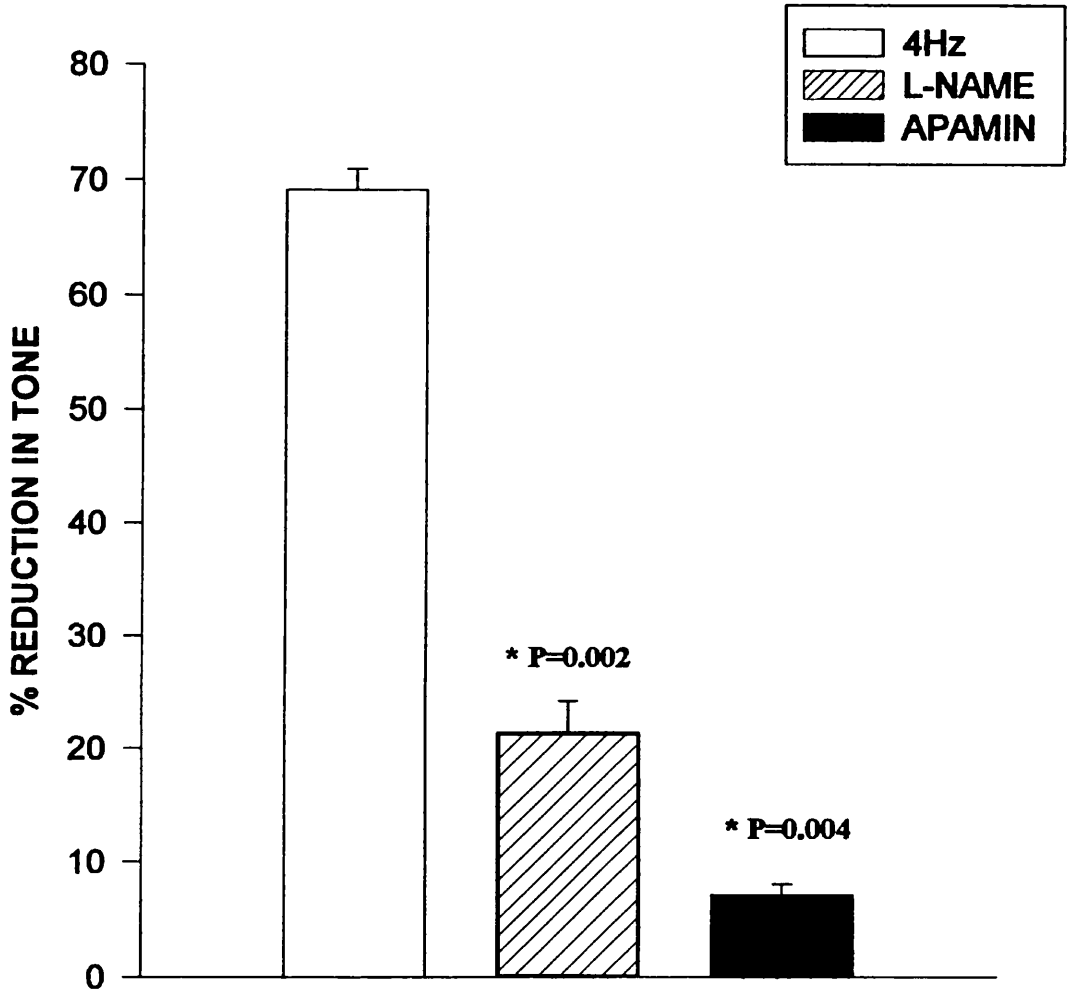


Fig 5.21 Histogram showing the response of control ileum (3 patients, 4 strips) to EFS (4Hz, 64 pulses, 0.05ms, supramaximal voltage) alone, in the presence of L-NAME (10^{-4}M) and in the additional presence of apamin ($5x10^{-7}$M). L-NAME alone significantly reduced the response to EFS which was then virtually abolished in the additional presence of apamin.

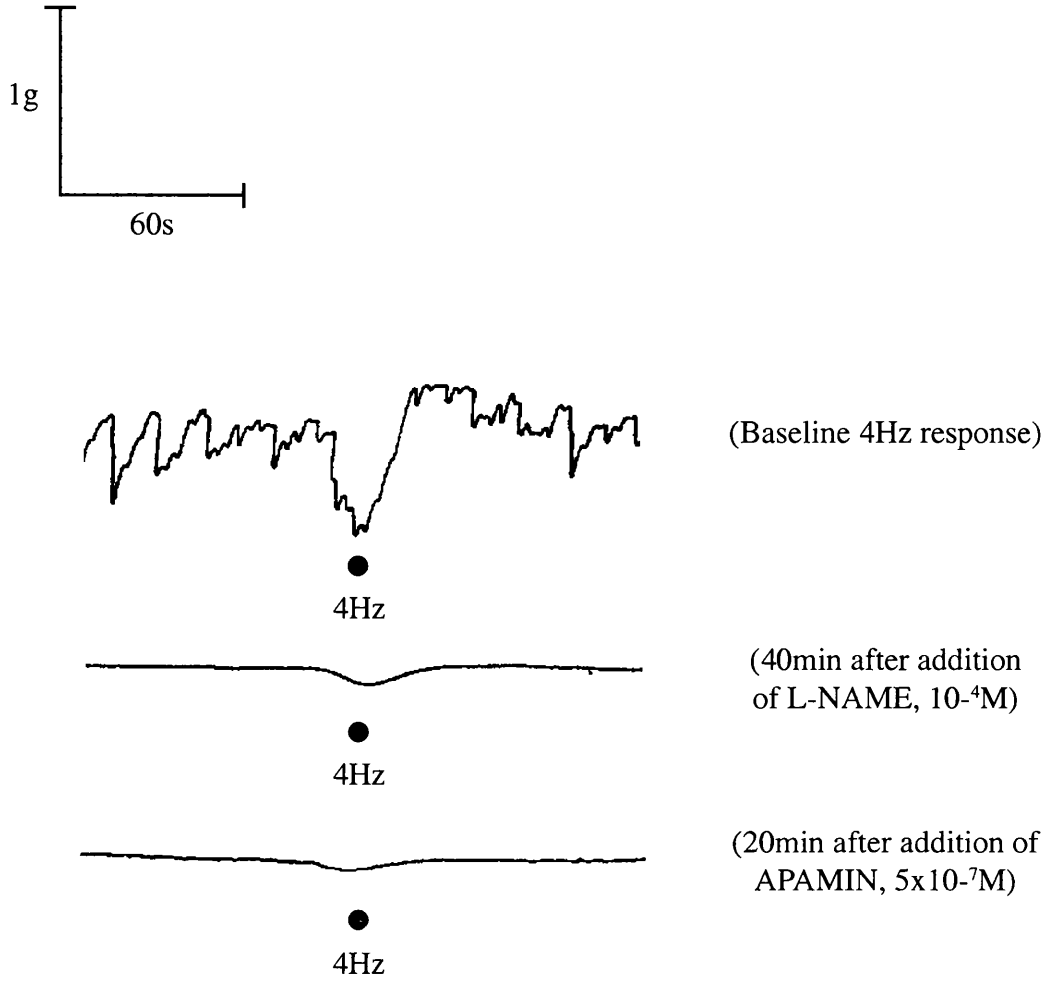


Fig 5.22 The effects of L-NAME (10^{-4}M), alone and after the addition of apamin ($5x10^{-7}$M) on the inhibitory responses of UC ileum to EFS (4Hz, 64 pulses, 0.05ms, supramaximal voltage). The 4Hz response was reduced by L-NAME alone. Together with apamin the response was abolished. Diagrams in chronological order moving downwards.

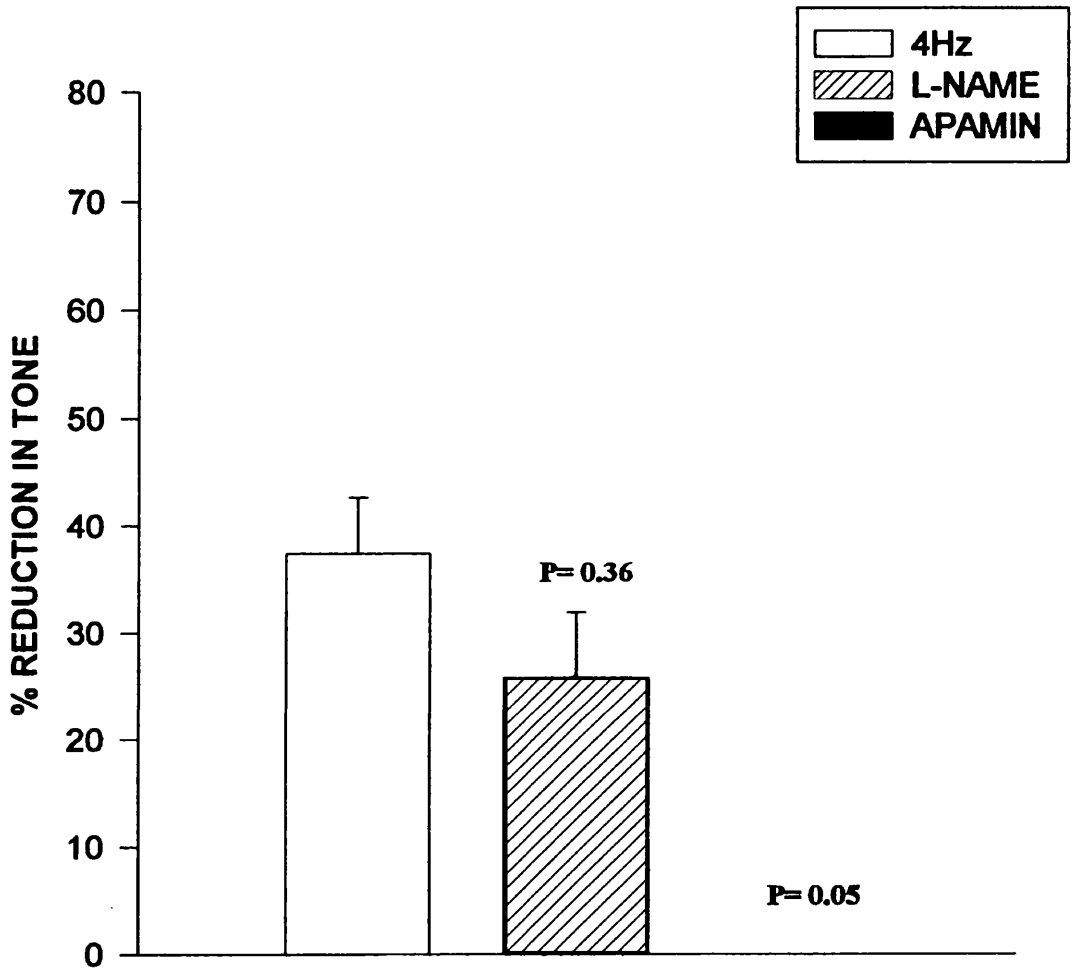


Fig 5.23 Histogram showing the response of ulcerative colitic ileum (3 patients, 4 strips) to EFS (4Hz, 64 pulses, 0.05ms, supramaximal voltage) alone, in the presence of L-NAME (10^{-4}M) and in the additional presence of apamin ($5x10^{-7}$M).
L-NAME alone reduced the response to EFS but not to a significant degree (*Cf.* control results, see Fig 5.21). The response was abolished in the additional presence of apamin.

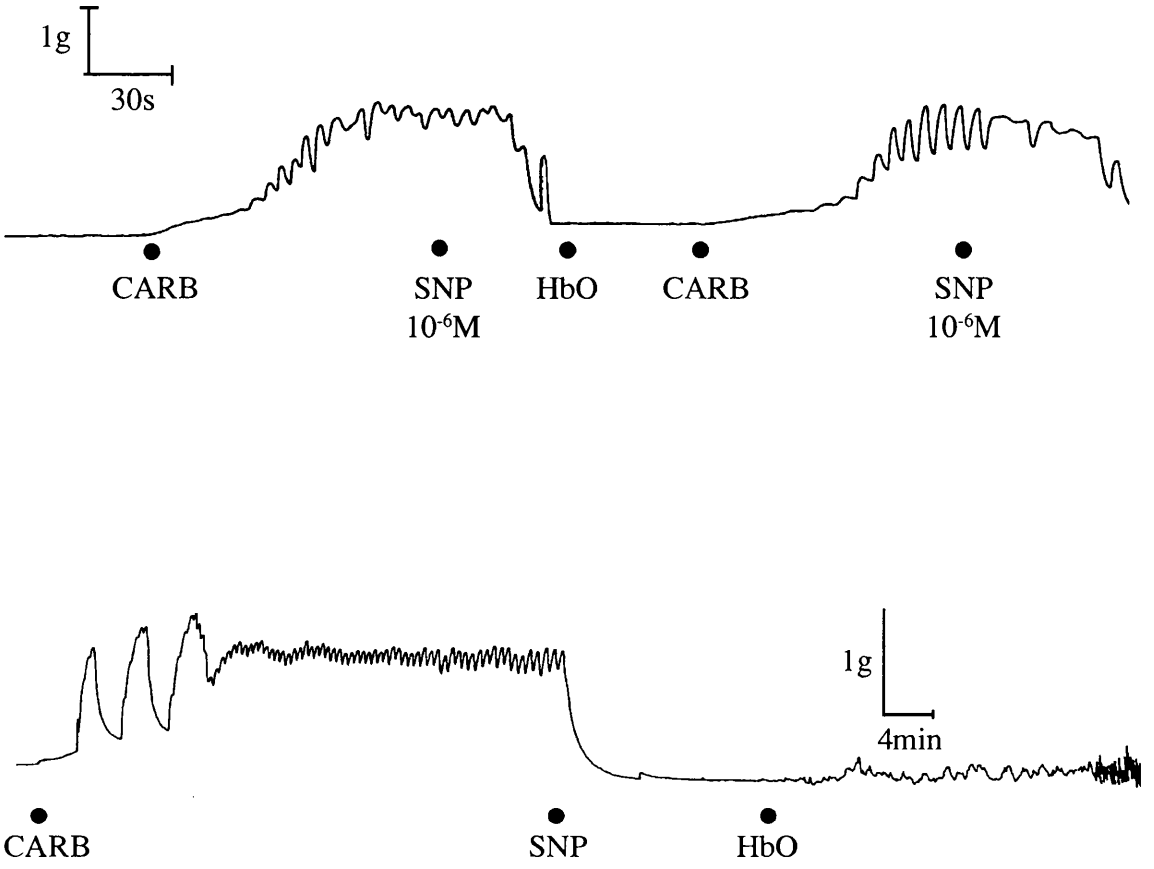


Fig 5.24 The effects of oxyhaemoglobin (● , HbO, $3x10^{-5}$M) on the relaxant responses of SNP (● , 10^{-6}M) in control ileum in the presence of carbachol-induced tone (● , 10^{-6}M). HbO was ineffective in preventing (top trace) or reversing (lower trace) the relaxant response to SNP, suggesting that extracellular release of NO was not involved in this process.

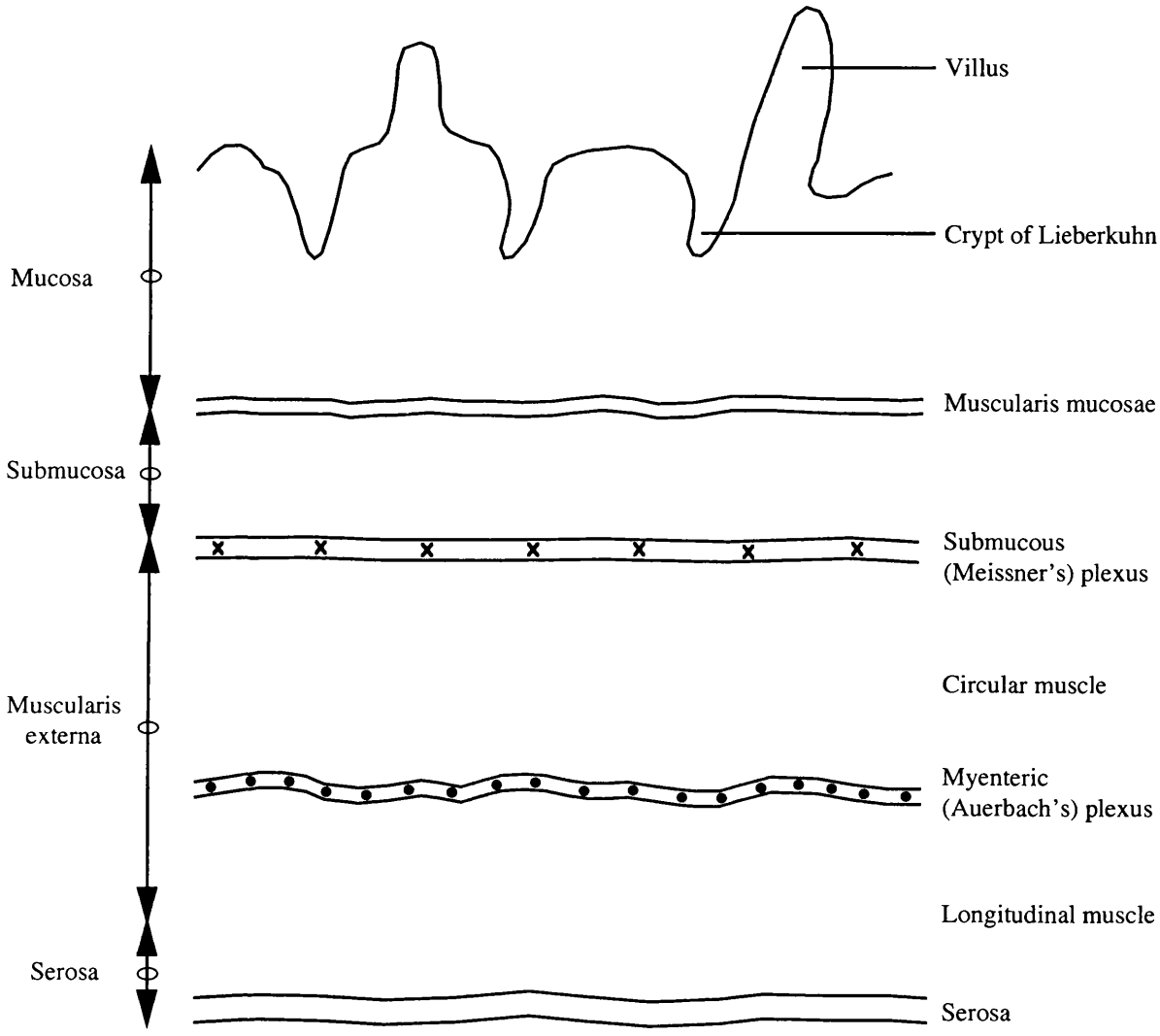
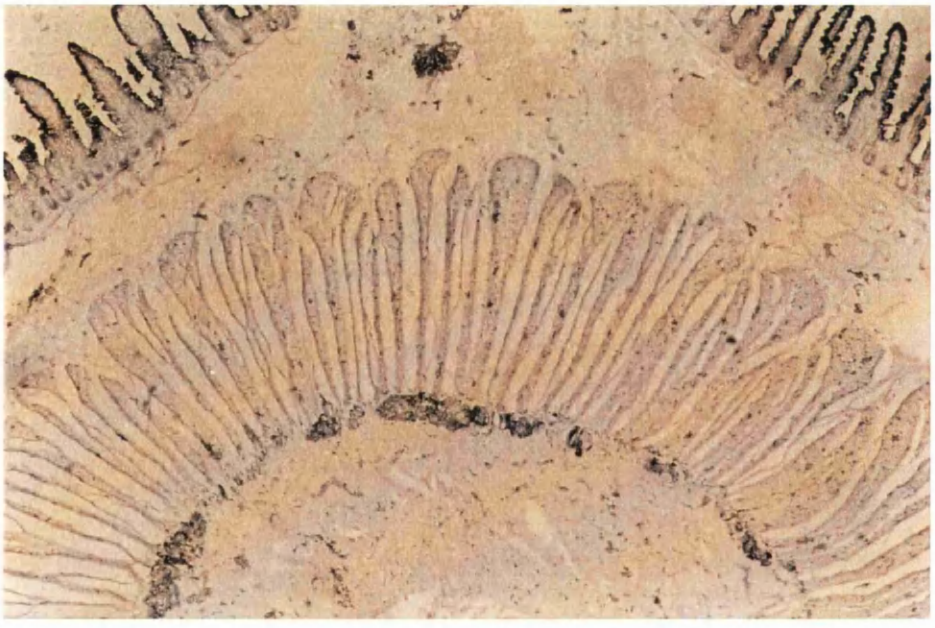
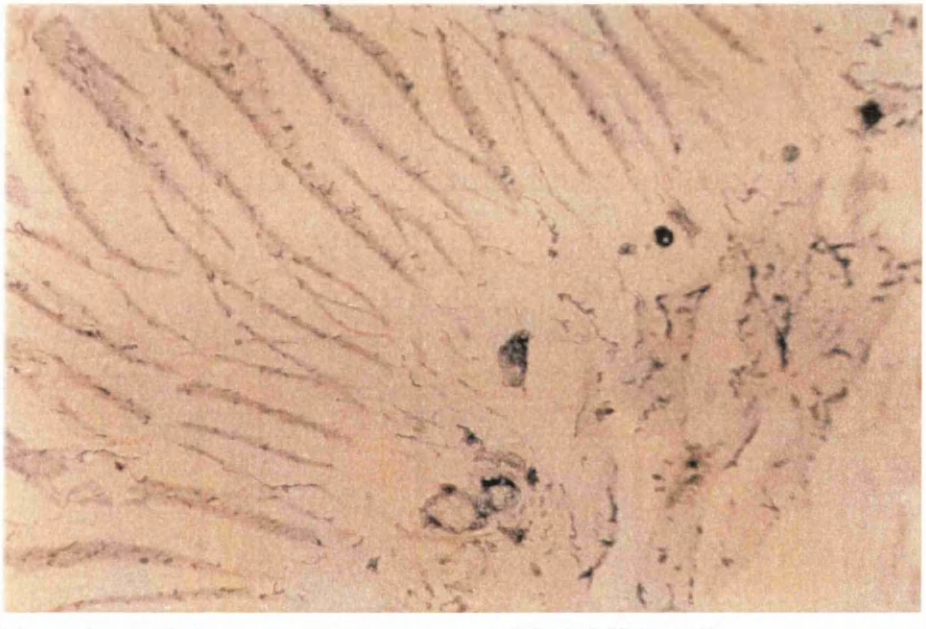


Fig 5.25 Schematic diagram of layers of human small bowel.

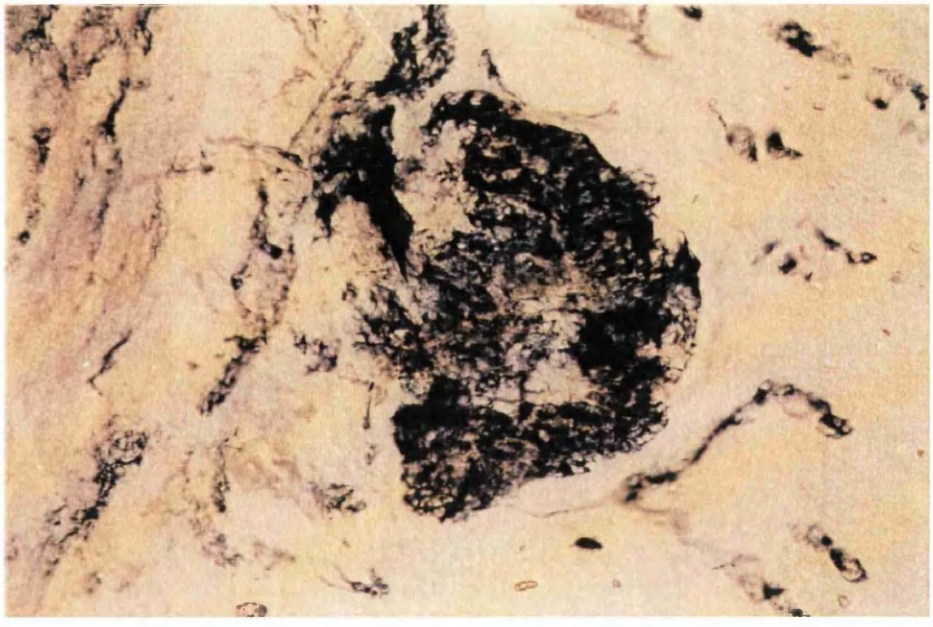


a): Control ileum, transverse section (NADPHd, x8): low magnification to show general appearances.

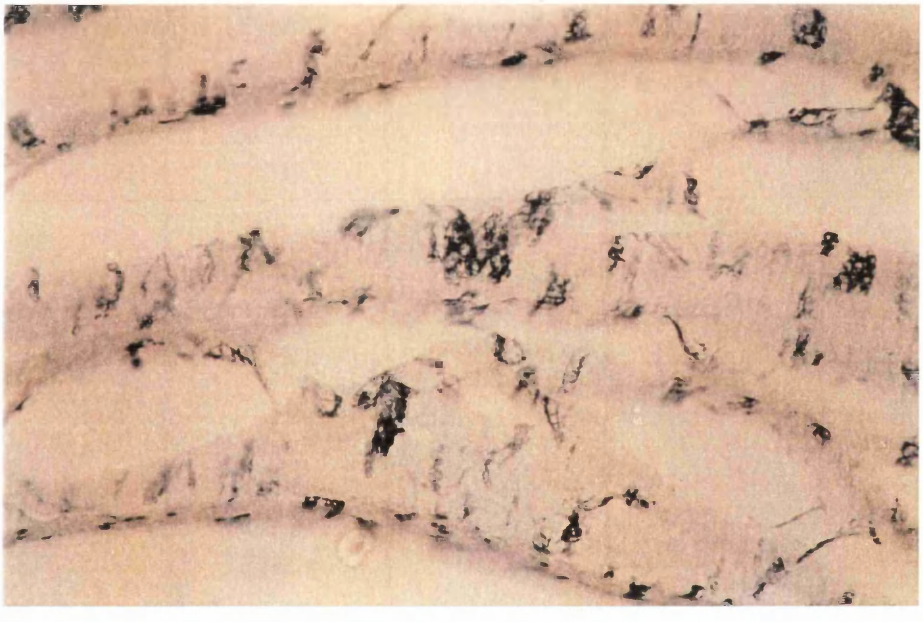


b): Control ileum, transverse section (NADPHd, x19).

Fig 5.26 Photomicrographs (a-d) of NADPH-diaphorase staining in control and ulcerative colitic samples of ileum. NADPH-diaphorase positive fibres have stained dark blue.



c): UC ileum, transverse section (NADPHd, x77).



d): UC ileum, transverse section (NADPHd, x77).

Fig 5.26 Photomicrographs (a-d) of NADPH-diaphorase staining in control and ulcerative colitic samples of ileum. NADPH-diaphorase positive fibres have stained dark blue.

diaphorase reaction were noted in the ganglion cells. In the muscularis externa, both the inner circular and outer longitudinal layers contained numerous thin nerve fibres which showed strong NADPH diaphorase activity. Interestingly, the distribution of NADPH-diaphorase-positive nerve fibres appeared even in both layers of the muscularis. This was a constant feature in all patient categories.

Vagotomy did not alter the concentration or distribution of NADPH-diaphorase activity; likewise, fulminant UC and previous colectomy for chronic UC had no visible effects on the NADPH-diaphorase staining characterisitics of terminal ileum. The rare opportunity to stain for NADPH-diaphorase-positive activity in 2 established IPAA patients with dysfunction similarly failed to show any striking differences with control or pre-pouch tissues.

5.4 DISCUSSION

5.4.1 SPONTANEOUS ACTIVITY, THE EFFECTS OF TEMPERATURE AND PERFUSED DRUGS

Ileal muscle strips under 1g of isometric tension in Krebs solution in standard organ bath apparatus elicited a variable degree of spontaneous mechanical activity. Using the Golenhofen apparatus, recordings of spontaneous extracellular electrical and mechanical activity were made from similar muscle strips but these recordings showed marked differences in the frequency and mean amplitude of the mechanical contractions. In general, Golenhofen recordings of spontaneous activity in the 3 patient groups showed that, with respect to simple organ bath measurements, spontaneous mechanical activity measurements had a greater frequency, stronger amplitude and shorter duration. These trends were seen in all 3 patient groups, reaching statistical significance in 5 of the 9 comparative measurements (see Table 5.13). Clearly, absolute values for spontaneous mechanical activity measurements relate to the recording method employed, but it is difficult to explain why these differences arose. One obvious reason may simply be that the Golenhofen apparatus is a more sensitive and accurate way of detecting every oscillation in tone generated by pacemaker activity. Organ bath recordings of spontaneous activity did not have simultaneous electrical activity measurements to verify whether every contraction was an individual mechanical event or instead the result of a series of smaller summated contractions. In addition, orientation of the ileal muscle strips differed in the two types of recording apparatus. Perhaps a vertically stretched strip of ileum generates different patterns of spontaneous mechanical activity to a strip stretched horizontally in the Golenhofen apparatus?

The Golenhofen experiments, together with the organ bath observations show that human ileum does not generate any appreciable levels of resting tone. Even under 1g of isometric tension, there is almost invariably a spontaneous relaxation to baseline levels. However, when carbachol is used to raise it, tone can

SPONTANEOUS MECHANICAL ACTIVITY PARAMETERS	ORGAN BATH	GOLENHOFEN
CONTROL		
Number of patients (strips analysed)	16 (99)	10 (10)
Frequency of spontaneous mechanical contractions (min^{-1})	4.39±0.66	9.30±0.96 **P=0.0005
Isometric tension of spontaneous contractions (g)	0.64±0.14	1.88±0.46 *P=0.0065
Duration of spontaneous contractions (s)	11.68±2.23	8.20±1.99 P=0.0853
UC		
Number of patients/ strips analysed	18 (160)	7 (7)
Frequency of spontaneous mechanical contractions (min^{-1})	3.40±0.61	8.57±0.78 *P=0.0010
Isometric tension of spontaneous contractions (g)	0.84±0.15	1.14±0.15 P=0.0841
Duration of spontaneous contractions (s)	16.1+2.77	7.86±1.07 *P=0.0423
ISTC		
Number of patients/ strips analysed	7 (37)	7 (7)
Frequency of spontaneous mechanical contractions (min^{-1})	4.54±1.16	9.14±1.53 P=0.0545
Isometric tension of spontaneous contractions (g)	0.56±0.21	1.43±0.13 *P=0.0021
Duration of spontaneous contractions (s)	18.3±4.34	6.07±0.52 *P=0.0205

Table 5.13: Summary of differences in spontaneous mechanical activity of human ileum as recorded in organ bath and Golenhofen apparatus.
Mann-Whitney *u* tests applied to compare UC and ISTC parameters with control subjects.

persist for upwards of 2h (see nerve-mediated experiments in section 5.3.2). The predominant pattern of contraction seen in organ bath and Golenhofen experiments is therefore phasic. The phasic and tonic components of gastrointestinal motility have been extensively summarised (see Golenhofen *et al*, 1992). In basic terms, smooth muscle is seen to have two different motor functions. Firstly, to produce phasic-rhythmical activity, predominantly for propulsion. Secondly, to produce tonic contractions, predominantly for reservoir contractions. The observations from the current series of experiments show a uniform pattern of spontaneous mechanical activity in the presence of almost zero background tone, in keeping with studies from anatomically similar sites in other species (see Golenhofen *et al*, 1992). Thus, human ileum used in IPAA has almost entirely propulsive properties at the time of ileal pouch construction. Interestingly, the ileal pouch subsequently has to adapt to take on the physiological role of a reservoir. The adaptive processes which must occur at a smooth muscle level have yet to be elucidated.

In human ileal smooth muscle the effects of cooling reduced the frequency of slow wave activity but interestingly did not alter resting tone, which largely remained at baseline. Stepwise cooling to 27.5°C never abolished slow wave activity in any of the strips analysed from the 3 patient categories. These observations suggest that this tissue, unlike other spontaneously active smooth preparations such as guinea-pig trachealis (see Small, 1982), is more resilient to temperature change.

The experiments above have noted that spontaneous mechanical activity in human ileum is Ca^{++}-dependent and the preliminary studies give some evidence as to how this complex process operates at an ionic level. Other workers have shown that an increase in the cytosolic concentration of free Ca^{++} is a fundamental requirement for activation of the contractile machinery in smooth muscle (Bolton, 1989). The source of this activation can be either intracellular or extracellular. Bolton proposed that excitatory stimuli promote the cellular influx of Ca^{++} either through voltage-operated channels (VOCs) or receptor-operated channels (ROCs),

(Bolton, 1989). Thus, Ca^{++} entering a smooth muscle cell through these channels could simply increase the concentartion of cytosolic free Ca^{++}. Alternatively, it could in turn stimulate Ca^{++} release from intracellular storage (sequestration) sites, creating an amplification of the initial excitatory stimulus. Further, a stimulant which activated cell surface receptors might evoke intracellular Ca^{++} release from sequestration sites by a process which did not involve VOCs or ROCs. A VOC, by definition is a channel which can admit Ca^{++} into the smooth muscle cell. Opening this channel is dependent on the membrane potential of the cell (Bolton, 1989). The VOC opens during the graded depolarization of smooth muscle cells which do not normally discharge action potentials. This opening corresponds with the upstroke of the smooth muscle action potential. The VOC is also readily blocked by organic inhibitors of Ca^{++} influx e.g. diltiazem and is relatively specific for Ca^{++}. In the experiments described above, KCl produced VOC opening when added to the extracellular fluid, and was ineffective when the tissue was pretreated with DTZ. From other simple organ bath experiments, KCl raised tone after prior exposure of the tissues to guanethidine, atropine, SNP or TTX. KCl-induced spasm was also unaffected when these agents were added to the preparation suggesting that at least the action of KCl was not dependent on adrenergic, muscarinic or nerve-mediated processes (traces not shown). This resistance of KCl-induced spasm suggests that it has a direct action on human ileal smooth muscle. Moreover, the fact that KCl spasm is produced in the presence of TTX, but not with diltiazem suggests that the VOCs which bring about KCl spasm conduct Ca^{++} rather than Na^{+}.

Carbachol was seen to operate through muscarinic receptors to raise tone. In addition, carbachol could exert an additive effect on background KCl-induced spasm (Fig 5.10). In organ bath experiments, carbachol in this tissue still produced its spasmogenic effect after pretreatment with diltiazem and was resistant to incubation with diltiazem, but not atropine. Both VOCs and ROCs would therefore appear to exist in human ileum and when activated can have additive spasmogenic effects.

The studies with diltiazem and SNP were also useful in suppressing the spontaneous mechanical contractions in human ileum. As outlined above, Golenhofen and colleagues have categorised phasic and tonic components of gastrointestinal motility (Goelhofen *et al*, 1992). They have described two different types of Ca^{++} channel which exist in the cell membrane and which underlie these contractions. Here the nomenclature becomes confusing, suffice to say that the Ca^{++} channels described by Golenhofen and colleagues correspond to the VOCs and ROCs of Bolton (Bolton, 1989). Thus, according to the work of Golenhofen and colleagues one activation process involves L-type calcium channels (LCA; DTZ-sensitive), and the other a non-L-type calcium channel (NLCA; SNP-sensitive). The experiments above show that although the pattern of contractions in human ileum is almost purely phasic, hence LCA-mediated, this activity can also be abolished by SNP, a specific antagonist of NLCA-generated tonic activity. Ca^{++} channels classed as LCA, DTZ-sensitive correspond with VOCs described above, while Ca^{++} channels which are NLCA, SNP-sensitive are thought to be predominantly controlled by chemical processes (ligand operated channels) and not by membrane potential changes of the smooth muscle cell. These Ca^{++} channels correspond with ROCs described above.

In the current experiments there appears to be an additive (synergistic) effect when VOCs (LCA) and ROCs (NLCA) are activated. Inhibition of spontaneous activity by SNP, which suppresses NLCA, also occurs but this effect is reversed by the tissue and does not subsequently suppress VOC (LCA) stimulation by KCl. The accepted mechanisms by which SNP may operate in this tissue are are challenged by the finding that HbO was ineffective in preventing or reversing SNP-relaxation in human ileum (see section 5.4.2).

Metronidazole, used commonly in clinical practice to treat pouchitis (Mortensen & Madden, 1993) may in theory cause resolution in symptoms not just by its antibacterial action, but also by altering or resetting irritable-type patterns of motility in the ileal pouch. On the basis of the Golenhofen experiments, in which

metronidazole was infused, there is no evidence to support this theory, since metronidazole had no effect on spontaneous pacemaker activity.

While no statistically significant differences in parameters were detected in the UC Golenhofen experiments compared with control measurements, UC strips showed a greater mean number of electrical spikes per spike burst, greater duration of electrical spike burst and greater mean electrical spike amplitude than control or ISTC subjects. The mean duration of spontaneous contractions in the UC strips was also shorter than in controls. Greater patient numbers in these experiments may have given data which would have exposed statistically significant differences in these parameters between control subjects and UC patients. Clearly. further studies are required to explore these aspects (see Section 6.3.2).

Using Spearman's rank correlation coefficients as described in Chapter 3, no statistically significant correlates could be demonstrated between the clinical features of the UC illness with regard to duration, patient age and nocturnal stool frequency on the one hand and the Golenhofen parameters measured on the other. Again, greater patient numbers would be required to investigate this further.

Perhaps the most surprising finding in the Golenhofen experiments was the marked differences in the ISTC patients compared with the others. This group had statistically fewer numbers of electrical spikes in each spike burst and shorter durations of electrical spike burst activity (see Fig 5.27). Thus the extracellular electrical activity recordings of the spontaneous pacemaker in ileal smooth muscle of ISTC is abnormal. This would support the view that ISTC is a pangastrointestinal motility disorder (Watier *et al*, 1983; Krishnamurthy *et al*, 1985; Kumar *et al*, 1989; Bassotti *et al*, 1996; Panagamuwa *et al*, 1994; Hemingway *et al*, 1996). Indeed, in one small bowel manometry study of patients with ISTC *in vivo* assessment of terminal ileal motility confirmed the presence of motor abnormalities in both fasting and postprandial states (Panagamuwa *et al*, 1994). There was an increase in phase II activity, most significantly during sleep, which may have interfered with the passage of chyme leading to constipation.

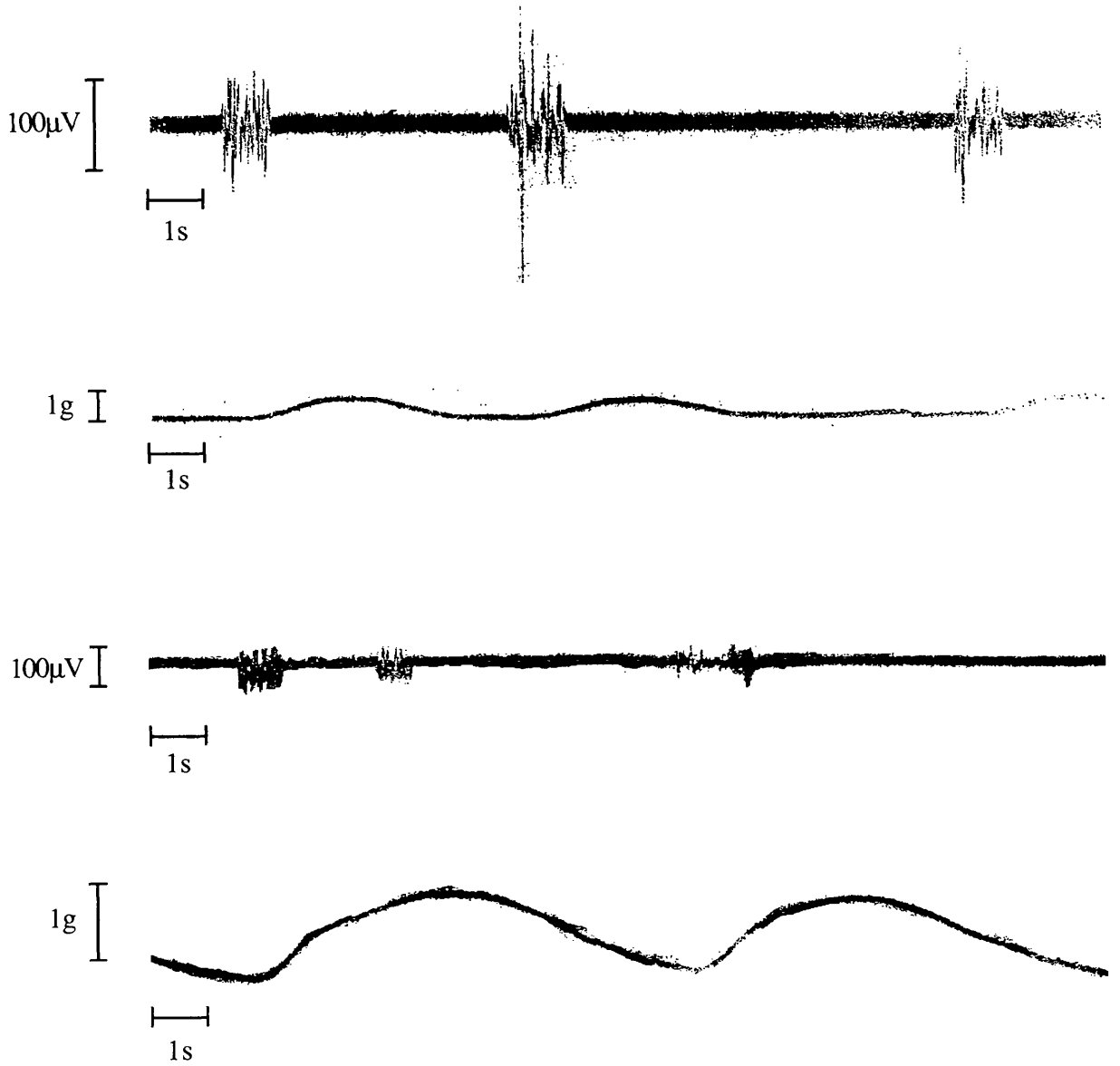


Fig 5.27 A comparison of the electrical (upper traces in each set) and mechanical activity in ileal tissue from control (top) and ISTC patients. There were fewer electrical spikes per burst in the ISTC patient and electrical spike burst activity was of shorter duration (Golenhofen recording).

Phase III fronts were also found to be of shorter duration in constipated patients with approximately one fifth of phase III activity fronts showing either retrograde propulsion or increased tonic and phasic activity. The constipated patients in this study also had a significantly shorter duration of postprandial activity. Whether these *in vitro* small bowel abnormalities are caused by the same process which produces delayed transit in ISTC is unclear.

From the above preliminary observations it is interesting to postulate that the intestinal pacemaker activity is abnormal in ISTC and modified in UC. In other words, these two conditions may be driven by a systemic process which alters the function and/ or number of ICC.

5.4.2 INHIBITORY NERVE STIMULATION AND THE EFFECTS OF DRUGS

Inhibitory nerve-mediated relaxation to EFS was demonstrated in control and UC patients. While these responses were non-adrenergic, the 2 examples of relaxations to EFS in the presence of guanethidine (10^{-6}M) and atropine (10^{-6}M) confirm that NANC processes nonetheless exist in UC ileum. It is almost certain that the inhibitory nerve-mediated responses which were studied throughout the remainder of the experiments were NANC relaxations.

NO, or a related nitroso compound has a role in inhibitory transmission in many tissues and species (see section 4) and is the main inhibitory transmitter in control human ileum (Campbell, 1993; Campbell *et al*, 1993). NO is synthesised from L-ARG by NOS. This process is inhibited by a number of L-ARG analogues with a substituted N-guanidino group (see review by Moncada, 1992). The present studies show that one such analogue, L-NAME, effectively blocked inhibitory nerve-mediated relaxations produced by EFS in control subjects but had a heterogeneous effect in ileum from UC patients awaiting IPAA. Incubation with L-ARG partially reversed the effects of L-NAME in the UC tissues examined indicating a role for the L-arginine-nitric oxide pathway in inhibitory relaxation of

the human terminal ileum in UC patients. Likewise, relaxations to SNP were unaffected by L-NAME in both controls and UC tissues making it unlikely that L-NAME exerts its effect at a location other than NOS.

In control tissues and those UC strips where L-NAME was effective in reducing inhibitory nerve-mediated relaxation its effects were identical whether or not EFS stimulation continued throughout the 40min incubation period. While a thiol derivative of NO may be stored and released from vesicles (Tøttrup *et al*, 1992) NO may alternatively be synthesised *de novo* on nerve stimulation, thus precluding the need for storage. NOS may be activated by Ca^{++} influx into the nerve terminal in response to an action potential (Luckhoff & Busse, 1990). The observation that L-NAME was effective whether or not EFS stimulation was present during the incubation period, therefore, suggests that L-NAME inhibition of nerve-mediated relaxation is not use-dependent and implies that NO, at least in human ileum is not released from a stored precursor as these stores would need time to become depleted before the effects of competitive antagonism of NOS were observed.

In the UC tissues, where L-NAME was either ineffective or weakly effective in reducing the nerve-mediated relaxation response to inhibitory EFS, one may postulate that this may have been due to an alteration in the potencies of other putative inhibitory neurotransmitters, such as VIP and ATP. Alternatively the ileal smooth muscle may have developed a relative resistance to the effects of NO generated by NANC nerve stimulation, perhaps as a result of the systemic effects of either chronic UC or the long-term medications used to treat the colitis. The samples in which neuronal responses were absent, yet the tissues still responded to carbachol and other exogenous drugs, are difficult to interpret. These tissues may simply have been traumatised by surgical dissection or transport, although surgical techniques and specimen retrieval procedures were no different than those used to obtain the control tissues studied in the same set of experiments. In hindsight, electron microscopy of such UC samples may have helped to exclude neuronal

damage. On balance, however, it is difficult to imagine that UC patients undergoing IPAA, with no unusual gastrointestinal symptoms manifesting preoperatively, could have ileal enteric neurons which are non-functioning. Nevertheless, these absent responses to inhibitory EFS highlight another difference between UC and control tissues.

Using intracellular recording techniques, apamin has been used to pharmacologically dissect 2 distinct NANC hyperpolarising responses (Niel *et al*, 1983; Bywater & Taylor, 1986). These reponses have been termed the fast and slow inhibitory junction potentials (IJPs) respectively (Crist *et al*, 1991; Crist *et al*, 1992). The fast IJP is sensitive to antagonism by apamin and best revealed in the presence of atropine. The slow IJP is best revealed when the fast IJP is blocked by apamin and a substance P-mediated excitatory potential blocked by substance P desensitisation. In guinea-pig ileum circular muscle using apamin and NOS antagonists, He and Goyal have suggested that the fast IJP is mediated by ATP; that NO is involved in the slow IJP which is mediated by VIP and NO acting in series; and that the hyperpolarising effects of VIP and the slow IJP are normally masked by overlying depolaristaion due to concomitant release of substance P by VIP (He & Goyal, 1993). Apamin was therefore used in the present study, albeit in basic organ bath recordings of inhibitory nerve-mediated responses, to investigate whether or not an apamin-sensitive component was contributing to the relaxations elicited by EFS in control and UC tissues. Clearly these measurements of inhibitory response are different from the precise, intracellular membrane potential recordings of single smooth muscle cells used by He and Goyal (He & Goyal, 1993). Also, anatomical and species-related differences must be remembered when comparing the present studies of human ileum with those of guinea-pig ileum. Nevertheless, the sequential incubation of L-NAME followed by apamin in 3 control subjects showed that the reponse to 4Hz inhibitory EFS was not abolished, but that a residual nerve-mediated component was left (Fig 5.20). This may suggest that although NO is the main inhibitory transmitter in control ileum a second apamin-

sensitive component contributes to the overall relaxation response. In the UC strips studied with L-NAME and apamin, the nerve-mediated relaxations in 3 patients were abolished after incubation with L-NAME then apamin (Fig 5.22). Thus, at least in these 3 UC patients the enteric inhibitory nerve-mediated response would appear to be mediated by NO and another apamin-sensitive neurotransmitter (see Rae & Muir, 1996).

From these studies NO has been shown to be the main inhibitory neurotransmitter in control samples of human terminal ileum. A more complex picture emerges in UC ileum. The contributions of other neurotransmitters to the nerve-mediated inhibitory responses in both types of tissue are as yet unestablished. Likewise, the mechanisms which bring about the heterogeneity of inhibitory responses in the ileum of UC patients undergoing IPAA are unknown, but are presumably related either directly or indirectly to the disease process itself and/ or the drugs used to treat UC prior to surgical intervention.

5.4.3 SNP-INDUCED RELAXATION

While inhibitory relaxation in control ileum can be elicited by stimulating enteric neurons which synthesise and release NO as the principal inhibitory transmitter, SNP does not produce its relaxation through the same nerve-mediated mechanisms. Further, SNP relaxation is not dependent on K^+ channel opening, as demonstrated by the ineffectiveness of K^+ depolarising solution or apamin to modify its effect. HbO, which has a high affinity for NO and scavenges it from extracellular media does not appreciably modify relaxant responses to SNP in human ileum.

Although these are only preliminary observations they lend weight to the comments made by other workers (Garcia-Pascual & Triguero, 1994), that SNP does not produce smooth muscle relaxation through NO released in the extracellular medium. The HbO used in these experiments can become unstable and ineffective but caution was taken to exclude this factor as a reason for HbO-

ineffectiveness in preventing SNP-induced relaxation, by using fresh HbO preparations for each experiment.

In human ileum, SNP-induced relaxation could potentially occur via other pathways distinct from that in which NO activates guanylate cyclase (Fig 4.1). Workers have shown that the relaxation produced by SNP may not be solely due to a rise in tissue cGMP content (Vidal *et al*, 1991; Hu *et al*, 1992). Indeed, different mechanisms from cGMP accumulation such as stimulation of prostaglandin synthesis, membrane hyperpolarisation and uncoupling of oxidative phosphorylation have been put forward as possible ways in which SNP may cause smooth muscle relaxation (Ahlner & Axelsson, 1987). Further studies are required to investigate this in human ileum (see section 6.3).

5.4.4 NADPH-DIAPHORASE HISTOCHEMISTRY

Immunohistochemistry, rather than histochemistry *per se*, would have appeared to have been the best method of detecting NOS in human ileum. Although other workers have used NOS antibody in rats (Bredt *et al*, 1990), prohibitive cost implications combined with difficulties in obtaining good NOS antibody which is sensitive enough to react in fixed tissue specimens, resulted in NADPH-diaphorase histochemistry being the technique of choice for these studies.

Colocalisation of NOS and NADPH-diaphorase in neurons of the guinea-pig intestine (Young *et al*, 1992) and canine colon (Ward *et al*, 1992) have been reported. In addition, several workers have shown that NOS and neuronal NADPH-diaphorase are identical (Hope *et al*, 1991; Dawson *et al*, 1991; Bredt *et al*, 1991; Grozdanovic *et al*, 1992; Aimi *et al*, 1993). Thus, with wide acceptance that the existence of NOS can be demonstrated by NADPH-diaphorase histochemistry, NADPH-diaphorase-positive neurons may be regarded as NO-producing neurons.

The photomicrographs (Fig 5.26 a-d) show that NO is abundant in the myenteric plexus of the human terminal ileum, as well as the mucosa and layers of the muscularis. NADPH diaphorase activity was however, notably scarcer in the

submucosa. Taken together, these results are in keeping with a role for NO as a major neurotransmitter in the human enteric nervous system. Similar patterns have been reported by other workers studying regions of the human gastrointestinal tract. These include the ICJ and caecocolonic junction (Faussone-Pellegrini *et al*, 1993), colon (Hanani *et al*, 1993) and rectum (O'Kelly *et al*, 1994).

These histochemistry studies have shown that in the ileal smooth muscle of 9 UC individuals and 6 other cases of mixed surgical diagnoses, there were no appreciable differences in the concentration or anatomical distribution of NO-producing neurons. These findings contrast with the differences between the two patient groups with respect to NO-mediated enteric nerve relaxation. In particular, there was no wide-ranging spectrum of staining in the UC patients, unlike the spectrum of responses noted when studying the effects of L-NAME on NO-mediated relaxation in the same individuals and in other UC patients. Thus, while the facility to generate NO exists in the terminal ileum of UC patients undergoing IPAA, the heterogeneity in responses to NO-mediated relaxation, as studied by the effects of L-NAME on nerve-mediated relaxation suggests that at some stage following generation of NO in ileal neurons, the cascade of events bringing about the relaxation response seen in control ileal smooth muscle is modified in the ileum of patients with UC.

CHAPTER 6

GENERAL DISCUSSION

6.1 COMMENTS

This thesis has looked at two very different areas of small bowel motility. In the first section attempts were made to characterise gross patterns of small bowel motility using an ambulatory manometry technique in prospective IPAA patients with UC. This contrasts with the second part of the project in which specific *in vitro* aspects of terminal ileal motility were examined in control and UC patients at the time of surgical resection and IPAA. For various practical and anatomical reasons which have been discussed in preceding chapters these two areas of study were essentially the only ones which could be pursued in human subjects with UC undergoing IPAA.

Although these two approaches are at opposite ends of the spectrum of motility investigations (see Chapter 2) and consequently examine small bowel motility in ways which are functionally and anatomically distinct, the following discussion attempts to evaluate the *in vivo* and *in vitro* studies in this thesis in terms of design and clinical relevance of the data obtained. The studies are also appraised, where applicable, against related work reported by other groups since this research began in 1992. Finally, those subjects who underwent both small bowel manometry and *in vitro* studies are re-examined to see if there are any obvious associations which emerge from the motility measurements for each individual.

6.1.1 SMALL BOWEL MANOMETRY STUDY

The small bowel manometry study described in Chapter 3 has several inherent deficiencies. Firstly, larger patient numbers, including control subjects, would have strengthened the validity of the observation that gross patterns of small bowel motility do not correlate with nocturnal stool frequency in intact UC patients prior to IPAA. To achieve this in reality would require either several years of study of consecutive UC patients in the one centre, or alternatively a standardised

manometry assessment of such patients carried out in several IPAA centres. Although not impossible, both of these approaches would require much in the way of funding and human resources. Secondly, although small bowel motility was assessed by manometry throughout its entire length, closer transducer spacing distally would have given more relevant information to compare with the *in vitro* findings of resected portions of terminal ileum. However, to have looked at manometry patterns over the entire length of the small bowel rather than at one specific region was entirely reasonable in this study, given the fact that the technique had never been used by the author previously, and that gross patterns of manometric activity are easier to interpret compared with those obtained from regional studies. Nevertheless, both types of manometric study i.e full length and regional (terminal ileum) are required in the study of UC patients undergoing IPAA. Longer diurnal periods of recording would have also been desirable to enable at least some comment to be made on MMC variables. Analysis was impossible in the present study because of the intense phase II activity which occurred postprandially, in what was a short overall recording period of 59 hours. This highlights the major deficiency of small bowel manometry, and has been encountered by other workers examining established IPAA patients (see Stryker *et al*, 1985; Groom *et al*, 1994). In my opinion, therefore, small bowel manometry can only be usefully applied to investigate patterns of *nocturna*l small bowel activity.

Since this research commenced in October 1992, there have been no other reports of small bowel manometry studies in prospective or established IPAA patients. Although the St Mark's study was published in 1994 (Groom *et al*, 1994), the original work contained in this paper was first presented in March 1992 in abstract form (Groom *et al*, 1992).

6.1.2 SMALL BOWEL *IN VITRO* STUDIES

Any *in vitro* study of the small intestine, in patients undergoing IPAA, has obvious limitations. Firstly, the region of interest must be confined to the most distal portion of terminal ileum which is normally sacrificed at the time of mesenteric arcade division in preparation for reservoir construction. Intestine proximal to this region is preserved and therefore impossible to obtain for laboratory analysis. Secondly, the quantity of ileum which is obtained is insufficient to study in apparatus designed to examine the effects of drugs on whole tissue peristalsis (see Trendelenburg, 1915; Grider, 1989). Thirdly, any tissue obtained in this fashion must be analysed with the knowledge that it has been exposed to anaesthetic agents and a variety of long-term anti-inflammatory preparations such as 5-aminosalicylic acid derivatives and steroids. Finally, the presence of inflammatory sequelae secondary to the disease process, such as backwash ileitis (Gustavsson *et al*, 1987), must be acknowledged. Taking these aspects into account, one may make several observations regarding the current study.

Although the samples of ileum obtained from patients were small, thus making peristalsis studies impossible, adequate quantities were available for Golenhofen apparatus, organ bath and histochemical studies. The muscle strips represented whole tissue samples, containing both inner circular and outer longitudinal layers of the muscularis propria. While it is possible to dissect these two layers and study them independently (see Hara *et al*, 1986), for preliminary assessment of the tissue it was decided to retain both layers, albeit in a longitudinal direction. Orientation in the axis of the circular layer, and studies of both isolated inner and outer muscle layers would need to be examined in future studies (see section 6.3).

All tissues obtained for *in vitro* analysis in this study were exposed to a variety of anaesthetic agents (see Table 5.4), and these must be considered when interpreting the data. However, both control and UC patients alike were exposed to

a broadly similar anaesthetic technique. While certain drugs such as atropine can have profound effects on human gut motility when assessed *in vitro* (A MacDonald, personal communication) there were no instances when this drug was administered to the cases studied in this thesis. The long-term effects of previous systemic steroids and 5-aminosalicylic acid derivatives on ileal motility are unknown, but clearly these drugs too may have affected the subsequent motility characteristics of the ileal strips examined. From the present study it is clear that certain *in vitro* motility characteristics of the ileum are altered at the time of IPAA in patients with UC. Whether this is due to the disease process itself, the anti-inflammatory medications or a combination of both is clearly clinically relevant and will require further investigation. Another factor to consider in this study is that all the tissue samples were exposed to diathermy currents prior to resection. Thus, for reasons including preoperative medication, anaesthetic agents, drugs administered perioperatively and operative factors such as diathermy, inherent deficiencies will exist in any studies of human gut tissue obtained for *in vitro* analysis until a reliable animal model is developed which simulates UC. Animal models which have been used to study chronic colitis (Morris *et al*, 1989; Allgayer *et al*, 1989; Grisham *et al*, 1991) and chronic ileitis (Miller *et al*, 1993) have debatable relevance to the clinical conditions of UC and CD. Indeed, "most of the induced models (of intestinal inflammation) are merely models of induced inflammation in the colon, not of UC", (Warren, 1994).

There were no examples of "backwash ileitis" amongst the patients studied in this project. This is a condition seen in up to 11% of patients undergoing restorative proctocolectomy (Gustavsson *et al*, 1987). Larger patient numbers may have yielded examples of this condition for examination but there is no evidence in the literature to suggest that backwash ileitis is related to pouch function or indeed the subsequent development of pouchitis (Gustavsson *et al*, 1987). Indeed, one doubts if the findings of non-specific inflammatory changes in the terminal ileum

at the time of reservoir construction have any relationship to long-term pouch function.

The use of ileum from patients undergoing staged IPAA could also represent a relative weakness in this study since patients with ileostomies may have possible structural and functional differences relating to their previous surgery and not to the residual disease process in their rectal stumps. Further work would be necessary to establish if this is true. Indeed, ideally one would want to study samples of tissue from patients with no previous history of gastrointestinal surgery and/or colectomy at the time of IPAA. In clinical pactice, however, this is not easy and the IPAA patients used in this study represent the typical range of staged and intact subjects presenting to an established tertiary referral centre (see Appendix).

Has there been any related research in inflammatory bowel disease carried out since this research project commenced? During the last four years several groups have examined the role of the L-arginine-nitric oxide pathway in inflammatory bowel disease and in animal models of intestinal inflammation. Middleton and colleagues compared NO production in rectal biopsies from patients with active UC, quiescent UC and normal controls (Middleton *et al*, 1993). The assay technique employed was based on the simple fact that NO is produced by NOS with the liberation of equimolar quantities of L-citrulline. As noted in Chapter 4, NO is highly reactive and very difficult to measure directly in tissue. L-citrulline assay was therefore performed as an indirect means of quantifying NO production. The study showed that citrulline concentrations were significantly increased relative to other amino acids in the rectal biopsy specimens from patients with active UC. The authors speculated that increased mucosal NO generation may be a contributory factor in the distension seen in severe colitis and at worst, toxic megacolon, because the same group had shown previously that NO in low concentrations relaxes colonic circular smoth muscle (Middleton & Hunter, 1991). Increased NO production and the associated augmented mucosal blood flow seen

in areas of intestinal inflammation could, however, represent a defensive reaction against some unidentified damaging agent (Su *et al*,1989).

Further studies investigated the activity of cNOS and iNOS in colonic mucosa and muscle samples from patients with active UC (n=6), CD (n=4) and in control, noninflamed subjects (n=11). (Boughton-Smith *et al*, 1993). This group found that active UC is associated with a substantial elevation of iNOS. Activity of NOS was unchanged in the colonic muscle fraction of UC patients when compared with control samples, suggesting that iNOS is not expressed in smooth muscle or associated neuronal tissue. Thus, the apparent anatomically specific induction of iNOS in UC mirrors the mucosal location of the inflammatory process. Increased amounts of NO produced from the mucosa or submucosa after induction could diffuse to the muscle layers underneath, akin to NO diffusing from endothelium to vascular smooth muscle and hence could relax enteric smooth muscle which would contribute to the impaired motility response seen in toxic megacolon. The four cases of CD, on the other hand , failed to show an increase in mucosal levels of cNOS and iNOS despite histological evidence of acute inflammation. In addition, CD patients had a reduction in the cNOS levels in the muscular layers. Whether or not this was due to deficiencies in the concentration or activity of the enzyme in the smooth muscle cells or fibres was not clear. These differences in NOS activity between CD and UC patients could contribute to the differences in motility seen between the two conditions and the pathological vascular changes seen in the former (Wakefield *et al*, 1989). Furthermore, these differences could be due to differences between the two disease states in terms of composition and penetration of inflammatory infiltrate or to excessive mucosal production of cytokines such as interleukin (IL) -4 or IL-10 which have been reported to inhibit induction of iNOS activity (Cunha *et al*, 1992). On this point, the balance of stimulatory to inhibitory cytokines may determine the expression of NOS in UC and CD (Boughton-Smith, 1994). In a review in 1994, Boughton-Smith speculated that if NOS induction occurred in UC but not CD, then the therapeutic potential for selective inhibitors of

induced NOS in inflammatory bowel disease may be different for the two conditions (Boughton-Smith, 1994).

In UC, inhibitors of iNOS may be beneficial where increased NOS could be contributing to the mucosal vasodilation and increased vascular permeability. Moreover, the obvious benefits of eliminating toxic megacolon which could be driven by increased quantities of NO (Guslandi, 1993) would be appealing. These agents have been tested in animal models. L-ARG antagonists ameliorated chronic ileitis, induced by trinitro-benzenesulfonic acid, in guinea-pigs (Miller *et al*, 1993a) while enhanced generation of NO promoted mucosal injury, but was anti-inflammatory under physiological conditions. Under acute conditions, (luminal acetic acid and casein in rabbits and luminal deoxycholite in neonatal piglets and adult rabbits) NO may contribute to the functional repair of the epithelial barrier (Miller *et al*, 1993b).

In CD, on the other hand, where there is an underlying vascular pathology, removal of a vasodilator and antiaggregatory substance, such as NO, may exacerbate vascular perfusion and be detrimental. NO donors, or agents increasing local NO production or stimulating guanylate cyclase activity, may have a therapeutic value in CD and are reportedly useful in animal models of acute intestinal damage (Boughton-Smith *et al*, 1990; Boughton-Smith *et al*,1992).

Thus, the few human studies carried out to date to evaluate NO in inflammatory bowel disease have focussed on colonic NOS activity and enzyme isoforms. Importantly, they have contained only small numbers of subjects. In patients with UC undergoing IPAA, the role of NO in the NANC neurotransmitter mechanisms of the residual ileum from which the reservoir is fashioned was unexamined until the work in this thesis was carried out.

6.1.3 CORRELATES AND PREDICTIONS?

From the preliminary studies descibed in this thesis it would have been encouraging to have demonstrated any correlation between the *in vivo* and *in vitro* data on one hand and the clinical features of the patient on the other. Table 6.1 shows the individual patients from whom data were available to attempt to examine this relationship.

Meaningful analyses were possible only in 6 intact UC patients who had undergone small bowel manometry and L-NAME studies. The clinical factors assessed included age, duration of history of UC and nocturnal stool frequency. The small bowel manometry nocturnal indices were assessed together with an arbitrary score for whether the patients' tissues were strongly affected by L-NAME (score 3); weakly affected by L-NAME (score 2); unaffected by L-NAME but exhibiting neuronal relaxation (score 1); or no demonstrable neuronal relaxations (score 0). Fisher's exact test showed that of the 9 parameters i.e 8 nocturnal small bowel manometry parameters, (see Table 3.7) and the score for the tissue response to incubation with L-NAME, assessed against the patients' clinical features, the number of nocturnal DCCs and the tissue response to incubation with L-NAME showed associations with the duration of UC, in both cases P=0.100. While these data did not reach statistical significance they are nevertheless interesting and could be investigated in future studies with greater patient numbers. It is interesting to speculate that those patients with the shortest history of UC coming forward for IPAA have a more aggressive form of colitis which caused the absent neuronal relaxations noted in the organ bath, while the more chronic UC patients adjusted to their illness by altering the balance of inhibitory neurotransmitters in the non-inflamed small bowel. On this basis, the absence of DCCs in these patients could be similarly explained. Alternatively, and perhaps more plausible, is the possibility that the tissues with absent neuronal relaxations were in some way traumatised by retrieval for the *in vitro* experiments (see section 5.4.2).

PATIENT	SEX	AGE	UC DURATION	STOOL FREQUENCY	TOTAL MMCs	DCCs	EFFECT OF L-NAME
ZS	M	30	72	0	12	5	3
JBRD	M	48	24	7	12	0	0
AR	M	24	18	1	7	0	0
WF	M	35	84	1	7	4	3
RK	M	29	132	1	8	6	2
DK	M	29	48	4	7	0	0

Table 6.1: Clinical details of 6 intact ulcerative colitic (UC) patients assessed by preoperative ambulatory small bowel manometry and *in vitro* ileal smooth muscle pharmacology studies of nitric oxide-mediated inhibitory neuronal responses.
Duration of UC expressed in months. Stool frequency refers to nocturnal pattern as do total migrating motor complexes (MMCs) and discrete clustered contractions (DCCs).
Antagonistic effects of L-NAME (10^{-4}M) scored from 0-3 (see text).

The data obtained from the present studies, which were preliminary investigations of small bowel motility in UC, in an attempt to predict long-term IPAA function could not be statistically analysed given the small numbers available. However, while no scoring system is currently available to predict long-term function in prospective IPAA patients, this could be achieved by having access to large numbers of patients in the form of a prospective multi-centre trial. Such a preoperative scoring system would be particularly useful clinically if it included easily measured parameters such as patient age, duration and extent of UC, previous colonic resection for UC, response to previous medical therapies, number of episodes of UC exacerbation requiring in-patient treatment and if female, any significant obstetric history. Simple physiological measurements could also be included such as gastric emptying and anal sphincter manometry profiles, the latter perhaps being combined with an endoanal ultrasound assessment of the sphincter mechanism. However, attempts to develop a scoring system using the *in vivo* and *in vitro* assessment techniques employed in this thesis would be extremely difficult to routinely carry out and to reproduce in other centres.

6.2 CONCLUSIONS

This thesis was undertaken to investigate two principal aspects of small bowel motility in UC patients undergoing IPAA. Solid state manometry assessed the *in vivo* motility patterns of the small bowel over its entire length in prospective patients, while *in vitro* experiments were confined to the most distal portions of terminal ileum discarded at the time of ileal reservoir construction.

Small bowel manometry showed varying patterns of nocturnal motility in 7 intact patients with no demonstrable correlation between the various motility parameters and stool frequency. The former included number of MMCs, MMC cycle length, MMC mean duration, MMC mean velocity, number of non-propagating MMCs, number of DCCs and number of PPCs. As in previous manometry studies of established IPAA subjects (Stryker *et al*, 1985; Chaussade *et al*, 1989; Groom *et al*, 1994) patient numbers in the present study were small; this was a major disadvantage but had to be accepted in this pilot study. However, the study gave useful information on the small bowel motility patterns of intact UC patients prior to IPAA, which have hitherto never been available. The present study also demonstrated major weaknesses in the use of ambulatory manometry as a means of assessing motility patterns along the length of the whole small bowel. These include patient discomfort, intubation difficulties, length of time required to achieve successful catheter positioning, time-consuming analysis of traces and considerable expense associated with the catheter plus equipment for data storage and analysis.

In vitro experiments, on the other hand, were possible in greater patient numbers and allowed a comparison of UC patients with controls undergoing elective procedures for non-obstructing, non-inflammatory cancer resections of the right colon. Organ bath and Golenhofen apparatus recordings of spontaneous activity using controls and UC patients showed that differences in apparatus yielded differences in the values recorded. The Golenhofen apparatus was the more

sensitive and detected subtle oscillations in tone with corresponding extracellular electrical activity providing an electrical basis for the contractions seen. Organ bath studies showed that UC strips exhibited spontaneous contractions which were weaker, but more frequent than controls. A similar pattern was noted in Golenhofen experiments. Whether this represents the chronic exposure of ileum in UC patients to the systemic effects of an inflamed colon remains unestablished. There was no obvious relationship between the extent of UC (i.e total versus left-sided UC) and the pattern of spontaneous activity, nor any instances of backwash ileitis. Golenhofen experiments confirmed that both control and UC ileal smooth muscle specimens have VOC and ROC. Spontaneous activity in both patient groups was also temperature-sensitive and blocked by the calcium channel antagonist diltiazem as well as SNP. L-NAME had no effect on ileal smooth muscle spontaneous activity in either patient group. Golenhofen experiments in ISTC patients showed that the intestinal pacemaker generated spontaneous mechanical activity via less intense, shorter duration electrical activity than its control counterpart. These differences may be due to factors, as yet unestablished which modify the number and/ or the function of the ICC.

NO-mediated inhibitory relaxation in carbachol-induced tone (10^{-6}M- $3x10^{-6}$M) and guanethidine (10^{-6}M) was non-adrenergic and was the predominant pattern of relaxation under these conditions. In contrast, varied responses to L-NAME were noted in 18 UC patients (11 intact, 7 staged). Only 5 of these patients (4 intact, 1 staged, 10 strips) responded similarly to L-NAME as controls, where L-NAME was effective in abolishing neuronal relaxation. In 8 patients (3 intact, 5 staged, 23 strips) L-NAME was either only weakly effective or ineffective. A further 5 patients (4 intact, 1 staged, 25 strips) had no demonstrable neuronal relaxation responses to EFS. NADPH-diaphorase staining confirmed NOS presence in representative samples from both groups. Thus, while some patients with UC have NO-containing nerves mediating relaxation in a similar fashion to ileum from control subjects, others appear to have a comparatively defective NO

pathway. These latter individuals may be utilising alternative neuronal mechanisms and will require future investigation.

Studies to look at how SNP, a classic NO-donor relaxed human ileal smooth muscle, challenged the idea that SNP liberates extracellular NO to produce its action. HbO, which scavenges NO from extracellular media had no appreciable effect on SNP relaxation in samples of control ileum (3 patients, 3 strips).

The differences noted between control and UC samples of ileum in these *in vitro* experiments merit further investigation and suggest that the visibly "normal" ileum from which the IPAA is constructed in UC patients may have different motility characteristics from control counterparts i.e FAP pouch patients. Studies to investigate small bowel motility in ISTC patients and also the mechanism of action of SNP-induced relaxation in ileal smooth muscle are also warranted.

6.3 FUTURE WORK

Today, small bowel manometry is used only as a research tool. Indeed, as recently as March 1997 at the British Society of Gastroenterology Spring Scientific Meeting, a symposium on gastrointestinal motility reviewed available techniques for investigating every region of the human gut *except* for the small bowel. This is most probably due to the relative inaccessibility of the midgut region to catheterisation. In addition, the limited number of papers available in the scientific literature on the *in vivo* and *in vitro* properties of the non-diseased human small bowel reflect our poor understanding of the motility characteristics of this region of the gut. The motility of the small bowel in UC is even less well understood and much more work is therefore required to investigate this in control subjects and UC patients undergoing IPAA. While *in vivo* studies are dogged by the anatomy of the region which makes intubation of the distal small bowel difficult, samples of ileum from patients undergoing elective surgical procedures can, on the other hand, be obtained for *in vitro* analysis with relative ease and regularity.

This thesis has reported preliminary studies of small bowel motility in patients with UC undergoing IPAA. It has only touched the surface of a complex and largely unknown area of human gut function and much could be written about the various directions which future research might take in this field. The following is therefore only a précis of specific research areas which would be logical to examine in light of the work described above, in both control subjects and UC patients.

6.3.1 SMALL BOWEL MANOMETRY

While acknowledging the difficulties with small bowel manometry as a means of *in vivo* assessment of motility, the work outlined in Chapter 3 is nevertheless only the third study of its kind to investigate IPAA patients, and the first to assess such patients preoperatively. One obvious extension of the work in

Chapter 3 would be to repeat the study in the same patients once established pouch function (more than 12 months following ileostomy reversal- see Appendix) had occurred. This would allow assessment of the effects of reservoir construction on small bowel motility. Further monitoring of the progression of MMCs into the pouch, along with measurements of pouch capacity and emptying characteristics may yield comparable values to those reported in the St Mark's study (Groom *et al*, 1994). This study would have the added advantage over previous ones of being able to use each patient as his/her own preoperative control. However, the problems of adhesions preventing catheter migration, which were encountered in the present study would have to be borne in mind, although interestingly these were not reported by the St Mark's group.

Terminal ileal manometry is possible (Hammer *et al*, 1993), and as mentioned above would be useful in characterising the precise manometric properties of the terminal ileum and ileocaecal region in UC patients prior to IPAA. The specialised motility characteristics of these regions have been comprehensively reviewed by Phillips and colleagues (Phillips *et al*, 1988) and are well documented in control subjects (Kerlin & Phillips, 1982; Quigley *et al*, 1984; Nasmyth & Williams, 1985) but remain unknown in UC patients. Clearly this is an important area which requires further investigation.

Fuller evaluation of small bowel manometry as a clinical research tool is also required. Studies looking at patient discomfort during this investigation with pain score assessment as well as cost analysis of the technique would help to define its role in the evaluation of surgical patients. Assessment of recently introduced computer programmes to facilitate data analysis would also be worthwhile.

Finally, if one hypothesises that chronic UC modifies motility in non-inflamed segments of the gut then it would be reasonable to study oesophageal motility using either water-perfused (which is our preferred system at Glasgow Royal Infirmary) or solid-state manometry. This would have the advantages of

being easier to perform than small bowel manometry involving equipment which was cheaper and more widely used in hospitals throughout the country.

6.3.2 *IN VITRO* STUDIES

The main thrust of any work in the area of human small bowel motility must, in my opinion, be targetted at *in vitro* assessment of tissue obtained from control patients and subjects with pathological conditions.

As mentioned in section 6.1 various types of tissue orientation have yet to be assessed in the investigation of ileal motility in health and in UC. It would be particularly interesting to examine the properties of the isolated inner circular and outer longitudinal layers of the muscularis propria given that ICCs are predominantly located in the myenteric plexus and circular muscle layer of the human small bowel (Hagger *et al*, 1997).

Most *in vitro* studies of the type described in this thesis tend to excise the mucosa before muscle strips are suspended in the organ bath. By constructing larger electrodes it would be possible to accommodate mucosal-clad strips in the stimulating electrode and would permit studies of the effects of the presence and absence of ileal mucosa on underlying enteric muscle function.

The pilot studies described in this thesis could lead to various other in-depth projects. One obvious example would be to progress from the extracellular recording techniques described in Chapter 5 to intracellular aspects of spontaneous activity and the effects of various drugs on nerve-mediated inhibitory and excitatory responses (see review by Bolton, 1989; Hara *et al*, 1986). This would help to clarify the neurotransmitters thought to be contributing to fast and slow IJPs in human ileum from controls and UC patients. Thereafter, patch-clamp analysis of dispersed human ileal smooth muscle cells from control and UC patients would be the state-of-the-art technique to use to examine the ionic mechanisms underlying spontaneous and evoked responses in these tissues.

Biochemical assays of cGMP levels in ileal strips after SNP-induced relaxation would help to clarify whether this intracellular messenger was involved in processes which would suggest that SNP generates NO *per se* (Garcia-Pascual & Triguero, 1994).

Other clinical conditions in which ileal samples should be examined at the time of surgical resection include CD, in light of work comparing colonic samples from patients with UC and CD (Boughton-Smith *et al*, 1995), and also patients with obstructing right colon cancers undergoing emergency right hemicolectomy. The latter study would be especially interesting since manometry studies in IPAA pouches have raised the suggestion that the ileal reservoir causes a functional small bowel obstruction (Chaussade *et al*, 1989). One could hypothesise that differing degrees of obstruction, produced by the ileal reservoir, could cause varying patterns of small bowel motility proximal to the pouch, with functional consequences in terms of stool frequency and urgency. *In vitro* studies of ileal samples, from patients with poor pouch function would be useful but, although access to tissue from the most severe cases of pouch dysfunction which ultimately require excision or defunctioning would be possible, these occur too sporadically in practice to generate large patient numbers for analysis.

The hypothesis underlying this thesis has been that small bowel motility may be modified in UC patients undergoing IPAA. In other words, inflammation of the colon may in some way bring about motility changes in more proximal, histologically normal, non-inflamed areas of the gut. Of great relevance to this project have been recent *in vitro* studies which investigated the immunomodulation of enteric neuromuscular function in animal models of gastrointestinal inflammation (see review by Collins, 1996). A clear causal relationship now exists between the presence of mucosal inflammation and changes in sensory-motor function. Notwithstanding the inherent drawbacks of animal models of acute intestinal inflammation outlined above, one exciting study has shown that in a rat model of colitis a marked suppression of release of ^{3}H-noradrenaline (a

radiolabelled enteric neurotransmitter) occurs not only in the inflamed distal colon but also in the noninflamed transverse colon and terminal ileum (Jacobson *et al*, 1993). As Collins has pointed out (Collins, 1996), this may provide a possible functional correlate of the ultrastructural abnormalities noted in enteric nerves at noninflamed sites in patients with Crohn's disease (Dvorak *et al*, 1979) and the altered intestinal motility patterns seen in UC patients (Manousos & Salem, 1965). Thus, the relatively new field of immunophysiology may in future years help to clarify the effects of UC on small bowel motility before IPAA. However, extensive descriptions of the various immunological and ultrastructural studies which would be of great interest to perform in samples of ileum from UC and CD patients are beyond the scope of this discussion.

6.3.3 CLINICAL STUDIES OF POUCH DYSFUNCTION

The Appendix to this thesis, like its anatomical namesake, may on first inspection appear unnecessary. However, this thesis evolved from a simple project, initiated in 1992, which was designed to audit operative and postoperative experience with the technique of TSRP as well as long-term functional outcome. The findings of that audit project are summarised in the Appendix. One contribution which a database such as the Appendix can make, in terms of directing future research, is its ability to identify those established IPAA patients with pouch dysfunction.

As experience at Glasgow Royal Infirmary has increased with TSRP, and with steadily rising numbers of IPAA patients who achieve long-term function, it is becoming increasingly clear that many patients with problematic pouch function cannot be conveniently labelled as having "pouchitis". The Oxford group have recently described a problem-solving approach to IPAA dysfunction (Thompson-Fawcett *et al*, 1997). Classification of the non-pouchitis group of conditions contributing to functional problems include small bowel dysfunction, pouch abnormalities and outlet problems. As patient numbers increase nationwide, a

multi-centre study incorprating audit databases similar to that summarised in the Appendix, would generate adequate patient numbers for meaningful statistical analysis and would also provide the opportunity to study the small bowel motility and transit characteristics of those individuals falling into the small bowel dysfunction category. Retrospective assessment of preoperative factors in these individuals such as age, duration of illness, types and duration of medical therapies, previous surgery, extraintestinal manifestations of UC, stool patterns and presence or absence of IBS would be obvious variables to measure if attempts were made to develop a scoring system which may ultimately predict pouch dysfunction.

Clinical studies including prospective small bowel manometry assessment of patients with pouch problems, along the lines of the St Mark's study (Groom *et al*, 1994) could be performed. Small bowel transit and pouch evacuation characteristics could be assessed by scintigraphic techniques, along with pouchmetrography (Levitt *et al,* 1992) and standard anal manometry. Although difficult to perform, this type of comprehensive clinical assessment is possible and has been performed on a small number of IPAA patients at Glasgow Royal Infirmary.

The studies described in this thesis shed some interesting new light on differences which exist in the so-called "normal" ileum of patients with UC. This research project has therefore lent support to the comments of Aufses Jr who stated in 1986 that:

"patients who had the operation (IPAA) performed for polyposis (FAP) had fewer bowel movements than the patients who had an operation for UC. This implies that the function of the remaining small bowel is different in the two sets of patients". (see Becker & Raymond, 1986).

The IPAA procedure is no longer in its infancy as a surgical treatment for UC. From the foregoing it is clear that little is known, however, about the pathophysiology of pouch function and dysfunction, let alone the motility

characteristics of the proximal small bowel and terminal ileum used to fashion the reservoir. Future *in vitro* and clinical studies on the functional constituents (i.e. proximal small bowel, terminal ileum and anal sphincter mechanism) of the IPAA, both before and after reservoir construction are therefore needed if the growing population of IPAA patients is to receive effective treatment for pouch dysfunction.

APPENDIX

TOTALLY STAPLED RESTORATIVE PROCTOCOLECTOMY AT GLASGOW ROYAL INFIRMARY 1988-1995

BACKGROUND: Totally stapled restorative proctocolectomy (TSRP) has simplified IPAA but few reports exist concerning experience with the technique. A retrospective study of operative and functional data in a consecutive series of patients undergoing TSRP was undertaken.

METHODS: Case-note review with functional assessment by clinical interview and/or postal questionnaire.

RESULTS: TSRP with "J" pouch formation was attempted in 103 patients between 1988 and 1995 (ulcerative colitis 87; familial adenomatous polyposis coli 9; slow transit constipation 6; hereditary non-polyposis colorectal cancer 1). Three technical failures resulted in 100 patients available for assessment. Average operating time was 200 mins, intraoperative blood loss 360ml and hospital stay 12 days. There was no operative mortality. Loop ileostomy formation and subsequent reversal in all but 5 cases (95%) was associated with 29 complication episodes, requiring further surgical intervention in 6 (6%). Six pouches were excised and 2 defunctioned with temporary ileostomy formation (8%). Five patients were rediagnosed as Crohn's disease, of whom 4 underwent subsequent pouch excision (80%). Pouchitis in the absence of Crohn's disease occurred in 8 individuals (8%). In 60 patients with at least 12 months established function, average day/night stool frequencies were x5 during the day and x1 overnight. Functional evaluation in 49 (81.7%) revealed 21 using regular antidiarrhoeal medication (42.8%); urgency in 17 (34.7%); and total continence by day/ night in 37 (75.5%) and 35 (71.4%) respectively. Fifty-three patients (88.3%) were satisfied with overall long-term outcome.

CONCLUSION: TSRP is safe, has simplified a technically difficult operation and gives good long-term functional results.

Restorative proctocolectomy (RP) is now the treatment of choice for patients with ulcerative colitis (UC) requiring elective surgery (Williams, 1989) and is being used increasingly as a surgical option for the management of familial adenomatous polyposis coli (FAP), (O'Connell & Williams, 1991).

The initial descriptions of the operation which were complicated and technically demanding (Parks & Nicholls, 1978; Utsunomiya *et al*, 1980), involved retention of a long rectal stump with mucosectomy and a handsewn pouch-anal anastomosis. Although the early procedures suffered from high complication rates (Parks & Nicholls, 1978; Utsunomiya *et al*, 1980; Fonkalsrud, 1980) progressive simplification of the operative technique (Nicholls *et al*, 1984; Johnston *et al*, 1987; Keighley, 1987; Pescatori, 1988; Lavery *et al*, 1989; Slors *et al*, 1989; Miller *et al*, 1990; Oresland *et al*, 1990; Mowschenson *et al*, 1993) has reduced the morbidity associated with RP. Arguably the simplest form of RP uses stapling instruments to fashion the pouch and pouch-anal anastomosis without performing an endoanal mucosectomy. While totally stapled restorative proctocolectomy (TSRP) is technically easier to perform than earlier RP procedures, there are few reports of its use and little is known about long-term function. We describe our operative experience with TSRP and report on the long-term functional outcome.

PATIENTS AND METHODS

Consecutive patients who underwent TSRP with formation of a pelvic ileal J reservoir between 1988 and 1995 were studied. Preoperative diagnosis in these 103 patients was UC in 87, FAP in 9, idiopathic slow transit constipation (STC) in 6 and one case of hereditary non-polyposis colorectal cancer (HNPCC). Eighteen of the 87 patients with UC underwent semi-urgent surgery to treat fulminant

disease (21%). Twenty seven patients (26%) had previously undergone total colectomy with rectal stump preservation (21 with UC, 2 with FAP and 4 with STC).

The operative technique involved diathermy dissection of the rectum, including the mesorectum, to the level of the upper anal canal with division approximately 1cm above the dentate line using a stapling instrument (Ethicon RL 30). Care was taken to identify and preserve the presacral nerves. An ileal pouch was fashioned in the "J"-configuration using 2-3 firings of a linear stapling device (Ethicon PLC 75) ensuring that the apex of the "J" reached the upper anal canal without tension. Anastomosis of the ileal pouch to the anal canal was then performed by the double-staple technique, using a detached head stapling instrument (Ethicon CDH 29). Operative manoeuvres such as mucosal proctectomy, endoanal retraction and anal eversion were avoided. In all but 5 cases a temporary loop ileostomy was fashioned (5%); ileostomy was avoided when technical difficulties prevented formation of a tension-free stoma. Patients received intraoperative subcutaneous heparin (5000I.U) and intravenous antibiotics (cefotaxime 1G, metronidazole 500mg) for 5 days postoperatively.

All patients were followed at a designated clinic at 3 monthly intervals in the first year and 6 monthly thereafter. Patients with functioning pouches for more than 12 months after closure of ileostomy underwent detailed functional assessment, similar to that used by Weinryb and colleagues (Weinryb *et al*, 1995), by interview at an out-patient clinic and/or by postal questionnaire.

Operative mortality was defined as death within 30 days of surgery. Anastomotic leakage was defined clinically with features of pyrexia, lower abdominal pain and purulent discharge passed per anum. Contrast studies were only performed when clinically indicated. The degree of continence was graded as

normal, minor incontinence or major incontinence using standard clinical criteria (Nicholls & Pezim, 1985). Urgency of stool was defined as the inability to defer defaecation for a minimum of 30 minutes (Nasmyth *et al*, 1986a). Patients were considered to have pouchitis on the basis of diarrhoea with or without bleeding, the sigmoidoscopic appearances of pouch inflammation and the absence of specific pathogenic organisms in the pouch effluent. Confirmation of the diagnosis was made by histological analysis of pouch mucosal biopsies (Moskowitz *et al*, 1986).

RESULTS

TSRP was attempted in 103 cases but in 3 patients double-stapling of the reservoir to the anal canal failed, necessitating conversion to a handsewn pouch-anal anastomosis. The technical reasons for these conversions are summarised in Table A.1.

There were no operative deaths. One patient committed suicide 4 months after surgery. The remaining 99 patients (53 males), median age 31years (range 9-59years) were available for assessment (Fig A.1)

The median follow-up was 31months (range 12-77months). The median duration of surgery for TSRP in the 100 patients was 200mins (range 70-285mins), median operative blood loss 360ml (range 50-2000ml) and the median duration of hospital stay was 12days (range 7-74days). Elective closure of loop ileostomy was performed at a median of 85days (range 9-660days) after TSRP.

EARLY COMPLICATIONS

Two pouches were excised during the early postoperative period; one because of ischaemia and the other because of haemorrhage.

PATIENT	AGE	DIAGNOSIS	TECHNICAL REASON
Male	**34yrs**	**UC**	**Inadvertent omission to fire gun after bowel division**
Female	**25yrs**	**UC**	**Staple line opened during endoanal insertion of CDH stapling instrument**
Male	**45yrs**	**UC**	**Rectal thickening precluded satisfactory cross-stapling**

Table A.1. Details of attempted totally stapled restorative proctocolectomy (TSRP) cases converted intraoperatively to handsewn pouch-anal anastomoses. UC, Ulcerative colitis; CDH, Curved detached head

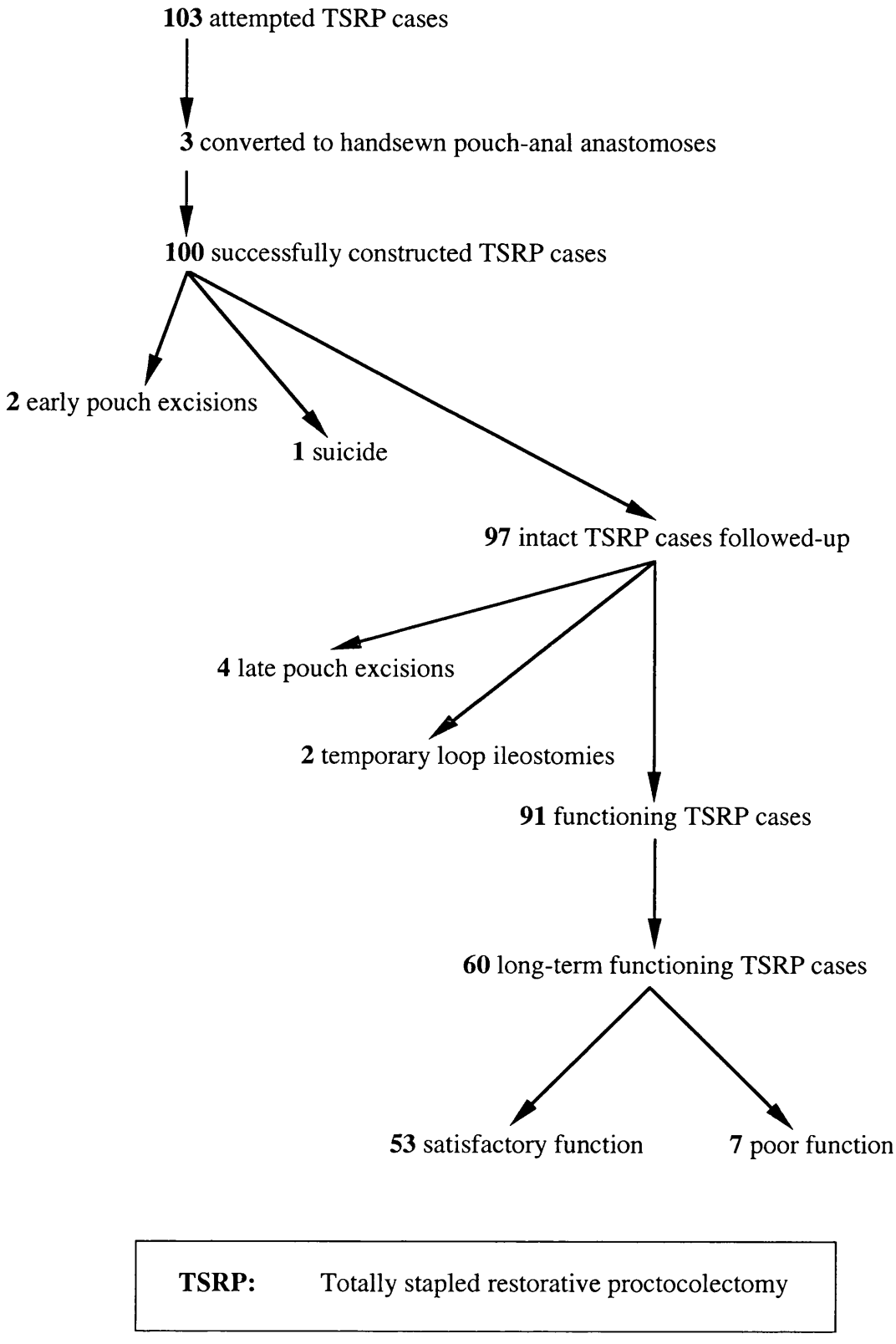


Fig A.1 Outcome of 103 patients undergoing totally stapled restorative proctocolectomy.

Six patients developed clinical evidence of pouch/anastomotic leakage and/or pelvic abscess formation. In 4 of the 6 cases there was no covering ileostomy. Two of these 4 patients required formation of a loop ileostomy; the remaining 4 healed with antibiotic treatment and nutritional support.

Pulmonary thromboembolism occurred despite prophylaxis in 2 patients; both fully recovered.

LATE COMPLICATIONS

Complications associated with formation of a loop ileostomy are given in Table A.2. Although 29 complication episodes occurred only 6 patients required further surgery.

Pouch-anal stenosis occurred in 5 patients (3 females) and was treated by dilation under day-case general anaesthetic. One patient developed a rare complication of complete pouch prolapse at the anus which was corrected by abdominal "pouchopexy".

Crohn's disease and pouchitis

Five patients (1 male) initially considered to have UC have been shown subsequently to have Crohn's disease (CD) in the ileal pouch. Retrospective detailed review of the original colectomy specimen showed UC in 4 and indeterminate colitis in 1, whilst the anastomotic "doughnuts" showed no evidence of CD. In 4 of these patients pouch excision was performed subsequently because of poor function and/or pouchitis.

Eight patients developed clinical and histological evidence of pouchitis in the absence of CD. Three individuals had more than one episode. Treatment was medical in 7, while 1 patient required a temporary ileostomy.

COMPLICATION	n
POST-POUCH CONSTRUCTION	
Intestinal obstruction (conservative treatment)	3
Ileostomy obstruction (laparotomy)	1
Ileostomy retraction	3
Parastomal abscess	1
Excoriation at stoma site	1
High output ileostomy	7
TOTAL	16
POST-REVERSAL OF LOOP ILEOSTOMY	
Anastomotic leak (conservative management)	2
Anastomotic leak (ileostomy revision)	2
Intestinal obstruction (conservative treatment)	7
Intestinal obstruction (laparotomy)	1
Incisional hernia	1
TOTAL	13

Table A.2: Complications associated with loop ileostomy.
n, number of patients.

Pouch function

A temporary ileostomy was created in 1 patient because of poor pouch function. Of the 60 patients who fulfilled the criteria for long-term pouch follow-up, 49 completed a detailed questionnaire to assess pouch function. The remaining 11 patients were assessed, in detail, during regular out-patient review. Approximately two thirds of patients with established pouch function were, by their own assessment, leading normal lives. In contrast 7% of patients had a poor functional result and were unable to work.

Three UC patients (2 males, 5%) had increased pouch frequency, in the absence of both pouchitis and pouch-anal stenosis, on serial examinations under anaesthetic. These individuals were labelled as suffering from irritable bowel syndrome (IBS), after exclusion of occult small bowel Crohn's disease using radiolabelled white cell scans and after measuring haematological/ biochemical indices. Small bowel dysmotility, either related to IBS *per se* or as a result of UC, was suspected to have been a contributing factor in these patients' symptoms.

The long-term functional outcome for the 3 patient subgroups completing the detailed questionnaire is shown in Table A.3. Clearly, the majority of the patients had a preoperative diagnosis of UC (39 patients, 79.6%) and hence a statistically meaningful comparison of outcome measurements with the 7 FAP and 3 STC patients is not possible. The data for FAP pouches, however, show that the stool frequencies and continence in this group tend to be superior to those of their UC counterparts, along with a lower incidence of urgency. Interestingly, the impact of IPAA on previously asymptomatic FAP patients creates more lifestyle disadvantages, as one would expect, than in UC patients who have longstanding chronic symptoms prior to undergoing TSRP. Three UC patients (7.7%) reported

LONG-TERM FUNCTIONAL PARAMETER	UC		FAP		ISTC	
	n = 39		n = 7		n = 3	
	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
STOOL FREQUENCY	6 (2-16)	1 (0-4)	6 (1-8)	1 (1-2)	5 (1-9)	1 (1-2)
CONTINENCE*						
Total control	30 (76.9)	27 (69.2)	6 (85.7)	6 (85.7)	1 (33.3)	2 (66.7)
Minor incontinence	8 (20.5)	11 (28.2)	1 (14.3)	1 (14.3)	2 (66.7)	1 (33.3)
Major incontinence	1 (2.5)	1 (2.5)				
USE OF PADS*						
Never	32 (82.0)	26 (66.7)	5 (71.4)	5 (71.4)	2 (66.7)	1 (33.3)
Sometimes	3 (7.7)	5 (12.8)	2 (28.6)	1 (14.3)	1 (33.3)	2 (66.7)
Frequently	3 (7.7)	2 (5.1)		1 (14.3)		
Always	1 (2.5)	6 (15.4)				
ANTIDIARRHOEAL MEDICATION*						
Never	17 (43.6)		3 (42.8)		2 (66.7)	
Occasional	5 (12.8)		1 (14.3)			
Regular x1 medication	6 (15.4)		3 (42.8)			
Regular x2 medications	9 (23.1)				1 (33.3)	
Regular x3 medications	2 (5.1)					
URGENCY*						
None	25 (64.1)		6 (85.7)		1 (33.3)	
Urgency	14 (35.9)		1 (14.3)		2 (66.7)	
FLUID/ FLATUS DISCRIMINATION*						
Yes	31 (79.5)		5 (71.4)		1 (33.3)	
No	8 (20.5)		2 (28.6)		2 (66.7)	

Table A.3: Long-term functional outcome in 49 patients undergoing totally stapled restorative proctocolectomy (TSRP). UC, ulcerative colitis; FAP, familial adenomatous polyposis; ISTC, idiopathic slow transit constipation. Stool frequencies expressed as median (range). * Values expressed as number of patients (% total). ***Continued overleaf.***

LONG-TERM FUNCTIONAL PARAMETER	UC	FAP	ISTC
	n = 39	n = 7	n = 3
DIETARY ADJUSTMENT*			
None	10 (25.6)	3 (42.8)	1 (33.3)
Small changes	17 (43.6)	3 (42.8)	2 (66.7)
Moderate changes	5 (12.8)	1 (14.3)	
Significant dietary restrictions	2 (5.1)		
Very substantial alteration	5 (12.8)		
PERIANAL IRRITATION*			
Never	8 (20.5)	2 (28.6)	1 (33.3)
1-2 episodes per week	17 (43.6)	3 (42.8)	2 (66.7)
Every day	8 (20.5)	1 (14.3)	
Painful, cracked areas	3 (7.70		
Open, raw bleeding areas	4 (10.2)	1 (14.3)	
LIFESTYLE DISADVANTAGES*			
None	16 (41.0)	1 (14.3)	1 (33.3)
Undisturbed professional life, small changes to social life	11 (28.2)	3 (42.8)	1 (33.3)
Undoubtedly affected professional and social life	6 (15.4)	2 (28.6)	1 (33.3)
Definitely restricted professional and social life	3 (7.7)	1 (14.3)	
Unable to work, almost impossible to leave home	3 (7.7)		

Table A.3 contd: Long-term functional outcome in 49 patients undergoing TSRP.

that TSRP had caused no lifestyle disadvantages and indeed had improved the quality of their social and professional lifestyles.

Genitourinary function

Three men developed retrograde ejaculation whilst 1 patient had partial erectile failure after TSRP. One female patient noticed reduced libido, whilst a further 3 women experienced pouch leakage during intercourse.

It was noted that 76% of the women with established pouch function who had regular menstrual cycles detected a marked increase in pouch stool frequency just prior to and during menstruation.

Two patients had children following TSRP. Both were delivered by caesarian section.

DISCUSSION :

Restorative proctocolectomy has evolved since the first description of the procedure in adults in 1978 (Parks & Nicholls, 1978). Initially it was a time-consuming and technically difficult operation which involved performing a long mucosectomy with excision of the anal transitional zone (ATZ). In addition the handsewn pouch-anal anastomosis required prolonged anal dilatation. The operation has since been progressively simplified with regard to pouch configuration and the avoidance of a long rectal stump. The relatively recent availability of stapling instruments has allowed the operation to be further simplified. Operative time has been reduced and prolonged anal canal dilatation avoided. TSRP might therefore be expected to yield improved functional results particularly with regard to continence and night leakage.

The present study shows that the results of TSRP for UC are variable. Moreover, from the small numbers of FAP pouches available for long-term assessment TSRP can be safely performed for this autosomal dominant condition with good functional outcome. In general, FAP patients undergoing TSRP have lower 24-hour, daytime and nocturnal stool frequencies than UC patients undergoing TSRP. Total continence is excellent for both patient groups with a lower incidence of urgency in FAP patients. Like Aufses Jr, we too believe that the functional difference amongst the two groups suggests that there is an inherent difference in the function of the remaining small bowel in each disease category (see Becker & Raymond, 1986). We no longer perform TSRP for ISTC because of variable outcome and this experience has been reported by other workers (Keighley *et al*, 1993). Although only 3 ISTC patients were reviewed in this series it is interesting to note that 2 (66.7%) had urgency. This may well be a manifestation of either a panenteric dysmotility (Bassotti *et al*, 1996) or IBS.

The data from the present study confirm that TSRP can be performed safely with short operative times, minimal blood loss and low morbidity. Further, there is excellent long-term pouch function with a median stool frequency of x5 per day and x1 per night. This is in keeping with comparable series of TSRP patients (Wexner *et al*, 1991; Keighley *et al*, 1993; Reissman *et al*, 1995). While one would not anticipate major differences in pouch frequency between studies of handsewn and stapled pouches a difference in continence rates might be expected. In particular TSRP appears to avoid the troublesome complication of night leakage associated with the traditional operation. In the present series 75% of patients were totally continent by day and only 26% reported minor night leakage. In contrast, in a study of 389 patients who underwent handsewn pouch-anal anastomosis with

mucosectomy, Pemberton and colleagues reported nocturnal soiling rates of 56% and 44% in females and males respectively (Pemberton *et al*, 1987).

The leakage rates may be related to either the mucosectomy with excision of the anal transition zone or to prolonged anal dilatation. In a prospective pilot study comparing stapled and traditional techniques the Leeds group found that 92% of patients were completely continent after TSRP compared to only 58% in the handsewn group (Johnston *et al*, 1987). Moreover, whilst anal manometry showed normal anal canal pressures after TSRP, these were reduced in the handsewn group. Similar results have been reported in a retrospective comparative study from the Cleveland Clinic (Tuckson *et al*, 1991). Others have suggested that TSRP is at least comparable to the handsewn operation (Seow-Choen *et al*, 1994; Gozzetti *et al*, 1994) in terms of functional outcome. In an isolated but important prospective randomised trial of 32 patients from St Mark's Hospital, London (Seow-Choen *et al*, 1991), it was suggested that stapled pouches gave an inferior functional result to handsewn pouches. This study however, did not comment on the important complication of night leakage which makes patients miserable by irritating the perianal skin. Those authors also had considerable experience and skill in the handsewn technique of pouch-anal anastomosis.

In the present series the clinical anastomotic leak rate was comparatively low at 5%. This compares favourably with other series where the leakage rates range from 8-16% (Sugerman & Newsome, 1994; Seow-Choen *et al*, 1991; Luukkonen & Jarvinen, 1993) and may be attributed to the use of a defunctioning ileostomy in the majority of pouch patients. Indeed, in the present series pelvic sepsis occcurred predominantly in those patients who were not defunctioned. The use of a diverting ileostomy does however, have associated morbidity including dehydration, small bowel obstruction and risk of anastomotic leakage at closure.

These complications have been reported by others (Metcalf *et al*, 1985; Feinberg *et al*, 1987; Schoetz *et al*, 1986; Grobler *et al*, 1992) and occurred in our series (see Table 2). It is for this reason that the use of ileostomy has been abandoned or used only in selective cases by several groups (Mowschenson *et al*, 1993a-b; Sugerman & Newsome, 1994; Matikainen *et al*, 1990; Jarvinen & Luukkonen, 1991; Winslet *et al*, 1991; Sagar *et al*, 1992) . While acknowledging these arguments we continue to use routine loop ileostomy whenever technically possible, since pelvic sepsis is such a serious complication. Indeed, our experience with loop ileostomy for temporary faecal diversion after TSRP has, like others (Khoo *et al*, 1994), been favourable with only 6% of patients requiring surgery to correct ileostomy-related problems.

Other recognised technical complications of TSRP include the development of pouch-anal stenosis (Wexner *et al*, 1991; Seow-Choen *et al*, 1991) and pouch-vaginal fistula (Groom *et al*, 1993; Keighley & Grobler, 1993). In the present series the division of the upper anal canal was performed with a 30mm diameter stapling instrument confirming full mobilisation and resection of the rectum. If the 30mm instrument cannot be easily accommodated this indicates inadequate dissection with residual rectum and necessitates further mobilisation. In the present series there have been no pouch-vaginal fistulae.

The major late complication of restorative proctocolectomy is pouchitis which in the present series occurred in 13% of patients (5 with CD). This is comparable to other series (Keighley *et al*, 1993; Sugerman & Newsome, 1994; Gozzetti *et al*, 1994; Lohmuller *et al*, 1990; de Silva *et al*, 1991) where the incidence varies from 12-30%. Such variability may relate to differences in the definition of pouchitis. It has been suggested that pouchitis may be secondary to ischaemia (Levin *et al*, 1992) and since stapled pouch-anal anastomoses are under

less tension than handsewn anastomoses this may explain our relatively low figure for pouchitis. We also found that pouchitis may indicate occult CD (Keighley *et al*, 1993) with 5% of patients (38% of all pouchitis cases) having histologically confirmed CD. Review of the original colectomy specimens, even with the benefit of "hindsight" showed UC in 4 and indeterminate colitis in the fifth. This suggests that even if a staged operation had been performed, CD would not have been diagnosed. As such these data do not support the suggestion that patients should be treated by initial subtotal colectomy to avoid the risk of performing restorative proctocolectomy in the presence of occult CD (Keighley *et al*, 1993; Lucarotti *et al*, 1995). We do however, use a staged approach when the patient has other features suggestive, but not diagnostic of CD e.g. mouth ulcers or recurrent perianal sepsis. Given the inferior functional outcome after staged procedures (Zenilman *et al*, 1990) the use of on-table retrograde endoscopy of the small bowel may be a superior method of identifying occult CD unrecognised by standard preoperative investigations (Lescut *et al*, 1993).

Arguably the greatest putative risk of TSRP is the retention of a short anal canal mucosal cuff with the risks of colitis, dysplasia and carcinoma. In a study from the Cleveland Clinic, Florida the incidence of inflammation and dysplasia in retained mucosa after TSRP for UC was assessed (Schmitt *et al*, 1992). In 35% of patients the distal doughnut revealed UC at the time of pouch construction. At subsequent biopsy 43% of these patients had persistent UC. No dysplasia was identified in any of these biopsies. Only one patient with evidence of UC on "doughnut" and/or biopsy had symptoms attributable to active UC (2%). In another series there have been 3 reported cases of symptomatic colitis from the anal canal after TSRP (Curran & Hill, 1992) and this may prove to be the major long-term problem with the technique. Leaving a short anal canal cuff in patients with FAP is

perhaps more controversial. However, this appears to be an academic rather than practical argument since the risk of carcinoma in an easily surveyed 1cm cuff will be small when compared with the benefits of superior functional outcome and the high risk of premature death in these patients from tumours at other sites. There have been no reports to date of carcinoma arising in the retained cuff of mucosa after TSRP.

Although this is not a randomised comparison of operative techniques the results of the present study confirm that TSRP has simplified a difficult operation and gives good long-term functional results, with particular regard to night leakage. Future careful follow-up is required, however, to ensure that there are no long-term complications associated with the short cuff of diseased mucosa which this technique leaves behind.

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