CLINICAL AND EXPERIMENTAL STUDIES ON GASTROINTESTINAL ANASTOMOSES AND COLORECTAL CANCER

Ahmet Murat Akyol

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Western Infirmary, Glasgow

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P.O. Box 1346
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DECLARATION

I declare that the composition of this thesis is entirely my own work and that it has not been submitted previously for another degree. With the exception of a few of the references, which are acknowledged overleaf, all the books and papers cited were consulted by me personally.

The multi-centre trial described in the first section of the thesis took five years to complete and 13 consultant surgeons contributed patients into this study. During the first three years two successive Research Fellows were responsible for data collection and co-ordination. In the final two years of the study I took over the data collection and the co-ordination and supervision of the trial. It was my responsibility alone, with the exception of statistical assistance, to compile the data from the entire trial period and to perform the definitive analysis.

Other clinical studies presented in the following sections of the thesis and all the experimental work were designed and conducted by me personally, except for the processing of tissue samples for histopathology.

SUMMARY

The first section of this thesis discusses the role of stapling techniques in surgical practice. A prospective controlled clinical trial is described, where surgical stapling techniques were compared with conventional manual suturing techniques in the construction of gastrointestinal anastomoses. The following section deals with work on recurrence of colorectal cancer following surgical treatment. Two clinical studies are presented in this section, where "anastomotic techniques" and "anastomotic leaks" are examined respectively in relation to tumour recurrence. The final section of the thesis describes experimental studies in a rodent model, which were designed and conducted to investigate the association between anastomotic leaks and peri-anastomotic tumour growth.

Suturing or stapling in gastrointestinal surgery

Between April 1985 and April 1989 1,161 consecutive patients undergoing surgery under the care of 13 consultant surgeons throughout the West of Scotland and Highland regions were studied prospectively. All patients had operations that entailed the construction of a gastrointestinal anastomosis. If, at the time of surgery suturing and stapling techniques were considered equally appropriate, the method of anastomotic construction was determined by randomisation. Methods of data collection, bowel

preparation, antibiotic prophylaxis, anastomotic materials and anastomotic techniques were standardised by the study protocol. Four hundred and ninety six patients received sutured and 508 received stapled anastomoses. In the remaining 157 patients randomisation was considered inappropriate. All patients were followed until death or discharge from the hospital. The incidence of clinically evident anastomotic dehiscence was 3.3% in patients with sutured anatomoses, compared with 4.7% in the stapled group ($p < 0.22$). Sub-clinical (radiologically detected) leaks were encountered with a significantly higher frequency in the sutured group (14.4% versus 5.2%; $p < 0.05$). Surgical stapling also afforded significantly quicker anastomoses and operations (Mean anastomosis time $\pm$ SEM: 28.1 $\pm$ 0.7 versus 14.3 $\pm$ 0.5 minutes, $p< 0.001$; Mean operating time $\pm$ SEM: 115.5 $\pm$ 2.4 versus 103.9 $\pm$ 2.2 minutes, $p< 0.001$). With regard to other important outcome measures such as operative mortality, incidence of infective complications, recovery of gastrointestinal function and duration of hospital stay, suturing and stapling techniques produced comparable results. Further detailed analyses are presented, where the randomised and non-randomised patients were stratified according to anastomotic technique and various surgical categories. These data are hoped to provide guidance to surgeons in their selection of anastomotic technique.

Anastomotic techniques and recurrence of colorectal cancer

Recently some concern has been expressed in the literature regarding a potential adverse influence on the recurrence of rectal cancer associated with the use of stapling techniques. Prompted by these reports, the effect of anastomotic technique on the incidence of recurrence following potentially curative resections was studied in 294 patients. One hundred and forty two of these patients had their anastomoses randomised to suturing and 152 to stapling. By the end of the second post-operative year the incidence of tumour recurrence ($\pm$ SEM) was 29.4% (4.4%) in the sutured group, compared with 19.1% (3.9%) in the stapled group ($p< 0.05$). Cancer specific mortality was also significantly higher in patients with sutured anatomoses (22.3% $\pm$ 4.1% versus 10.9% $\pm$ 3.0% at 24 months, $p< 0.01$). Further analysis revealed that the influence of anastomotic technique on recurrence and mortality rates was independent of tumour stage and other co-variates.

These results suggest that in patients undergoing potentially curative resections for colorectal cancer, the use of stapling instruments for anastomotic construction may be associated with a significant reduction in recurrence and cancer specific mortality rates compared with conventional manual suturing techniques. Potential explanations for this previously unreported observation are discussed.

Anastomotic leaks and tumour recurrence

Long term clinical consequences of anastomotic leaks in patients undergoing surgery for malignant disease has not been studied before. This study was undertaken to investigate the influence of anastomotic leaks on the incidence of tumour recurrence following potentially curative resections for colorectal cancer. For the purposes of the study patients with both clinically evident and radiologically demonstrated leaks were considered together. One hundred and sixty seven patients were studied, who were assessed both clinically and radiologically for anastomotic integrity following potentially curative resections for left sided colonic or rectal cancer. Thirty two of these patients had a clinical and/or a radiological leak. In the remaining 135 patients there was no evidence of an anastomotic leak in the post-operative period. At the end of a mean follow-up period of 24.7 months (range 10-56 months) 15 of the 32 patients with leaks developed tumour recurrence (46.9%), compared with 25 of the 135 in the no leak group (18.5%, $p< 0.001$). The proportion of patients who had died as a result of cancer at 24 months post-operatively ($\pm$SEM) was 36.9% ($\pm$ 9.7%) in the "leak" group, compared with 12.6% ($\pm$ 3.3%) in the "no leak" group ($p< 0.001$). The association between anastomotic leaks and recurrence or mortality remained highly significant after adjusting for the influence of tumour stage in a multiple regression analysis ($p= 0.003$ and $p=0.001$ respectively). As a potential explanation for these results, it was postulated that an enhanced escape of

exfoliated intra-luminal tumour cells in the presence of an anastomotic leak could be responsible for the higher incidence of local recurrence observed. This hypothesis was tested in an experimental model, which constitutes the subject of the final section of the thesis.

Experimental studies

These studies were designed to investigate the relationship between the integrity of large bowel anastomoses and local tumour growth and to examine the mechanisms responsible for any observed effect of anastomotic leaks.

A model of sub-lethal anastomotic leaks in end-to-end descending colon anastomoses was developed in the Fischer F344 rat, which was validated by testing for air tightness intra-operatively and by contrast radiography post-operatively.

In the first set of experiments the growth pattern of tumours in groups of rats with and without leaks were examined, following per-operative intra-rectal instillation of 7.5×10^3 Mtln3 cells. Animals in the control group had the same procedure at the time of laparotomy without any anastomosis. Post-mortem examinations 21 days later revealed that in the absence of a bowel anastomosis intra-rectal instillation of tumour cells did not result in tumour growth in any animal. In contrast, when an

anastomosis was performed, a variable proportion of the animals developed peri-anastomotic and widespread intra-abdominal tumours. The incidence of tumour growth was dependent on the integrity of the anastomosis. Animals with anastomotic leaks had significantly higher incidence of tumours compared with those which had no leaks ($p<0.001$).

In another set of experiments the growth pattern of circulating tumour cells was investigated in the presence and absence of anastomotic leaks. 5×10^5 Mtln3 cells were injected into the left ventricle via the carotid artery three days after the construction of descending colo-colostomies (with and without leaks). The animals were sacrificed at day 21. In the control group, intracardiac injection of Mtln3 cells did not result in tumour growth in normal bowel in any animal. In contrast, the presence of an anastomosis almost always induced tumour growth locally, irrespective of whether or not there was a leak. The growth pattern of tumours in other tissues did not differ between the animals in the control group and those with anastomoses, again irrespective of anastomotic integrity.

SECTION I

SUTURING OR STAPLING IN GASTROINTESTINAL SURGERY

CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

1.1 INTRODUCTION

Since the earliest days of abdominal surgery, mechanical devices of various kinds have been used as aids in performing intestinal anastomoses. The main principle employed by these devices was to invert and compress bowel edges together which resulted in sloughing of the compressed margins while external to this the bowel healed and remained united [1]. The plates, clips or buttons made of various materials would come loose in the lumen and were passed with faeces. With the possible exception of the Murphy button [2], none of these devices gained widespread popularity and none bears any resemblance to modern surgical stapling instruments.

1.2 DEVELOPMENT OF STAPLING INSTRUMENTS

The development of surgical stapling instruments is commonly attributed to the Institute of Experimental Surgical Apparatus and Instruments in Moscow [3,4]. Although the surgical stapling instruments currently in use in the Western world are derivatives of the Soviet instruments developed in this Institute, the earliest example of a mechanical instrument that resembles a modern stapler was invented in 1908 by Hultl, a prominent surgeon in Budapest [5]. Named the Fischer-Hultl stapler after its manufacturer and inventor, this instrument consisted of a large crushing type forceps with a cog wheel, gear rod and a moving crankshaft to deliver the U-shaped steel wires. Two of the principles employed in the design of this

instrument prevail to date. One is the "B" shape closure of the staples and the second is the application of two double staggered rows of staples. Despite the instant success that the instrument met, it also had its shortcomings. It was expensive, cumbersome and heavy. Another young surgeon in Budapest, von Petz, developed a modification of the Fischer-Hultl stapler in 1921 which was lighter and more practical. The von Petz instrument soon replaced the Fischer-Hultl stapler in most institutions and remained in use until fairly recently [6]. Two of the new design features employed in the von Petz stapler, flat and heavy silver wire staples in a single file, have been abandoned in the construction of modern stapling instruments in favour of fine steel wire staples and double staggered rows.

1.2.1 THE SOVIET STAPLING INSTRUMENTS

The Fisher-Hultl and the von Petz staplers inspired several surgeons who developed slightly modified instruments operating on the same basic principles [1,7]. None of these however gained widespread use. A major step in the development of modern stapling instruments was the undertaking of a systematic programme at the Scientific Research Institute for Experimental Surgical Apparatus and Instruments (*NIIEChAI*) in Moscow [1,3]. In this institute, which opened in 1951, a range of ingenious surgical stapling instruments were developed in the 1950s. These may be divided into several groups:

i. Instruments effecting a linear closure (the prototype being named UKL stapler) which applied a double staggered row of staples in a number of different patterns intended for different sites. A modification of this basic linear stapler (UTL) worked in two steps and applied a second layer of staples inverting the first staple line.

ii. Instruments designed to construct side-to-side gastrointestinal anastomoses (NZhKA). The prototype of this series of instruments consisted of two limbs, one housed the staples and a knife and the other the anvils. The two halves of the instrument were inserted into the lumen of the loops of bowel to be anastomosed and were mated and locked. Pushing the knife through drove in the staples in two rows and divided the stapled tissue midway between the staple lines.

iii. Instruments creating circular, inverting, end-to-end anastomoses (PKS, SPTU, KS). In the construction of an anastomosis with these instruments, one end of the bowel was secured to the end of the cylindrical shaft of the instrument and the other bowel end to the detachable nose cone of the instrument by means of purse-string sutures. These two bowel ends were then brought together by turning a knob on the handle of the instrument. Squeezing the handle fired a circular row of staples and drove home a circular knife which cut through two purse-stringed ends of the bowel just inside the circular staple line, hence creating an inverted end-to-end anastomosis.

iv. In the same institute in Moscow, Russian surgeons have also developed a considerable variety of other stapling instruments to create vascular anastomoses, clip vessels in continuity, ligate major bronchial and pulmonary vascular trunks, staple ribs, sternum, fractured bones, affix corneal grafts, etc. [1].

The Soviet staplers designed for use in gastrointestinal surgery represent the first instruments creating true anastomoses as well as simple linear closures. They all operated on the same two-step action whereby the tissues are approximated first to the required degree and the staples are then driven home to join the tissues together. Unlike the Fischer-Hultl or the von Petz instruments, these stapling devices did not crush tissues and required no additional sutures to invert an everted staple line. An everted mucosa-to-mucosa anastomosis was however contradictory to the longstanding surgical dictum of serosa-to-serosa approximation and in the earlier days of their application everted staple lines were almost always oversewn [8].

1.2.2 AMERICAN STAPLING INSTRUMENTS

Despite the revolutionary innovation they brought to gastrointestinal and thoracic surgery, the Soviet stapling instruments had a number of drawbacks. They were manufactured individually by hand, so their parts were not interchangeable.

Most of the instruments necessitated the staples to be hand-loaded one by one. For those instruments that accepted a cartridge, a complicated disassembly was required to replace an expended cartridge. Each instrument accepted a single size of cartridge with a single arrangement of staples so that a large variety of these instruments needed to be kept in each operating theatre. Furthermore the multiple delicate moving parts in the design of the instruments created difficulties in cleaning and maintenance and rendered them susceptible to breakage.

The pioneers of surgical stapling in the United States were Mark Ravitch and Felicien Steichen. Both these surgeons were introduced to the Soviet instruments during their visits to Russia and gained further experience with their use experimentally and clinically in the United States[1,3,4,8]. In the mid 1960s, with technical advice and recommendations from Ravitch and Steichen, United States Surgical Corporation (Norwalk, Connecticut) manufactured the first American designed surgical stapling instruments. Initially these instruments were limited to TA and GIA series instruments which operated on the same principles as the Soviet UKL and the NZhKA staplers. They were however lighter, better balanced and were provided with presterilised, colour-coded disposable cartridges. The cartridge replacement was an easy procedure requiring no disassembly of the instrument and each instrument accepted cartridges with various patterns and sizes of staples. In

addition, all the fine moving parts, staple-driving fins and knife blades were incorporated into the disposable cartridge leaving the basic instrument as a simple, trouble-free compression device. The American design also reaffirmed the use of fine staple materials and double staggered rows of staples. In the early 1970s the equivalent of the Soviet SPTU circular stapling instrument was introduced by United States Surgical Corporation as the EEA stapler. There has since been further refinements in surgical stapling instruments with totally disposable models and an extended range of cartridges having different sizes and patterns of staples, however all stapling instruments currently in use in gastrointestinal surgery are fundamentally derivatives of the original TA, GIA and EEA series. United States Surgical Corporation remains a large supplier of surgical stapling instruments in North America, Western Europe and the Far East. At least one other large company manufacturing surgical suture materials, Ethicon Corporation, have since launched their own brand of visceral stapling intruments [9].

TA instruments

The TA instruments are used to effect everting linear closures by inserting two double staggered lines of staples 30, 55 or 90 mm long. There are two staple sizes 3.5 and 4.8 mm, accommodating for the different thicknesses of tissues being stapled. The TA30 in addition has a special cartridge loaded

with finer, closely spaced staples in two or three rows for stapling across large vessels in procedures like lobectomy, pneumonectomy, nephrectomy, etc. [10]. The TA instruments are suitable for closure of all parts of the gastrointestinal tract. One of their most important applications however is in the various techniques in which pulmonary parenchyma is sealed prior to incision or excision [11]; hence giving the instrument its name, the Thoraco Abdominal stapler. More recently absorbable staples have been developed using a lactide - glycolic copolymer. Cartridges of these absorbable staples fitted to a TA 55 instrument (Polysorb 55 stapler) allows application of stapling techniques in areas where stainless steel is contra-indicated, especially in gynaecological and urological procedures [12,13].

GIA Instruments

The GIA stapler inserts two 50 mm long double staggered rows of staples and during the same process divides the tissue between the staple lines. Originally the GIA instrument, modified from its Russian predecessor, was intended for the construction of anatomic side-to-side inverting anastomoses. The two limbs of the instrument are inserted through small openings into the lumen of the organs to be anastomosed. When the instrument is closed and fired it converts these two openings into one, as the anastomosis is constructed. This single opening can either be closed manually or by an application of the TA instrument. In

addition to performing side-to-side anastomoses, the GIA instrument is also used to transect and simultaneously close the ends of every portion of the gastrointestinal tract. A true anatomic end-to-end everted anastomosis in the small bowel or colon is also feasible with the linear stapling instruments [14,15]. Steichen however developed an easier technique to construct a functional end-to-end anastomosis with GIA and TA instruments [16]. Except for situations where the intraluminal circular stapling instruments are better suited, mainly anastomoses of the oesophagus and the rectum, the functional end-to-end anastomosis has become the fundamental technique for stapled intestinal reconstruction.

Further modifications of these basic linear stapling instruments have been developed which considerably facilitate certain surgical procedures like the construction of ileal reservoirs, jejunal pouches, gastroplasties for morbid obesity etc [17-20].

EEA Instruments

The EEA series staplers, being inspired by the Soviet SPTU instrument, are designed to create inverting, anatomic end-to-end circular anastomoses. Although the initial EEA gun, which was loaded with a disposable cartridge has been almost completely replaced by more refined, totally disposable models, they both operate on the same principle. The anvil at the end of the central rod of the instrument is detachable. The ends of

bowel to be anastomosed are secured to the anvil and to the end of the rod by means of purse-string sutures. The anvil is then attached to the central rod and tissues are apposed to the appropriate extent. Firing the gun from the handle joins the tissues by inserting two staggered circular rows of staples and just inside the staple lines a circular knife cuts the tissues completing the anastomosis. The EEA stapler can be used to perform end-to-end anastomoses in any part of the gastrointestinal tract. However its principal indications are in the construction of rectal anastomoses, particularly deep in the pelvis where manual suturing poses difficulties, and anastomoses of parts of the gastrointestinal tract with the oesophagus (21-23).

1.3 CLINICAL EVALUATION OF THE SURGICAL STAPLING INSTRUMENTS

1.3.1 Early studies

The pioneering work of Ravitch and Steichen in the 1960s and early 1970s stimulated a wave of enthusiasm in surgical stapling. The earlier studies in the literature were confined to reviews by a number of authors of their preliminary experience with stapling instruments. Between 1967 and 1971 Steichen and Ravitch operated on 218 patients using stapling instruments with 17 complications and one death (24). Latimer and his colleagues studied 104 patients with 112 stapled gastrointestinal operations. Four deaths and 18 complications were encountered in this series. Only five of these

complications were stapler related [25]. Fain and associates reported 165 low anterior resections carried out at the Moscow Proctological Institute using the SPTU instrument with 2.4% mortality and 3.6% anastomotic leak rate [26]. Lawson et al., reported three deaths and four stapler related complications in a series of 113 stapled gastrointestinal procedures [27]. Goligher reported four clinical and four radiological leaks in 62 stapled low anterior resections [28]. Heald in a similar series of 60 patients reported six clinical and three radiological leaks with the circular staplers [29]. Smith reported the results of a survey of the American Society of colon and Rectal Surgeons [30] where data from 243 surgeons using the EEA stapler on 3,594 operations was summarised. Postoperative anastomotic dehiscence was reported in 2.5% of these cases. In addition, on 352 occasions (9.8%) a defect in the anastomosis was noted and dealt with intra-operatively.

Despite the initial enthusiasm that the stapling instruments received, relatively few investigators have attempted to compare surgical stapling with conventional manual suturing techniques and of these comparative studies the majority have been retrospective.

The experience with stapling instruments in the Soviet Union was reported by Gritsman [31] from Moscow in 1966. In a large series consisting of 1,663 patients, the mortality after stapled

gastric resections was 1% for benign disease and 3.6% for gastric cancer. This was compared with the figures that Gritsman accumulated from the data in the literature on 42,528 gastrectomies for peptic ulcer and 10,358 resections for cancer (excluding total gastrectomies) where the mortality was 3.2% and 10.4% respectively. Chassin et al., in 1978 reported a study of 812 consecutive gastrointestinal procedures [32]. Two hundred and ninety six of these procedures were sutured and these were compared with 472 subsequent stapled procedures. The complication rates related to the anastomotic technique were 4.4% in the sutured and 4.1% in the stapled groups. Weil and Scherz [33] in 1981 reviewed 545 Billroth II gastrectomies in an attempt to compare the results of stapled and sutured cases. One hundred and eighty two patients had staples used for at least one anastomosis. There were no complications out of 71 stapled gastroenterostomies and four leaks occurred in 160 stapled duodenal stumps (2.5%). In the sutured group complication rates for duodenal stump closure and gastroenterostomies were 4.7% and 3.5% respectively. Lowdon and his colleagues [34] reviewed 481 upper gastrointestinal procedures on 310 patients. Two hundred of these procedures were stapled and 281 sutured. The cumulative incidence for a number of anastomosis related complications was 21% in the sutured group compared with 16% in the stapled group. The only significant difference between the two groups, in favour of the stapled group, was in the incidence of duodenal stump leaks.

Scher et al., [35] reported a similar comparative study in 242 consecutive patients undergoing colonic resections. There were 155 patients with sutured anastomoses and 87 with stapled anastomoses. A total of six clinical leaks were encountered, three in each group. They found no difference between the groups in terms of operating time, return of gastrointestinal function or duration of hospital stay.

1.3.2 Prospective Controlled Studies

The first prospective randomised comparison of suturing and stapling techniques appears to have been carried out in the Soviet Union, which apart from the work described in this thesis remains the largest such study to date [36]. This study was reported by Kabanov in 1973 where the Russian stapling instruments were compared with manual sutures in 826 patients undergoing gastric surgery for ulcer disease. There were 415 patients in the sutured group while 411 operations were performed with the stapling instruments. Bleeding occurred in 2.9% of the stapled patients and 1.9% of the sutured cases. Incidence of duodenal stump leakage was 0.6% in the stapled group compared with 2.2% in the sutured group. No leaks occurred in stapled gastroenterostomies and seven leaks (1.6%) in manual gastroenterostomies. The incidence of wound infection and subdiaphragmatic abscesses were 0.7% and 0.07% for the stapled cases compared with 1.9% and 1.9% for the sutured cases. Kabanov concluded that the use of stapling instruments

resulted in significantly lower post-operative complications and mortality compared to manual suturing in gastric surgery.

The first prospective controlled study of suturing and stapling techniques in the Western literature was reported by Reiling and his colleagues [37]. Patients with all gastrointestinal anastomoses were randomised into this study. After recruiting 100 patients however (50 sutured and 50 stapled), the study was terminated prematurely because all the authors felt that staplers were more time efficient. There were no post-operative leaks or haemorrhage. Three patients with stapled anastomoses had intra-operative disruption of the anastomosis, which were recognised and rectified. No differences were observed between the two groups in terms of operating time, recovery of gastrointestinal function or hospital stay.

A year later Beart and Kelly from the Mayo Clinic reported a prospective randomised evaluation of the EEA stapler for colorectal anastomoses [38]. Seventy patients were randomised, 35 into each of the sutured and stapled arms of the study. The only significant difference between the two groups in the study was a shorter anastomosis time for staplers. Three intra-operative complications were encountered in the stapled group which required defunctioning colostomies in two occasions and resulted in the loss of rectum in the third patient. Ten additional low colorectal anastomoses, which the authors thought could not have been sutured, were not randomised.

The following year Brennan et al., from Scarborough published a controlled trial of anastomotic techniques in all large bowel surgery [39]. They compared the Russian SPTU circular stapler with a single layered suturing technique in 103 patients. Three patients allocated to stapling had sutured anastomoses due to technical failures and were excluded from analysis. There were three clinical and two radiological leaks among 50 sutured cases, compared with five clinical and two radiological leaks in the stapled group. Suturing afforded statistically significant advantages in terms of wound infection rate and post-operative hospital stay.

McGinn and his colleagues from Southampton reported another prospective randomised study evaluating anastomotic techniques for low colorectal anastomoses [40]. Sixty patients underwent sutured anastomoses in this study whereas 58 were randomised to receive stapled anastomoses with the EEA or the ILS (ILS Proximate, Ethicon Ltd., Edinburgh) circular staplers. In the sutured group there were two clinical leaks and four radiological leaks with no mortality. In comparison, four of the attempted stapled anastomoses resulted in technical failures. There were seven clinical leaks, 14 radiological leaks and one death in the remainder of the stapled group. Apart from the differences in the leak rates, the two techniques were shown to be comparable in terms of operating time, incidence of wound infections and post-operative hospital stay. The

unusually high stapled leak rates in this study might be partially explained by the relative inexperience of the authors with the stapling instruments which is admitted in the paper.

A year later Everett and his co-workers [41] from Cambridge reported a prospective controlled comparison of stapling and suturing for left sided large bowel anastomoses. There were 50 patients in each arm of the study, however in six patients a stapled anastomosis could not be performed satisfactorily. The leakage rates were very similar between the two groups (two clinical leaks in each group; seven stapled and six sutured radiological leaks). Stapling afforded significantly quicker operations. No other significant differences were noted between the two groups.

Didolkar and his colleagues [42] from Baltimore compared sutures and staples in 88 patients with advanced cancer undergoing large or small bowel anastomoses. The only difference between the sutured and stapled groups was quicker anastomoses with staples.

More recently Seufert et al reported a prospective randomised trial comparing sutured and stapled oesophagojejunostomies following total gastrectomy in 80 patients [43]. Operating time and post-operative hospital stay were similar for both groups. Two patients, one in each group died and one stapled

oesophagojejunal anastomosis dehisced. In addition five patients (3 sutured and 2 stapled) had duodenal stump leaks, five developed pancreatic fistula (2 sutured and 3 stapled), three (1 sutured and 2 stapled) had intra-abdominal bleeding, five developed intra-abdominal abscesses (1 sutured and 4 stapled) and six (2 sutured and 4 stapled) relaparotomies were required.

In summary, the majority of the early reports in the literature suggest that stapling technology represents a feasible alternative to conventional suturing techniques in gastrointestinal surgery. However due to their uncontrolled, retrospective nature these studies do not provide an assessment of the relative merits of suturing and stapling. Prospective controlled studies are relatively few in number and they have focused on specific areas of gastrointestinal surgery. Furthermore, these studies have produced some conflicting results and the small sample sizes has limited their power to reach firm conclusions. Therefore, there seems to be a need for a prospective randomised trial to better define the potential role of surgical stapling in gastrointestinal surgery.

CHAPTER 2

PATIENTS AND METHODS

2.1 INTRODUCTION

The discussion in the preceding chapter highlighted the lack of rational guidance regarding the role of surgical stapling in gastrointestinal surgery. This study was undertaken to address this important gap in the scientific literature. The specific aims of the investigation were to compare surgical stapling techniques with conventional manual suturing techniques in the construction of emergency and elective gastrointestinal anastomoses with regard to immediate post-operative outcome. To fulfil these aims a multicentre, prospective, randomised clinical trial was conducted where patients undergoing surgery amenable to both stapling and suturing techniques were randomly allocated to one or the other group and were prospectively followed until discharge from hospital.

2.2 PARTICIPATING UNITS AND SURGEONS

Patient recruitment into the study commenced in April 1985, with the participation of seven consultant surgeons in three hospitals throughout the West of Scotland. The trial was co-ordinated centrally from the University Department of Surgery, Western Infirmary, Glasgow, where four of the seven participants were also based. The two other centres were the surgical units of two district general hospitals, one in Greenock and one in Ayrshire. During the course of the trial three other consultant surgeons from these hospitals, three from Raigmore Hospital in Inverness and one from the Royal Alexandra Hospital

in Paisley joined the study bringing the total number of contributors to 13. Patient recruitment was terminated in April 1989.

2.3 PATIENTS

Any patient under the care of the participating surgeons who was scheduled to undergo elective or emergency gastrointestinal surgery was considered for eligibility. The pre-requisite for randomisation was that the operation involved the construction of a gastrointestinal anastomosis which was suitable for either anastomotic technique. All patients were studied irrespective of whether the operation was performed by the responsible consultant or by one of his junior staff.

2.4 DOCUMENTATION OF DATA

All data were collected prospectively and recorded on standard patient information documents in a format suitable for computer storage and analysis. Methods of data collection and unambiguous definitions of all recorded peri-operative variables were agreed upon prior to the commencement of the trial. Participating units were visited frequently by the study co-ordinator to ensure uniformity of data collection and recording. Any inconsistencies were resolved in discussion with the individual investigators. Regular meetings for the participants during the course of the trial were also arranged, where any problems in the conduct of the study were discussed,

resolved and participants were updated about the overall progress. Anonymity of the patients was assured and information stored in the computer complied with the Data Protection Act.

To ascertain the accuracy of the recording of data and its transcription into computer, a 10% random sample of the study population was selected after the termination of patient recruitment and the principal variables recorded in these randomly selected documents were checked against the original hospital records.

2.5 STANDARDISATION

Anastomotic materials and techniques were standardised by the study protocol. Sutured anastomoses were constructed using 2/0 braided polyamide suture material (Nurolon, Ethicon Ltd., Edinburgh) in a single layer interrupted fashion. For gastric, small bowel and ileo-colic anastomoses an alternative two layered suturing technique was also allowed by the study protocol using full thickness continuous 2/0 polyglycolic acid (Dexon Plus, Davis and Geck, Gosport, Hampshire) for the inner layer and 2/0 polyamide for the outer sero-muscular layer. All stapled anastomoses were constructed using the TA, GIA and EEA series of Auto Suture stapling instruments (Auto Suture Company UK, Ascot). Anastomoses involving the oesophagus and the rectum were constructed in an inverted end-to-end fashion with the EEA circular stapling instrument [44,45].

Gastroenterostomies, biliary-enteric anastomoses and enteric by-pass procedures were done in a side-to-side fashion with the linear GIA and TA stapling instruments [21,46]. Continuity following resections of small bowel or intra-peritoneal large bowel was established by functional end-to-end anastomoses, again using the GIA and TA instruments [16].

The study protocol also dictated peri-operative antibiotic prophylaxis and pre-operative bowel preparation. Patients undergoing upper gastrointestinal procedures received a single dose of 1.5 g intravenous Cefuroxime with the induction of anaesthesia. For large bowel surgery the antibiotic prophylaxis consisted of 1 g of Cefotaxime and 500 mg of Metronidazole administered intravenously at the time of induction of anaesthesia and repeated twice at eight hourly intervals thereafter.

In elective large bowel surgery patients were restricted to a low residue liquid diet (Nutrauxil, Kabi Vitrum Ltd., Middlesex) for 72 hours prior to surgery. In the absence of an obstructing lesion they were also given 500 mls of 10% Mannitol solution orally on the afternoon of the day preceding surgery.

2.6 RANDOMISATION

Allocation of patients into one of the two arms of the trial was done by drawing a sealed envelope in the operating theatre. Each participating surgeon was assigned an equal number of envelopes indicating suturing or stapling. The randomisation procedure was further stratified to four surgical categories; namely oesophageal, upper gastrointestinal, colonic and colorectal. These arbitrarily chosen categories were defined as follows :

Oesophageal: Patients having any anastomosis involving the oesophagus.

Upper GI : Patients with anastomoses involving the stomach, small bowel or extra-hepatic biliary system but not the oesophagus or the large bowel.

Colonic : Patients with anastomoses involving all parts of the large bowel except the rectum.

Colorectal : Patients having any anastomosis involving the rectum below the peritoneal reflection.

Between these four surgical categories, an arbitrary order of "risk" was assigned starting from oesophageal anastomoses followed by colorectal, colonic and upper gastrointestinal anastomoses in descending order. For patients undergoing operations that entailed multiple anastomoses the randomisation envelope drawn was chosen from the 'highest risk' category and

all anastomoses were constructed in the fashion indicated by this single randomisation. For the purposes of the analysis such patients were considered in the group relating to the highest risk anastomosis. For instance a patient undergoing a small bowel resection and right hemicolectomy was considered in the colonic surgery group.

The randomisation took place at the time of surgery, only once the surgeon was satisfied that either anastomotic technique was feasible and equally appropriate. When one or other anastomotic technique was considered to confer a particular advantage on a patient, randomisation was not carried out and the electively chosen anastomotic technique was used. These non-randomised patients were also studied in an identical fashion but were considered as a separate group.

2.7 PRE-OPERATIVE DATA

As detailed in Table 2.1 and Table 2.2, data recorded pre-operatively consisted of simple anthropometric parameters, results of routine haematological and biochemical investigations and some nutritional indices.

PRE-OPERATIVE VARIABLES

Anthropometric Data

Age

Sex

Height

Weight

Recent weight loss

Quantity and duration of weight loss

TABLE 2.1

PRE-OPERATIVE VARIABLES

Haematological and Biochemical Data

Haemoglobin (Hb)

Mean corpuscular volume (MCV)

White blood cell count (WBC)

Serum albumin

Serum transferrin

Leucocyte ascorbic acid (LAA)

TABLE 2.2

2.8 INTRA-OPERATIVE DATA

The information recorded on each patient at the time of surgery is summarised in Table 2.3.

INTRA-OPERATIVE DATA

Grade of surgical and anaesthetic staff
Nature of surgery (emergency or elective)
Anastomosis time
Operating time
Surgical complications
Anaesthetic complications
Abdominal drains
Use of Neostigmine

TABLE 2.3

For patients with colorectal anastomoses the distance of the anastomosis from the anal margin was documented as estimated by the operator at the time of surgery and the use of a defunctioning stoma was recorded.

Anastomosis time was defined as the time taken from the end of dissection until a complete anastomosis had been achieved. For patients with multiple anastomoses, the time taken to complete each anastomosis was recorded separately. Operating time was defined as the time taken from the commencement of the skin incision until the completion of the skin closure.

Eleven anastomotic sites throughout the gastrointestinal tract, specified in the patient information document are outlined in Table 2.4.

SITE OF ANASTOMOSES IN THE GI TRACT
Oesophageal
Gastric/gastroduodenal
Gastrojejunal
Pyloroplasty
Duodenal stump closure
Biliary enteric
Entero enteric
Ileocolic
Colo colic
Colorectal
Colostomy closure

TABLE 2.4

2.9 POST-OPERATIVE DATA

The variables recorded during the post-operative period are summarised in Table 2.5.

POST-OPERATIVE DATA
Anastomotic integrity
Recovery of gastrointestinal function
Morbidity
Blood transfusion
Tumour stage and grade
Day of discharge or death

TABLE 2.5

One of the key outcome measures of the study was anastomotic integrity. A clinical leak was defined as anastomotic dehiscence confirmed by re-operation or post-mortem, appearance of bowel contents from drains, development of an entero-cutaneous fistula or development of systemic sepsis associated with peritonitis. For easily accessible anastomoses the assessment of anastomotic integrity also included contrast radiography. A water soluble radiological contrast medium (Gastrografin; Schering, FRG) was used for this purpose. Contrast swallows or enemas were carried out between the 4th and 14th post-operative days. A radiological leak was defined as any extravasation of the contrast medium in the region of the anastomosis in the absence of any of the criteria for a clinical leak.

For patients undergoing upper gastrointestinal procedures an estimation of the return of gastrointestinal function was made by recording the first day that the patients' oral intake exceeded 1000 mls. In large bowel surgery the corresponding assessment was made by recording the first day that the patient passed flatus or stool.

Operative mortality was defined as those deaths occurring within 30 days of the surgical procedure [47].

Infective complications that developed during the post-operative period were recorded and numerically graded by means of a sepsis score system modified from Elebute and Stoner [48]. Other information recorded post-operatively included the presence of malignant disease, patients' duration of hospital stay and blood transfusion requirements.

2.10 STATISTICAL METHODS

Transcription of data from the standard patient record forms to computer was performed centrally in Glasgow. An ICL mainframe computer based at the University of Glasgow was used with the BMDP software package for data storage and analysis [49]. Statistical comparisons were made by the "Chi^2 test" for non-continuous variables and the "student's t test" for continuous variables. In the assessment of the predictive value of contrast radiography, Fisher's exact test was used for the comparison of the results in the sutured and stapled groups. Clinical leak rates and mortality were analysed using logistic regression to investigate the effects of technique (suturing versus stapling) and category (oesophageal, upper gastrointestinal, colonic, colorectal). Anastomosis and operating times were analysed using two way analysis of variance to investigate the effects of technique and category as above. Confidence intervals were obtained for the leak and mortality rates in the stapled groups relative to the corresponding rates in the sutured groups using the technique described by Morris and Gardner [50].

CHAPTER 3

RESULTS

3.1 INTRODUCTION

Between April 1985 and April 1989 1,161 patients were studied. In 157 of these patients (13.6%) randomisation was considered inappropriate. The results presented relate to those patients whose anastomoses were randomised to suturing or stapling. The non-randomised group of patients are considered separately at the end of this chapter.

3.2 DISTRIBUTION OF PATIENTS

There were 1,004 patients who had their anastomoses randomised to either suturing (n= 496) or stapling (n= 508) at the time of surgery.

The distribution of the sutured and stapled cases in each of the four surgical categories is illustrated in Table 3.1.

DISTRIBUTION OF RANDOMISED PATIENTS

SURGICAL CATEGORY	PATIENTS		
	Sutures	Staples	Total
Oesophageal	25	27	52
Upper gastrointestinal	150	150	300
Colonic	208	220	428
Colorectal	113	111	224
ALL PATIENTS	**496**	**508**	**1004**

TABLE 3.1

There were six patients among the 508 in the stapled group of the study who had their anastomoses sutured for a variety of reasons (3 instrument/technical failures, 2 unsatisfactory anastomoses requiring reconstruction and 1 randomisation error). None of these patients developed anastomotic complications. In accordance with "intention to treat" principle these patients were considered with the stapled group for the purposes of the analysis.

Table 3.2 shows the distribution of the anastomoses stratified into 11 anastomotic sites.

LOCATION OF ANASTOMOSES WITHIN THE GASTROINTESTINAL TRACT

ANASTOMOTIC SITE	NUMBER OF ANASTOMOSES		
	Sutures	Staples	Total
Oesophageal	25	27	52
Gastric/Gastroduodenal	43	42	85
Gastrojejunal	85	106	191
Pyloroplasty	3	4	7
Duodenal stump closure	35	53	88
Biliary-enteric	30	22	52
Entero-enteric	95	90	185
Ileocolic	128	138	266
Colocolic	71	73	144
Colorectal	113	111	224
Colostomy closure	19	15	34
ALL ANASTOMOSES	647	681	1328

TABLE 3.2

Mean number of anastomoses per patient was 1.3 in both the sutured and the stapled groups.

3.3 PATIENT CHARACTERISTICS

The distribution of the recorded pre-operative variables between the sutured and stapled groups is outlined in Table 3.3 and Table 3.4.

PATIENT CHARACTERISTICS
Anthropometric Data

	SUTURES n= 496	STAPLES n= 508
Age (mean ± SD)	63.7 ± 15.8	65.3 ± 14.5
Sex (male/female)	231/265	238/270
Height (cms) (mean ± SD)	164.6 ± 10.2	164.8 ± 9.4
Weight (kg) (mean ± SD)	60.9 ± 13.8	62.7 ± 13.8
> 10% weight loss	22.0%	22.6%
Malignant disease	66.5%	66.0%

TABLE 3.3

PATIENT CHARACTERISTICS
Haematological and Biochemical Data
(Mean ± SD)

	SUTURES n= 496	STAPLES n= 508
Haemoglobin (g/dl)	12.9 ± 2.0	12.9 ± 2.1
WBC (x 10^9/l)	8.9 ± 3.6	8.9 ± 3.6
MCV (fl)	87.2 ± 7.4	87.3 ± 6.8
Albumin (g/l)	37.8 ± 5.5	37.5 ± 5.5
Transferrin (g/l)	2.6 ± 0.7	2.6 ± 0.8
LAA (fmol/l)	1.3 ± 0.8	1.2 ± 0.9

TABLE 3.4

3.3.1 Grade of surgical staff

The grade of the operator for the surgical procedures in the two groups is outlined in Table 3.5.

GRADE OF SURGEON

OPERATOR	SUTURES n= 496	STAPLES n= 508
Consultant	267	301
Senior Registrar	77	79
Registrar	133	125
SHO	19	3

TABLE 3.5

A consultant was present, either as the operator or supervising junior staff, in 401 of the 496 operations in the sutured group (81%). The corresponding ratio was 420 out of 508 for the stapled cases (83%).

3.3.2 Nature of surgery

Similarly there was no major difference between the two groups in the number of patients undergoing emergency or elective operations (Table 3.6).

NATURE OF SURGERY

	SURGERY	
	Emergency	Elective
SUTURED		
Oesophageal	0	25
Upper GI	26	124
Colonic	29	179
Colorectal	5	108
TOTAL	60	436
STAPLED		
Oesophageal	0	27
Upper GI	24	126
Colonic	39	181
Colorectal	3	108
TOTAL	66	442

TABLE 3.6

3.4 ANASTOMOTIC INTEGRITY

Forty out of the 1,004 randomised patients developed clinically evident anastomotic leaks (4.0%). Sixteen of these leaks were encountered in patients with sutured anastomoses (3.3%), compared with 24 in the stapled group (4.7%). This difference is not statistically significant (x^2= 1.4, 1 d.f., p= 0.22). Nevertheless, the leak rate observed in the stapled group was 1.46 times that observed in the sutured group (95% confidence intervals: 0.79 - 2.72). Figure 3.1 illustrates the distribution of the sutured and stapled clinical leaks between the four surgical categories. Leak rates were higher for patients with stapled anastomoses in three of these four categories, with the exception of the "colonic" category. Multiple regression analysis revealed no significant differential effect of the anastomotic technique on leak rates between the four surgical categories (x^2 for interaction= 5.88, 3 d.f., p= 0.12). In Figure 3.2, the ratio of stapled to sutured leaks in each of the four surgical categories and their corresponding confidence intervals are plotted against a logarithmically scaled axis.

In the upper gastrointestinal surgery group there were two clinically evident leaks among 150 patients with sutured anastomoses, whereas eight of the 150 patients with stapled anastomoses developed clinical leaks. This discrepancy was largely due to the difference between the results of sutured and

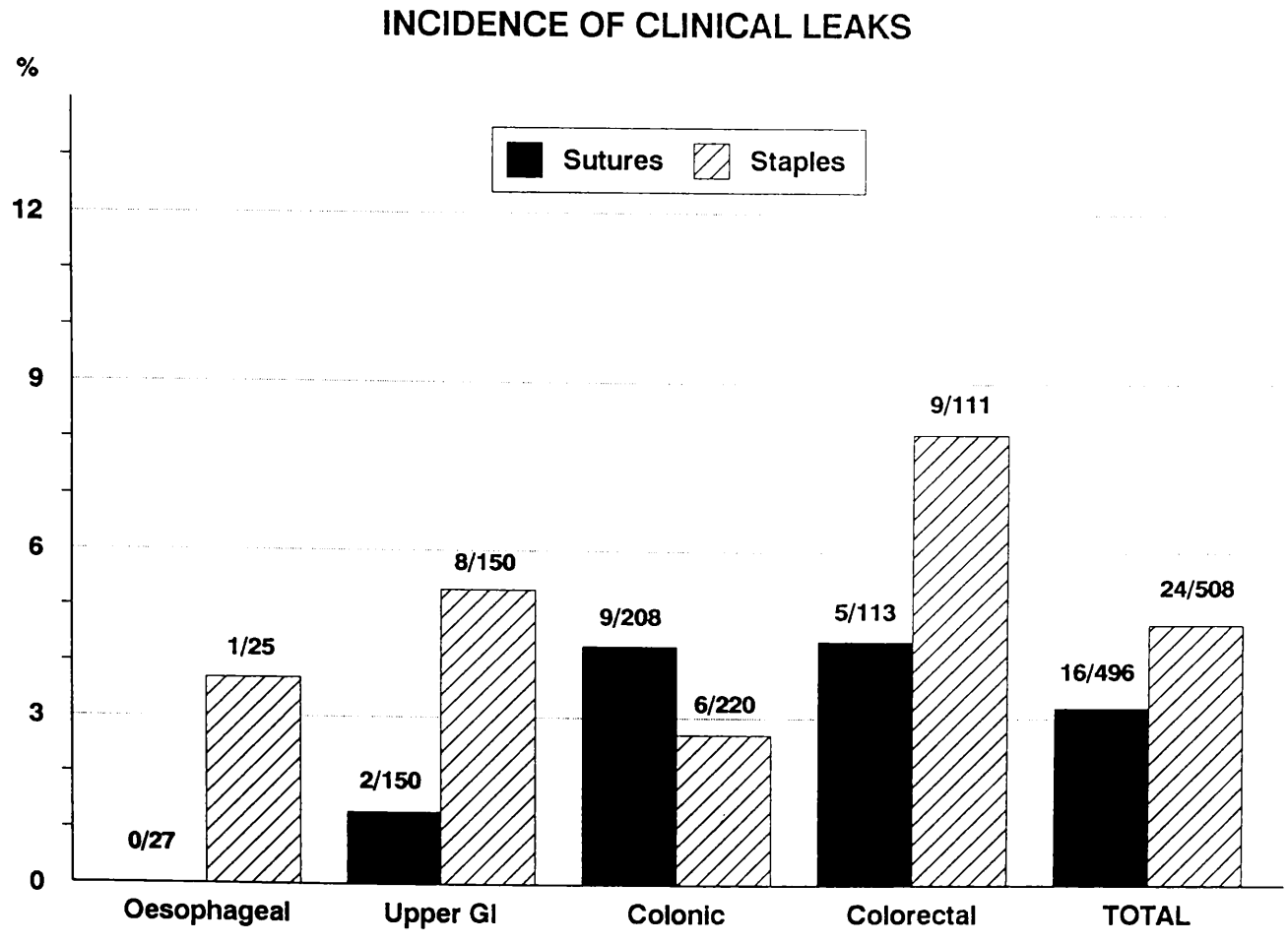

INCIDENCE OF CLINICAL LEAKS
%
Sutures
Staples
12
9
6
3
0
0/27
1/25
2/150
8/150
9/208
6/220
5/113
9/111
16/496
24/508
Oesophageal
Upper GI
Colonic
Colorectal
TOTAL

Figure 3.1

RATIO OF STAPLED/SUTURED LEAKS

& 95 % CONFIDENCE INTERVALS

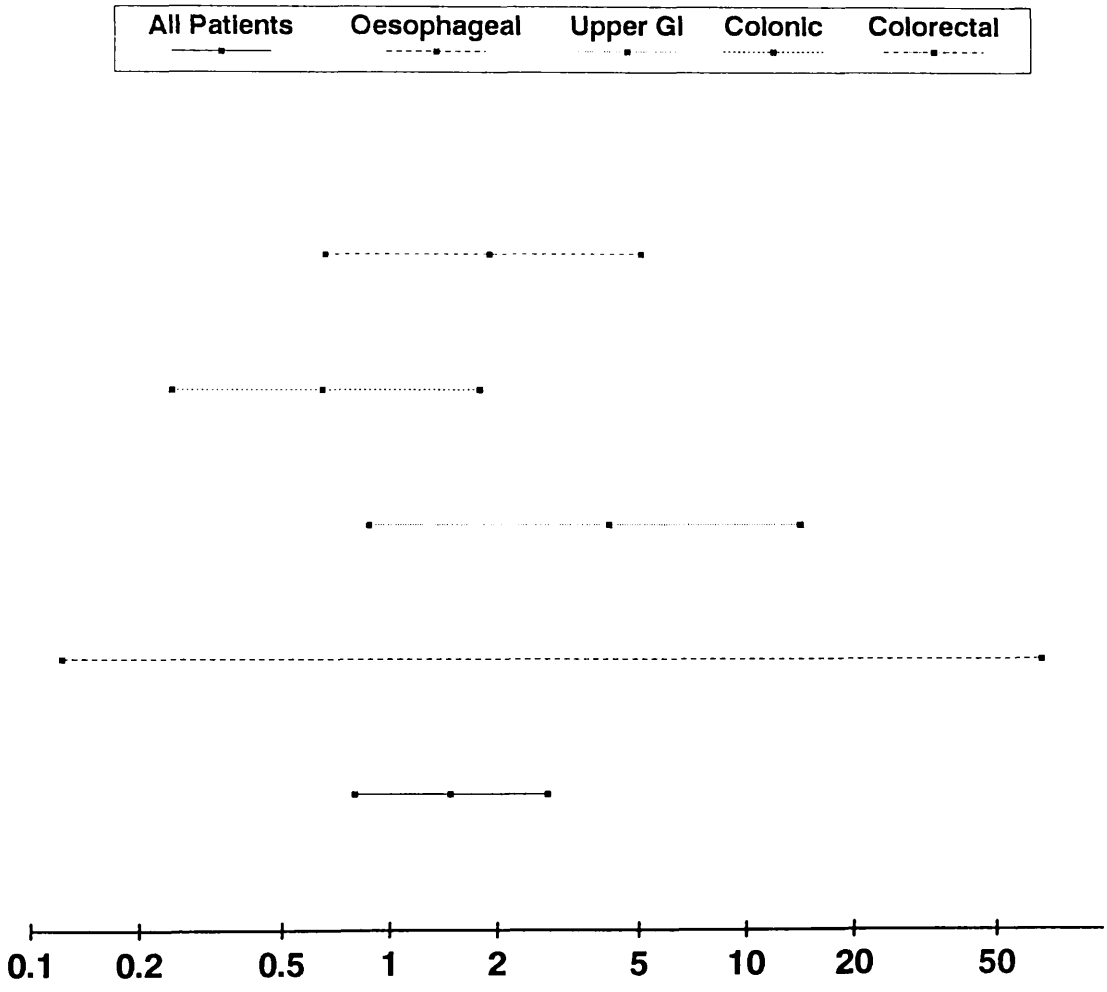


Figure 3.2

stapled duodenal stump closures. No anastomotic dehiscence occurred following gastro-enterostomies, proximal gastric closures of gastrectomies, pyloroplasties or biliary-enteric anastomoses. Two patients with small bowel anastomoses, one in each group, developed clinical leaks. The remainder of the leaks in the upper gastrointestinal surgery group occurred from duodenal stumps. Seven of the 53 stapled duodenal stumps leaked, compared with one of the 35 sutured stumps.

3.4.1 Nature of surgery and leaks

The incidence of anastomotic disruption was not higher in emergency surgery (Table 3.7). Only three clinical leaks occurred in emergency operations, one in the sutured and two in the stapled group.

CLINICAL LEAKS IN ELECTIVE AND EMERGENCY OPERATIONS

SURGERY	INCIDENCE OF CLINICAL LEAKS	
	Sutures	Staples
Emergency (n= 126)	1.7%	3.0%
Elective (n= 878)	3.4%	5.0%

TABLE 3.7

3.4.2 Grade of surgeon and leaks

There was no association between the grade of surgeon and the incidence of anastomotic dehiscence. In the sutured group the presence or absence of a consultant at the time of surgery did not make any difference to the leak rates (3.3%). In the stapled group the incidence of anastomotic leakage was 4.5% when a consultant was present and 5.7% when they were absent.

3.4.3 Temporal Distribution of Leaks

Eight hundred and twenty nine (83%) of the randomised patients in the study were managed by seven of the participants, while the remaining six consultants contributed 175 patients (17%) between them. The period that each participant spent in the trial was divided into two equal halves and the anastomotic leak rate in the first half of the study was compared with that in the second half (Table 3.8). A similar analysis was repeated after excluding those participants who contributed a relatively small number of cases. Data in Table 3.8 shows that in both analyses more leaks were seen to have occurred in the second half of the study and the difference was more marked for the stapled group.

TEMPORAL DISTRIBUTION OF CLINICAL LEAKS

	NUMBER OF LEAKS DURING THE STUDY			
	FIRST HALF		SECOND HALF	
	Sutures	Staples	Sutures	Staples
All participants	7	8	9	16
7 larger contributors	6	7	9	14

TABLE 3.8

The implications of these results in terms of a learning curve effect with surgical stapling instruments are discussed in Chapter 4.

3.5 RADIOLOGICAL LEAKS

All patients with oesophageal anastomoses and 186 of those with colorectal anastomoses were subjected to post-operative contrast radiography.

Gastrografin swallows revealed only one radiological leak, which was detected in a patient with a sutured oesophageal anastomosis.

For patients with rectal anastomoses, the incidence of radiologically detected leaks was 14.4% (13 out of 90) in the sutured group compared with 5.2% (5 out of 96) in the stapled group ($x^2 = 5.32$, 1 d.f., $p < 0.05$).

Patients who developed clinically evident anastomotic disruption after having a leak demonstrated on contrast radiography were considered as clinical leaks and were not included in the above analysis. In other words patients with radiological leaks suffered no morbidity and they all had an uneventful post-operative recovery.

3.5.1 PREDICTION OF EARLY POST-OPERATIVE MORBIDITY BY CONTRAST RADIOGRAPHY

A separate analysis was undertaken to investigate the value of post-operative contrast radiography in predicting clinical leaks. Because of small patient numbers and only one clinical leak in the oesophageal group, it was not feasible to meaningfully assess the results of Gastrografin swallows. The investigation was therefore limited to the study of Gastrografin enemas in large bowel surgery.

Two hundred and thirty three patients were studied, all of whom had Gastrografin enemas after a colorectal or a left sided colonic anastomosis. Forty of these patients had a leak demonstrated by contrast radiography (Table 3.9). Only 12 of these 40 patients however developed a clinical leak. Furthermore, the total number of patients with a clinically evident leak was 23. The radiological investigation however was only able to predict 12 of these.

CLINICAL AND RADIOLOGICAL LEAKS

		CLINICAL LEAK		
		YES	NO	TOTAL
RADIOLOGICAL LEAK	YES	12	28	40
	NO	11	182	193
	TOTAL	23	210	233

TABLE 3.9

Table 3.10 outlines further characteristics of contrast radiography with respect to the prediction of a clinical leak.

PREDICTION OF POST-OPERATIVE MORBIDITY BY CONTRAST RADIOGRAPHY

(n = 233)

Specificity	:	86.6%
Sensitivity	:	52.2%
Accuracy	:	83.3%
Predictive value for a negative result	:	94.3%
Predictive value for a positive result	:	30.0%

TABLE 3.10

Three patients in the study group developed signs of sepsis and a clinically evident leak within 12 hours of the radiological investigation, which may have been caused by the procedure.

There was no mortality associated with these three incidents. No other complications were encountered.

The value of post-operative contrast radiography was also assessed differentially for sutured and stapled anastomoses (Table 3.11). The specificity of the radiological investigation was significantly higher in the stapled group. However, this occurred at the expense of sensitivity and the predictive value for a positive result remained poor at 40% .

VALUE OF CONTRAST RADIOGRAPHY DIFFERENTIALLY FOR SUTURED AND STAPLED ANASTOMOSES

	ANASTOMOSES		
	SUTURES	STAPLES	"p"
Specificity	81%	92%	0.02
Sensitivity	60%	46%	0.68
Accuracy	79%	87%	0.11
Positive predictive value	24%	40%	0.31
Negative predictive value	95%	92%	0.76

TABLE 3.11

The clinical implications of these results are discussed in Chapter 4.

3.6 ANASTOMOSIS TIMES

Tables 3.12 to 3.22 below show the anastomosis times in the sutured and stapled groups for each anastomotic site. There were only seven patients with pyloroplasties and no attempt was made to compare the anastomosis time for sutures and staples in this group. For the remaining ten anastomotic sites the group sizes, mean anastomosis times, standard deviations, difference of means and 95% confidence intervals for the latter difference are given in each table.

ANASTOMOSIS TIME

OESOPHAGEAL ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 25)	Staples (n= 27)
Mean anastomosis time (minutes)	43.5	27.0
Standard deviation	12.6	9.9

Mean time saving with staplers: 16.5 minutes
95% confidence interval : 10.2 - 22.8

TABLE 3.12

ANASTOMOSIS TIME

GASTRIC CLOSURE OR GASTRODUODENAL ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 43)	Staples (n= 42)
Mean anastomosis time (minutes)	14.1	3.8
Standard deviation	7.0	4.1

Mean time saving with staplers: 10.3 minutes
95% confidence interval : 7.8 - 10.8

TABLE 3.13

ANASTOMOSIS TIME

GASTROJEJUNOSTOMIES

	Anastomotic Technique	
	Sutures (n= 85)	Staples (n= 106)
Mean anastomosis time (minutes)	21.5	9.1
Standard deviation	9.6	5.6

Mean time saving with staplers: 12.4 minutes
95% confidence interval : 10.0 - 14.8

TABLE 3.14

ANASTOMOSIS TIME

PYLOROPLASTIES

	Anastomotic Technique	
	Sutures (n= 3)	Staples (n= 4)
Mean anastomosis time (minutes)	7.3	7.3
"Range"	3.0 - 14.0	4.0 - 10.0

TABLE 3.15

ANASTOMOSIS TIME

DUODENAL STUMP CLOSURES

	Anastomotic Technique	
	Sutures (n= 35)	Staples (n= 53)
Mean anastomosis time (minutes)	11.1	2.4
Standard deviation	11.1	2.3

Mean time saving with staplers: 8.7 minutes
95% confidence interval : 5.3 - 12.2

TABLE 3.16

ANASTOMOSIS TIME

BILIARY-ENTERIC ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 30)	Staples (n= 22)
Mean anastomosis time (minutes)	17.5	8.9
Standard deviation	7.7	4.9

Mean time saving with staplers: 8.6 minutes
95% confidence interval : 5.1 - 12.1

TABLE 3.17

ANASTOMOSIS TIME

SMALL BOWEL ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 95)	Staples (n= 90)
Mean anastomosis time (minutes)	16.3	8.0
Standard deviation	12.0	5.9

Mean time saving with staplers: 8.3 minutes
95% confidence interval : 5.5 - 11.1

TABLE 3.18

ANASTOMOSIS TIME

ILEOCOLIC ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 128)	Staples (n= 138)
Mean anastomosis times (minutes)	22.0	8.7
Standard deviation	11.4	5.1

Mean time saving with staplers: 13.3 minutes
95% confidence interval : 11.1 - 15.5

TABLE 3.19

ANASTOMOSIS TIMES

COLOCOLIC ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 71)	Staples (n= 73)
Mean anastomosis times (minutes)	19.5	9.0
Standard deviation	8.8	6.3

Mean time saving with staplers: 10.5 minutes
95% confidence interval : 7.9 - 13.1

TABLE 3.20

ANASTOMOSIS TIMES

COLORECTAL ANASTOMOSES

	Anastomotic Technique	
	Sutures (n= 113)	Staples (n= 111)
Mean anastomosis times (minutes)	31.1	21.3
Standard deviation	14.1	12.4

Mean time saving with staplers: 9.8 minutes
95% confidence interval : 6.3 - 13.3

TABLE 3.21

ANASTOMOSIS TIMES

COLOSTOMY CLOSURES

	Anastomotic Technique	
	Sutures (n= 19)	Staples (n= 15)
Mean anastomosis times (minutes)	13.9	7.3
Standard deviation	6.7	5.7

Mean time saving with staplers: 6.6 minutes
95% confidence interval : 2.4 - 10.8

TABLE 3.22

Data in Tables 3.12 to 3.22 demonstrate that stapling techniques enabled quicker anastomoses than suturing at all anastomotic sites. The mean time saving with the staplers varied between 6.6 to 16.5 minutes per anastomosis. To compare the anastomosis times on a "per patient" basis, the times taken to construct each individual anastomosis were added to obtain "total anastomosis times" (in patients with multiple anastomoses). Figure 3.3 illustrates the "total anastomosis times" for the patients in the sutured and stapled groups. Use of stapling instruments was associated with an overall mean reduction of 13.8 minutes per patient in total anastomosis time compared with manual suturing (95% confidence intervals: 12.1 - 15.5 minutes, $p< 0.001$). Two way analysis of variance showed that the mean time difference in the construction of anastomoses, although consistently in favour of the stapled group, varies significantly between the four surgical categories ($p= 0.01$). The largest mean saving was observed for oesophageal anastomoses (24 minutes), with broadly similar savings in time in the other groups (13, 14 and 11 minutes in the upper gastrointestinal, colonic and colorectal groups respectively).

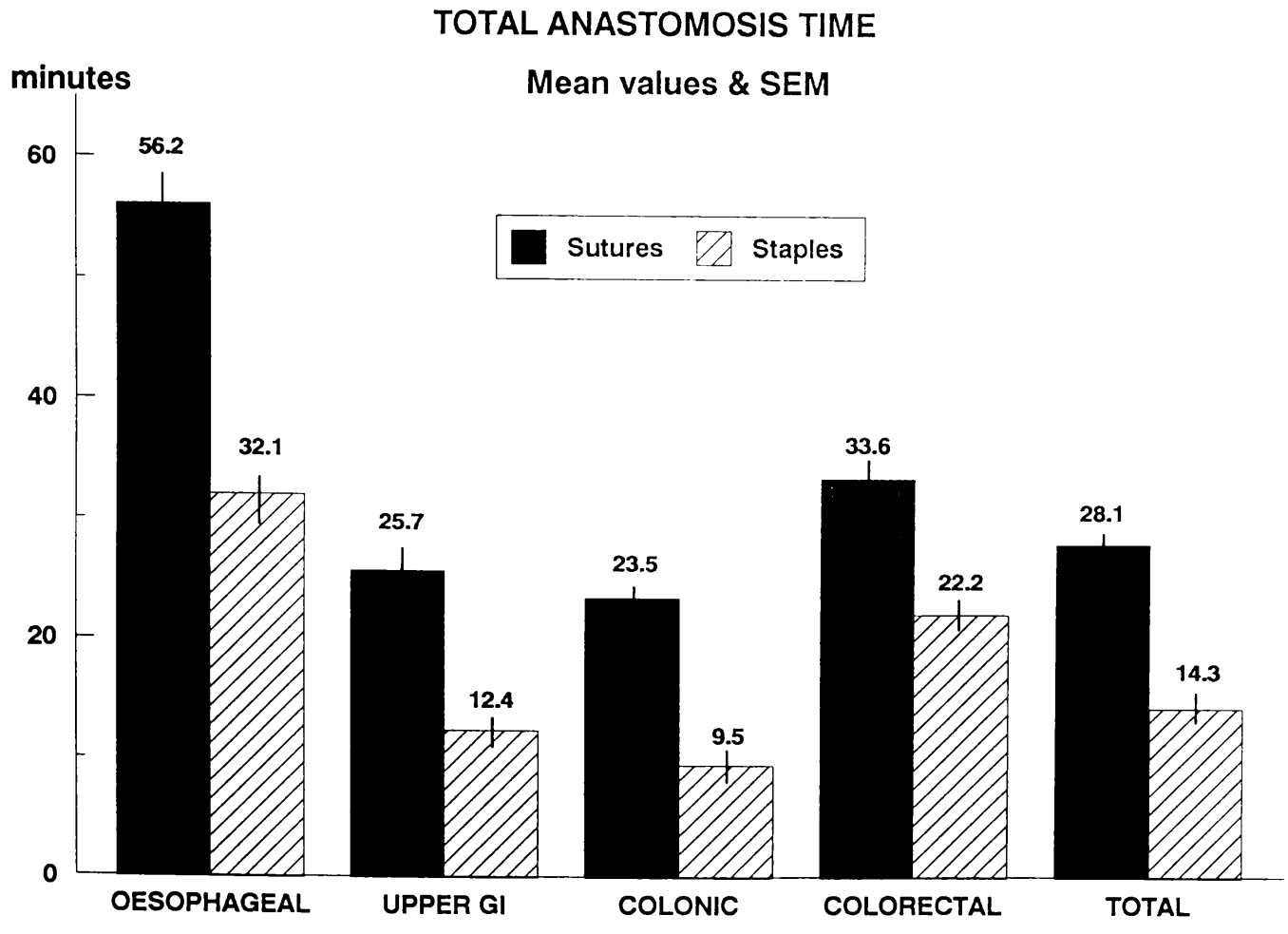

TOTAL ANASTOMOSIS TIME
Mean values & SEM
minutes
60
40
20
0
Sutures
Staples
56.2
32.1
25.7
12.4
23.5
9.5
33.6
22.2
28.1
14.3
OESOPHAGEAL
UPPER GI
COLONIC
COLORECTAL
TOTAL

Figure 3.3

3.7 OPERATING TIMES

The influence of the anastomotic technique on the length of time taken to complete an operation is summarised in Figure 3.4 . The overall mean operating time in the stapled group was significantly shorter than that in the sutured group (mean values $\pm$ SEM in minutes: 115.5 $\pm$ 2.4 versus 103.9 $\pm$ 2.2, $p< 0.001$). The mean time saving with staplers is thus 11.6 minutes (95% confidence interval: 5.2 - 18.0 minutes). Despite the significant overall difference between the sutured and stapled groups, greater variability in terms of operating times is reflected in the wide confidence interval. The two way analysis of variance revealed that the interaction between the anastomotic technique and surgical category in terms of the operating time was not significant ($p= 0.27$).

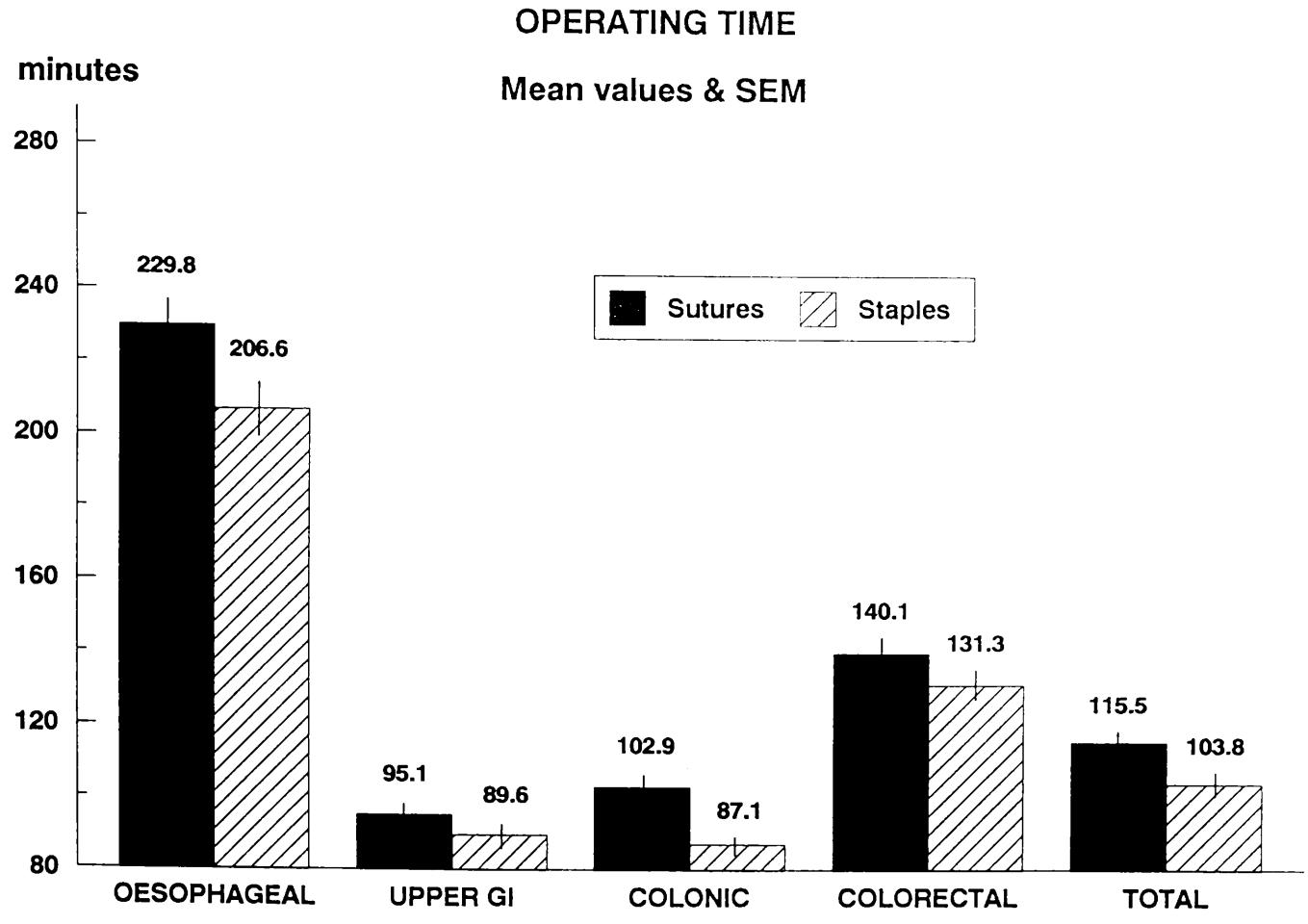

OPERATING TIME
Mean values & SEM
minutes
280
240
200
160
120
80
Sutures
Staples
229.8
206.6
95.1
89.6
102.9
87.1
140.1
131.3
115.5
103.8
OESOPHAGEAL
UPPER GI
COLONIC
COLORECTAL
TOTAL

Figure 3.4

3.8 INTRA-OPERATIVE COMPLICATIONS

In the stapled group 40 patients (7.9%) suffered complications related to anastomotic construction. On 29 occasions stapled anastomoses required additional sutures. Four of these were colorectal anastomoses where one or both "donuts" were incomplete and the anastomosis had to be reinforced with sutures. A similar reinforcement was required on ten side-to-side or functional end-to-end anastomoses where defects were noticed on the TA closure line. Intra-operative bleeding from a staple line necessitated underrunning with sutures on 23 stapled anastomoses (14 upper gastrointestinal and 9 lower gastrointestinal). Furthermore five patients, all of whom had undergone stapled upper gastrointestinal procedures required reoperation due to significant haemorrhage from the anastomosis. In one oesophageal and two colorectal anastomoses the EEA instrument or its sizer split the bowel. Other complications in the stapled group included two occasions where the stapling instruments misfired, one case where a kinked anastomosis had to be re-fashioned, one inadvertent stapling of a nasogastric tube and one case of small bowel perforation during the insertion of a GIA instrument.

In contrast to the stapled group, there were only three anastomosis related complications in the sutured group (0.8%). In one case the mesenteric and anti-mesenteric corners of an end-to-end ileocolic anastomosis were noted to have been

misorientated which required reconstruction. Another sutured anastomosis had to be re-fashioned due to ischaemia and one patient required reoperation due to a mesenteric haematoma causing external compression at the level of the anastomosis.

3.9 RECOVERY OF GASTROINTESTINAL FUNCTION

For upper gastrointestinal procedures, the proportion of sutured patients who tolerated an oral intake of greater than 1000 mls by the end of the fourth post-operative day was 53% (93 out of 175). The corresponding ratio for patients with stapled anastomoses was 51% (91 out of 177; $x^2 = 0.11$, 1 d.f., $p = 0.74$).

For large bowel surgery the assessment of the return of gastrointestinal function was based on recording the day that the patients passed flatus or stools post-operatively. By the end of the fourth post-operative day 75% of the patients had fulfilled this criterion in both the sutured and the stapled groups (241 out of the 321 sutured patients and 247 out of the 331 stapled patients; $x^2 = 0.02$, 1 d.f., $p = 0.89$).

3.10 MORTALITY

Fifty five of the 1,004 randomised patients in the study died within 30 days of surgery, giving an overall operative mortality rate of 5.5%. Twenty six of these patients were in the sutured group and 37 were in the stapled group. The difference between

the two groups with respect to operative mortality was not statistically significant (x^2= 1.43, 1 d.f., p= 0.23). Figure 3.5 shows the operative mortality rates for the sutured and stapled groups in each of the four surgical categories. The ratio of the mortality rate in the stapled group to that in the sutured group was 1.36 (95% confidence interval 0.81 to 2.29). Logistic regression analysis revealed no evidence of a differential effect of the anastomotic technique on mortality between the four surgical categories (x^2 for interaction= 2.71, 3 d.f., p= 0.44).

3.10.1 Anastomotic leaks and operative mortality

Of the 1,004 randomised patients 652 underwent large bowel surgery. In this group there were 29 clinical leaks and 28 deaths. Eight of the deaths occurred as a result of anastomotic dehiscence. The overall operative mortality in large bowel surgery therefore was 4.3% (28 out of 652) whereas mortality in the event of a leak from a large bowel anastomosis was 27.6%.

The remaining 352 patients in the study underwent upper gastrointestinal surgery. In this group there were 11 patients who developed a clinical leak and 27 who died. Five of the deaths were due to anastomotic dehiscence (operative mortality: 7.7%, mortality of anastomotic dehiscence: 45.4%).

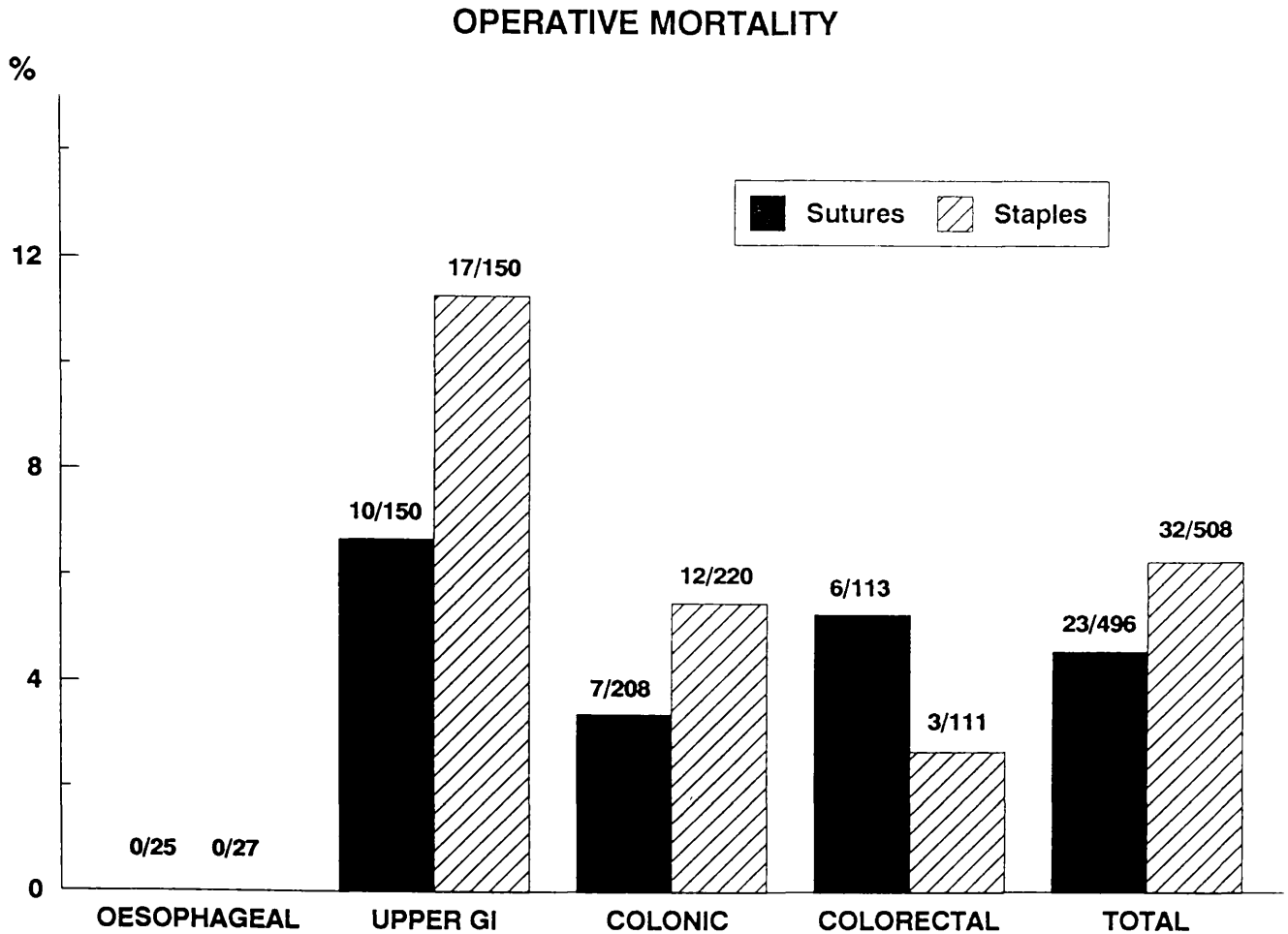

OPERATIVE MORTALITY
%
12
8
4
0
Sutures
Staples
17/150
10/150
12/220
7/208
6/113
3/111
32/508
23/496
0/25
0/27
OESOPHAGEAL
UPPER GI
COLONIC
COLORECTAL
TOTAL

Figure 3.5

3.11 INFECTIVE COMPLICATIONS

Wound infections were recorded with similar frequencies in the sutured and stapled groups (10.5% versus 11.8% respectively; x^2= 0.45, 1 d.f., p= 0.50). Their distribution in different surgical categories is illustrated in Table 3.23.

INCIDENCE OF WOUND INFECTION

SURGICAL CATEGORY	ANASTOMOTIC TECHNIQUE	WOUND INFECTION	(%)
Oesophageal	Sutures (n = 25)	0	(0.0)
	Staples (n = 2)	0	(0.0)
Upper GI	Sutures (n = 150)	17	(11.3)
	Staples (n = 150)	15	(10.0)
Colonic	Sutures (n = 208)	19	(9.1)
	Staples (n = 220)	29	(13.2)
Colorectal	Sutures (n = 113)	16	(14.2)
	Staples (n = 111)	16	(14.4)
TOTAL	Sutures (n = 496)	52	(10.5)
	Staples (n = 508)	60	(11.8)

TABLE 3.23

Similarly, there was no significant difference between the sutured and stapled groups when the mean sepsis scores were compared (Table 3.24).

SEPSIS SCORES

ANASTOMOTIC TECHNIQUE	SEPSIS SCORE (Mean ± SEM)
Sutures (n = 496)	3.98 ± 0.15
Staples (n = 508)	4.30 ± 0.18
x^2= 1.86, 1 d.f., p= 0.17	

TABLE 3.24

3.12 BLOOD TRANSFUSION

During their hospital admission for surgery, 130 patients in the sutured group (26%) received blood transfusions compared with 156 patients (31%) in the stapled group. The indications for blood transfusion were not recorded. In those patients who were transfused, the median number of units of blood given was three for both groups. Twenty-two patients in the sutured group and 24 patients in the stapled group received more than four units of blood.

3.13 ABDOMINAL DRAINS

Abdominal drains were used in 81%, 42% and 31% of the patients with colorectal, colonic and upper gastrointestinal anastomoses respectively. There were no major differences between the sutured and stapled groups in this respect (overall incidence: 48% for the sutured and 45% for the stapled group).

3.14 DEFUNCTIONING STOMAS

Temporary defunctioning stomas were performed in 35 of the 224 patients with colorectal anastomoses. In the sutured group 20 patients (17.7%) had defunctioning stomas, compared with 15 (13.5%) in the stapled group (x^2= 0.74, 1 d.f., p= 0.39).

3.15 NEOSTIGMINE

Three hundred and eighty eight patients (78%) in the sutured group had Neostigmine administered for the reversal of anaesthesia compared with the 385 patients (76%) in the stapled group (x^2= 0.84, 1 d.f., p= 0.36).

3.16 LEVEL OF COLORECTAL ANASTOMOSES

The height of the colorectal anastomoses from the anal margin were recorded as estimated by the operator at the time of surgery. The mean height ($\pm$ SD) was 10.7 $\pm$ 3.8 cm for patients with sutured anastomoses and 9.7 $\pm$ 4.1 cm for those with stapled anastomoses (x^2= 2.59, 1 d.f., p= 0.1).

Table 3.25 illustrates the distribution of the sutured and stapled colorectal anastomoses in three arbitrarily defined levels in the pelvis.

LEVEL OF COLORECTAL ANASTOMOSES

DISTANCE FROM ANAL MARGIN	ANASTOMOTIC TECHNIQUE Sutures (n= 92)	Staples (n= 94)
0 - 5 cm	9	18
5 - 10 cm	38	40
> 10 cm	45	36
x^2= 4.03, 2 d.f., p= 0.13		

TABLE 3.25

3.17 DURATION OF HOSPITAL STAY

Patients in the sutured and stapled groups were similar with respect to their day of discharge from the hospital. The mean hospital stay (± SD) in the sutured group was 13.6 ± 8.2 days compared with 14.1 ± 10.2 days in the stapled group (p= 0.4). 95 % confidence intervals for the difference between the group means in this respect are -0.7 to 1.72 days.

3.18 NON-RANDOMISED PATIENTS

Randomisation was considered inappropriate in 157 cases. The distribution of non-randomised patients in the surgical categories is demonstrated in Table 3.26.

DISTRIBUTION OF NON-RANDOMISED CASES

SURGICAL CATEGORY	ANASTOMOTIC TECHNIQUE	NUMBER OF PATIENTS	TOTAL
Oesophageal	Sutures	8	20
	Staples	7	
	Combined	5	
Upper GI	Sutures	15	57
	Staples	25	
	Combined	17	
Colonic	Sutures	18	30
	Staples	10	
	Combined	2	
Colorectal	Sutures	20	50
	Staples	27	
	Combined	3	
TOTAL	**Sutures**	**61**	**157**
	Staples	**69**	
	Combined	**27**	

TABLE 3.26

Twenty-eight percent of the oesophageal anastomoses in the study were considered unsuitable for randomisation. In contrast, ileocolic and colocolic anastomoses were randomised on 93.4% of the occasions. When an anastomosis was not randomised, manual suturing and surgical stapling techniques were used with similar frequencies (Table 3.26).

In terms of their pre-operative baseline characteristics the patients in the non-randomised group did not differ significantly from those in the randomised category (Table 3.27 and Table 3.28).

PATIENT CHARACTERISTICS

Anthropometric Data

	NON-RANDOMISED PATIENTS (n= 157)
Age (mean ± SD)	61.4 ± 16.6
Sex (male/female)	92/65
Height (cm) (mean ± SD)	167.3 ± 9.1
Weight (kg) (mean ± SD)	64.0 ± 15.2
>10% weight loss	33 (21.0%)
Malignant disease (%)	92 (58.6%)
Emergency surgery (%)	16 (10.2%)

TABLE 3.27

PATIENT CHARACTERISTICS

Haematological and Biochemical Data
(Mean Values ± SD)

	NON-RANDOMISED PATIENTS (n= 157)
Haemoglobin (g/dl)	13.2 ± 2.4
WBC (x 10^9/l)	9.4 ± 4.9
MCV (fl)	89.5 ± 6.9
Albumin (g/l)	38.3 ± 7.3
Transferrin (g/l)	2.7 ± 0.8
LAA (fmol/l)	1.5 ± 1.2

TABLE 3.28

The mean number of anastomoses per patient in the non-randomised group was 1.6. Table 3.29 shows the total number of anastomoses and their distribution according to anastomotic sites.

DISTRIBUTION OF NON-RANDOMISED ANASTOMOSES

ANASTOMOTIC SITE	NUMBER OF ANASTOMOSES
Oesophageal	20
Gastric/Gastroduodenal	18
Gastrojejunal	33
Pyloroplasty	5
Duodenal stump closure	13
Biliary enteric	18
Entero enteric	60
Ileocolic	19
Colocolic	9
Colorectal	50
Colostomy closure	4
TOTAL	**249**

TABLE 3.29

The total anastomosis time and the operating time for the non-randomised group (mean values & SEM) were 24.3 $\pm$ 1.4 minutes and 135.4 $\pm$ 5.0 minutes respectively. Fifteen patients developed clinically evident anastomotic dehiscence (9.6%) and 12 patients died within 30 days of surgery (7.6%).

CHAPTER 4

DISCUSSION

4.1 INTRODUCTION

Attitudes towards surgical stapling within the surgical community vary widely. Published data regarding the relative frequency with which stapling instruments are being used by general surgeons is hard to find. However a review of large patient series suggests that suturing remains the most commonly employed anastomotic technique [51-53].

At the time of their introduction, stapling instruments were presented as possessing certain benefits over manual suturing such as regular and accurate placement of the staples, reduced tissue manipulation and anastomotic oedema, quicker anastomoses and operations, quicker anastomotic healing, earlier return of gastrointestinal function and reduced post-operative hospital stay. These alleged benefits remain unsubstantiated by scientific data. Although many surgeons have adopted surgical stapling, at least in selected areas of application, those who maintain a more sceptical or conservative stance are possibly deterred by considerations such as cost, unproven benefits or potential complications and adverse effects. The participants of this study represented varying shades of this spectrum of opinions. They were united however in their concern about the lack of clear guidance in the literature and their willingness to rectify this.

4.2 STUDY DESIGN

The aim of the project was to assess the relative merits of surgical stapling and manual suturing techniques in gastrointestinal surgery. It was clear from the outset that the question had to be addressed by a prospective randomised clinical trial. The study sought to compare the two anastomotic techniques in relation to immediate post-operative outcome. Anastomotic integrity is one of the fundamental performance indicators in this comparison. As summarised in Table 4.1, the reported incidence of clinically evident anastomotic leaks in gastrointestinal surgery varies between 2-20%, more typically around 6-8%. It was calculated that to detect a difference between the true leak rates of 5% and 10%, at a 5% significance level with adequate statistical power (between 80-90%) around 1,000 patients would be required. This compelled us to design a multi-centre trial in order to achieve the necessary sample size within a reasonable time span.

In theory the process of randomisation distributes any extraneous variables equally so that the two groups in a randomised trial can be compared for the one variable that is under investigation and which constitutes the only deliberate difference in the study design. However in patients with gastrointestinal surgery certain variables other than the anastomotic technique - such as bowel preparation [54-57] and perioperative antibiotics [58-60] - have a well recognised

influence on the outcome. Despite randomisation these extraneous variables were standardised as much as possible in this study.

It is also well recognised that even in a given set of circumstances (standard bowel preparation, antibiotic prophylaxis, anastomotic materials and techniques) the outcome of a surgical procedure varies considerably from surgeon to surgeon [61]. When the surgeon related variability is large and the degree of variability applied to different techniques is inconsistent then the efficacy of a technique varies in an unpredictable way according to the operating surgeon. Thus any given outcome in a randomised clinical trial may not be a true reflection of the merits of the procedure under investigation, but may be, at least partly, a consequence of the variation between the participants. The phenomenon of surgeon-related variability was observed in this study as well. Few participants with small contributions had no clinical leaks, whereas for those who had larger number of patients the leak rates ranged between 1.5% to 11.3%. The randomisation method used was a deliberate attempt to ensure that each participant contributed equal numbers of sutured and stapled cases. This provision certainly limits the bias resulting from surgeon related variability. The complete elimination of this problem however is dependent on each individual participant having

consistent (ie equally good or equally bad) results with both suturing and stapling techniques, which is difficult to ascertain in clinical trials with multiple participants.

4.3 PARTICIPANTS AND PATIENTS

None of the participating surgical units in this trial were specialist institutions for gastrointestinal surgery and some of the consultants taking part in the study had their areas of expertise outwith gastrointestinal surgery, such as breast disease, urology, vascular surgery and transplantation surgery. All junior staff attached to the participating consultants contributed to the project and no attempt was made to alter the policies of the participating units with respect to the members of staff normally carrying out the operations. Thus operations were usually but not exclusively performed or supervised by consultant surgeons. The participating consultants had a variable degree of experience with surgical stapling prior to the commencement of the study. With the exception of one however, they were not regular stapler users. Therefore, there was little selectivity with regard to participating centres, surgeons and patients. In this regard we feel that this study is representative of the average surgical practice in the United Kingdom.

4.4 AUDIT OF DATA RECORDING

Controlled prospective randomised trials are undoubtedly one of the more valuable ways of acquiring knowledge. Despite their remarkable discriminative power however, they have a number of pitfalls which have to be taken into account in the design and the conduct of a study. The longer a trial continues the less easy it is to keep the background conditions steady, junior staff come and go and the protocol is subtly altered by unconscious drift [62]. Multicentre entry of patients helps accumulate data faster and somewhat alleviates these problems. However it also introduces a "noise" which may have a considerable impact on the findings [63]. To assess the accuracy of data collection in our trial we chose to re-examine the patient information documents of a randomly selected sample of patients after the completion of patient recruitment. The comparison of the data recorded in these documents with the original hospital records revealed few errors. In terms of key study variables, the anastomotic site had been entered incorrectly or incompletely on four occasions and the gender for one patient had been marked incorrectly. The results of this quality control exercise suggested that major inaccuracies in data collection that could have influenced the conclusions of the study have not occurred.

4.5 ANASTOMOTIC INTEGRITY

There were 40 patients in the study who developed anastomotic dehiscence. Although a 4% overall anastomotic leak rate in gastrointestinal surgery appears to compare favourably with the results of most published series, it has to be emphasised that direct comparisons are often misleading. For instance, Fielding et al., reported a prospectively studied series of 1,466 patients, where the incidence of clinically evident anastomotic dehiscence was 13% [64]. However, the study population in this report is quite different to ours. Patients in our trial had benign or malignant disease throughout the entire gastrointestinal tract, while Fielding et al., only studied patients with large bowel cancer. Also the protocol for the trial reported herein imposes an element of selectivity for the randomised cases, in that only anastomoses suitable for both suturing and stapling were considered. A further factor that may have favourably influenced the leak rates in our trial is the "audit-effect". This phenomenon refers to an improvement in the observed results when a surgical management strategy is being assessed in the context of a trial, especially when there is information feedback to the participating surgeons during the course of the trial [65].

Matheson and his associates from Aberdeen have consistently reported good results with a single layered suturing technique [66-69]. These results have often been quoted as the

gold standard for anastomotic integrity [70,71]. Between 1969 and 1974, Matheson and Irving carried out 205 elective gastrointestinal anastomoses with only one clinical leak (0.5%) [67]. During the same period three of their 20 emergency anastomoses dehisced (15%) [66,67]. In a follow-up to this, Matheson published a review of his experience in 116 elective and 20 emergency large bowel anastomoses between 1975 and 1979 [68]. Clinical leak rates were 5.3% for elective and 30% for emergency anastomoses. More recently Matheson et al., published results on 204 elective large bowel operations where only three clinically evident anastomotic leaks occurred (1.5%) [69]. It must be emphasised that these commendable results, unlike the study reported herein, are achieved by a single experienced surgeon with a specialist interest in this field of surgery practising a well established surgical technique.

Table 4.1 summarises 15 clinical studies reporting anastomotic leak rates in large bowel surgery. There is clearly a wide variation in the specific aims, methodology and patient populations between these studies and the majority refer to sutured anastomoses only. Nevertheless these results suggest that the anastomotic leak rate observed in our study is comparable to the available data in the literature.

ANASTOMOTIC LEAK RATES IN LARGE BOWEL SURGERY

Authors	No. of Patients	Clinical leaks	(%)
Everett, 1975 [72]	92 *	19	(20.6%)
Goligher et al., 1977 [73]	135 *	9	(6.7%)
Jonsell & Edelmann, 1978 [74]	165	14	(8.5%)
Fielding et al, 1980 [64]	1466	191	(13.0%)
Matheson et al, 1981 [68]	137	11	(8.0%)
Heald & Leicester, 1981 [75]	100 *	13	(13.0%)
Beart & Kelly, 1981 [38]	80 *	3	(3.8%)
Shorthouse et al, 1982 [76]	112 *	14	(12.5%)
Leff et al., 1982 [77]	106	9	(8.4%)
Brennan et al., 1982 [39]	100	12	(12.0%)
Kennedy et al, 1983 [78]	265	8	(3.0%)
McGinn et al, 1985 [40]	118 *	9	(7.6%)
Matheson et al, 1985 [69]	204	3	(1.5%)
Everett et al, 1986 [41]	94	2	(2.1%)
Canivet et al, 1989 [79]	373	35	(9.4%)

(*) = *Rectal anastomoses only.*

TABLE 4.1

4.5.1 SUTURED VERSUS STAPLED LEAKS

Any new anastomotic technique, before gaining universal acceptance, ought to be compared with the established norms of anastomotic construction. Arguably the most important criterion in this comparison is the incidence of anastomotic dehiscence. Such a comparison however is confounded by difficulties on at least two accounts. Firstly, the occurrence of anastomotic dehiscence is influenced by a number of other diverse factors, hence the investigation of any one variable requires a well controlled prospective trial. Secondly, anastomotic dehiscence is a relatively uncommon occurrence, therefore demonstrating any significant difference in its incidence as a result of a particular management policy requires a large number of patients. This fact is well illustrated in all of the previously published prospective randomised comparisons of suturing and stapling techniques [37-43] where the sample sizes were small and a "Type II" error [80] cannot be ruled out.

In this trial the total study population was determined by prior estimations of statistical power. Yet we demonstrated no statistically significant difference between surgical stapling and manual suturing techniques in terms of anastomotic security. It has to be emphasised however that the confidence intervals are consistent with anything from a 20% improvement to a 2.7 fold deterioration in the incidence of clinical leaks with the use of stapling instruments.

With regard to anastomotic disruption, perhaps the most striking difference between the sutured and stapled groups was the relative incidences of duodenal stump leakage. In an attempt to provide an explanation for the high incidence of leakage from stapled duodenal stump closures, hospital records of the seven patients with dehisced stapled stumps were reviewed. One of these patients had bled significantly from the gastrojejunostomy and required an emergency re-operation to achieve haemostasis. Another patient developed severe intra-abdominal sepsis following a stapled gastrectomy which was found to be due to a perforation in the transverse colon (of unexplained aetiology). In a third case electro-cautery was used on the stapled duodenal stump at the time of the operation to control bleeding, a practice which is not recommended by the manufacturers of stapling instruments. Whether any of these factors were important in the aetiology of the dehiscence of stapled duodenal stumps remains uncertain. Our results in this respect are at variance with the previously published reports. Lowdon et al., [34] reported eight leaks in 66 sutured duodenal stumps and one leak in 54 stapled stumps in a retrospective comparison. Weil and Scherz [33] reported 545 Billroth II gastrectomies where the incidence of duodenal stump leakage was 4.7% with sutures and 2.5% with staplers. Kabanov compared suturing and stapling techniques in a prospective randomised trial where 826 patients undergoing gastrectomies were studied [36].

Duodenal stump failure occurred in 0.6% of the stapled and 2.2% of the sutured closures. The explanation for the discordance between these results and our observations is not clear. However duodenal stump closures constitute a small proportion of our total study population and the observed difference between sutures and staples in this small sub-group is compatible with a chance effect.

Stapling techniques also resulted in a slightly, but not significantly, higher incidence of clinical leaks in patients with colorectal anastomoses. Fifteen of the 111 stapled colorectal anastomoses were defunctioned by a temporary proximal stoma whereas this ratio was 20 out of 113 for sutured colorectal anastomoses. It is accepted that a proximal stoma does not prevent anastomotic dehiscence. It may however alter the clinical consequences should anastomotic disruption occur. Hence it is conceivable that as a result of the strict definition of clinical leak employed in this study, the discrepancy between the use of defunctioning stomas in sutured and stapled groups may have influenced the observed frequency of clinical leaks from colorectal anastomoses.

4.5.2 RADIOLOGICAL LEAKS

Radiological assessment of an anastomosis may have a clinical value in the assessment of anastomotic stenosis or recurrence, or in the management of clinically evident anastomotic

dehiscence. It also provides useful clinical information for objective evaluation and comparison of new surgical techniques and for purposes of quality control and surgical audits. On the other hand demonstration of a sub-clinical leak in the early post-operative period will rarely alter a patient's management. The safety of such investigations has also been questioned. Matheson et al., abandoned radiological assessment of large bowel anastomoses in 1976 based on their concern about potential risks [68]. Haynes et al., in a review of their experience with 117 early post-operative contrast enemas for left sided colonic resections found a high incidence of serious complications including one death [81]. In our experience the investigation appeared safe. There were only three incidences where the radiological procedure may have contributed to the development of a clinical leak. We encountered no other complications associated with the technique. This is in agreement with the findings of Shorthouse et al., who reviewed 135 consecutive contrast enemas in St Mark's hospital and concluded that the investigation was safe [76].

In our experience the incidence of radiological leaks was significantly higher in sutured colorectal anastomoses compared to stapled ones. Although a difference between sutured and stapled anastomoses in this respect has not been reported before, this result is in agreement with the findings

of Goligher, who reported a very high radiological leak rate in his sutured rectal anastomoses (29%) [28,73]. McGinn et al., however found the reverse to be true. The radiological leak rates from their series were 24% for staples and 6.6% for sutures [40]. Both Brennan et al., [39] and Everett et al., [41] also included radiological assessment in their comparative studies of suturing and stapling. The radiological leak rates were very similar for both anastomotic techniques in these two studies.

In a separate analysis investigating the value of contrast radiography in the prediction of early post-operative morbidity, we found that radiography had disappointingly low sensitivity and specificity. The high predictive value for a negative result was only a reflection of the paucity of clinically evident leaks. For instance, if the incidence of clinical leak is 5%, any investigation attempting to predict this would have a negative predictive value of at least 95%. On the other hand the likelihood of developing a clinical leak when the enema had demonstrated a leak (predictive value for a positive result) was only 30% in our experience. These results suggest that apart from surgical audits or clinical trials assessing new anastomotic techniques, the routine use of contrast radiography in large bowel surgery to assess anastomotic integrity may not be justified. Nevertheless, Chapter 7 discusses further long-term implications of

sub-clinical anastomotic leaks in patients with colorectal cancer. The usefulness of contrast radiography therefore will be reconsidered in the light of the data presented in Chapter 7.

4.6 LEARNING CURVE EFFECT

As with any new technique, it is reasonable to assume that with the use of stapling instruments there might be a learning curve effect. This may have influenced the results achieved by staplers in our study. McGinn and his colleagues, in their prospective comparison of the two anastomotic techniques state that the participants had not had extensive experience with the stapling instruments at the commencement of their trial and they observed an improvement in the results of stapling in the second half of the study [71]. We also analysed our results to see if there was any evidence of a learning curve effect in our trial. Due to the different times that participants joined the trial, the four year study period was not divided into two equal halves. Instead we divided the time period that each individual spent in the trial into two equal halves. The data presented in Table 3.8 shows that in the latter half the incidence of stapled anastomotic leaks was considerably higher compared to the first half of the trial period. However these results are confounded by other factors and are not conclusive in refuting a potential influence of a learning curve effect. Firstly

all junior staff attached to the participating consultants contributed cases (and clinical leaks) to the study. Since most junior staff in surgical units rotate fairly frequently, irrespective of when they performed the operations they are more likely to have been relative beginners with surgical stapling instruments. This factor brings an imponderable bias while assessing a learning curve effect. Furthermore the participants of our trial had varying degrees of experience with staplers prior to commencement of the study and at least one of them had been a regular stapler user. When the results were analysed separately for each individual consultant, one participant was noted to have three stapled leaks amongst his first seven patients and none subsequently. No other identifiable trend was observed for the remaining consultants. In summary, we have not been able to demonstrate an influence from a learning curve effect with the use of stapling instruments in this study. The data however is not conclusive in this respect.

4.7 ANASTOMOSIS AND OPERATING TIMES

This study demonstrated a mean time saving of 13.8 minutes in the creation of gastrointestinal anastomoses when surgical stapling instruments are used instead of manual suturing. This is in accordance with the finding of Beart and Kelly [38] and Didolkar et al. [42] both of whom found a significant difference in anastomosis times in favour of staplers in their

respective studies. No other study of anastomotic techniques has attempted to measure or compare anastomosis times. Although the magnitude of difference in terms of anastomosis time is modest, in patients requiring multiple anastomoses the time saving may assume clinical relevance. Perhaps clinically more relevant however is the time taken to complete an operation. In this regard previous prospective controlled trials have produced variable results. Everett and his associates [41] demonstrated a significant time saving in operations where stapling instruments were used, compared to those where anastomoses were hand sewn. Brennan et al., [39] make no mention of anastomosis or operating times in their study. Other prospective controlled trials found no difference between sutured and stapled groups in terms of operating times [37,38,40,42]. In our experience surgical stapling instruments afforded quicker operations as well as quicker anastomoses compared with manual suturing. Furthermore the difference in operating times was consistently in favour of the stapled group in all four surgical categories and it was statistically very highly significant suggesting that it is a true phenomenon. Nevertheless the actual magnitude of the time saving was approximately 12 minutes per operation and whether this constitutes a significant advantage from the clinical point of view remains debatable.

4.8 MORTALITY

The overall operative mortality rate in this study was 5.5% and there was no significant difference between the sutured and stapled groups in this regard. Around one quarter of all the post-operative deaths occurred as a consequence of anastomotic complications. The high mortality of anastomotic dehiscence in gastrointestinal surgery has been highlighted by Fielding et al.,[64,82]. This contention is confirmed in our study where anastomotic dehiscence resulted in 28% and 45% mortality in lower gastrointestinal and upper gastrointestinal surgery respectively. Comparison of these results with quoted mortality rates in the literature is somewhat hampered by the use of different definitions of operative mortality, a problem which has been highlighted by Brown et al., recently [47]. Gritsman compiled data in the literature from different countries on over 50,000 gastric operations and found a mortality of 3.4% for benign disease and 10.4% for cancer [31]. A series from Belgium was recently reported by Canivet et al., [79] who reviewed 476 operations for large bowel cancer and found a 13.5% mortality rate. The corresponding rate for St Mark's Hospital was reported by Lockhart-Mummery et al., [83] to be 2.1%. By and large therefore the operative mortality rate in this series seems comparable to the experience of most other investigators. Although the operative mortality appeared to be higher for patients with upper gastrointestinal surgery than in large

bowel surgery, this probably was a reflection of a substantial proportion of patients with advanced gastric or pancreatic cancer. Nineteen of the 27 deaths in upper gastrointestinal surgery occurred in patients who underwent palliative procedures for advanced malignant disease.

4.9 DURATION OF HOSPITAL STAY

Suturing was shown to afford earlier discharge of patients from hospital compared to stapling in one study[39]. Other prospective controlled trials [38,40-43] demonstrated no difference between suturing and stapling in terms of postoperative hospital stay, which is in accordance with the findings of our trial. Our experience suggests that the day of discharge is a relatively poor indicator of post-operative recovery. In retrospect we feel that we should perhaps have also recorded the date that the patients were considered fit for discharge rather than the actual day they were sent home, since the latter decision was often based on social and domestic considerations.

4.10 NON-RANDOMISED PATIENTS

Over the four year study period 86.5% of the patients undergoing gastrointestinal anastomoses were considered suitable for randomisation. In the remainder of the operations there were a variety of reasons why the surgeons considered randomisation inappropriate. For most cases a mention was made

of these reasons in patient information documents, however they were not recorded in a standardised and computer coded fashion as part of the protocol dictated data collection. Therefore only some general comments are possible.

In some cases stapling was not feasible due to the nature of the anastomosis, such as hepatico-jejunostomy, choledocho-duodenostomy, oesophageal anastomoses in the neck, cysto-gastrostomy for pancreatic pseudo-cysts, etc. Inflammation, oedema and friability of the bowel or a marked disparity between the thickness of the bowel ends often deterred surgeons from using stapling instruments. On certain occasions (in particular oesophageal anastomoses) the organs involved were found to be too narrow to accomodate the smallest size EEA cone and suturing was chosen electively. Occasionally, when a junior surgeon unfamiliar with stapling techniques was operating without supervision they did not attempt randomisation. One of the frequent reasons for using staplers electively was the surgeons' concern about time saving in sick patients or those requiring multiple anastomoses. Another area where the surgeons felt that stapling techniques were more appropriate was low colorectal anastomoses when access to the pelvis was difficult.

The patients having non-randomised sutured or stapled anastomoses are, by definition, not comparable. Therefore no

attempt was made to analyse the results according to anastomotic technique in this group. However, as a whole the non-randomised patients did not differ significantly from the randomised group with regard to pre-operative variables. The higher clinical leak rate and the higher operative mortality in the non-randomised patients is presumably a result of the different nature of operations that they underwent, which was reflected by a higher proportion of the patients having multiple anastomoses and longer operating times.

4.11 ECONOMIC CONSIDERATIONS

Stapling instruments are undoubtedly more expensive than suture materials and the additional cost incurred by their use has been one of the frequent criticisms voiced against stapling. Reiling et al., in their prospective evaluation of the two techniques, observed a 68% increase in operating room charges when staplers were used [37]. McGinn and his colleagues calculated the cost of anastomotic materials to be over £5,000 for 58 operations where staplers were used, compared with £420 for 60 similar operations where the anastomoses were sutured [40]. Everett et al., also remarked that the cost of a stapled rectal anastomosis with the EEA instrument was substantially greater than that of a similar sutured anastomosis [41]. They add however that in view of the total cost of such a procedure they did not consider this to be a major issue. Based on figures obtained from the

pharmacy and the administration of one of the participating hospitals in our trial, we estimated the cost of stapling instruments to be approximately 5% of the total cost of care for a patient. Another factor that may be relevant in the cost analysis is the proportion of patients in whom a permanent stoma is avoided by the use of stapling instruments. Some authors have reported that between 10-60% of their patients have been spared permanent colostomies by the use of stapling instruments for low colorectal anastomoses[70,84,85]. Although the issue is controversial and it is not possible to provide direct evidence for these allegations, if some patients are in fact spared permanent colostomies the resultant saving in colostomy care appliances would more than offset the extra cost of stapling.

4.12 CONCLUSIONS

As the largest prospective randomised comparison of manual suturing and surgical stapling techniques yet reported, this trial has demonstrated that stapling instruments produce results comparable to that of suturing techniques in terms of important immediate outcome measures such as operative mortality, anastomotic security, incidence of infective complications, recovery of gastrointestinal function and duration of post-operative hospital stay. Of some concern was post-operative haemorrhage from stapled upper gastrointestinal anastomoses. Although this complication

occurred in a small number of patients it has also been observed by other investigators[6,24,70,80]. It would therefore seem prudent to advise extra caution in securing complete haemostasis for stapled upper gastrointestinal anastomoses. A further point that deserves attention is the alarmingly high incidence of anastomotic breakdown in duodenal stumps closed with staplers, for which no readily apparent explanation could be identified. It should be pointed out that in this respect the experience of previous investigators has been contrary to our findings [33,34,36] and our data are compatible with a chance distribution. In patients with colorectal anastomoses, stapling significantly reduced the incidence of sub-clinical anastomotic leaks. Although this did not have any short term clinical implications, its relevance in the long term remains to be determined. Surgical stapling also afforded significantly quicker anastomoses and operations. However the magnitude of the time saving is not large and is perhaps unlikely to translate into significant cost-efficiency.

It is hoped that this study will provide some guidance to surgeons in their selection of anastomotic technique. The final selection of anastomotic technique is likely to be multifactorial and will also take into account other considerations such as ease of anastomotic construction, cost, speed, personal preferences and training of junior surgical staff.

SECTION II

STUDIES ON COLORECTAL CANCER

CHAPTER 5

CARCINOMA OF THE COLON AND RECTUM

Backround and literature review

5.1 INTRODUCTION

Worldwide, colorectal cancer is ubiquitous among all ethnic groups and geographic regions. The incidence, however, varies sharply. Areas with the highest prevalence are New Zealand, Australia, North America and Western Europe, where the age-standardised incidence is around 50 per 100,000 of the population [87-89]. In these parts of the world where it is particularly prevalent, colorectal cancer is the second leading cause of cancer related deaths [90,91].

At the time of presentation patients with colorectal cancer fall into one of the following categories:

i. Tumour confined to its site of origin or to the local lymphatics.

ii. Spread of primary tumour to the liver along the portal venous system.

iii. Distant metastases associated with primary tumour, with or without liver metastases.

5.1.1 COLORECTAL CANCER WITH SYSTEMIC SPREAD

Like all other malignancies, the main determinant of outcome in colorectal cancer is the stage of disease at presentation. At the time of presentation, around a quarter of patients with colorectal cancer have widespread systemic metastases [92] and 50-65% of patients will have them by the time of their death [93,94]. At present no prospect of cure exists for

these patients. Three quarters of all patients presenting with metastases will die of their disease within 12 months and very few will survive beyond two years [94].

The role of radiotherapy in widespread systemic disease is naturally limited to palliation. As regards chemotherapy, a number of reviews quote response rates between 9-15% with single agent therapy and occasionally up to 40% response rate with combination chemotherapy [95-97]. Very few trials have shown any prolongation of survival [98].

5.1.2 COLORECTAL CANCER WITH LIVER METASTASES

In patients with colorectal carcinoma, the frequency of overt hepatic metastases at the time of presentation is between 15 and 25% [99,100]. The diagnosis of liver metastases is associated with a mean survival of 6-10 months [101-103]. Survival shows a direct relationship with the extent of liver involvement [100,104] and the differentiation of the metastatic tumour [105].

Around 5% of patients with hepatic metastases from colorectal cancer could be candidates for liver resection [106]. There is now good evidence that resection is associated with a major improvement in survival in these patients [103,106,107].

In theory, for patients with multiple unresectable metastases of colorectal cancer confined to the liver, regional chemotherapy techniques allow high local concentrations of the chemotherapeutic agent to reach the tumour with reduced systemic toxicity. However, earlier experiences comparing systemic versus intra-arterial chemotherapy have been disappointing or at best equivocal [108,109]. Two recent prospective randomised trials demonstrated significantly better response rates for intra-arterial chemotherapy [110,111]. However the improved response rate did not translate into significantly improved survival rates. Aigner developed a further technical refinement of regional chemotherapy [112]. His preliminary experience with this technique, named "isolated liver perfusion", in 46 patients, suggests that it might be superior to intra-arterial chemotherapy alone. However this technique has not been evaluated clinically in a large scale anywhere else in the world.

5.1.3 COLORECTAL CANCER: LOCAL DISEASE

It is evident from the discussion above that any improvement in the survival prospects for those patients in whom the spread of colorectal cancer has progressed beyond the local lymphatics is dependent upon the development of more effective treatment methods for advanced disease. In contrast, for truly local disease surgical treatment has a proven curative

role. At the time of presentation approximately 60% of patients fall into this category where they are amenable to a "curative" resection [113]. Only half of these patients however, will survive for five years [105-115]. Although most series report a favourable trend in survival from colorectal carcinoma in the last few decades, the reason for this appears to be earlier detection and improvements in operative mortality [83,116,117]. A number of studies [113-115,118-120] have shown that the long-term survival prospects of patients undergoing potentially curative surgery for colorectal cancer has remained more or less static over the last 30 years.

5.1.4 WHY DOES "CURATIVE" SURGERY FAIL TO CURE?

A possible explanation for the failure to cure local disease by surgery was suggested by Fisher and Turnbull [121]. These authors demonstrated cancer cells in the portal venous blood of eight of the 25 colon cancer patients they studied and postulated that these cells may have been scattered by operative manipulation. Based on this hypothesis, Turnbull adopted a technique of large bowel resection for cancer wherein the tumour was not dissected or handled in any manner until after its lymphatic and vascular pedicles were ligated and the bowel was divided at the sites elected for resection. To emphasise the type of technique, the name "no-touch isolation" was adopted. Turnbull compared 664 patients

operated on using the no-touch isolation technique at the Cleveland Clinic between 1953 and 1964, with 232 patients operated on during the same period in the same institution by five other surgeons using conventional techniques [122]. For all stages the cumulative survival rate was better for the no-touch isolation group, 51% compared to 35%. This was an uncontrolled study however, and due to incomparability of groups it fell short of providing conclusive evidence for Turnbull's hypothesis. In two retrospective reviews Stern and Schottenfeld at the Memorial Hospital in New York [123] and Ritchie at St Mark's Hospital, London [124] failed to demonstrate any influence of the no-touch isolation technique on patient survival. More recently Jeekel's group in the Netherlands undertook a prospective randomised trial to test the effect of no-touch isolation technique on the prognosis of patients undergoing surgery for colorectal cancer [125]. In the years 1979-1982, 236 patients entered this trial and Jeekel reported results with a mean follow-up of 58 months on these patients [126]. The proportion of patients who developed liver metastases was 7% in the no-touch isolation group, compared to 20% in those who had a conventional resection. However at 58 months the survival between the two groups was not different (65% versus 62%).

An alternative explanation for the failures of ostensibly curative surgery was suggested by Finlay and his colleagues [127]. These authors studied 43 patients undergoing surgery for colorectal cancer and subjected them to isotope liver scan, ultrasonography and computed tomography in the immediate post-operative period. Six of these 43 patients had macroscopically evident liver metastases at the time of surgery. Of the remaining 37, 11 developed liver metastases within two years of surgery. The post-operative CT scans had detected sub-clinical liver metastases in nine of these 11 patients. Based on these observations Finlay et al., suggested that around 30% of patients undergoing apparently curative surgery for colorectal cancer already possess occult liver metastases. Further support to this hypothesis came from another study published in the same year by Finlay and McArdle [128] where they demonstrated that in patients undergoing surgery for colorectal cancer CT findings at the time of surgery predicted the prognosis more accurately than pre-operative CEA status or tumour stage or grade.

5.2 LOCAL RECURRENCE OF COLORECTAL CANCER

The above discussion highlighted two factors which may partly be responsible for the 50% failure rate following potentially curative resections for colorectal cancer:

i. Wrongly classifying the operation as curative when there are occult liver metastases present.

ii. Converting localised disease to systemic disease by tumour cell embolisation at the time of surgery.

Neither of these mechanisms however explain the failures of surgical treatment due to local recurrence.

5.2.1 INCIDENCE OF LOCAL RECURRENCE

The wide variation in the reported incidence of local recurrence of colorectal cancer may partly reflect the lack of consensus regarding an exact definition. Recurrent cancer in the region of the anastomosis may be associated with initially extra-mural tumour growth which spreads to reach the mucosal surface of the bowel. Alternatively, a localised recurrence of the luminal aspect of the anastomosis may extend outwards and present as a tumour mass involving the entire thickness of the bowel wall associated with an extra-mural component. Clinical data seldom allow a clear cut distinction between these two possible modes of spread [129].

The reported incidence of local recurrence following curative surgery for colorectal cancer varies between 5-46%. In studies where 'second-look' laparotomies were used either routinely [130] or as indicated by a rise in CEA levels [131,132], the incidence of local recurrence was found to be between 30-45%. One autopsy study on 1008 patients who died after curative resections for colorectal cancer detected a 46% incidence of local recurrencePW[133]. With a strict clinical follow-up policy, Schiessel et al., detected 156 recurrences (22%) among 715 patients following curative surgery, of which 90 (13%) were local recurrences [134,135]. In a study from Malmo, Berge et al., reported a 34% incidence of recurrence (local and/or distant) among 639 patients [136]. The most comprehensive experience in the U.K. was reported by Phillips et al., who obtained complete follow-up information on 2,220 patients after curative surgery for colorectal cancer [137]. Local recurrence was detected in 309 (14%) of these patients.

In summary the exact incidence of local recurrence following curative surgery for colorectal cancer remains unclear. Data from post mortem studies and 'second look' laparotomies serve to illustrate the biological problem of local recurrence, the incidence of which may be as high as 46% . However, some patients reported to have a local component to their recurrent disease presumably do not have a clinical problem with the

local recurrence. A review of the data suggests that local recurrence alone presents as a clinical problem in 10-20% of patients undergoing curative surgery.

5.2.2 DISEASE FREE INTERVAL IN PATIENTS WITH LOCAL RECURRENCE

The majority of patients with local recurrence following "curative" surgery for colorectal cancer present within the first two years of the operation. In the experience of Berge et al., 70% of the 172 patients with recurrent colorectal carcinoma were diagnosed within 24 months of surgery [136]. The corresponding figure from the Massachusetts General Hospital series was 69% [138]. Stulc et al., [139] found median disease free interval between resection of the primary colorectal tumour and evidence of local recurrence to be 13 months and all recurrences in the 158 patients they studied had occurred within 27 months of surgery. The experience from the Large Bowel Cancer Project revealed that the risk of local recurrence increases rapidly to reach a peak between 9 and 12 months post-operatively and thereafter falls to a steady rate of between 0.75 - 1 % per each three month period [137]. The minimum follow-up in this study was three years and by this time 85% of local recurrences had been detected.

5.2.3 LOCAL RECURRENCE: PROGNOSIS

Local recurrence following "curative" surgery for colorectal cancer, once manifest is a major factor limiting survival. Welch and Donaldson, in an autopsy study of recurrent colorectal cancer reported that depending on the site of the primary tumour 20 to 35% of the deaths were attributable to local recurrence [93]. According to Moertel 50% of patients with colorectal cancer die from the local effects of recurrent cancer and 50% succumb to distant spread [140]. Taylor, in a post mortem study of a group of patients with colorectal cancer (only 25% of whom had a curative resection) found that three-quarters of all deaths were due to local recurrence [141]. Cass et al., attributed death from colorectal cancer to local recurrence alone in 60%, local and distant disease in 14% and distant metastases alone in 26% of the patients [142]. McDermott et al., in a review of 1,008 patients with rectal cancer reported an overall five year survival rate of 69% [120]. In the sub-group developing local recurrence however five year survival was 19%. Wenzl et al., found that among 121 patients with local recurrence of colorectal cancer, 60 were amenable to a second radical operation with the intention of cure [135]. Sixty five per cent of these patients however died within 35 months and no patient with palliative treatment was alive at the end of the 35 month period.

5.3 MECHANISMS OF LOCAL RECURRENCE

5.3.1 INADEQUATE LOCAL CLEARANCE

Wide margins of resection both proximal and distal to a tumour and wide clearance of the mesentry of a tumour bearing segment rarely poses a problem for intraperitoneal neoplasms of the large bowel. In contrast, for rectal carcinomas, 'safe' clearance margins have remained a matter of debate since rectal excision became surgically feasible. In 1908 Miles reported results in 57 patients undergoing perineal excision of rectum for carcinoma, of whom 95% developed local recurrence within three years of operation. [143]. These results prompted him to develop the technique of abdominoperineal resection which enabled tumour clearance in the 'zone of upward spread'. For nearly three decades abdominoperineal resection remained the most widely practiced operation offering scope for adequate tumour clearance in patients with rectal cancer. However, the prevailing concepts of the spread of rectal carcinoma were challenged in the 1930s [144-146], paving the way to Dixon [147] and Wangensteen [148] to develop the first sphincter preserving abdominal excisions of the rectum for rectal cancer. This approach, adopted by many eminent colorectal surgeons [149-152], was in turn followed by debate on safe distal resection margins while performing sphincter preserving rectal excisions. Initially concern regarding adequate distal

clearance and technical feasibility limited the application of anterior resection to carcinomas of the upper rectum. However several studies published in the 1960s and 1970s demonstrated that sphincter saving operations could also be safely employed for cancers of mid-rectum, with distal resection margins of approximately 2 cms, without any apparent increase in the incidence of local recurrence [83,153-156]. In 1983 Hughes et al., reported a pathological study investigating patterns of local spread on resection specimens from 42 patients with large bowel cancer [157]. Intra-mural tumour spread was seen in only two of these specimens, one extending distally for 2 cm in a lymphatic channel and the other spreading proximally on the serosal surface of a specimen from a palliative resection. In the same year Williams et al., published another pathological study [158] where 50 abdomino-perineal resection specimens were examined for the presence of microscopic distal intra-mural spread. There was no spread in 38 of these specimens. Seven revealed evidence of spread for 1 cm or less and only five had distal spread greater than 1 cm. Each of these five patients had poorly differentiated Dukes C adenocarcinomas. Williams concluded that distal intramural spread in colorectal carcinoma is rare, when it does occur it usually extends for less than 1 cm. and in those cases where its extent is greater, the tumours are poorly differentiated Dukes C or D lesions with poor prognosis [159]. There is convincing evidence in the literature that retrograde

lymphatic spread in rectal carcinoma is similarly rare and occurs only in patients with advanced tumours [160-162]. The pathological evidence therefore strongly refutes the theoretical risk of inadequate distal clearance by sphincter saving resections of the rectum, compared with the clearance obtained by total rectal excision. These observations have been confirmed by a number of clinical reviews which have shown sphincter saving operations to be as "curative" as abdomino-perineal resection for rectal cancer [163-168].

More recently however attention has been focused on the lateral spread of cancer and the importance of lateral clearance, thus reviving the potential role of inadequate tumour clearance in the development of local recurrence. Heald and his colleagues drew attention to microscopic tumour deposits in the mesorectum of five patients with colorectal cancer [169]. Based on this observation they postulated that wide excision of the mesorectum is crucial in the prevention of local recurrence of rectal cancer. Adhering to this surgical principle Heald published local recurrence rates of 2.6% and 3.3% in two recent reviews of his own series [170,171]. Durdey et al., also demonstrated the importance of lateral clearance on 52 rectal excision specimens examined [172]. They identified tumour in lateral excision margins in 20 of these specimens. Eleven of these 20 operations had been classified as curative at the time of surgery and in 15

of these 20 patients local recurrence had become manifest after a median follow-up of 23 months. Keighley and his colleagues found that cytological smears from the four quadrants of the pelvis and imprint cytology of the tumour bed are reliable indicators of inadequate tumour clearance [173]. They studied 60 patients of whom 12 were found to have positive malignant cytology. At a mean follow-up of 16.5 months seven of these 12 patients had developed local recurrence.

In summary, adequate local clearance of colorectal cancer is fundamental for the prevention of local recurrence. In terms of distal clearance, there is substantial evidence that a margin of 2 cm is adequate. Lateral clearance however has received less recognition and may account for a proportion of the local recurrences following ostensibly curative resections. Nevertheless inadequate tumour clearance alone is not sufficient to explain all local recurrences. In carcinomas of the large bowel more proximal to the rectum satisfactory resection margins are achieved without difficulty and yet local recurrence can occur. Furthermore local recurrence has been described following the resection of early tumours where the possibility of inadequate clearance should not arise. It is therefore likely that in the development of local recurrence mechanisms other than inadequate tumour clearance also play a role.

5.3.2 IMPLANTATION METASTASIS

Attention has been drawn to implantation of tumour cells as a potential mechanism for recurrence at the turn of the century by Ryall[174,175] and Mayo[176]. Since then there has been numerous case reports of colorectal cancer deposits on raw surfaces such as haemorrhoidectomy wounds, anal fissures and fistulae, sites of circumanal purse-string sutures or excision of benign mucosal lesions of the bowel [177-181].

Clinical Evidence

The early evidence in support of implantation metastasis largely relied on clinicopathological studies of patients who developed local recurrence. Wheelock et al., [182] followed 90 patients who had undergone curative resections for rectal or sigmoid cancers by sigmoidoscopy. Ten cases of tumour recurrence at the suture-line were detected. In eight of these patients the primary tumour was Dukes A and in nine the distal resection margins were at least 3.5 cm from the lower border of the tumour. The authors suggested that implantation was a more likely explanation for these recurrences than incomplete resection. Lofgren and his colleagues [183] reported a study of 108 patients with local recurrence following anterior resection. In 76 of the specimens they could find no evidence of incomplete resection, lymphatic or vascular permeation to explain the recurrence and postulated that implantation was the responsible mechanism in

these cases. As the number of reports on the potential risk of implantation of cancer cells grew, it became established practice in some centres to isolate the tumour bearing segment of bowel with tapes and/or to irrigate the bowel lumen with various cytotoxic agents. The introduction of these measures to kill or prevent the dissemination of exfoliated cancer cells resulted in a reduction in the incidence of local recurrence, thus providing more compelling evidence for the validity of the implantation concept. Goligher [184] reported four local recurrences after 35 operations for colorectal cancer where no washouts were used. In 102 operations where he used mercury bichloride washouts only two patients developed local recurrence. Keynes [185] published the experience of St Mark's Hospital with mercury bichloride washouts in anterior resection. The incidence of local recurrence was reduced from 13% to 2.6% by the adoption of this policy. The experience of Southwick et al., from Chicago was very similar [186]. This group of authors adopted a policy of isolating the tumour bearing segment of bowel with occlusive tapes before mobilisation and irrigating the bowel lumen with 0.25% sodium hypochloride. These measures completely prevented suture line recurrence in a series of 101 patients with a minimum one year follow-up, compared with a 16% incidence of local recurrence in their own historical series [187].

Experimental evidence

Numerous experimental studies using a variety of malignant cell lines have revealed that tumour cells are capable of implanting themselves and causing tumour growth at a suture line in the large bowel [188-192]. However Rosenberg in 1978 published work questioning the viability of the exfoliated cells in patients with colorectal cancer, thus casting doubt on the validity of the implantation metastasis theory [193]. He compared the viability of colorectal cancer cells prepared from homogenate suspensions of primary tumours in patients to that of the exfoliated cancer cells. Exfoliated cells were obtained by pre-operative colonic lavage (in vivo) or from the resected specimens (ex vivo). Homogenate suspensions were prepared from the middle of the resected tumour specimen. Viability was assessed by the demonstration of non-specific esterase activity, trypan blue exclusion, tritiated thymidine uptake and growth potential in tissue culture. All of the 37 in vivo and ex vivo lavage specimens were shown to contain tumour cells on smears. None of the cells in these samples however revealed any evidence of being alive in any of the viability assays. In contrast, well over half of the cells obtained from the tumour homogenates were demonstrated to be viable (23 out of 25 capable of excluding trypan blue, 6 out of 10 with esterase activity, 8 out of 12 showing uptake of radiolabelled thymidine and 7 out of 16 growing in culture).

Rosenberg was subsequently able to reproduce these results using a dimethylhydrazine induced rat colonic carcinoma model (194). Exfoliated cells from these experimentally induced tumours were found to be non-viable whereas cells from tumour homogenates produced implantation metastases in syngeneic animals. In the light of the evidence contradicting the implantation metastasis theory, Rosenberg reviewed 16 well documented cases of anastomotic recurrence that occurred between 1955 and 1973 in Leeds, from Goligher's personal series. In 14 of these cases he was able to attribute the recurrence to incomplete resection. One was found to be a second primary tumour which left only one case of local recurrence for which he was unable to provide an alternative explanation other than implantation metastasis.

In 1984 Umpleby et al., published work that contradicted Rosenberg's findings and renewed the interest in implantation metastasis (195). They obtained exfoliated colorectal cancer cells by pre-operative lavage of patients with carcinoma and by the irrigation of the resection margins of tumour bearing specimens. Assessed by morphology and by their ability to exclude trypan blue and hydrolyse fluorescein/diacetate, they demonstrated that 52 of the 74 specimens examined contained viable tumour cells (74% of lavage specimens and 69% of those from resection margins). The same group of authors

subsequently investigated the proliferative and metastatic capacity of exfoliated human colorectal cancer cells [196]. They found that six out of 17 cell suspensions injected intravenously into nude mice as xenografts resulted in tumour growth. More recently Skipper et al., from Southampton harvested cells from washings of colorectal tumours in patients and succeeded in growing them in cell culture [197].

In summary, there is considerable evidence that malignant cells are desquamated from primary tumours into the lumen of the large bowel in patients. It has been shown that these cells are viable and are capable of implantation into exposed sub-mucosal, muscular or serosal surfaces and they have the potential to proliferate and cause local tumour growth. Further studies are required to determine the exact role of this mechanism in the development of local recurrence following colorectal cancer surgery in man.

5.3.3 METACHRONOUS CARCINOGENESIS

Around a quarter of the local recurrences occur more than two years after primary resection for large bowel cancer [136,138]. Neither inadequate tumour clearance nor implantation metastasis seem plausible mechanisms to explain these late recurrences. Metachronous carcinogenesis has been suggested as an alternative explanation. Little direct

evidence exists to incriminate metachronous carcinogenesis in the development of local recurrence. Nevertheless certain established clinico-pathological facts regarding large bowel cancer in man and a number of experimental observations lend support to the concept.

Clinical Evidence

Epithelial neoplasia of the large bowel is a multifocal disease. Adenomas are multiple in around a third of the patients at the time of diagnosis [198,199] and synchronous carcinomas are present in 2-3% of patients at the time of presentation [198,200]. Following the resection of one large bowel cancer, 1-2% of patients will develop a metachronous cancer [198,200]. Moreover, the presence of synchronous adenomas at the time of resection for carcinoma doubles the risk of developing metachronous cancer [201]. The well recognised malignant predisposition in patients with familial polyposis coli [198] and ulcerative colitis [202,203] also supports the concept of an unstable epithelium. In 1951 Goligher, Dukes and Bussey [162] reported 15 patients with unexpected local recurrences following surgery for colorectal cancer and commented that a second primary tumour developing in the region of the anastomosis may have been responsible for some of these recurrences.

Experimental evidence

Consistent with the concept of altered biological properties at anastomotic sites is the demonstration of abnormal mucus production around colonic tumours. Filipe demonstrated that an increase in sialomucins at the expense of the normally predominant sulphomucins is associated with premalignant change in the bowel [204]. The abnormal mucin pattern extends for a considerable distance from the tumour edge into morphologically normal mucosa and reaches the resection margins in 15% of the resected specimens [205,206]. This alteration in mucus production has also been shown to occur in carcinogen induced experimental tumours [207,208]. It is unclear whether these changes are primary events in carcinogenesis or simply epiphenomena. They have been reported in inflammatory conditions [209] and after simple by-pass procedures in experimental models [210] suggesting that they may only be markers of hyperplasia. Of clinical interest however is the increased incidence of tumour recurrence when the sialomucin dominant pattern in the bowel mucosa extends into resection margins [211,212]. Habib et al., showed a significantly increased incidence of local recurrence and reduced survival associated with sialomucins at resection margin in a prospective study of 250 patients [213].

Animal studies using chemical carcinogens have consistently shown an increased yield of bowel tumours at the site of an anastomosis. Using azoxymethane in a rat model Williamson and his colleagues [214] from Bristol demonstrated that large bowel shows limited proliferative or hyperplastic response to partial resection and this does not render it more susceptible to carcinogenesis except in the region of suture lines. The same phenomenon has been shown at sites of other anastomoses [215-217] or merely the placement of a suture into the bowel wall [218] and also with different carcinogens [219-220].

In summary, epithelial neoplasia of the large bowel is a multi-focal disease and patients with resected bowel cancers remain at a higher risk to develop further tumours. Some indirect clinical and experimental evidence suggests that the site of an anastomosis may be more susceptible to metachronous carcinogenesis compared to the remainder of the bowel. This mechanism may be responsible for some of the local recurrences seen in patients following "curative" surgery for colorectal cancer.

CHAPTER 6

ANASTOMOTIC TECHNIQUES AND RECURRENCE OF COLORECTAL CANCER

6.1 BACKGROUND

The safety of stapled restorative resections for rectal carcinoma has recently been questioned. The contention that the use of circular staplers results in an increased incidence of local recurrence was derived from several retrospective reviews published in the early 1980s. Hurst and his colleagues reported 11 recurrences among 34 patients (32%) who had stapled anastomoses following anterior resection for carcinoma of the lower and mid rectum[221]. All operations in this series were potentially curative, however 10 of the 11 recurrences occured after resection of locally advanced tumours and the authors suggested that the use of stapling instruments for low colorectal anastomoses be reserved for earlier tumours. Anderberg et al reported results on 38 patients with rectal cancer who had anterior resections and stapled anastomoses [222]. Nine local recurrences (24%) were detected in the follow-up of these patients. Reid et al[223] in another series observed eight pelvic recurrences in 27 patients (30%) when anterior resection was followed by a stapled anastomosis. Bisgaard et al[224] also reported 35 patients who had undergone anterior resection and stapled anastomosis for rectal cancer, of whom seven developed local recurrence.

In contrast with these observations, other investigators examining the influence of anastomotic technique on the outcome of colorectal cancer failed to identify an adverse effect

associated with stapling. Ohman and Svenberg reported 20 patients with stapled rectal anastomosis who did not develop local recurrence after a follow-up period ranging from 4-40 months[225]. Subsequently Ohman reported a review on 96 patients with colorectal cancer, where the crude survival rate after a three year mean follow-up was 76% for those with stapled reconstructions and 53% for those who had sutured anastomoses[226]. More recently Kennedy et al[227]., reported an overall recurrence rate (regional and distant) of 36% after a mean follow-up of 44 months in 63 patients with stapled colorectal anastomoses. These authors add that the results compare favourably with the recurrence rate prior to the introduction of stapling techniques in their unit. The first study comparing suturing and stapling techniques in a consecutive patient series was published by Rosen and his colleagues[228]. In this report follow-up results on 119 patients operated on by a single surgeon were reviewed. 76 patients had stapled and 43 had sutured anastomoses. The choice of anastomotic technique was left to the discretion of the surgeon and was influenced by technical feasibility. Fourteen patients were excluded from analysis before completing 24 months follow-up due to death with distant metastases (no local recurrence) or death with no evidence of tumour and 11 patients were lost to follow-up. Of the remaining patients, 22 developed local tumour recurrence. 16 of these were in the stapled group and 6 were in the sutured group. Disease-free survival at 24

months was 55.3% for patients with stapled anastomoses and 69.8% for those with sutured anastomoses. The results were analysed in three categories according to the site of the tumour; lower, middle and upper rectum. The advantage observed for sutured patients was confined to those with tumours located in the middle rectum. The authors concluded that the likely explanation for the observed difference was patient selection. Wolmark et al reported another review assessing the effect of stapling techniques on tumour recurrence and patient survival[53]. They reviewed 1,292 patients with colorectal cancer who were included in the National Surgical Adjuvant Breast and Bowel Project in Pennsylvania, of whom 337 had stapled and 955 had sutured anastomoses. No difference was observed between the sutured and stapled patients with regard to survival, disease-free survival or local recurrence.

The potential influence of stapling techniques on the long-term outcome of patients with colorectal cancer has not been examined by prospective controlled studies. Furthermore, previous studies, as discussed above, have often been based on small patient series and have produced conflicting results. This study was undertaken in an attempt to bring some clarification to this controversial issue. The aim of the study was to determine the relative frequency of tumour recurrence and disease-free survival rates in patients undergoing sutured and stapled anastomoses after resections for colorectal cancer.

6.2 PATIENTS AND METHODS

All patients in this study have been subjects of the prospective controlled trial comparing manual suturing and surgical stapling techniques reported in "Section I". 463 consecutive patients underwent surgery for colorectal cancer between April 1985 and April 1989, under the care of 13 surgeons in five hospitals in Scotland. On 33 occasions it was considered inappropriate to randomise the anastomotic technique, leaving 430 patients who were randomly assigned to receive either sutured or stapled anastomoses following resection. 96 of these resections (22.3%) were palliative, which were excluded from further analysis. Of the 334 patients who had potentially curative resections, 12 (3.6%) died within 30 days of surgery and follow-up data was incomplete on 28 (8.4%) patients. The remaining 294 patients constitute the study group.

Sutured colo-colic and colo-rectal anastomoses were carried out using a single layer of interrupted 2/0 polyamide (Nurolon, Ethicon Ltd., Edinburgh, Scotland) suture material. Ileo-colic anastomoses were performed either in the same fashion or in two layers using an inner layer of continuous 2/0 polyglyolic acid (Dexon Plus, Davis & Geck, Gosport, Hampshire, England) and an outer layer of 2/0 polyamide. All stapled anastomoses were performed with the TA, GIA or EEA series surgical stapling instruments (Auto Suture Company UK, Ascot, England).

The TNM classification, as adopted by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC) was used for tumour staging [229], which is summarised below:

Primary Tumour (T)

TX	Primary tumour cannot be assessd
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour invades submucosa
T2	Tumour invades muscularis propria
T3	Tumour invades through the muscularis propria into the subserosa or into pericolic or perirectal tissues.
T4	Tumour perforates the visceral peritoneum or directly involves other organs or structures.

Regional Lymph Nodes (N)

NX	Regional lymph nodes cannot be assessd
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 pericolic or perirectal lymph nodes
N2	Metastasis in 4 or more pericolic or perirectal lymph nodes
N3	Metastasis in any lymph node along the course of a named vascular trunk

Distant Metastasis (M)

MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Staging was done after surgical exploration of the abdomen and pathological examination of the resected specimens. The stage groupings used (the corresponding Dukes stages are given in brackets) were as follows:

Stage 0	Tis	N0	M0	
Stage I	T1,T2	N0	M0	- **(Dukes A)**
Stage II	T3,T4	N0	M0	- **(Dukes B)**
Stage III	Any T	N1,N2,N3	M0	- **(Dukes C)**
Stage IV	Any T	Any N	M1	

Curative surgery was defined as removal of all macroscopically evident disease at the time of surgery with tumour free resection margins on histological examination. Two patients, both with stapled anastomoses, who had excision of solitary liver metastases at the time of resection of the primary tumour were considered as having had a curative operation. Deaths occuring within 30 days of the surgical procedure were considered as operative mortality. Local recurrence was defined as clinical, histological or post-mortem evidence of recurrent carcinoma at or in the region of the anastomosis. Patients developing tumour spread to other intra-abdominal sites, lung, liver, bones etc., during follow-up were classified as distant recurrences. Disease free interval and survival was measured from the time of resection. The estimation of the cumulative probability of recurrence and cancer specific mortality rates

were performed by the life table method of Kaplan and Meier (230). Comparison of recurrence and cancer specific mortality rates between the sutured and stapled groups was done by log rank test. Cox's proportional hazards regression model was used for multivariate adjustment for the influence of covariates on recurrence and mortality rates simultaneously (231).

6.3 RESULTS

The study population consisted of 142 patients with sutured and 152 patients with stapled anastomoses. Patient characteristics for the sutured and stapled groups are outlined in Table 6.1 .

Table 6.1

PATIENT CHARACTERISTICS
(Mean values ± Standard Deviation)

	SUTURES (n= 142)	STAPLES (n= 152)
Age	69.6 ± 11.3	68.7 ± 11.3
Sex (male/female)	69/73	69/83
Weight (kg)	62.5 ± 12.7	63.7 ± 10.8
Height (cm)	165.2 ± 10.2	164.9 ± 8.3
Haemoglobin (g/dl)	12.7 ± 2.1	12.8 ± 2.0
WBC (x 10^9/l)	8.4 ± 2.9	8.4 ± 2.6
Albumin (g/l)	37.7 ± 5.1	38.5 ± 4.6
Operating time (min)	119.8 ± 40.9	103.3 ± 41.6

Forty-five patients in the sutured group (31.7%) and 48 patients in the stapled group (31.6%) received peri-operative blood transfusions (during the same admission when surgical resection was performed). 109 of the patients had restorative rectal excisions for carcinoma of the rectum or recto-sigmoid. 112 patients had right hemicolectomy and ileo-colic anastomosis for right sided colonic tumours. The remaining 72 patients had segmental bowel resections and colo-colic anastomoses.

The distribution of anastomotic sites in the two groups is shown in Table 6.2 .

Table 6.2

ANASTOMOTIC SITE

ANASTOMOSES	SUTURES (n= 142)	STAPLES (n= 152)
Colorectal	52	57
Colocolic	39	34
Ileocolic	51	61

The stage and grade of the tumours for the patients in the two groups are outlined in Table 6.3 .

Table 6.3

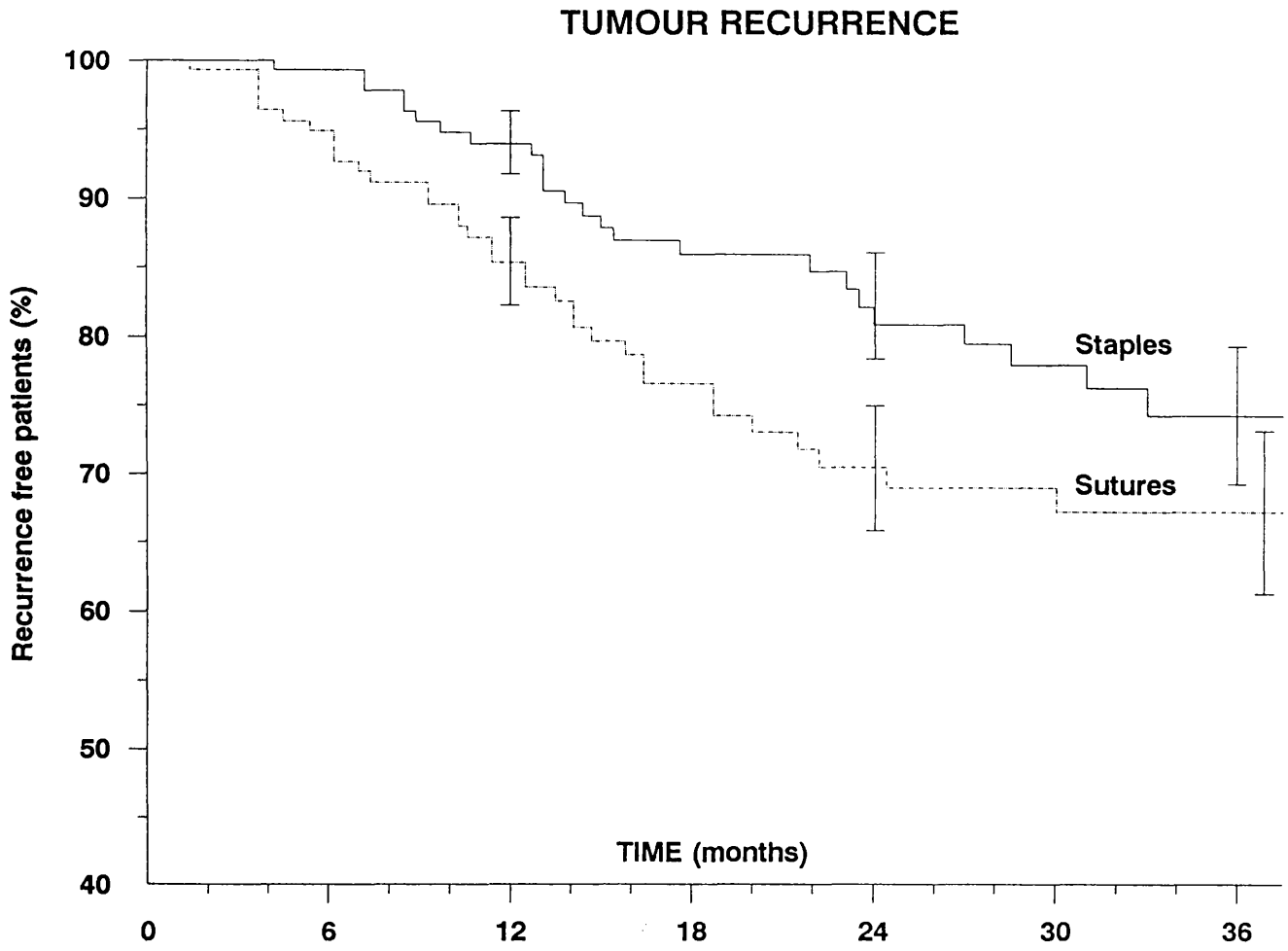
TUMOUR STAGE & GRADE

	SUTURES (n= 142)	STAPLES (n= 152)
TUMOUR STAGE		
Stage I	15	20
Stage II	76	88
Stage III	51	42
Stage IV	0	2
TUMOUR GRADE		
GX Cannot be assessed	5	6
G1 Well differentiated	8	23
G2 Moderately differentiated	101	105
G3/G4 Poorly differentiated	28	18

Follow-up for the patients ranged between 11-54 months. The median follow-up was 21.0 months in the sutured group and 21.2 months in the stapled group.

The cumulative probability of tumour recurrence is illustrated in Figure 6.1, expressed as the proportion of recurrence free patients plotted against time during the follow-up period. The incidence of tumour recurrence in patients with sutured anastomoses was significantly higher than for those with stapled anastomoses ($p< 0.05$). 29.4% of the patients in the sutured group had recurrence diagnosed by the end of the second post-operative year (SEM: 4.4%), compared with 19.1% in the stapled group (SEM: 3.9%).

Figure 6.2 illustrates the cumulative probability of cancer specific death in the two groups. Higher incidence of tumour recurrence in the sutured group was paralleled by a significantly higher cancer specific mortality ($p< 0.01$). By the end of 24 months, 22.3% of the patients with sutured anastomoses had died as a result of cancer (SEM: 4.1%) compared with 10.9% in the stapled group (SEM: 3.0%).



NUMBER OF PATIENTS AT RISK								
	Staples	152	139	114	86	62	47	29
	Sutures	142	128	95	71	49	38	26

Figure 6.1

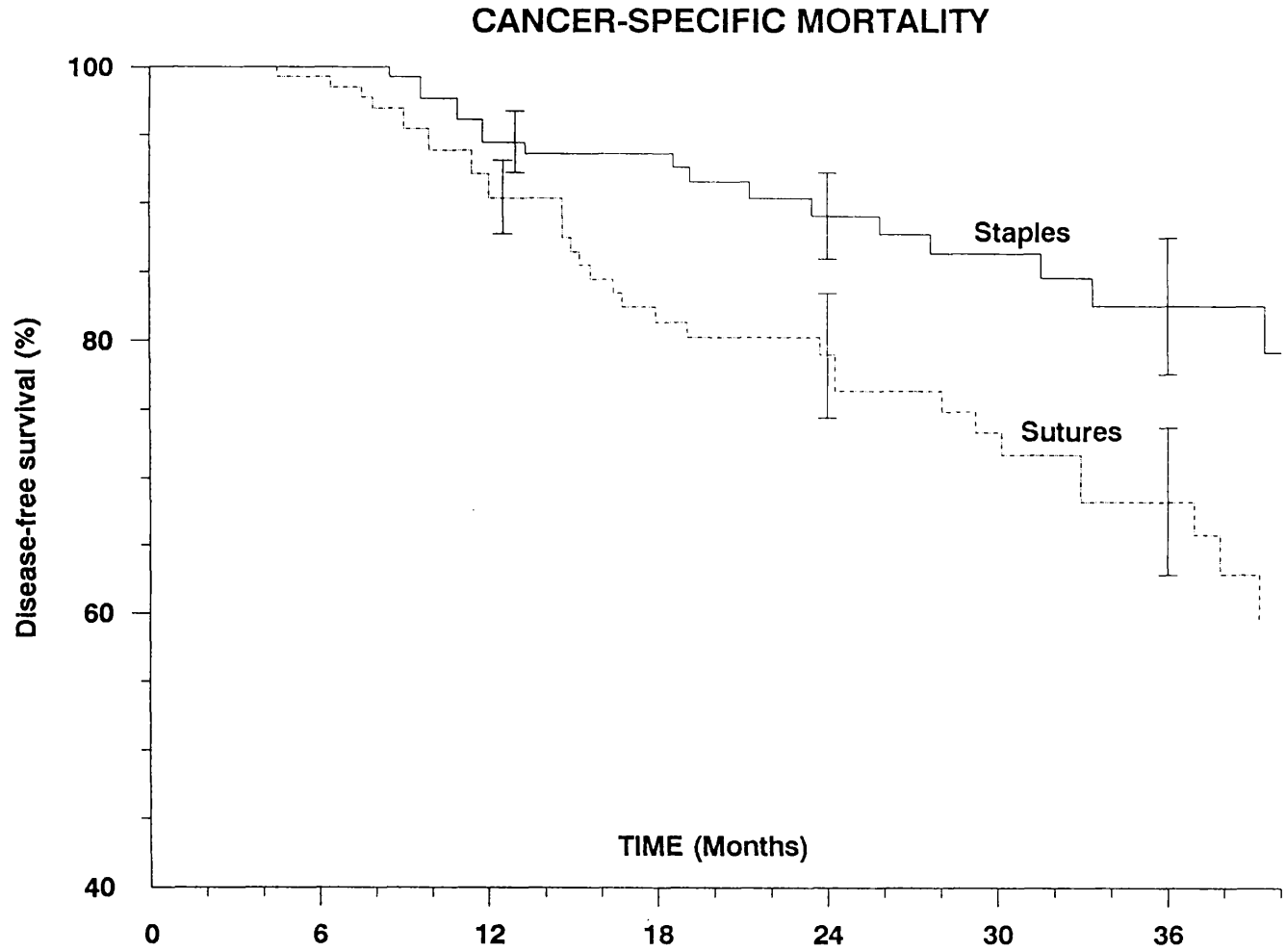

CANCER-SPECIFIC MORTALITY
100
80
60
40
Disease-free survival (%)
Staples
Sutures
TIME (Months)
0
6
12
18
24
30
36
NUMBER OF PATIENTS AT RISK
Staples 152 139 114 86 62 47 29
Sutures 142 128 95 71 49 38 26

Figure 6.2

Table 6.4 shows the number and site of recurrences in each group differentially for tumour stage.

Table 6.4

NUMBER AND SITE OF RECURRENCES IN SUTURED AND STAPLED GROUPS TABULATED BY TUMOUR STAGE

		RECURRENT TUMOUR			
		Local	Local + Distant	Distant	All recurrences
SUTURED GROUP					
Stage I	(n= 15)	2	0	0	2
Stage II	(n= 76)	7	7	4	18
Stage III	(n= 51)	7	8	2	17
TOTAL	(n=142)	16	15	6	37
STAPLED GROUP					
Stage I	(n= 20)	0	0	1	1
Stage II	(n= 88)	6	6	2	14
Stage III	(n= 42)	4	1	5	10
Stage IV	(n= 2)	1	0	0	1
TOTAL	(n=152)	11	7	8	26

In order to identify and adjust for the influence of all the co-variates that were associated with the outcome, Cox's regression analysis was used. The effect of age, sex, pre-operative haemoglobin and serum albumin levels, anastomotic site, blood transfusion, tumour stage, tumour grade and anastomotic technique were examined in relation to outcome. In univariate analysis, only tumour stage and anastomotic

technique were seen to be significantly associated with recurrence (Table 6.5). Male sex was also associated with a higher incidence of recurrence, which had a borderline significance in univariate analysis. The difference between sutured and stapled groups in terms of recurrence rate remained statistically significant ($p< 0.05$) after correcting for tumour stage in the multiple regression model (Table 6.6).

Table 6.5

RELATIONSHIP BETWEEN PERI-OPERATIVE VARIABLES AND CANCER RECURRENCE - UNIVARIATE ANALYSIS

Variables	"p"
Age	0.30
Sex	0.053
Stage	0.001
Grade	0.07
Haemoglobin	0.53
Albumin	0.88
Tranfusion	0.97
Anastomotic technique	0.027
Anastomotic site	0.44

Table 6.6

ASSOCIATION BETWEEN TUMOUR RECURRENCE AND PERI-OPERATIVE VARIABLES - COX REGRESSION ANALYSIS

Variables	Regression coefficient	Standard error	"p"
Age			0.60
Sex			0.051
Stage	0.683	0.218	0.001
Grade			0.38
Haemoglobin			0.75
Albumin			0.53
Tranfusion			0.93
Anastomotic technique	- 0.546	0.263	0.035
Anastomotic site			0.71

The "relative hazard" of recurrence (in the stapled group relative to the sutured group) was 0.58 (95% confidence intervals: 0.35 - 0.97). In other words there was a 42% reduction in the incidence of tumour recurrence associated with the use of stapling instruments (95% confidence intervals: 3% - 65%).

Univariate analysis with regard to disease free survival revealed that tumour stage, grade, anastomotic technique and patients' gender had statistically significant associations with cancer specific mortality (Table 6.7). In the multiple regression model, sex and tumour grade no longer remained significant independent predictors of poorer disease free survival. However the significantly higher cancer specific mortality for patients with sutured anastomoses remained unaltered after correcting for tumour stage (Table 6.8).

Table 6.7

RELATIONSHIP BETWEEN PERI-OPERATIVE VARIABLES AND CANCER SPECIFIC MORTALITY - UNIVARIATE ANALYSIS

Variables	"p"
Age	0.06
Sex	0.04
Stage	0.0001
Grade	0.04
Haemoglobin	0.47
Albumin	0.63
Tranfusion	0.89
Anastomotic technique	0.01
Anastomotic site	0.46

Table 6.8

ASSOCIATION BETWEEN CANCER SPECIFIC MORTALITY AND PERI-OPERATIVE VARIABLES - COX REGRESSION ANALYSIS

Variables	Regression coefficient	Standard error	"p"
Age			0.18
Sex			0.06
Stage	0.952	0.249	0.0001
Grade			0.34
Haemoglobin			0.73
Albumin			0.59
Tranfusion			0.72
Anastomotic technique	- 0.695	0.294	0.015
Anastomotic site			0.75

The "relative hazard" of cancer specific mortality (stapled versus sutured) was 0.5 (95% confidence intervals: 0.28-0.89); i.e. the cancer specific mortality in the stapled group was 50% of that in the sutured group (95% confidence intervals: 11% - 72%).

6.4 DISCUSSION

This study has demonstrated a significant reduction in the incidence of tumour recurrence when stapling instruments were used for anastomotic construction instead of conventional manual suturing techniques in colorectal cancer surgery, which has not been reported before. Previous studies investigating the influence of anastomotic technique on recurrence and survival rates have reported either no difference between suturing and stapling [53,226,227] or have shown a poor outcome associated with stapling techniques [221-224,228]. All studies reporting high incidences of tumour recurrence in patients with stapled anastomoses have assessed the use of circular staplers in restorative resections for rectal carcinoma. This might suggest that the increasing use of stapling instruments has resulted in an inappropriate abandonment of abdominoperineal resection in favour of restorative resections in the management of rectal cancer. Surgical staplers have probably accelerated the rate with which sphincter saving procedures were adopted, however the retreat from total rectal excisions towards sphincter saving operations was well underway before the introduction of the circular stapling instruments. This change in the surgical management of rectal cancer was founded on extensive histopathological and clinical evidence which demonstrated that restorative rectal excisions were as "curative" as abdominoperineal resection[83,153-168].

It has been suggested that stapling instruments may allow a colorectal anastomosis to be performed at a lower level than would be possible by suturing techniques[38,84,85]. Conceivably, the effort to perform a low stapled anastomosis in such cases might lead to a compromise in the extent of tumour clearance. This contention has been expressed as a potential mechanism by which stapling techniques could adversely affect recurrence rates in patients with rectal cancer[222].

In theory there are mechanisms other than compromised tumour clearance, by which anastomotic technique could have an influence on the prognosis of colorectal cancer. In this context, the scraping of tumour cells by the intraluminal stapler gun and their deposition at the site of the anastomosis[228], increased intraluminal trauma predisposing to the implantation of exfoliated tumour cells[232], or delayed mucosal healing of stapled anastomoses[233] have been postulated as potential mechanisms by which stapling could have an adverse influence. The extent of resection for intra-peritoneal large bowel tumours would not be expected to differ when either sutures or staplers are used for establishing recontinuity. Therefore, if any of the above mechanisms have an effect, it would be expected that the results with stapled reconstructions following resection of intra-peritoneal colonic tumours would be worse than the results achieved by suturing. Of the reports in the literature comparing stapling and

suturing techniques, the only one that includes colonic (intra-peritoneal) tumours is the previously discussed study by Wolmark et al[53]. In this study survival, disease-free survival and recurrence rates were very similar after 856 sutured and 255 stapled colonic anastomoses.

The study presented herein is the only report in the literature where the influence of anastomotic technique on the outcome of colorectal cancer has been studied in randomised patient groups. In uncontrolled and non-randomised studies, it would be expected that anatomic considerations that encourage the use of staplers are also likely to be associated with a higher risk of tumour recurrence. The discordance between the results of our study and other previous reports (all of which were retrospective and uncontrolled) could be explained by this "patient selection" effect, which is likely to have biased the results of the previous studies. In contrast, the randomisation process used to determine the choice of anastomotic technique in our study should, in theory, have resulted in an even distribution of all extraneous variables between the sutured and stapled groups. As would be anticipated from the randomised nature of the study, the sutured and stapled groups were well matched in terms of the recorded peri-operative variables. Furthermore, we found that the only significant determinants of recurrence or mortality were tumour stage and anastomotic technique and the disease free survival

advantage in favour of the stapled group remained unchanged after correcting for tumour stage. However it is acknowledged that the study population represents a sub-group selected from a larger cohort of patients and the approach falls short of a prospective randomised evaluation of stapling and suturing techniques in relation to the outcome of colorectal cancer. It is therefore not possible to rule out a "patient selection" bias that may have operated in favour of the stapled group in the study.

Anastomotic materials have been implicated as a potential influence on local tumour recurrence in animal models. Phillips and Cook examined anastomotic tumour growth in rats which were given dimethylhydrazine to induce tumours two months after suturing the bowel with various suture materials [234]. The highest incidence of anastomotic tumours was associated with monofilament steel wire sutures. These results do not necessarily imply that stainless steel is a more potent promoter of tumours. They might reflect the persisting local proliferative instability [235], due to more prolonged retention of steel wires at the anastomosis compared with other suture materials. This instability may act as a promoter during the initiation phase of carcinogenesis. McGregor [236] found that stainless steel was associated with significantly fewer tumours compared with polyamide or polyglycolic acid when the carcinogen injection preceded suture implantation in an animal

model. Calderisi and Freeman also reported an experimental study where the carcinogen administration preceded caecal insertion of one of six different types of suture materials in a rat model [237]. They found that slowly absorbed or non-absorbable suture materials promote tumour induction locally at the site of suture insertion and multifilament steel wire sutures were associated with the highest number of tumours. One should be wary of drawing clinical conclusions from these studies. In man colorectal carcinogenesis is a very slow process and it is generally agreed that if metachronous tumours develop at the site of an anastomosis, they do so many months after surgery [173]. There is no evidence in the literature to suggest that in the clinical setting anastomotic materials will be associated with metachronous carcinogenesis. On the other hand some experimental evidence suggests that suture materials may have a role in the implantation of tumour cells at an anastomosis. O'Dwyer et al[238], in an in-vitro assay demonstrated that tumour cells adhere differentially to various suture materials. McGregor et al[239], showed that this adherence was significantly greater to polyamide or polyglycolic acid than to stainless steel. Furthermore, in an animal model stainless steel was shown to entrap and transfer significantly fewer intraluminal tumour cells compared with braided suture materials[239].

All sutured anastomoses were constructed using braided polyamide and/or polyglycolic acid in our patients. Differing properties of stainless steel and braided suture materials with respect to tumour cell entrapment or adherence may, at least in part, have been responsible the poorer results observed in patients with sutured anastomoses in our study.

In conclusion, our results suggest that the use of stapling instruments in colorectal cancer surgery could be associated with a reduction in the incidence of local recurrence and cancer specific mortality, by as much as 50%. The mechanisms leading to this reduction in this study remain unclear, however if confirmed by other studies, these observations are likely to have important clinical implications. Further studies are required to clarify the role of anastomotic techniques and materials on the long-term outcome of patients with colorectal cancer.

CHAPTER 7

ANASTOMOTIC LEAKS AND RECURRENCE OF COLORECTAL CANCER

7.1 INTRODUCTION AND AIMS

Anastomotic dehiscence is a well recognised cause of intra-abdominal sepsis and operative mortality in patients undergoing large bowel surgery. In this context, the healing process in intestinal anastomoses and various factors associated with anastomotic leaks have been studied extensively [8,51,54,58,240]. However there has been no published reports investigating the long-term clinical consequences of anastomotic dehiscence following surgery for malignant disease. The current study addresses this issue. The specific purpose of the investigation was to determine whether there is an association between anastomotic leaks and tumour recurrence in patients with colorectal cancer.

7.2 PATIENTS AND METHODS

Patients in this study were selected from the database of the prospective randomised clinical trial described in Section I. The cohort chosen consisted of those patients who have had potentially curative resections for colorectal cancer, followed by a complete assessment of anastomotic integrity. Curative resection was defined as complete removal of all macroscopicaly evident disease at the time of surgery with tumour free resection margins on histological examination. Investigation of anastomotic integrity was considered as complete only when clinical assessment was supplemented by contrast radiography performed in the early post-operative period. Methods of data collection, surgical techniques and contrast enemas are described in Chapter 2. As detailed in Chapter 2, during the trial period contrast radiography was routinely performed only in easily accessible anastomoses (namely left sided colonic and colorectal anastomoses). Therefore, no patient with carcinoma of the right colon was included in this analysis. A clinical leak was defined as an anastomotic dehiscence confirmed by re-operation or post-mortem, appearance of faecal material from drains, development of a colo-cutaneous fistula or development of systemic sepsis associated with peritonitis in the post-operative period. Any extravasation of the radiological contrast medium detected on radiography was considered as a radiological leak. 180 patients fulfilled the entry criteria into the study, i.e. a

curative resection for primary colorectal carcinoma followed by clinical and radiological assessment of anastomotic integrity. Six of these patients died within 30 days of the operation and were excluded from the analysis. A further seven patients were excluded (none of whom had anastomotic leaks) because of incomplete follow-up. The remaining 167 patients constitute the study population.

Staging of tumours was done after surgical exploration of the abdomen and pathological examination of the resection specimens. The TNM classification, as described in Chapter 6, was used for staging[229]. Local recurrence was defined as clinical, histological or post-mortem evidence of recurrent carcinoma at or in the region of the anastomosis. Tumour spread to other intra-abdominal sites, liver, lung, bones etc. were considered as "distant recurrences". Survival and disease free intervals were measured from the time of resection. The estimation of cumulative probabilities of tumour recurrence and cancer specific mortality in patients with and without leaks was done by Kaplan and Meier's life-table analysis.[230]. The incidence of tumour recurrence and the mortality rates for the patients with and without leaks were compared using the log rank test. The correction of mortality and recurrence rates for the distribution of tumour stage in the two groups was done by Cox's proportional hazards regression model [231].

7.3 RESULTS

The study population was divided into two groups based on the assessment of anastomotic integrity. In 135 patients there was no evidence of a clinical or a radiological leak in the post-operative period. These patients constituted the "No leak" group. Among the remaining 32 patients ("Leak" group), contrast enemas revealed a radiological leak on 30 occasions and 15 patients developed clinically evident anastomotic leaks.

Patient characteristics for the two groups are outlined in Table 7.1.

TABLE 7.1.

PATIENT CHARACTERISTICS

	LEAK (n= 32)	NO LEAK (n= 135)
Age (Mean ± SD)	68.4 ± 10.9	69.2 ± 9.1
Sex (Male/Female)	18/14	67/68
Anastomotic technique (Sutures/Staples)	19/13	59/76
Operating times (mins) (Mean ± SD)	123.3 ± 40.9	129.0 ± 50.6
Peri-operative blood transfusion(%)	47 %	26 %

Table 7.2 outlines the stage of tumours for the patients in the two groups.

TABLE 7.2

DISTRIBUTION OF TUMOUR STAGE

TUMOUR STAGE	LEAK (n= 32)	NO LEAK (n= 135)
Stage I	2 (6%)	20 (15%)
Stage II	22 (69%)	77 (57%)
Stage III	8 (25%)	38 (28%)

Similarly, Table 7.3 demonstrates the distribution of tumour grade between the two groups.

TABLE 7.3

DISTRIBUTION OF TUMOUR GRADE

TUMOUR GRADE		LEAK (n= 32)	NO LEAK (n= 135)
GX	Cannot be assessed	0 (0%)	6 (4%)
G1	Well differentiated	3 (9%)	19 (14%)
G2	Moderately differentiated	23 (72%)	94 (70%)
G3/G4	Poorly differentiated	6 (19%)	16 (12%)

At the end of a mean follow-up period of 24.7 months (range: 10-56 months) tumour recurrence was detected in 40 patients. Fifteen of the patients with recurrence were in the "leak" group (46.9%) and 25 were in the "no leak" group (18.5%). The site of recurrences in the groups are shown in Table 7.4.

TABLE 7.4

SITE OF TUMOUR RECURRENCE

RECURRENT TUMOUR	LEAK (n= 32)	NO LEAK (n= 135)
Local	9	8
Local and distant	4	9
Distant	2	8
ALL RECURRENCES	15	25

The life table analysis of the cumulative probability of tumour recurrence in the two groups is illustrated in Figure 7.1. By the end of the 24th post-operative month 49.4% (SEM: 9.8%) of patients with leaks developed recurrence, compared with 16.7% (SEM: 3.7%) of those in the "no leak" group. The incidence of tumour recurrence, assessed by the log-rank test, was significantly higher for patients with leaks ($p< 0.001$).

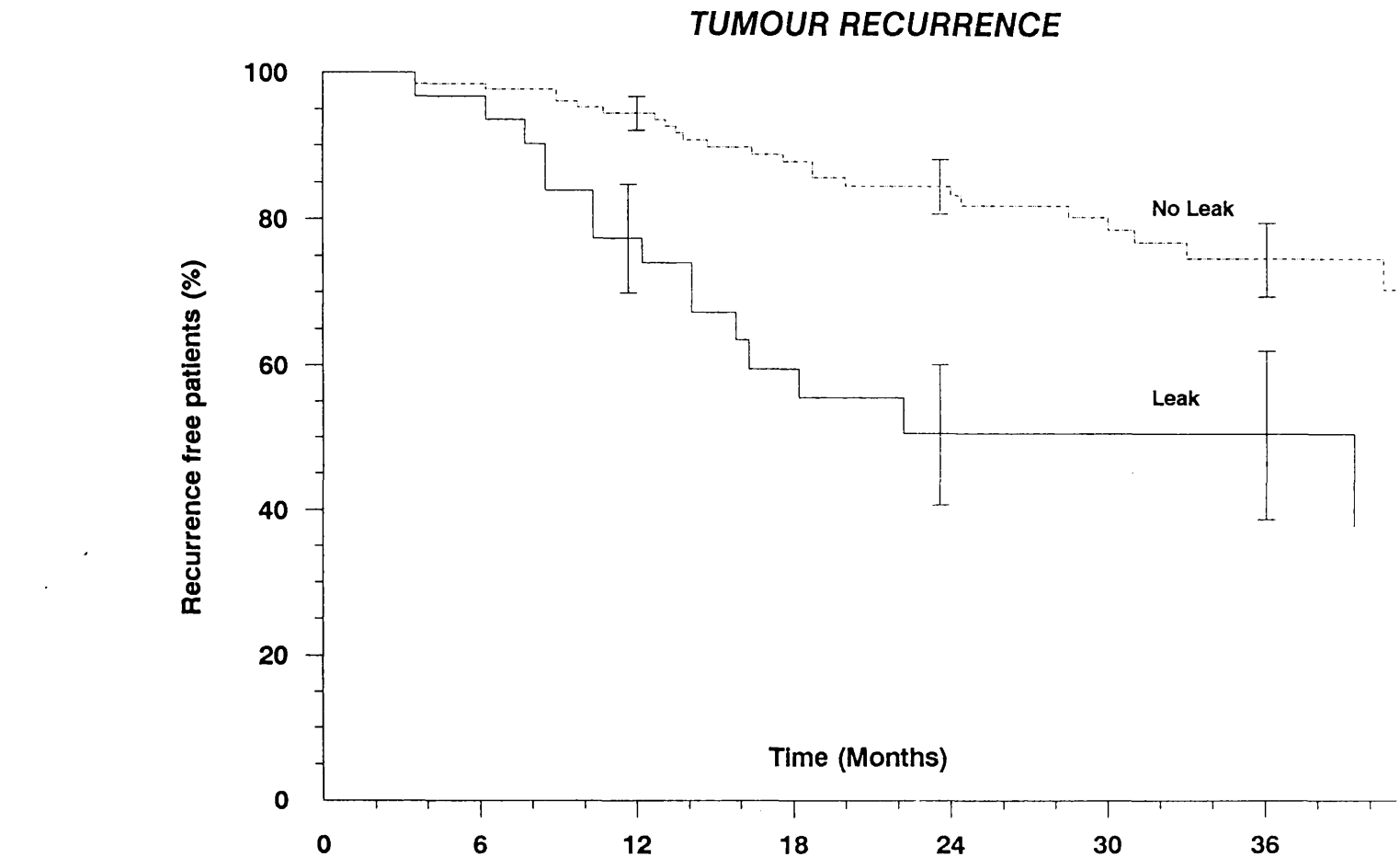

TUMOUR RECURRENCE
Recurrence free patients (%)
100
80
60
40
20
0
No Leak
Leak
Time (Months)
0
6
12
18
24
30
36
NUMBER OF PATIENTS AT RISK
NO LEAK 135 129 109 86 62 46 24
LEAK 32 30 23 15 8 6 5

Figure 7.1

A similar life-table analysis with regard to cancer specific mortality in the two groups is illustrated in Figure 7.2. Patients with leaks had a significantly higher cancer-specific mortality compared with their counterparts who did not have leaks ($p< 0.001$). By the end of the second post-operative year the proportion of patients ($\pm$ SEM) who had died as a result of cancer was 36.9% ($\pm$ 9.7%) in the "leak" group and 12.6% ($\pm$ 3.3%) in the "no leak" group.

There were two patients with Stage I tumours in the "leak" group. Neither of these patients developed tumour recurrence and both were alive at the time of analysis. In the "no leak" group there were 20 patients with Stage I tumours. Two of these patients had local recurrence detected at 20 and 49 months post-operatively and a third patient developed liver metastases at 33 months. For those patients with Stage II and III carcinomas, the cumulative probability of recurrence differentially for tumour stage is illustrated in Fig 7.3.

A similar life table curve with regard to cancer-specific mortality is illustrated in Fig 7.4. These two analyses demonstrate that the disease free survival advantage, in favour of patients with no leaks, remains unaltered when the patients are stratified according to tumour stage.

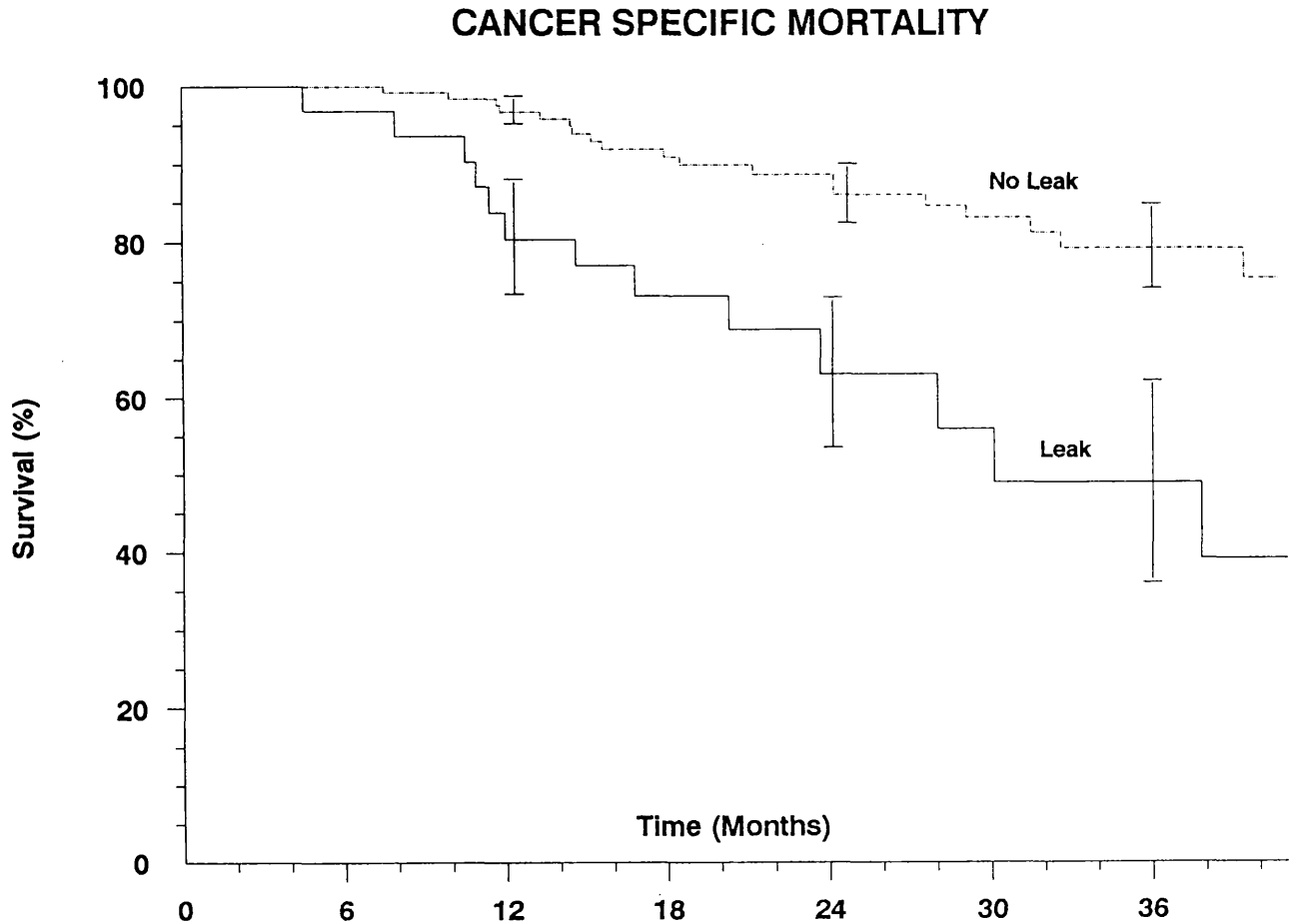

CANCER SPECIFIC MORTALITY
100
80
60
40
20
0
Survival (%)
No Leak
Leak
Time (Months)
0
6
12
18
24
30
36
NUMBER OF PATIENTS AT RISK
NO LEAK 135 132 112 89 67 51 27
LEAK 32 30 24 19 11 8 6

Figure 7.2

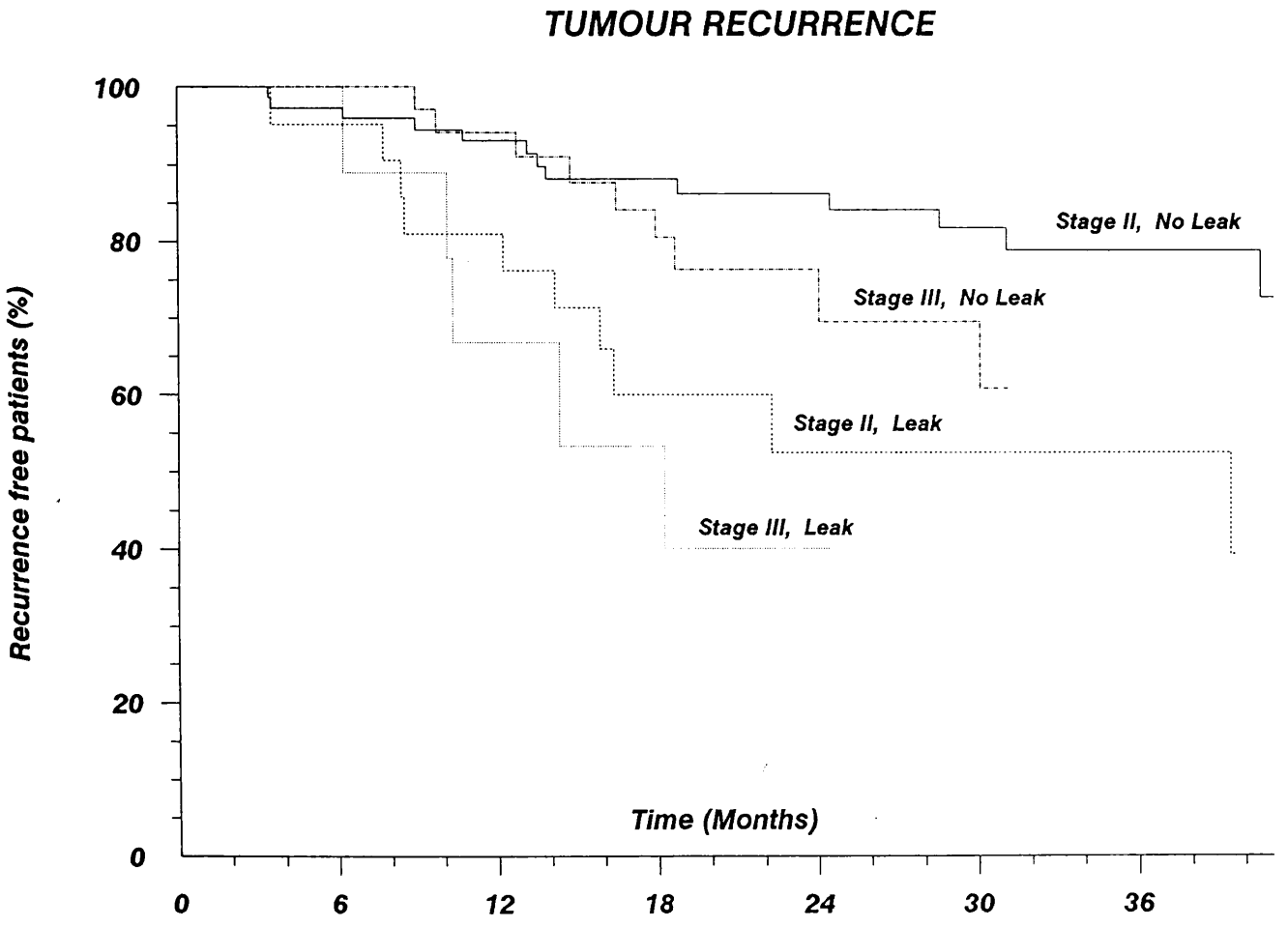

TUMOUR RECURRENCE
Recurrence free patients (%)
100
80
60
40
20
0
Stage II, No Leak
Stage III, No Leak
Stage II, Leak
Stage III, Leak
Time (Months)
0
6
12
18
24
30
36

Figure 7.3

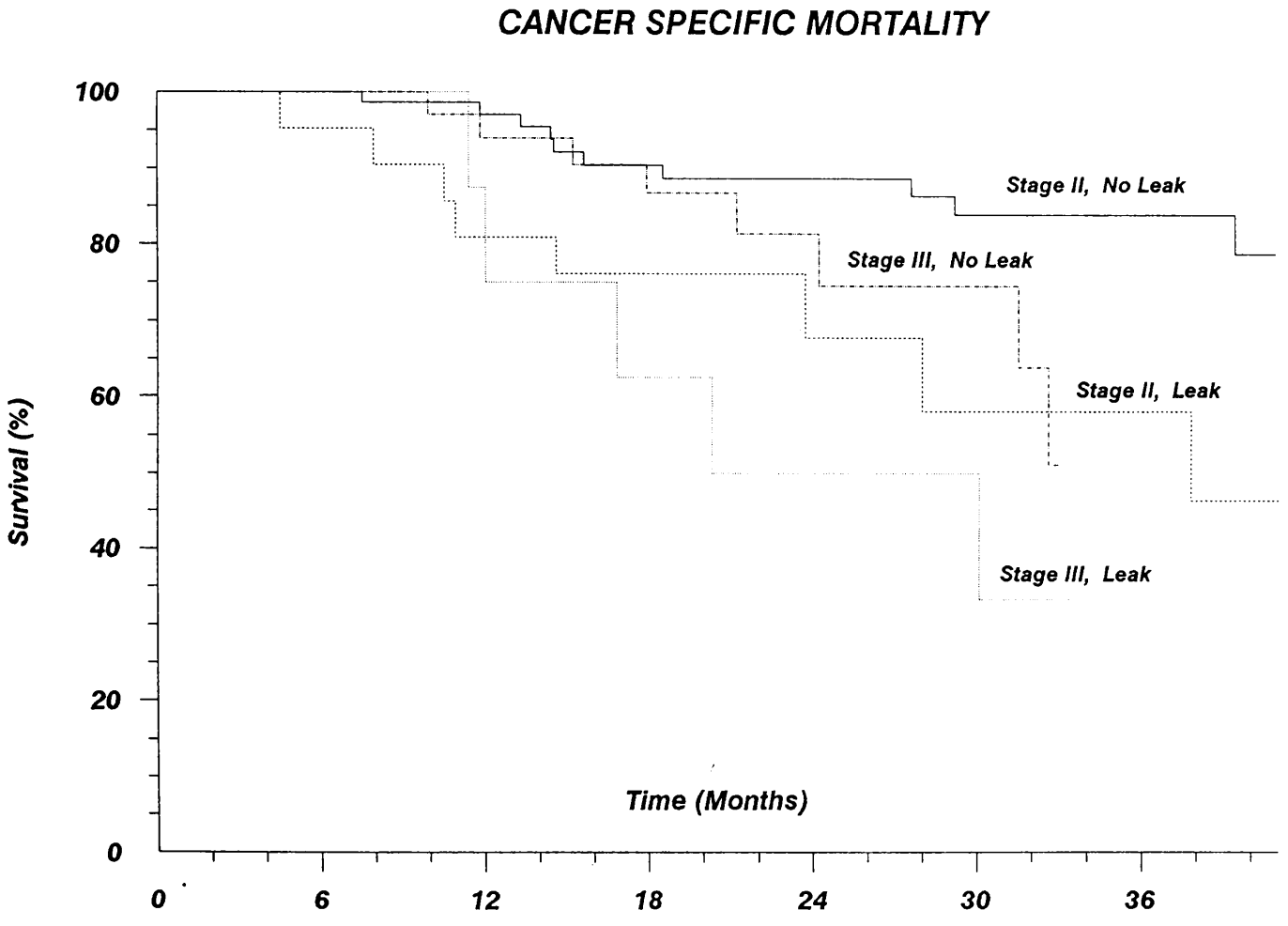

CANCER SPECIFIC MORTALITY
100
80
60
40
20
0
Survival (%)
Stage II, No Leak
Stage III, No Leak
Stage II, Leak
Stage III, Leak
Time (Months)
0
6
12
18
24
30
36

Figure 7.4

Cox's regression analysis was used to adjust for the potential influence of tumour stage on the overall results. The incidence of tumour recurrence and the cancer-specific mortality remained significantly higher for patients with leaks after correcting for tumour stage (p= 0.003 and p= 0.001 respectively).

In a separate analysis the relative association of clinical and radiological leaks with the outcome was investigated (Table 7.5).

TABLE 7.5

RECURRENCE AFTER CLINICAL OR RADIOLOGICAL LEAKS DIFFERENTIALLY

	ANASTOMOTIC LEAKS	
RECURRENT TUMOUR	**Radiological only (n= 17)**	**Clinical ± Radiological (n= 15)**
Local	4	5
Local and distant	2	2
Distant	0	2
None	11	6

There were 15 patients who had clinically evident anastomotic leaks. Thirteen of these patients had also had a leak demonstrated on radiography. Nine recurrences (60%) were detected in this sub-group. The remaining 17 patients in the

"leak" group had a leak demonstrated radiologically without any evidence of clinically evident anastomotic disruption. Six of these patients (35.3%) subsequently developed tumour recurrence.

7.4 DISCUSSION

This study has demonstrated a previously unreported association between anastomotic leaks and tumour recurrence in patients undergoing surgical resection for colorectal carcinoma. Higher incidence of tumour recurrence in patients who developed post-operative anastomotic leaks was also paralleled by a higher cancer specific mortality. Our findings also suggest that the adverse influence of anastomotic leaks on prognosis is independent of tumour stage. However, patients with anastomotic leaks are, by definition, not comparable to those who recover from surgery without any anastomotic complications. Other considerations that dictate a poor outcome such as tumour fixity, vascular and lymphatic invasion, lengthy and difficult resections etc. could be expected to occur more frequently in the group with leaks. Therefore these findings could potentially be a reflection of the differences in the two groups of patients being compared. On the other hand, one could postulate that in the presence of a post-operative anastomotic leak, exfoliated intra-luminal tumour cells gain enhanced and continuing access to pericolic tissues resulting in a higher incidence of local recurrence. Current evidence suggests that at the time of surgery patients with colorectal cancer have viable tumour cells present in the bowel lumen and that these cells are capable of implanting themselves and causing tumour growth [195-197]. Ranbarger et al., in 1982 reported a review of 200 patients undergoing

curative abdominoperineal resections for rectal carcinoma [241]. In 49 of these patients, iatrogenic perforation of the rectum occurred at the time of surgery, resulting in an increase in the incidence of local recurrence from 34% to 57%. When Dukes B cancers were taken separately, the recurrence rate was 8.1% in patients without surgical injury and 25.9% in those with perforation (p= 0.01). Slanetz from New York reported 174 curative resections for colorectal cancer during which disruption of the bowel wall occurred [242]. In 67 of these patients the cancer itself was inadvertently perforated, while in the remainder the injury to the bowel wall occurred some distance away from the tumour but still within the resection specimen. The overall five year survival in these 174 patients was 29%. The 67 occasions where the tumours were disrupted at the time of surgery were associated with five year survival rates of 14% in the colon and 9.3% in the rectum. The incidence of local recurrence in the event of tumour disruption was 65%. Neither this report by Slanetz, nor the study by Ranberger et al., is directly comparable to our study. The factor associated with a poor outcome in these studies was intra-operative tumour perforation whereas we investigated the influence of immediate post-operative anastomotic leaks. Nevertheless these observations lend support to the contention that tumour cells extravasated from the bowel lumen in patients with colorectal cancer may lead to implantation metastasis.

Sperling et al., reported a study on 13 patients who had undergone large bowel resections for perforated tumours [243]. Contrary to the results mentioned above, the 5 year survival in this small group was 65.7%. The failure of implantation metastases to develop after spontaneous perforation of large bowel cancers has been attributed to infection. Vink in 1954 reported an experimental study using the Brown-Pearce carcinoma in rabbits [244]. This work demonstrated that when the animals received no pre-operative antimicrobial drugs or bowel preparation the risk of tumour implantation at large bowel anastomoses was significantly reduced. In 1960 Cohn and Atik repeated essentially the same experiment and confirmed that the presence of infection in this model significantly inhibits tumour implantation at large bowel anastomoses [245]. Herter and Slanetz reported a clinical study in 1968 where 222 patients with anterior resections for rectal cancer were reviewed [190]. The incidence of suture-line recurrence in this group was 9.5% when pre-operative bowel preparation was combined with antibiotics, while in patients who received no antibiotics the corresponding incidence was 1.6%. Despite this apparently increased incidence of tumour recurrence, the authors concluded that the benefits of peri-operative antibiotic prophylaxis and bowel preparation far outweigh its potential risks and proposed that the risk of implantation metastasis be dealt with by the adoption of other mechanical and chemical

measures. If infection does indeed have an antagonistic effect on tumour implantation, this would suggest that escape of tumour cells from the lumen of a "clean" bowel (such as an immediate post-operative anastomotic leak) may have more serious consequences than spontaneous perforation of tumours.

The only previously published reference to anastomotic leaks in relation to local tumour recurrence appears in a report by Phillips et al., summarising the results of the Large Bowel Cancer Project [137]. These authors reported 1,645 patients who had curative resections for colorectal carcinoma with an anastomosis. Anastomotic dehiscence occured in 133 of these patients. There was no difference in the incidence of local recurrence between patients with leaks (19.5%) and those without (18.6%). The discordance between Phillips et al's., results and our observations might be due to the differences in the definition of leaks and the different patient populations studied. In the large bowel cancer project no radiological studies were carried out, hence the leaks refer to clinically evident anastomotic disruptions only. On the other hand our study was restricted to patients whose anastomoses were easily accessible for assessment by contrast radiography, which effectively excluded all cancers in the right colon.

In our study , the overall number of patients with leaks was small. Furthermore some patients had both radiological and clinical leaks. It is, therefore, difficult to determine the relative contribution of radiological leaks and clinical leaks differentially on the observed results. Nevertheless, there were 17 patients in the study who had radiological leaks only and six of these patients developed tumour recurrence (35.3%). This incidence was still more than twice as high as that observed in patients with no leaks.

In conclusion, this study has demonstrated an association between clinical and sub-clinical anastomotic leaks and recurrence of colorectal cancer. Patient selection appears to be a plausible explanation for this relationship, whereby leaks would occur more frequently in those patients who already have a poor prognosis. Alternatively anastomotic leaks may lead to a higher incidence of tumour recurrence as a result of implantation metastasis. Further experimental work which was designed and conducted to test this latter hypothesis constitutes the subject of the next section of the thesis.

Irrespective of the explanation for the results, the observations presented in this study may have potential clinical implications. They suggest that post-operative contrast radiography, in conjunction with clinical assessment

of anastomotic integrity might identify a group of "high risk" patients, who might benefit from closer follow-up after surgery for colorectal cancer.

The results also add another dimension to the findings of the clinical trial reported in Section I of this thesis, where patients with sutured colorectal anastomoses were shown to have significantly higher incidence of radiological leaks than those with stapled anastomoses. Furthermore, when viewed in conjunction with the study presented in the previous chapter, these results raise the possibility that the influence of anastomotic leaks and anastomotic techniques on the recurrence of colorectal cancer could be inter-related. We have shown that stapled colorectal anastomoses have a significantly lower radiological leak rate compared to sutured ones. Assuming that leaks do lead to a higher recurrence rate, it seems possible that poorer recurrence rates associated with suturing techniques could be a result of the higher incidence of radiological leaks from such anastomoses. Conversely, it could be hypothesised that anastomotic technique was the main determinant of recurrence and the higher incidence of recurrence associated with leaks was, in fact, a function of the anastomotic technique, since a higher proportion of leaking anastomoses were sutured. A further possibility is that the influence of anastomotic leakage and anastomotic technique on tumour recurrence are independent of

each other. The two issues have been addressed by two separate studies carried out on different patient populations in this thesis. Hence it is not possible to provide a direct answer to the questions raised. Nevertheless, with the available data, an attempt was made to examine several peri-operative variables in relation to tumour recurrence, after excluding those patients who were known to have clinical or radiological leaks. For this purpose age, sex, pre-operative haemoglobin and albumin levels, tumour site, stage, grade and anastomotic technique were entered as simultaneous co-variates into Cox's proportional hazards regression model. Anastomotic technique lost its statistical significance as an independent predictor of tumour recurrence in this analysis. However, even after excluding leaks and adjusting for other co-variates, patients with stapled anastomoses had a significantly lower cancer specific mortality ($p< 0.05$). Admittedly this analysis artificially selects out a sub-group of patients from randomised patient groups. Furthermore, the fact that patients with right sided colonic cancers were not subjected to contrast radiography is not taken into account. Hence such patients are assumed not to have a sub-clinical leak, without the knowledge of their radiological leak status. Further studies are required to clarify the potential influence of (and the relative contributions by) anastomotic techniques and anastomotic leaks on the long term outcome of patients undergoing surgery for colorectal cancer.

SECTION III

EXPERIMENTAL STUDIES

CHAPTER 8

ANASTOMOTIC LEAKS
AND PERI-ANASTOMOTIC TUMOUR GROWTH

8.1 INTRODUCTION

A previously unreported association between anastomotic leaks and recurrence of colorectal cancer in man has been described in the clinical studies presented in Chapter 7. This section of the thesis describes experimental studies in a rodent model, formulated to investigate the relationship between integrity of large bowel anastomoses and local tumour growth and to examine the mechanisms responsible for any observed effect of anastomotic leaks.

In the initial phase, the experiments were aimed at developing a reliable and reproducible model of sub-lethal anastomotic leak in the rat. Following this, peri-anastomotic tumour growth was investigated in groups of rats with and without leaks in the presence of intra-luminal tumour cells. Finally, to determine whether anastomotic leaks could enhance local tumour growth through mechanisms other than the escape and implantation of intra-luminal tumour cells, growth patterns of systemic circulating tumour cells were investigated in the presence and absence of anastomotic leaks.

8.2 MATERIALS AND METHODS

In vitro studies described in this section were performed in the laboratories of the University of Glasgow, CRC Medical Oncology Department. Animal experiments were carried out at the University Department of Surgery, Western Infirmary, Glasgow.

8.2.1 Tumour cells

The tumour cell line used for all experimental studies was the Mtln3 clone of a rat carcinoma 13762 NF. The parent cell line for the Mtln3 tumour was developed and characterised as a chemically induced transplantable mammary adenocarcinoma in the Fischer rat by Segaloff [246]. The Mtln3 clone was isolated from spontaneous lung metastases of this tumour by Neri et al [247].

8.2.2 Culture medium

The culture medium used to grow the cells (F10/DMEM-FCS) was prepared with 22.5 mls of Ham's F10 solution (Gibco, Paisley, Scotland) and 22.5 mls of Dulbecco's DMEM medium (Gibco, Paisley, Scotland) in 400 mls of distilled water, supplemented by 2mM l-glutamine and 10% v/v foetal bovine serum. Antibiotics were not added to the culture medium.

8.2.3 Cell culture methods

Mtln3 cells were stored in liquid nitrogen in 1 ml plastic vials containing 10^5 - 10^6 cells suspended in F10/DMEM-FCS. To grow cells in culture, the frozen samples were defrosted rapidly and the contents of the vial were transferred into 25 cm^2 tissue culture flasks (Falcon; Becton Dickinson, Oxford, England). 5 mls of culture medium was added and 5% CO_2 in air was passed through the flasks for 30 seconds. They were incubated at 37°C for 24 hours, checked for contamination and the culture medium was replaced every 24 hours until the cells were confluent. Once confluent, cells were passaged by removing the medium, incubating them with trypsin/EDTA (0.25 % / 1 mM) (Gibco, Paisley, Scotland) for 5 minutes, removing the trypsin, resuspending the loosened cells in F10/DMEM-FCS and distributing them into further flasks in required concentrations. Each batch of frozen cells was passaged no more than six times to avoid phenotypic drift of cells. (248).

8.2.4 Growth characteristics of Mtln3 cells

To determine in vitro growth characteristics of Mtln3 cells, the wells of two 24 well cell culture plates (Linbro, Flow Laboratories, Irvine, Scotland) were filled with 10^4 cells suspended in 1 ml of F10/DMEM-FCS, using 1 ml per well. The wells in each plate were numbered consecutively and they were kept at 37°C in a 2.5% CO_2 incubator. Culture medium in

wells was replaced every 24 hours and starting from day 1 contents of three consecutive wells were counted every day for twelve days. To obtain counts, medium from the three wells to be counted was aspirated and cells were incubated with 0.5 mls of trypsin/EDTA solution for 5 minutes. The trypsin was then diluted with 0.5 mls of F10/DMEM-FCS in each well. This suspension was further diluted 1/10 in phosphate buffered saline (PBS) before counting at day 1. As the cells grew in number, this latter dilution was increased in a stepwise fashion from 1/20 at Day 3 to 1/200 by Day 7. The contents of each well were counted three times in an electronic counter (Coulter Electronics, Luton, England), obtaining nine counts for each 24 hour period. The mean of these nine values was used to plot the growth curve.

8.2.5 Clonogenic Assay

A clonogenic assay was performed to determine whether the radiological contrast medium used in the animal experiments had any toxic effect on the Mtln3 cells. The initial assay was carried out with Gastrografin (Schering, Burgess, West Sussex, England). The Gastrografin solution was filtered through a 0.2 micron bacteriological filter before being tested in the clonogenic assay. However all experimental dishes containing Gastrografin dilutions were seen to be contaminated, indicating that sterilisation of the contrast medium by filtering was not feasible. Accordingly a pre-

sterilised, similar radiological contrast medium (Urografin 325, Schering) was used both in the clonogenic assay and for contrast radiography in the animal experiments. In addition to undiluted Urografin 325, 1/2, 1/4 and 1/100 dilutions were assessed by the clonogenic assay. Two 25 cm^2 tissue culture flasks were used for each dilution. One ml of 7.7 x 10^4 cells/ml Mtln3 cell suspension and 9 mls of F10/DMEM-FCS was added to each flask and they were incubated at 37^oC in an atmosphere of 2.5% carbon dioxide in air for 48 hours. Cells in the control flasks were then trypsinised, resuspended, counted and diluted to a concentration of 10^3 cells/ml. Cells in the experimental flasks were exposed to the respective concentrations of Urografin (diluted in F10/DMEM-FCS) for 20 minutes. Following this all experimental flasks were also trypsinised and diluted, <u>without counting</u>, by the same dilution factor used for the control flasks. One ml aliquots of these diluted suspensions from each flask were transferred into Petri dishes, (60 cm Nunclon, Gibco, Paisley, Scotland) using four dishes for each flask. Four mls of F10/DMEM-FCS was added to each Petri dish and they were incubated at 37^oC in 2.5% carbon dioxide in air for 10 days. At the end of the incubation period, culture medium was removed from the Petri dishes and they were washed twice with PBS to remove remaining debris. Each dish was then incubated twice for five minutes in Methanol for fixation and left to dry for 24 hours. They were then stained with "crystal violet" and individual colonies in each dish were counted.

8.2.6 Assessment of cell viability

Growth of cells in culture medium for the animal experiments were carried out in the same manner as described for the in-vitro experiments. However the cell counts and adjustment of concentrations were done by a haemocytometer instead of the Coulter Counter and at each count the proportion of viable cells was assessed by the Trypan Blue exclusion method[203].

8.2.7 Experimental animals

Fischer F344 rats were used for all the animal experiments. This is an inbred rat strain which is syngeneic with the Mtln3 tumour cell line. The animals were obtained from Harlan-Olac Ltd. (Bicester, England) or were bred in the animal house of the University Department of Surgery, Western Infirmary, Glasgow, using breeding pairs obtained from the same institution. The animals weighed between 170-290 g at the start of the experiments. A mixture of male and female rats were used during the preliminary phase while the anastomotic leak model was being developed. For the remainder of the experiments male rats were used exclusively. The animals were kept in the specifically designated area of the laboratories of the Department of Surgery. They were housed in groups of two, three or four, in polypropylene cages. Food and water were made available ad libitum, including the immediate pre-operative and post-operative periods. The diet consisted of "Biosure" commercial animal food (Special Diet Services, Manea, Cambridgeshire, England).

8.2.8 Anaesthesia

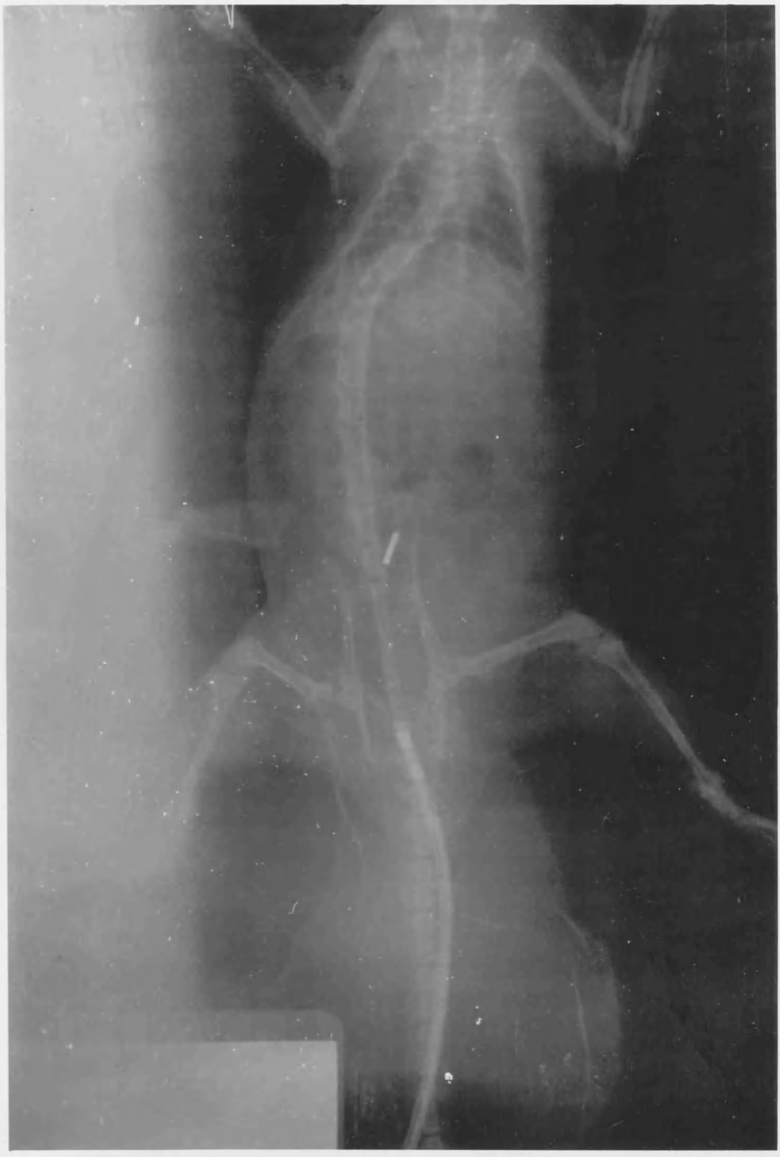
All procedures including Urografin enemas were carried out under general anaesthesia. Anaesthesia was induced by 5% Halothane (Halothane M-B, May & Baker Ltd., Dagenham, England) in glass chambers for 30-45 seconds and maintained by intra-peritoneal injection of a combination of Midazolam (Hypnovel, Roche Products Ltd., Welwyn Garden City, England) and Hypnorm (Janssen Pharmaceutical Ltd., Grove, Oxford, England). The anaesthetic combination consisted of equal parts of Hypnorm (diluted 1/2 in sterile water) and Midazolam (diluted 1/2 in sterile water) and was used at a dose of 0.3 mls/100 g body weight.

8.2.9 Contrast radiography

Contrast radiography to assess anastomotic integrity was performed under general anaesthesia in the supine rat. The radiological contrast medium (Urografin 325) was administered trans-anally into the rectum via a non-sterile 7F Swan-Ganz catheter (Figure 8.1). The balloon of the catheter was inflated with 1 ml of air to prevent reflux of the contrast medium. A hydrostatic manometer system was incorporated into the injection circuit and the pressure during the instillation of Urografin was monitored. A total of 1.5 - 3 mls of contrast medium was used to outline the distal large bowel and the injection pressure was kept below 40 cm H_2O. Contrast injections were monitored fluoroscopically using a Siemens

Figure 8.1

Contrast radiography Pre-injection radiograph



The tip of the Swan-Ganz catheter is seen in the rectum. The radioopaque titanium clip marks the level of the anastomosis.

"Siremobil 2" mobile screening unit. Sample radiographs were taken at 40 kV and 70 mA with 0.12 second exposure time. Any extravasation of the contrast medium from the anastomosis (marked by a titanium clip) observed during fluoroscopy was recorded as a leak. Before accepting an anastomosis as water-tight the distal large bowel was filled with contrast for a minimum of 2 cm beyond the anastomosis and intermittent screening was continued for one minute.

8.2.10 Histopathology

At the time of autopsy, each animal had a segment of large bowel containing the anastomosis excised, for histological examination. Animals exhibiting macroscopic tumour growth in other tissues had these nodules sampled also, for histological confirmation of the diagnosis. All specimens were fixed in 10% buffered formol saline and processed for paraffin embedding. Five micron paraffin sections were cut from each tissue block. The sections were stained with haematoxylin and eosin for light microscopic examination.

8.3 EXPERIMENTAL PROTOCOL AND SURGICAL PROCEDURES

Three sets of experiments were carried out as detailed below. For all experiments laparotomies were performed through midline abdominal incisions and wound closures were effected in two layers using continuous 4/0 silk (Ethicon Ltd., Edinburgh, Scotland).

8.3.1 Development of anastomotic leak model

Two different surgical procedures were investigated with respect to the establishment of an anastomotic leak model. The first technique involved the fashioning of a longitudinal colotomy along the anti-mesenteric border of the descending colon. The length of this incision was standardised to 16 mm by using scaled calipers. This colotomy was then closed using either 5 or 8 interrupted, full thickness 5/0 silk sutures (Ethicon Ltd., Edinburgh, Scotland) in two groups of animals in an attempt to achieve reproducible models for suture lines with and without leaks. The level of the suture line was marked with a 3.7 mm titanium clip (Premium Surgiclip 9.0", Auto Suture Co., U.K. Ascot, England) attached to the lowermost suture. To control for the potential influence of suture material on tumour cell implantation, the actual number of sutures used was the same in all animals. To achieve this, the colotomy closure was effected by five sutures in the group intended for leaks, however, three other sham sutures were inserted and tied as a loose loop. In the group intended for

a water-tight suture line, all of the eight sutures inserted were tied in a normal fashion to approximate the colotomy. All animals were subjected to contrast radiography 24 hours post-operatively to check whether or not they had leaks.

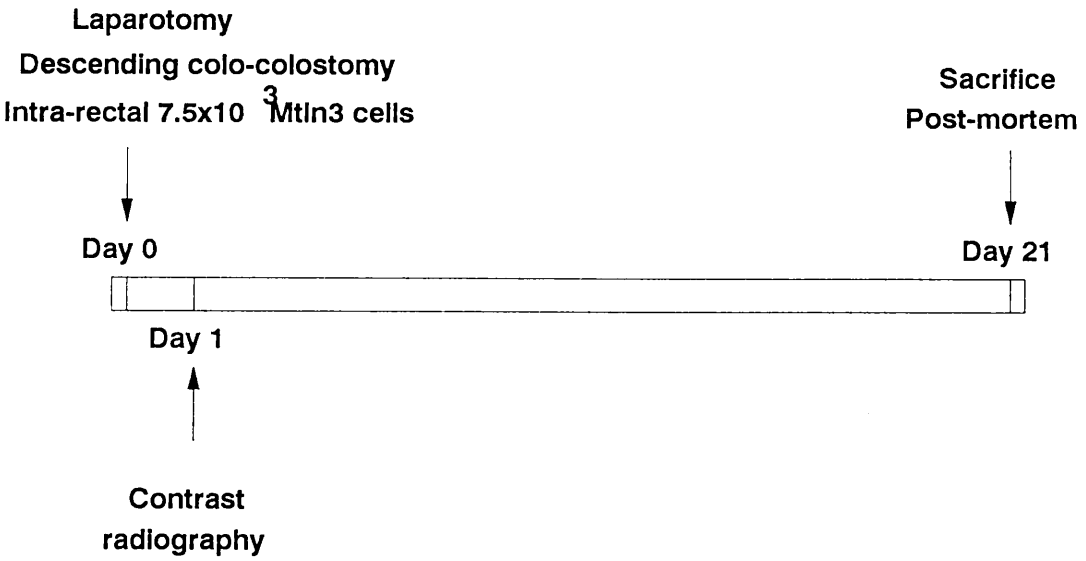
The second surgical procedure tested in an attempt to develop an anastomotic leak model was an end-to-end large bowel anastomosis. The descending colon was transected with scissors taking care not to damage the mesenteric vessels. The bowel ends were then re-anastomosed without any resection in an end-to-end fashion using either 4 (for the "leak") or 7 (for the "no-leak" group) interrupted full thickness 5/0 silk sutures. As in the previous experiment, animals in the leak group had only four sutures used for the anastomosis, but also had three additional sham sutures inserted and tied in a loose loop. Anastomoses were marked with a 3.7 mm titanium clip attached to one of the sutures as described previously (Figure 8.1). All anastomoses were tested intra-operatively for air-tightness. For this purpose the abdominal cavity was filled with warm saline until the anastomosis was completely submerged. Air was then insufflated into the rectum via the plastic tubing of a 19 gauge butterfly needle (from which the needle had been removed). The cannula was inserted trans-anally and advanced for 2 cm in the rectum. Clamping the colon proximal to the anastomosis was not necessary to achieve distention of the bowel with air, however peri-anal skin was

pinched around the catheter to prevent air escape during insufflation. In the group intended for "no leak" if the anastomosis was seen to be leaking air, additional sutures were inserted until an air-tight anastomosis was achieved. All animals also underwent contrast radiography 24 hours post-operatively as described above and they were killed at 14 days post-operatively. Post-mortem examinations were performed on all animals at the time of sacrifice.

8.3.2 Intraluminal tumour cells and tumour growth

These experiments were designed to determine whether an anastomotic leak had any bearing on peri-anastomotic tumour growth in the presence of viable intraluminal tumour cells. Three groups of rats were used for this purpose. A schematic representation of the experimental protocol is given in Figure 8.2. Animals used as the control group had a simple laparotomy and laparotomy closure at day 0 without any bowel anastomosis. The animals in the "leak" and "no leak" groups had end-to-end descending colon anastomoses constructed in the fashion described above. The "leak" and "no leak" status of the anastomoses in the respective groups were ascertained by checking for air-tightness per-operatively. Following this all animals including the control group had 7.5×10^3 Mtln3 cells suspended in 0.2 mls of F10/DMEM-FCS instilled into the rectum via a 19F butterfly cannula tubing inserted trans-anally at the time of surgery while the abdomen was still

INTRALUMINAL TUMOUR CELLS & ANASTOMOTIC TUMOUR GROWTH EXPERIMENTAL PROTOCOL



Control Group:	No anastomosis
Leak Group:	Anastomoses with intra-operative air leak and post-operative radiological leak
No Leak Group:	Air tight anastomoses and no radiological leaks

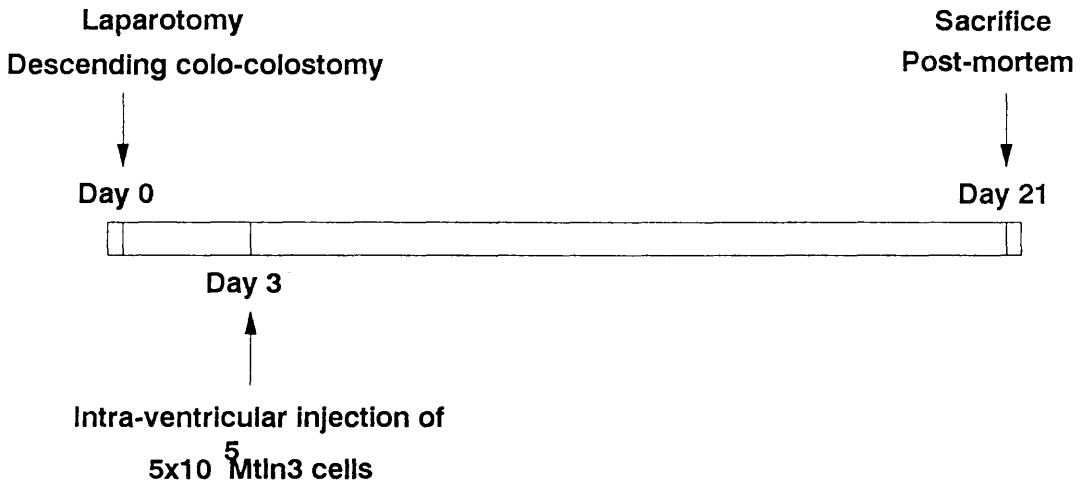
Figure 8.2

open. All animals were allowed to recover from surgery and 24 hours post-operatively they were subjected to contrast radiography as described above. This was followed by an observation period of three weeks during which the animals were examined daily. Those showing obvious signs of ill-health were sacrificed early and a post-mortem was carried out. Otherwise all animals were killed and post-mortems were performed at day 21 post-operatively.

8.3.3 Circulating tumour cells and tumour growth

Four groups of animals were used in these experiments. The experimental protocol is summarised in Figure 8.3. The control group had a laparotomy only day 0 without any bowel anastomosis. The "leak" and "no leak" groups had end-to-end descending colon anastomoses as described in the previous experiments. The fourth group ("double anastomoses") of animals had two large bowel anastomoses performed during the same procedure at day 0. One of these was an air-tight anastomosis of the descending colon carried out in the same fashion as for the anastomoses in the "no leak" group. The second anastomosis, which was also end-to-end, was performed more proximally in the descending colon just beyond the splenic flexure. It was intended for leak and constructed in the manner described for the "leak" group.

CIRCULATING TUMOUR CELLS AND ANASTOMOTIC TUMOUR GROWTH EXPERIMENTAL PROTOCOL



Control Group: No anastomosis

Leak Group: Anastomoses with air leak

No Leak Group: Air tight anastomoses

Figure 8.3

None of the animals had any intra-luminal tumour cells injected in this experiment. They were all allowed to recover from the initial surgical procedure. Three days later animals in all groups were anaesthetised again and through an incision in the neck the right carotid artery was exposed. It was isolated between two 4/0 silk ligatures and opened. A 2FG cannula with an external diameter of 0.63 mm (Portex Ltd., Hythe, Kent, England) was inserted into the carotid artery and advanced retrogradely into the left ventricle. 5×10^5 Mtln3 cells in 0.4 mls of F10/DMEM was injected into the left ventricle via this cannula and flushed in with saline. The cannula was removed, the carotid artery was tied off and the skin closed. The animals were allowed to recover and were observed for another 18 days. As in the previous experiments any animal becoming unwell before day 21 was sacrificed and an autopsy was done. Otherwise all animals were killed at day 21 and autopsies were performed.

8.4 RESULTS

8.4.1 In-vitro growth characteristics of Mtln3 cells

Table 8.1 demonstrates the number of cells (counted three times for each well) at each day throughout the growth curve experiment. The growth curve for Mtln3 cells plated in in-vitro culture is illustrated in Figure 8.4. The mean of the nine counts for each day, as illustrated in Table 8.1, was used to plot the growth curve. The initial lag phase before Mtln3 cells began to multiply was less than 24 hours. This was followed by a phase of exponential growth until the cells reached a saturation concentration of approximately 10^6 cells/ml at day 9. The multiplication of cells then reached a plateau which was followed by cell death.

Table 8.1

GROWTH CURVE of Mtln3 CELLS - CELL COUNTS

	Total no. of cells/well	Mean cell count
Day 1 (x 20)	106 / 104 / 121 347 / 326 / 313 282 / 249 / 261	4.66×10^3
Day 2 (x 20)	1493 / 1475 / 1398 1404 / 1394 / 1425 1298 / 1152 / 1114	2.68×10^4
Day 3 (x 40)	1057 / 1001 / 1004 751 / 755 / 761 707 / 715 / 709	3.32×10^4
Day 4 (x 40)	599 / 566 / 546 633 / 603 / 644 886 / 910 / 867	2.78×10^4
Day 5 (x 40)	10001 /10399 / 9855 11712 /11804 /11995 9464 / 9633 / 9682	4.20×10^5
Day 6 (x200)	4308 / 3694 / 3680 3277 / 3216 / 3285 3147 / 3125 / 3165	6.87×10^5
Day 7 (x 200)	3465 / 3564 / 3632 3263 / 3164 / 3349 3925 / 3870 / 3897	1.43×10^6
Day 8 (x 400)	740 / 834 / 758 795 / 774 / 914 765 / 748 / 710	3.14×10^5
Day 9 (x400)	2889 / 2936 / 2964 3120 / 3116 / 3068 2641 / 2584 / 2515	1.15×10^6
Day 10 (x400)	3047 / 3046 / 3039 3041 / 2992 / 2925 3202 / 3313 / 3154	1.23×10^6
Day 11 (x400)	2600 / 2679 / 2602 3027 / 2931 / 2986 2931 / 2903 / 2786	1.10×10^6
Day 12 (x400)	733 / 758 / 689 859 / 807 / 803 805 / 894 / 875	3.20×10^5

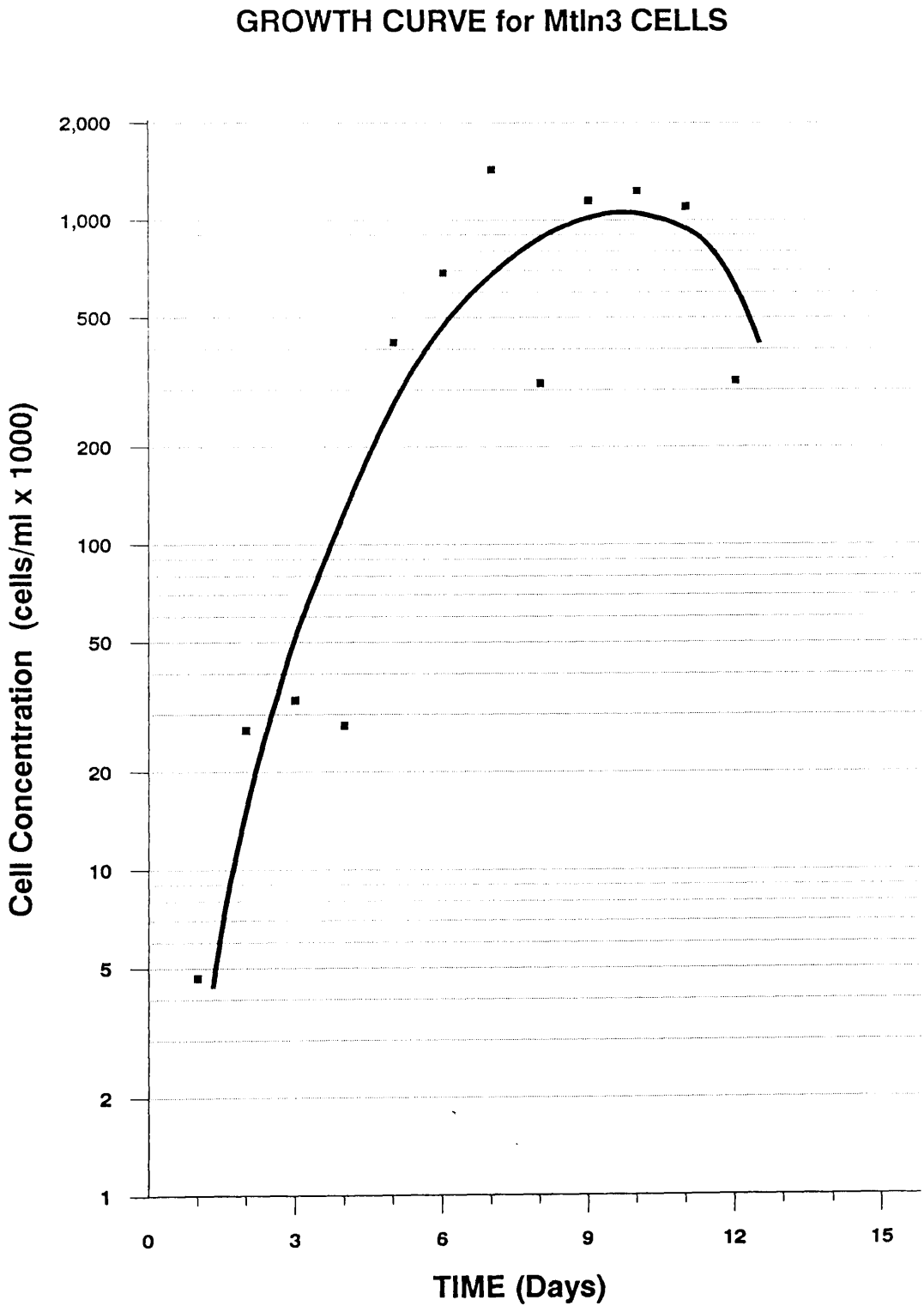

GROWTH CURVE for MtIn3 CELLS
Cell Concentration (cells/ml x 1000)
2,000
1,000
500
200
100
50
20
10
5
2
1
0
3
6
9
12
15
TIME (Days)

Figure 8.4

8.4.2 The effect of radiological contrast medium on Mtln3 cells

A clonogenic assay was used to determine whether Urografin had any toxic effect on Mtln3 cells. As detailed earlier, cells exposed to 1/1, 1/2, 1/4, and 1/100 dilutions of Urografin 325 were compared with control dishes in the final colony counts. Eight Petri dishes were counted for each concentration giving a total of 40 dishes including the controls. All dishes contained more than 200 colonies, some of which were confluent which made accurate quantification impossible.

8.4.3 Development of anastomotic leak model in the rat

No animal in any of the experiments developed peritonitis, sepsis or died as a result of anastomotic dehiscence.

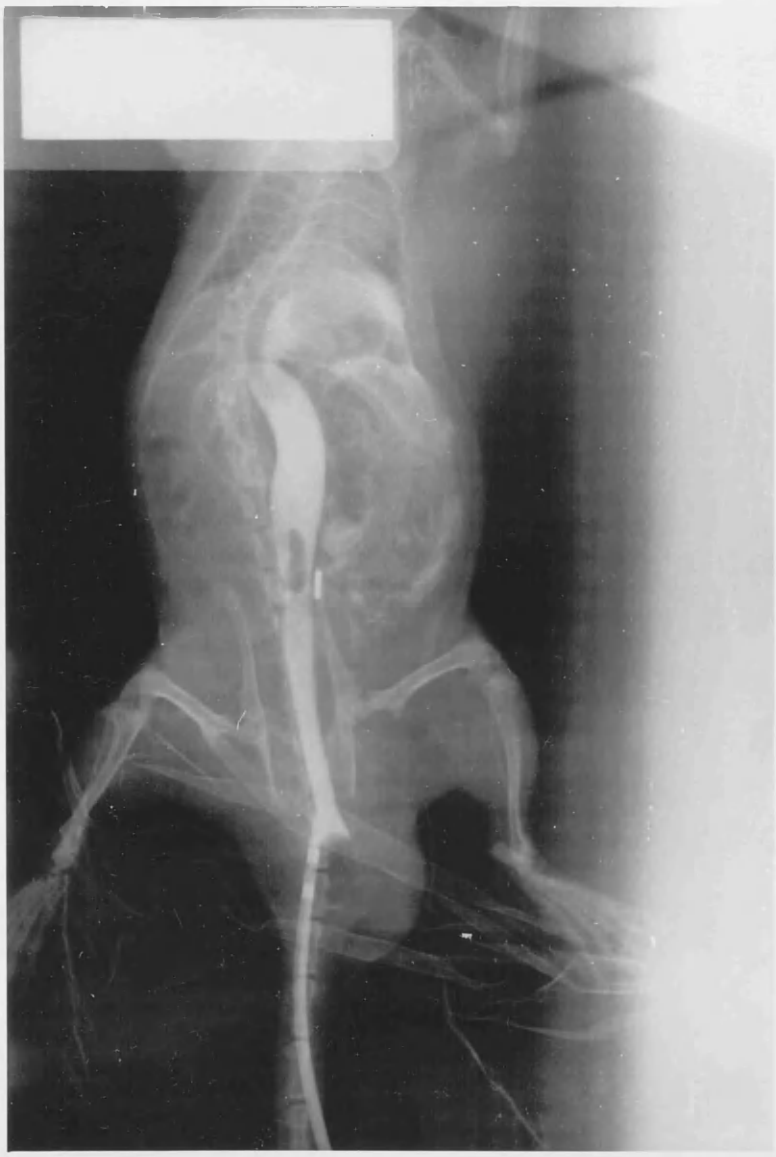
In an attempt to produce consistently "leaky" and consistently "water-tight" anastomoses in the animals, the first model tested was a longitudinal colotomy of a standard length, closed in two different manners in two groups of animals. The first group in this experiment consisted of 16 rats, where the suture line was re-approximated with the intention of producing a leak. Seven of these animals (44%) had a leak demonstrated on radiography 24 hours post-operatively. In the "no leak" group where the suture lines were intended to be water-tight there were also 16 animals. Two of these died under anaesthesia. Of the remaining 14 animals, three (21%)

were shown to have a leak by Urografin enemas 24 hours post-operatively. All animals had autopsies performed 14 days post-operatively. The only notable feature on autopsies was some flimsy adhesions around the anastomosis irrespective of whether or not a leak had been demonstrated radiologically. These results indicate that, in terms of anastomotic integrity there is a poor correlation between the intention at the time of surgery and the Urografin enema findings. In other words, closing a 16 mm descending colotomy with the two different surgical techniques employed, does not result in predictable and consistent patterns of "leaky" and "water-tight" anastomoses. This model was therefore abandoned for subsequent experiments.

The next anastomotic model tried consisted of end-to-end descending colon anastomoses. In order to have an objective intra-operative measure for the assessment of the anastomotic integrity, these anastomoses were tested for air leaks at the time of surgery. Sixteen animals had anastomoses created with the intention of producing a leak. One animal in this group died per-operatively. All the remaining animals had air leaks demonstrated by intra-rectal air insufflation at the time of surgery. Twenty four hours later 14 of the 15 animals in this group were shown to have radiological leaks (Figure 8.5). In the "no leak" group (n= 16) four animals required one additional suture to render the anastomosis air-

Figure 8.5

Contrast radiography - Radiological leak

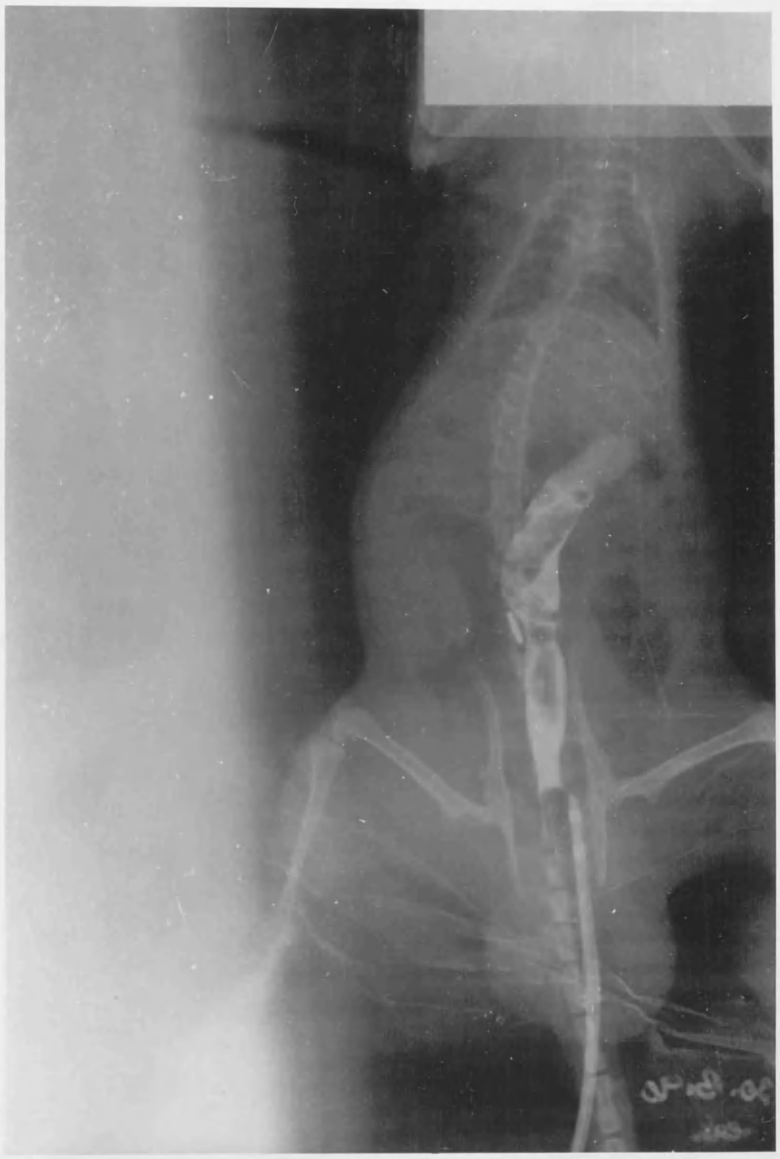


Radiograph is taken immediately after the leak from the descending colon anastomosis is demonstrated on fluoroscopy. A large amount of free intra-abdominal contrast medium is seen, mainly in the left upper quadrant of the abdomen.

Figure 8.6

Contrast radiography

Radiologically intact anastomosis



There is slight narrowing of the colon at the level of the anastomosis. Contrast medium has outlined the bowel for at least 2 cm beyond the anastomosis and there is no evidence of extravasation.

tight. An air-tight union was achieved with seven sutures per anastomosis in the remainder of the group. Urografin enemas 24 hours later revealed water-tight anastomoses in all 16 animals (Figure 8.6). In all subsequent experiments this model of end-to-end descending colo-colostomies with and without leaks was used.

8.4.4 Intraluminal tumour cells and tumour growth

A total of 58 animals were used for this experiment. The control group consisted of 10 animals which had laparotomies without any anastomosis, followed by intra-rectal instillation of tumour cells. The "leak" and "no leak" groups comprised 24 animals each. In both groups there was 100% agreement between the results of intra-operative "air leak" testing and post-operative contrast radiography. One animal in the "leak" group and two in the "no leak" group died under anaesthesia on day 1, during contrast radiography. Two other animals both in the "leak" group died at days 18 and 20 post-operatively. At post-mortem they were both seen to have widespread omental and mesenteric tumour deposits and large tumour masses at the level of anastomosis causing obstruction. The remaining animals in all groups were sacrificed at day 21 and autopsies were carried out. The results (excluding anaesthetic/ operative deaths) are summarised in Table 8.2.

Table 8.2

INTRALUMINAL TUMOUR CELLS AND TUMOUR GROWTH

EXPERIMENTAL GROUPS (Excluding operative deaths)	NUMBER (%) OF ANIMALS WITH TUMOURS
Leak group (n=23)	13 (57%)
No Leak group (n=22)	2 (9%)
Control group (n=10)	0 (0%)

Injection of 7.5×10^3 tumour cells trans-anally did not result in tumour growth in any animal in the absence of a bowel anastomosis. When an anastomosis was present in the descending colon, injection of intra-rectal tumour cells gave rise to peri-anastomotic and widespread intra-abdominal tumours in a variable proportion of animals dependent on the integrity of the anastomoses. The difference between the "leak" and "no leak" groups in this regard was statistically significant ($Chi^2 = 11.4$, 1 d.f., $p < 0.001$). Out of the 23 animals with anastomotic leaks 13 (57%) developed macroscopically obvious peri-anastomotic tumour masses

associated with smaller but widespread nodules of tumour disseminated inside the peritoneal cavity, mainly on the bowel mesentery, omentum and serosal surfaces of small and large bowel (Figure 8.7). Histological examination confirmed that the anastomotic masses and the intra-abdominal nodules consisted of sheets of deeply basophilic tumour cells, showing marked nuclear pleomorphism and prominent nucleoli. (Figure 8.8 and Figure 8.9). No animal had tumour in the lungs or liver. The pattern of growth was similar for the "no leak" group, however it was only observed in two animals (9%).

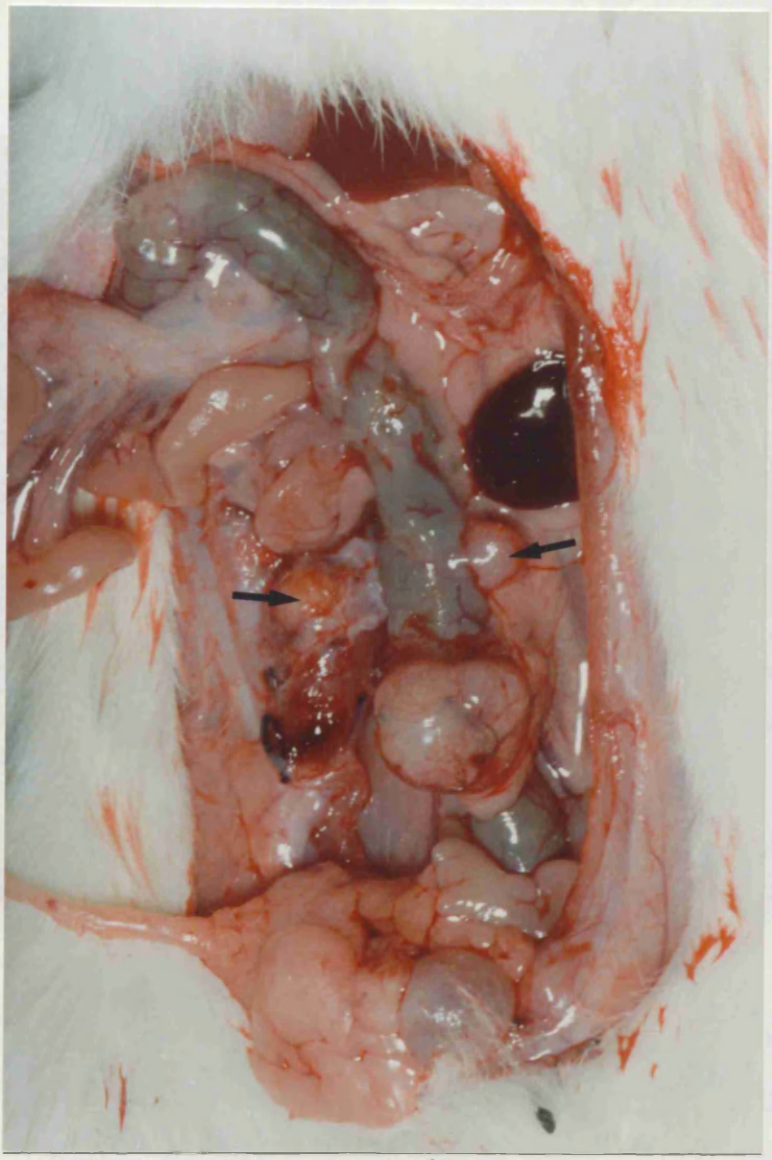
8.4.5 Circulating tumour cells and tumour growth

Four groups of animals were used for this experiment. Group A consisted of 14 animals where the anastomoses were shown to leak air per-operatively on day 0. Group B also comprised 14 animals, which had air tight anastomoses constructed on day 0. One animal in this group died during carotid cannulation on day 3. The animals in Group C (n=8) had double anastomoses as described earlier and Group D (n=8) consisted of control animals which had laparotomy only. Three animals in Group A, four in Group B and two in Group C were sacrificed between days 15-20 when they became clinically unwell with widespread tumour. The remaining animals survived until day 21 when they were killed.

Figure 8.7

Anastomotic and intra-abdominal tumour

Operative photograph

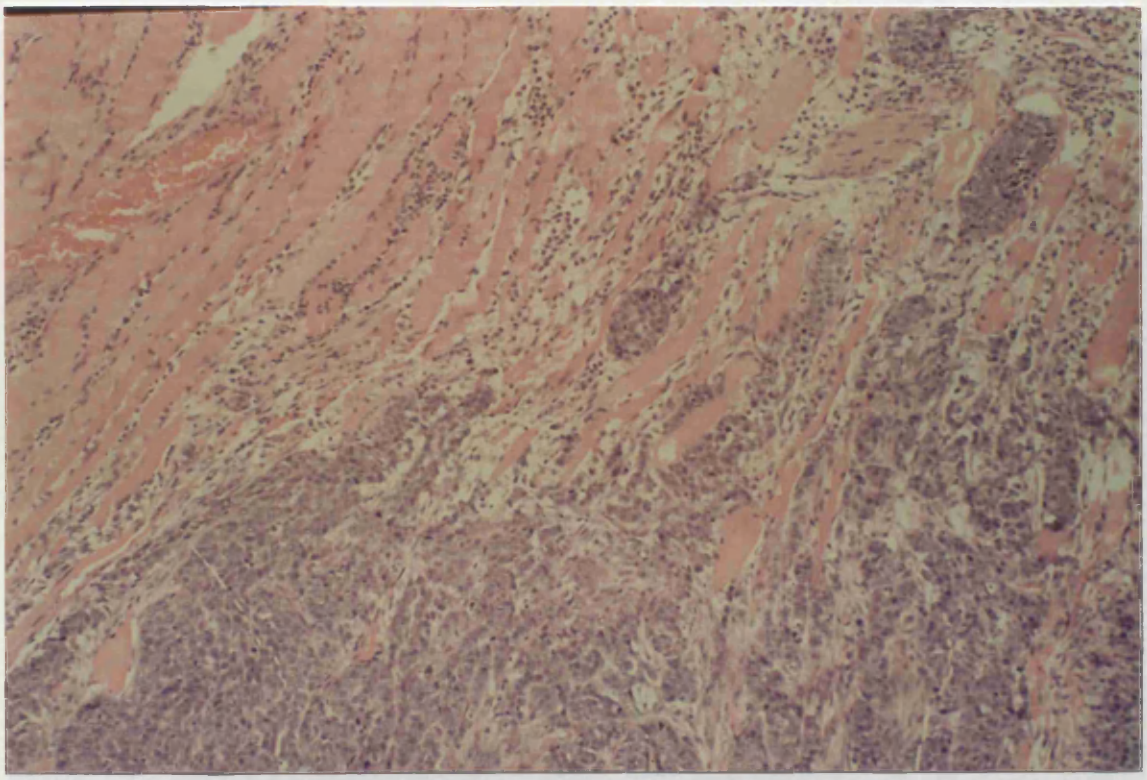


There is a large nodule of tumour at the site of the descending colon anastomosis. In two areas anastomotic sutures can be seen through the tumour, encased by the mass. Two further tumour nodules are highlighted by arrows.

Figure 8.8

Mtln3 tumour at large bowel anastomosis

Photomicrography (H.E., x60)

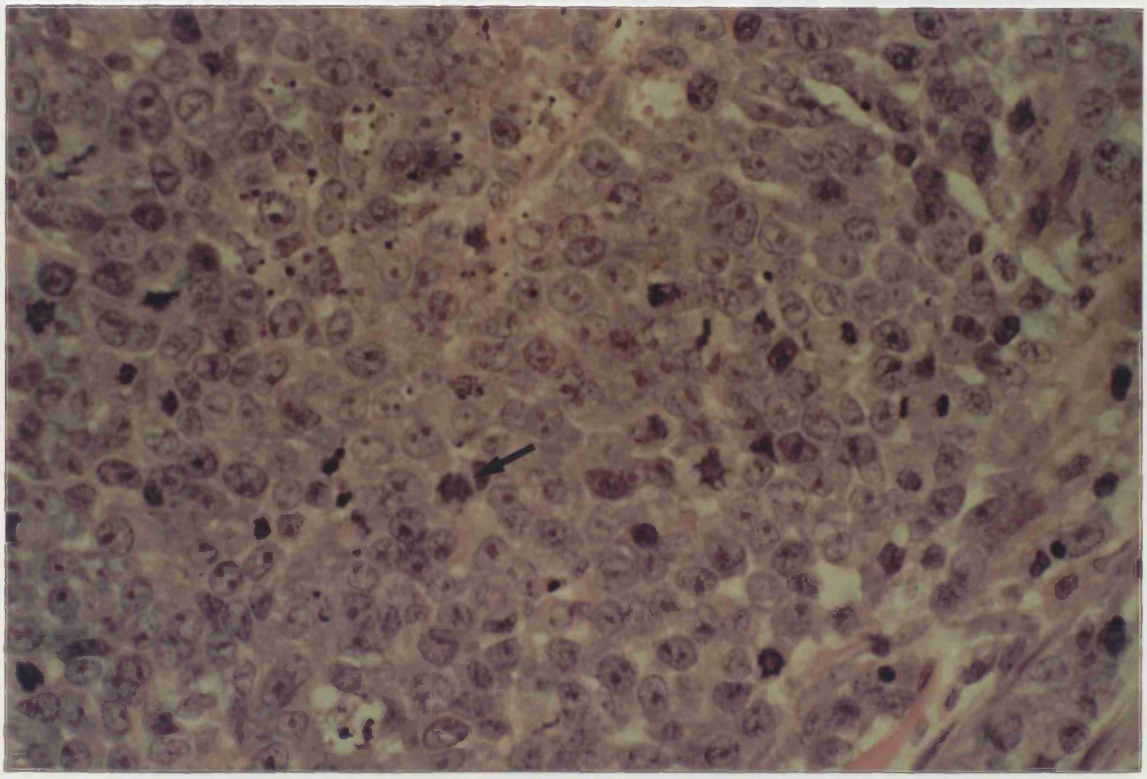


Sections from an anastomotic nodule, showing tumour which is infiltrating through the smooth muscle of the lamina propria.

Figure 8.9

Mtln3 tumour at large bowel anastomosis

Photomicrography (H.E., x200)



Sheets of anaplastic tumour cells are seen at high power on light microscopy. There is marked nuclear pleomorphism and frequent mitotic activity. The arrow points to an abnormal mitosis.

Post-mortem examinations revealed a similar pattern of tumour growth in Groups A, B and C, which contrasted sharply with the findings in Group D (Table 8.3). In the absence of a bowel anastomosis injection of 5×10^5 Mtln3 cells into the left ventricle resulted in widespread and uniformly sized nodules of tumour growth in the lungs, diaphragm and throughout the abdomen (Figure 8.10 and Figure 8.11), with the notable exception of the intestines. In Groups A, B and C the pattern of systemic tumour spread was similar to that in the control group. However in contrast with the refractory nature of the intestines to support tumour growth in the control group, the largest tumour nodules were almost always found to be located at anastomotic sites in Groups A, B and C (Figure 8.12 and Figure 8.13).

Table 8.3

DISTRIBUTION OF TUMOUR GROWTH FOLLOWING INTRA-VENTRICULAR INJECTION of Mtln3 CELLS

EXPERIMENTAL GROUPS	SITE OF TUMOUR		
	Anastomosis	Other sites	No tumour growth
Group A (n=14)	12/14	12/14	2/14
Group B (n=13)	12/13	12/13	1/13
Group C (n=8)	7/8	8/8	0/8
Group D (n=8)	0/8	7/8	1/8

Figure 8.10

Tumour in small bowel mesentery

Operative photograph

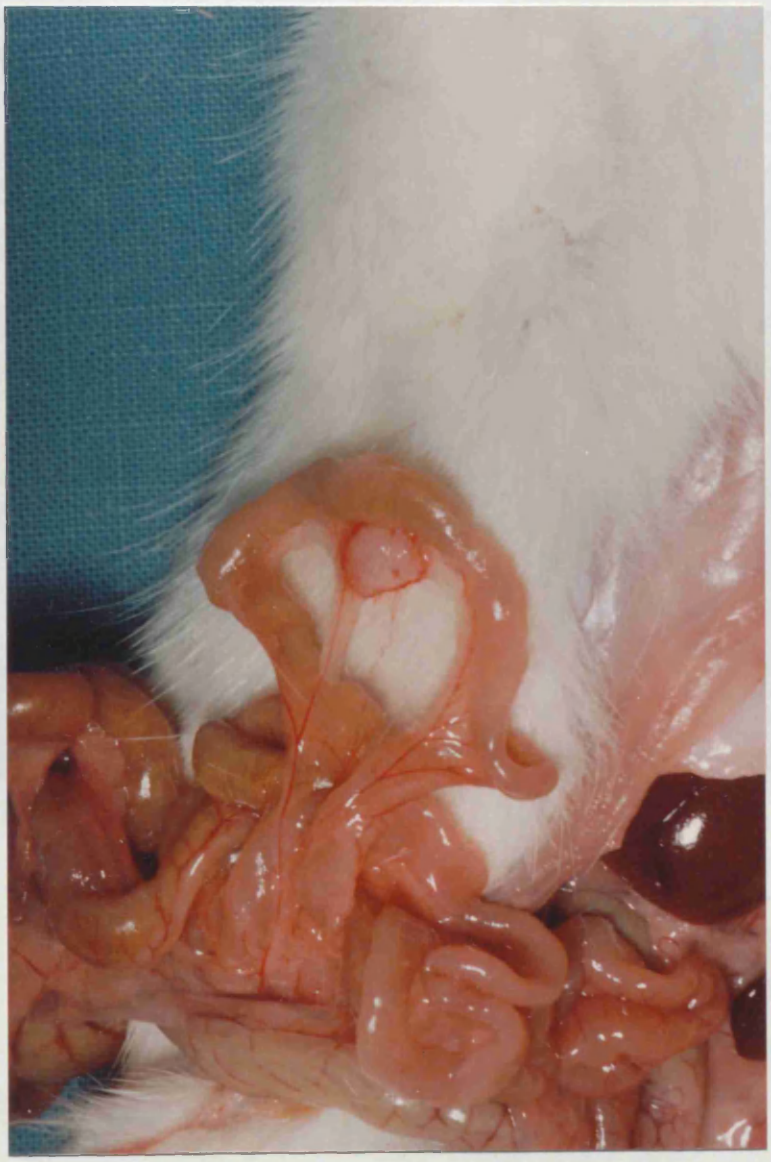
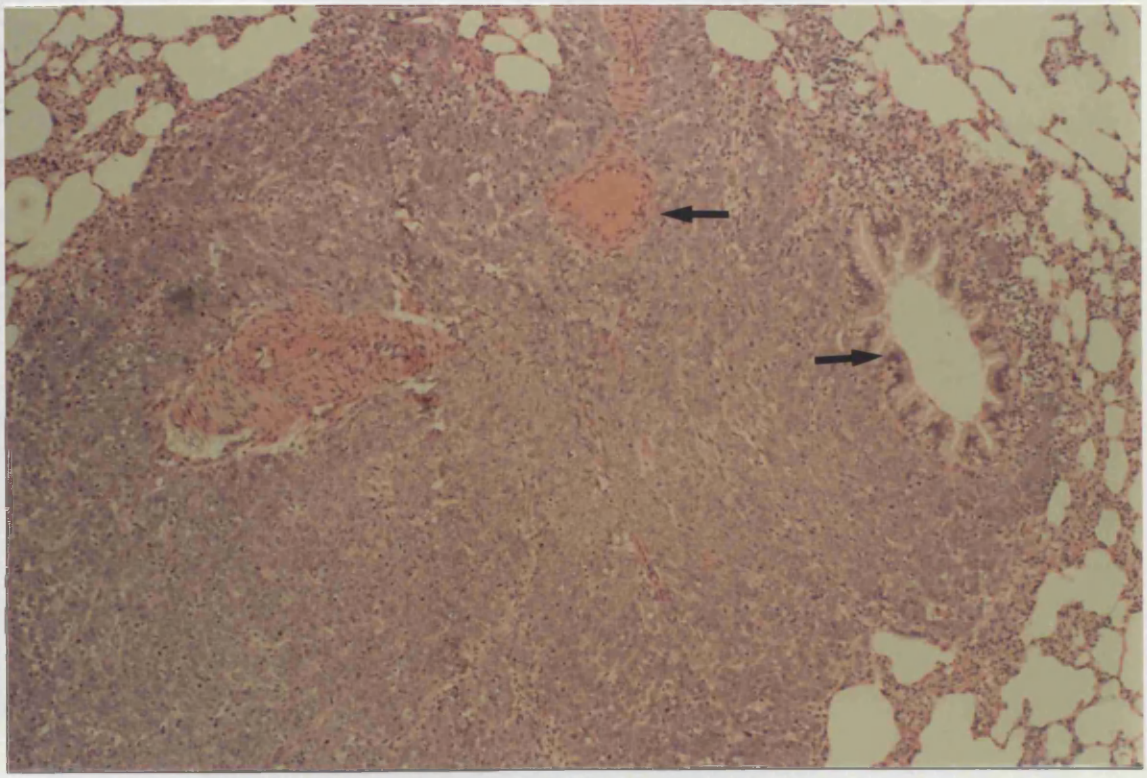


Figure 8.11

Mtln3 tumour in lung

Photomicrograph (H.E., x40)

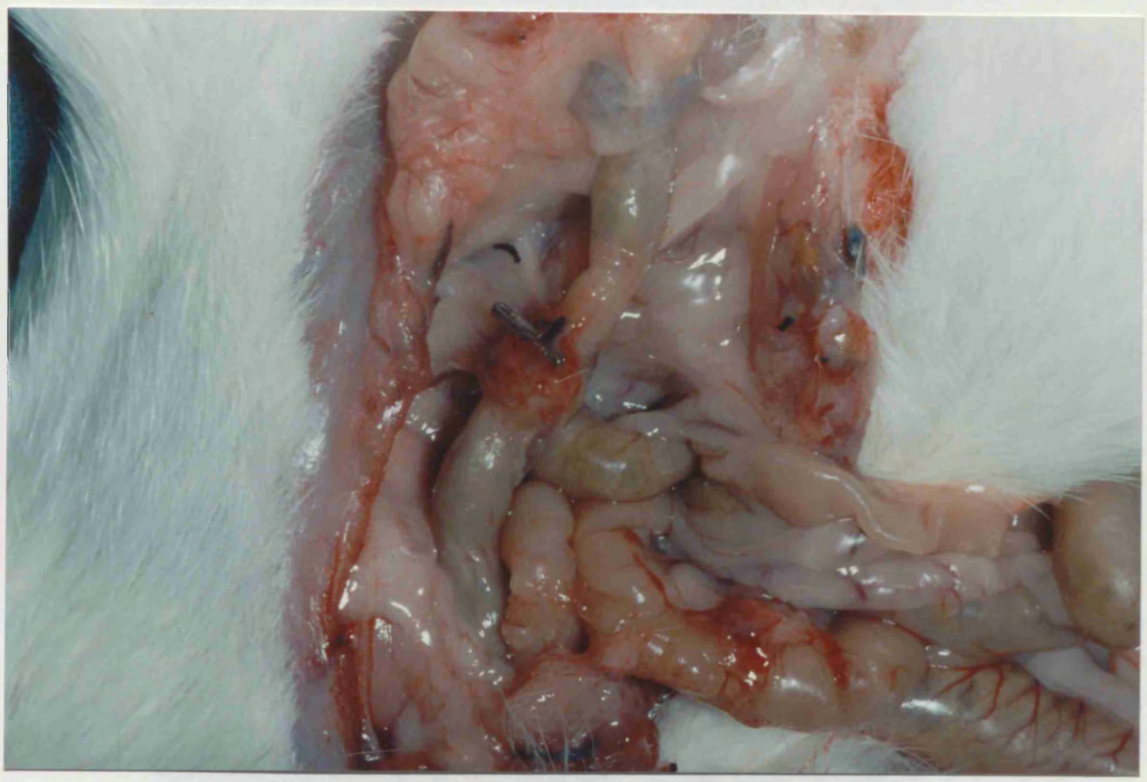


Tumour nodules in the lung have a propensity to grow in a peri-bronchial and peri-arterial location. A bronchus and an artery are highlighted by arrows.

Figure 8.12

Anastomotic tumour

Operative photograph

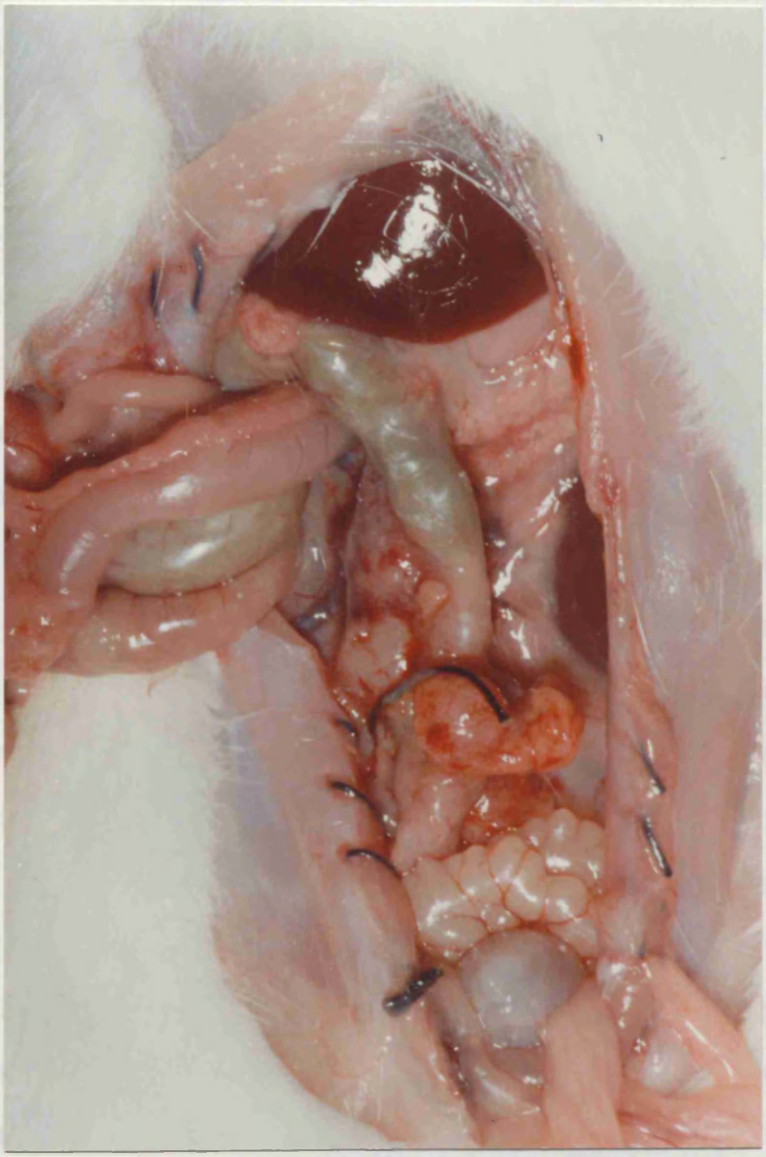


Early tumour growth at the site of the descending colon anastomosis. The titanium clip attached to one of the anastomotic sutures is visible adjacent to the tumour.

Figure 8.13

Anastomotic tumour

Operative photograph



Large tumour mass at the site of the descending colon anastomosis.

8.5 DISCUSSION

8.5.1 In vitro experiments with Mtln3 cells

In the clonogenic assay the initial incubation period was intended to allow the cells to reach the exponential growth phase before exposing them to Urografin. The growth curve experiment demonstrated that the cells would be well inside the exponential growth phase at the end of 48 hours of incubation. In the assay performed with Urografin 325, accurate quantification of the colonies was not possible due to large numbers of often confluent colonies in all dishes. However for the purposes of this study, these results demonstrate that Urografin has no direct cytostatic or cytotoxic effect on Mtln3 cells in any of the concentrations tested. In clinical practice Gastrografin is a more commonly employed radiological contrast medium for the assessment of anastomotic integrity, however the chemical composition of both substances are similar (*Urografin 325: sodium diatrizoate 40% w/v and meglumine diatrizoate 18% w/v, 325 mg/ml iodine. Gastrografin: sodium diatrizoate 10% w/v and meglumine diatrizoate 66% w/v, 370 mg/ml iodine.*). It seems unlikely therefore that the contrast medium used for radiography would have any influence on the in vivo viability of intraluminal tumour cells either in the experimental or in the clinical setting.

8.5.2 Intraluminal tumour cells and tumour growth

Current clinical and experimental evidence suggests that tumour cells are capable of implantation and growth on intestinal anastomoses [185-192, 195-197]. Exactly how this implantation takes place however is not known. There is general agreement in the literature that the normal colonic mucosa is not susceptible to tumour cell implantation [187,249,250]. Our results with intra-luminal tumour cells in the control animals are in accordance with this contention. On the other hand tumour cells readily implant on serosal surfaces, injured mucosa or exposed sub-mucosal and muscular layers of bowel[177-181, 188-193]. The suture materials used to construct the anastomosis may have a role in dragging tumour cells into the layers of the bowel wall or to the serosal surface. This has been shown to occur in experimental models [239,251]. Furthermore it has been demonstrated that the use of iodised sutures can significantly reduce the incidence of anastomotic tumour growth in experimental models [189,252]. Some recent work has demonstrated that tumour cells adhere in a variable manner to different suture materials[238,239]. This suggests that the anastomotic material may act as a nidus for the implantation of tumour cells in the anastomosis. It has also been shown that there is a variable potential among different suture materials to entrap and transfer intra-luminal cells to the anastomosis[239].

As well as anastomotic materials, the technique of anastomotic construction may also play a role in the implantation of tumour cells. Waltzer and Altemeier [253] studied closed and open anastomotic techniques in a rabbit model in relation to implantation of intra-luminal tumour cells and showed that tumours develop in 57% of open and 7% of closed anastomoses. Broyn and Helsingen reported another experimental study where imnplantation of Walker carcinosarcoma cells was assessed in inverted and everted anastomoses in rats [254]. No difference between the two techniques was observed in this study.

In our experiments all anastomoses were constructed using the same suture material and the same surgical technique. Therefore neither of these factors could be incriminated for the observed differences between the groups. The only deliberate difference between the experimental groups was the integrity of the anastomoses, which has not been previously studied in relation to peri-anastomotic tumour growth. We demonstrated that in the presence of intra-luminal tumour cells, a significantly higher proportion of animals with anastomotic leaks developed tumours compared to those animals which had air-tight anastomoses. It might be expected that an anastomotic leak would result in an altered biological environment in the region of the anastomoses such as a more prominent inflammatory response, changes in the local microcirculation, increased concentrations of various growth factors, etc. Although it is conceivable that such local

changes in the region of a leaking anastomosis could enhance implantation and growth of intra-luminal tumour cells, the pattern of tumour growth in the animals suggests that Mtln3 cells are capable of growing on serosal surfaces quite remote from the influence of any local peri-anastomotic change. This implies that, the mechanism responsible for the higher incidence of tumour growth in animals with leaks is likely to be enhanced and continuing escape of tumour cells from the lumen, rather than any alteration in the microenvironment as a result of the leak.

As a result of the clinical studies presented in the previous chapter, a hypothesis was put forward whereby the high incidence of recurrence in patients with leaks was ascribed to implantation metastasis. An association between tumour implantation and anastomotic leaks in the experimental work appears to be consistent with this hypothesis. However one must be wary of extrapolating too much from an animal model. It is debatable whether an anastomotic dehiscence or a radiological leak in the clinical situation is analogous to the deliberate leak model developed and used for these experiments. Furthermore the intra-luminal concentrations and the biological behaviour of Mtln3 cells and the host responses in this experimental model may have little resemblance to the situation in patients with colorectal cancer.

In summary, we have demonstrated that in an anastomotic leak model in the F344 rat, the integrity of the anastomosis is significantly associated with the risk of anastomotic and intra-abdominal tumour growth in the presence of intra-luminal Mtln3 tumour cells. Our results suggest that enhanced escape of the tumour cells from the bowel lumen in the presence of a leak is the responsible mechanism for the higher incidence of tumour growth observed in this model. Further studies are required to determine whether similar mechanisms play a role in colorectal cancer surgery in man.

8.5.3 Circulating Tumour Cells and Tumour Growth

It has been demonstrated that in experimental models locally implanted tumour cells grow preferentially at sites of injury[255]. The localisation of tumour growth in injured tissues also occurs when tumour cells are injected into the systemic circulation in animals[256,257]. Clinical observations demonstrating recurrent colorectal cancer at the sites of polypectomies or peri-anal wounds[177-181] also provide indirect evidence that tissue injury enhances the ability of that tissue to support tumour growth. Murphy et al., recently reported a study where patterns of tumour growth were investigated in rats which had been given intravenous, intra-portal and intra-arterial tumour cell injections[258]. These authors demonstrated that some tissues including the intestines were resistant to tumour growth despite receiving

tumour cells in the systemic circulation in a ratio proportionate to the fraction of the cardiac output they receive. The tumour cell line and the animals in our experiments differed from those studied by Murphy et al. Nevertheless we observed a broadly similar growth pattern in our control group when tumour cells were injected systemically. Certain tissues like the omentum, mesentery, lungs, abdominal wall, diaphragm and adrenals were found to be frequent sites of tumour growth while the bowel was always refractory. However when tumour cells were injected into the systemic circulation in the presence of a large bowel anastomosis, a large majority of the animals developed peri-anastomotic tumours. Similar observations have been reported by Skipper et al.,[259], who studied patterns of tumour growth when tumour cells are injected before, at the time of or at various intervals after the creation of intestinal anastomoses. The influence of anastomotic leaks on the ability of the bowel to support the growth of circulating tumour cells has not been investigated before. We found no difference between the animals with and without leaks in terms of anastomotic tumour growth. The mechanisms by which an anastomosis promotes local implantation of either intra-luminal or circulating tumour cells remain unknown. However, the demonstration that anastomotic integrity has no bearing on the local growth of circulating tumour cells suggests that the implantation and growth of intra-luminal and circulating

tumour cells are governed by different mechanisms. In the case of tumour cells reaching the site of an anastomosis by systemic circulation, the main factor enhancing the ability of the tissue to support tumour growth appears to be surgical trauma and the presence of a leak has little or no contribution to this enhancement. However, it is possible that the aggressive nature of the Mtln3 cells used in a relatively high dose may have concealed a difference between the "leak" and "no leak" groups in the circulating tumour cell experiments. Further experiments with other tumour cell lines at various doses may help to clarify this issue.

Although the presence of malignant cells in the systemic circulation of patients with colorectal cancer has been demonstrated before [260-262], there is no evidence to suggest that arterial delivery of these tumour cells to a large bowel anastomosis plays a role in anastomotic recurrence in man. Therefore the clinical implications of our findings with circulating tumour cells in this model remain uncertain.

GENERAL COMMENTS AND CONCLUSIONS

Surgical stapling techniques have emerged as increasingly popular alternatives to conventional manual suturing over the last two decades. However there is little evidence in the scientifific literature to place the role of this new technology into perspective, in comparison with the conventional methods of anastomotic construction. The importance of such evidence is perhaps brought to the fore further, by the recent initiatives regarding cost containment in the provision of health services. While increasing attention is being focused on the application of medical technology as a major contributor to the rise in health care expenditures, clearly the additional cost incurred by the introduction of any innovation has to be weighed against its clinical effectiveness.

The clinical trial described in the first section of this thesis aimed to provide detailed and conclusive information regarding the relative merits of suturing and stapling techniques. A review of the literature and prior statistical estimates suggested that a prospective randomised trial would require approximately 1,000 patients, to have adequate statistical power. This estimate regarding the necessary sample size also explains why previous studies comparing suturing and stapling techniques have fallen short of being conclusive.

In a prospectively studied population of over 1,000 randomised patients, the incidence of clinically evident anastomotic dehiscence was found to be comparable between the sutured and stapled groups. This study represents the largest published series of its kind and yet the confidence intervals with respect to clinical leaks were relatively wide, mainly reflecting the paucity of a clinical leak with either technique. The two anastomotic techniques produced comparable results in terms of other important measures of outcome such as operative mortality, frequency of infective complications, recovery of gastrointestinal function and duration of post-operative hospital stay. A statistically significant advantage that emerged in favour of stapling was the reduced anastomosis and operating times. Although the magnitude of the time saving, in the study groups taken as a whole, was clinically rather modest in certain circumstances where operating time is critical or when the operation entails multiple anastomoses this could be an important consideration in the choice of anastomotic technique. More detailed analysis of the results from the study revealed certain other trends which may be of assistance in guiding surgeons in the choice of anastomotic technique. For instance, attention was drawn to the infrequent but potentially important complication of haemorrhage from the staple line in upper gastrointestinal anastomoses. Another finding which gave rise to some concern was the high incidence of stapled duodenal stump leaks. This is in contrast with the

previously published experience of other investigators and no satisfactory explanation, apart from a possible "chance occurence", could be found for this observation. In patients undergoing colorectal anastomoses, the findings of this study suggest that the use of stapling instruments result in a significantly lower incidence of radiological leaks compared with suturing. Although this was not of any consequence in the early post-operative period, another analysis presented in the following section of the thesis links anastomotic leaks (including radiological leaks) to a higher incidence of tumour recurrence following resections for colorectal cancer. It seems therefore that further studies are required to clarify the clinical significance of radiological leaks in patients undergoing surgery for malignant disease.

Parallel with this clinical trial, a follow-up study was initiated for patients undergoing potentially curative resections for colorectal cancer. The follow-up data, in conjunction with the database of the original trial, enabled the examination of the influence of anastomotic techniques on the long term outcome in patients with colorectal cancer. The use of stapling instruments has been associated with a potential increase in the incidence of recurrence of rectal cancer following surgery. However, evidence in support of this contention has only come from retrospective studies. Such studies are likely to be affected by a selection bias, which

limits their ability to reach scientifically valid conclusions. The analysis presented in Section II of the thesis represents the only published work where the influence of anastomotic techniques on colorectal cancer was examined in randomised patient groups. In contrast with the previously published observations, these results suggest that in patients undergoing potentially curative resections for colorectal cancer the use of stapling techniques may be associated with a significant reduction in recurrence and cancer specific mortality rates compared with suturing.

The mechanisms by which the use of staplers result in a lower incidence of recurrence in this study remain unclear. Some experimental evidence exists to suggest that stainless steel, compared with braided suture materials, possesses different properties with respect to the entrapment and transfer of free intraluminal tumour cells into the bowel wall. It has also been shown that tumour cells adhere to stainless steel in significantly fewer numbers, compared with their adhesion to braided suture materials. It seems possible therefore that the observed difference between the sutured and stapled patient groups in this study could be a function of anastomotic materials rather than the technique associated with the construction of a sutured or stapled anastomosis.

A significant reduction in the incidence of tumour recurrence and cancer specific mortality, associated with the use of stapling instruments is a previously unreported and potentially important observation. Further studies are required to see if these results can be confirmed and to investigate the influence of anastomotic techniques on the outcome in patients with colorectal cancer.

Current evidence suggests that in the development of local recurrence of colorectal carcinoma, mechanisms other than incomplete resection such as implantation metastasis or metachronous carcinogenesis may also have a role. However no previous study has examined anastomotic leaks as a potential influence on the long term survival prospects of patients following resections for colorectal cancer. One of the clinical studies presented in Section II of this thesis addressed this issue. The cohort of patients chosen consisted of those who had undergone potentially curative resections for colorectal cancer, followed by clinical and radiological assessment of the integrity of the anastomosis. The results revealed that anastomotic leaks were associated with a significantly higher incidence of tumour recurrence and cancer specific mortality, even after correcting for tumour stage. It might be expected that certain factors associated with a high recurrence rate, such as tumour fixity, vascular and lymphatic invasion, lengthy and difficult resections could also occur

more frequently among patients with leaks. Hence a "selection bias" in the analysis might account for the apparent association between anastomotic leaks and tumour recurrence. On the other hand it seems not unreasonable to postulate that in the presence of a post-operative leak, intra-luminal tumour cells gain enhanced access to pericolic tissues resulting in a higher incidence of local recurrence. An experimental model of anastomotic leak in the rat was developed to test this latter hypothesis.

In the first set of experiments presented in Chapter 8, it was shown that intact bowel mucosa is refractory to the implantation and growth of intraluminally instilled tumour cells. This is in accordance with previously published observations. In the presence of an air-tight and water-tight anastomosis (validated by intra-operative testing and by contrast radiography respectively), intra-luminal tumour cells were able to give rise to peri-anastomotic and widespread intra-abdominal tumours in a small proportion of the animals. On the other hand, a "leaky" anastomosis resulted in tumour growth in a significantly larger proportion of the animals. The pattern of tumour growth in the animals and the significant difference between the "leak" and "no leak" groups suggest that, in this model the factor responsible for the enhancement in the capacity of intraluminal malignant cells to cause tumour growth was anastomotic leakage. This observation

supports the proposed hypothesis which had been put forward as a result of the previously discussed clinical studies. However one must be wary of extrapolating too much from studies on animal models. The biological behaviour of the tumour, the host responses and the experimental anastomotic leak model used in this study are unlikely to be directly comparable to the clinical situation with colorectal cancer surgery in man.

There is scope for further experimental work with an anastomotic leak model using different tumour cell lines and various anastomotic materials. However, the clarification of the relationship between anastomotic techniques/anastomotic leaks and recurrence of colorectal cancer will also require prospective audits and randomised clinical trials.

LIST OF PRESENTATIONS TO LEARNED SOCIETIES ORIGINATING FROM THE WORK DESCRIBED IN THIS THESIS

Akyol AM, Mcgregor JR, Galloway DJ, Sugden BA, Munro A, Bell G, George WD.
A prospective randomised trial to compare sutures and staples in the construction of colorectal anastomoses.
Surgical Research Society, January 1989, Canterbury.

Akyol AM, Galloway DJ, McGregor JR, Murray WR, Bell G, Sugden BA, Logie JRC, George WD.
The value of post-operative contrast radiology in the assessment of colonic and colorectal anastomotic integrity.
British Society of Gastroenterology, April 1989, Bradford.

Akyol AM, Galloway DJ, Murray WR, George WD, Morrice JJ, Sugden BA, Munro A, Walsh PV.
A prospective randomised trial to compare surgical stapling and manual suturing techniques in large bowel surgery.
Europen Society of Surgical Research, May 1989, Brussels.

Akyol AM, Galloway DJ, Murray WR, George WD, Morrice JJ, Sugden BA, Munro A, Walsh PV.
A prospective randomised trial to compare surgical stapling and manual suturing techniques in large bowel surgery.
Scottish Society for Experimental Medicine, Nov 1989, Aberdeen

Akyol AM, Galloway DJ, Morrice JJ, Munro A, Morran C, George WD.
The choice of anastomotic technique in large bowel surgery.
XIIIth Biennal Congress of International Society of University Colon and Rectal Surgeons, June 1990, Graz, Austria.

Akyol AM, McGregor JR, Bell G, Walsh PV, Sugden BA, George WD.
The prognostic value of contrast radiology following colorectal anastomoses.
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Akyol AM, Galloway DJ, McGregor JR, Sugden BA, Bell G, Munro A, George WD.
Sutures or staplers in gastrointestinal surgery - A prospective randomised comparison.
Surgical Research Society, July 1990, Southampton.

Akyol AM, Galloway DJ, Murray GD, McGregor JR, George WD.
Anastomotic leaks and tumour recurrence.
British Association of Surgical Oncologists, July 1990, Gateshead.

Akyol AM, Galloway DJ, Murray GD, George WD.
Peri-operative blood transfusions. A risk factor for infection?
West of Scotland Surgical Association, October 1990, Glasgow.

Akyol AM, Murray GD, George WD.
Blood transfusions and infective complications in surgical patients.
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Blood transfusions and recurrence of colorectal cancer.
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