CLINICAL AND EXPERIMENTAL STUDIES OF GASTRO-INTESTINAL ANASTOMOTIC TECHNIQUES

by

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DECLARATION

I declare that the composition of this thesis is entirely my own work and that it has not previously been submitted for consideration for a Higher Degree. All references have been personally consulted with the exception of a few, mainly historical references where copies were either not readily available or could not be traced. These latter cases are all indicated in the reference section by citing an appropriate source of reference which has been consulted.

The majority of the work for this thesis was carried out while I was employed as a full-time Research Fellow in the University Department of Surgery, Western Infirmary, Glasgow from the 1st August 1985, to the 31st July, 1987. During that period of time, I was responsible for the organisation and coordination of the clinical project described in this thesis, and for the collation of the data. Since August 1987, the data collection has been continued by a further Research Fellow but I declare that, with the exception of expert statistical assistance, I have been solely responsible for the analysis of the results.

I further certify that the experimental studies were my own design although I am indebted to my senior colleagues for their valuable advice on certain aspects. All animal operating, tissue sampling, tissue culture, stathmokinetic counting, and other experimental work was carried out personally except where acknowledged overleaf.

SUMMARY

Introduction

Recent years have witnessed the introduction and increasing application of surgical stapling instruments in gastro-intestinal surgery. This thesis is directed towards an investigation of the potential implications of this new technology with respect to both immediate and long term post-operative outcome. Clinical and experimental studies were carried out and these are considered separately.

Clinical Studies

Despite the increasing use of automated stapling devices for the construction of gastro-intestinal anastomoses, few attempts have been made to objectively compare these instruments with conventional manual suturing methods. In an attempt to clarify the role of stapling in modern surgical practice, a multicentre controlled clinical trial was designed with the aim of comparing the immediate clinical results associated with sutured and stapled anastomotic techniques.

A total of 682 consecutive patients undergoing a wide range of procedures were studied. Six hundred of these patients were randomised at laparotomy to have either sutured or stapled anastomoses. The remaining 82 patients had the type of anastomosis electively decided by the surgeon for a variety of reasons and the two groups were considered separately. Pre-operative bowel preparation, anti-microbial prophylaxis, and anastomotic materials were standardised throughout, and all data were recorded prospectively. Pre-operative data comprised anthropometric, haematological,

biochemical and nutritional indices. Details recorded at operation included the site and mode of construction of all anastomoses, and records of the anastomosis and total operating times. Post-operative data included an assessment of anastomotic integrity, gastro-intestinal function, and infective complications.

Sutured and stapled groups were subsequently found to be comparable with respect to their pre-operative characteristics. Stapling was associated with a significant reduction in anastomosis time compared with manual suturing for all anastomotic sites studied. In most cases total operating time was also significantly shorter in the stapled group. There was a tendency for stapling to be associated with a lower incidence of clinical anastomotic dehiscence in colonic and colorectal surgery whereas the opposite was the case in the upper gastro-intestinal tract. Neither of these differences, however, achieved statistical significance although the incidence of asymptomatic radiological leaks in the colorectum was significantly lower in the stapled group. The two techniques were similar with respect to the return of gastro-intestinal function, the incidence of infective post-operative complications, and the duration of post-operative hospital stay.

The most striking features of the non-randomised group were the elective use of staples for reconstruction following low anterior resection in cases where a sutured anastomosis was deemed impossible, and for procedures involving multiple anastomoses such as Roux-en-Y reconstruction after Polya gastrectomy.

The potential economic implications of surgical stapling are discussed.

Experimental Studies

For many surgeons, the introduction of the circular stapling instrument has enabled them to construct anastomoses deeper within the pelvis than was previously possible with traditional manual suturing methods. Combined with a general trend away from abdomino-perineal excision of the rectum for rectal cancer in favour of sphincter preserving procedures, this has led to a much greater proportion of patients with this disease undergoing low anterior resection. There has, however, been some concern regarding a possible association between stapled colorectal anastomoses and an increased incidence of local recurrence of colorectal carcinoma. The experimental work carried out for this thesis was designed to explore the possible mechanisms of local recurrence and to investigate the potential influence of the choice of anastomotic suture material. Two distinct studies were performed as follows:

1. Anastomotic Sutures and Colorectal Carcinogenesis

This study was designed to assess the possible promoting or protecting influences of 3 commonly used anastomotic suture materials (polyamide, polyglycolic acid, stainless steel) on experimental colorectal carcinogenesis. Male swiss albino rats received 12 consecutive weekly subcutaneous injections of the hydrazine carcinogen, azoxymethane, in a dose of 10mg/kg/week. Control animals received a course of normal saline injections over the same time period. At operation, carried out 4 weeks following the final injection, one of two procedures was performed. Half the animals had

a 2cm longitudinal colotomy fashioned along the anti-mesenteric border of the distal descending colon which was immediately repaired with 8 interrupted full-thickness sutures of the appropriate material. The remaining animals had simple transmural implantation of the same number of sutures along the corresponding length of the bowel. Sacrifice was at two distinct time intervals, 4 and 12 weeks post-operatively, at which time the number and distribution of colonic tumours was assessed.

Irrespective of whether the sutures were simply implanted into the colon or used to repair a colotomy, stainless steel was consistently associated with significantly fewer animals developing peri-anastomotic tumours compared with either polyamide or polyglycolic acid. There were no differences between the various materials with respect to the number of animals exhibiting large bowel tumours distant from the anastomotic site. In an attempt to identify the mechanisms responsible for this differential tumour incidence, dynamic cell population studies were performed but no consistent effects related to the type of suture material were observed.

2. Anastomotic Suture Materials and Implantation Metastasis

Current evidence suggests that viable tumour cells are present in the operative field during colorectal cancer surgery and their implantation may be responsible for local recurrence. Anastomotic sutures may have a role in this mechanism, either by acting as a nidus for the adherence of the free tumour cells or by directly implanting tumour cells into the colonic wall.

Four anastomotic materials (braided polyamide, braided polyglycolic acid, monofilament stainless steel, monofilament polypropylene) were compared with respect to their ability to entrap and transfer free Mtln3 carcinoma cells from the colonic lumen of the rat. The findings were consistent; the two braided materials transferred significantly greater numbers of tumour cells compared with the monofilament materials. When the firm adherence of the Mtln3 cells to the same four materials was assessed using an in vitro assay, the results were similar. The Mtln3 cells adhered in significantly greater numbers to polyamide and polyglycolic acid than to steel and polypropylene and this finding was supported by in vivo tumour growth studies.

Experimentation with an animal model of implantation metastasis served to confirm previously reported requisites necessary for tumour cell implantation but was unable to discriminate between the various suture materials.

PART I

PREFACE

Chapter 1

Gastro-Intestinal Anastomotic Techniques

1.1 An Introduction to Intestinal Surgery

Throughout history, generations of surgeons have been fascinated by the healing of intestinal wounds. The regular practice of intestinal suturing is, however, comparatively recent. Abdominal operations prior to the end of the nineteenth century were largely restricted to attaching diseased or injured bowel to the external surface in order to produce a faecal fistula rather than attempting to repair the wounded bowel. It is only within the last 100 years that intestinal resection and anastomosis has been attempted with any degree of regularity. Over this period of time a variety of techniques have been described with the aim of achieving the reliable union of two bowel ends. Traditionally, most attention has focused on manual suturing methods with emphasis on accurate wound closure, serosa-to-serosa apposition and the importance of the submucosal layer for strength. The universal adoption and refinement of such suture techniques, and the recognition of the other essential pre-requisites for successful wound healing meant that intestinal suturing could be practised with acceptably low complication rates.

In recent years modern surgical stapling instruments have emerged as an alternative means of constructing the majority of gastro-intestinal anastomoses. Their introduction, however, was immediately surrounded by controversy with certain aspects of stapling challenging the accepted surgical principles of intestinal suturing. The widespread clinical application of these intruments has since confirmed the reliable healing of stapled gastro-intestinal anastomoses in man but the arguments continue as regards their efficacy compared with traditional manual suturing methods. Added to this are the economic implications with stapling instruments being

vastly more expensive than conventional suture materials. This suturing versus stapling debate has been allowed to persist largely because of the lack of objective comparisons between the two anastomotic techniques. While the surgical literature has witnessed a plethora of retrospective reviews and anecdotal reports, few controlled prospective randomised studies have been performed. As a result, there remains little rational guidance as regards the place of stapling in routine surgical practice.

1.2 Anastomotic Techniques and Local Recurrence of Colorectal Cancer

Further controversy surrounding the use of staples has been aroused in the field of colorectal cancer surgery. Large bowel cancer is the second most frequent cause of cancer related death in the Western World and there has been little change in the overall prognosis of this disease over the past 30 years. One of the most important factors limiting improvements in survival following apparently curative surgery is the incidence of local tumour recurrence. Not only is it a major cause of mortality, the development of local recurrence is associated with substantial morbidity.

The incidence of local recurrence is greater with rectal cancer than it is with intra-peritoneal colonic carcinoma and it is generally accepted that the risk of recurrence is inversely proportional to the level of the tumour in the rectum. The surgical management of rectal cancer has undergone change over the past three decades, there being a gradual shift away from combined abdomino-perineal resection towards restorative procedures with preservation of the anal

sphincters. Most attention has been focused on low anterior resection particularly for lesions of the middle rectum. Although a matter of contention, it is probably fair to state that for the surgical community as a whole, the circular stapling instrument allows colorectal anastomoses to be constructed at a lower level in the pelvis than was previously possible with manual suturing methods. In consequence, the introduction of the circular stapler accelerated the trend towards low anterior resection for middle rectal cancer.

There have since been reservations, however, regarding the long term outcome associated with stapled low anterior resection following a number of reports of alarmingly high rates of local recurrence. While the clinical evidence is conflicting and there is little to suggest that any increase in the incidence of local recurrence is directly attributable to the technique of anastomosis, the controversy persists. Added to this, some recent experimental work has suggested that stainless steel staples may themselves promote colorectal carcinogenesis.

1.3 Outline of Thesis

This work described in this thesis comprises clinical and experimental studies designed to address both the functional and oncological implications of surgical stapling. A prospective randomised controlled clinical comparison of suturing and stapling techniques is described in Part II which attempts to clarify the respective merits of suturing and stapling techniques in the construction of anastomoses throughout the gastro-intestinal tract.

This is followed in Part III by an experimental investigation of the mechanisms of local recurrence and how these may be influenced by the choice of anastomotic suture material. Finally, Part IV comprises a series of statements which summarise the more salient points of the literature review and the findings of the clinical and experimental projects.

PART II

CLINICAL STUDIES

Chapter 2

Mechanical Intestinal Suturing

2.1 Introduction

Dehiscence of a gastro-intestinal anastomosis appreciably increases operative mortality and for survivors it significantly enhances morbidity and prolongs post-operative hospital stay (1). Such are the consequences of anastomotic dehiscence that the integrity of the anastomosis can be regarded as perhaps the single most important surgical factor influencing immediate post-operative outcome. This is particularly pertinent in the fields of rectal and oesophageal surgery where the risks of anastomotic dehiscence are higher than they are for intra-peritoneal procedures (2).

Fielding and his colleagues (1) have illustrated the deleterious effects of colorectal anastomotic dehiscence on patient well-being. They reported the findings of a large multicentre study of anastomotic integrity following large bowel cancer surgery which involved a total of 84 Consultant General Surgeons distributed between specialist institutions and district hospitals throughout the United Kingdom. The overall incidence of clinical anastomotic leakage was 13% with the incidence for extra-peritoneal rectal anastomoses being significantly higher than for intra-peritoneal colonic anastomoses (18.7% versus 10.8%). Post-operative mortality was three times greater in the group of patients who developed an anastomotic leak and hospital stay was doubled.

Clearly, there may be many variables contributing towards anastomotic dehiscence (3,4,5,6). The well established surgical maxims of avoidance of tension, adequate blood supply, accurate suture placement with edge-to-edge apposition, freedom from active disease, and avoidance of distal obstruction must never be ignored. Hawley has proposed that peri-anastomotic infection is the single most

important aetiological factor after these essential pre-requisites (7). Denied the protecting influence of the peritoneum, localised infection is more frequent around an extra-peritoneal rectal anastomosis and Hawley suggests that it is this which is responsible for the higher leakage rates associated with these anastomoses. Faecal contamination must therefore be minimised but whereas the per-operative administration of appropriate systemic antibiotics is of proven benefit in reducing wound sepsis (8,9), the efficacy of intestinal anti-microbial agents in limiting peri-anastomotic infection and facilitating healing is uncertain (10). Similarly, the role of mechanical bowel preparation is controversial (5,11,12) and excellent clinical results with a policy of no mechanical preparation have recently been reported (13). Irrespective of the aetiology of anastomotic dehiscence, however, the end result is an imbalance between collagen degradation and the synthesis of new collagen. This in turn leads to weakness of the submucosal connective tissue, the layer established as the true holding layer of an intestinal anastomosis (14).

Despite all these factors, the influence of surgical skill on anastomotic leak rates must not be forgotten. The surgeon-related variable is a well established phenomenon in clinical practice (15) and in the Large Bowel Cancer Project the individual Consultant Surgeon appeared to be the single most important independent variable influencing anastomotic integrity (1). The incidence of clinical anastomotic leakage for different Consultant Surgeons varied between 0.5% and greater than 30%, a sixty-fold difference.

Surgical technique is therefore of the utmost importance in determining sound anastomotic healing. Clearly, the study of this must take into account the efficacy of the methods used to construct the anastomosis.

2.2 The History of Intestinal Suturing

Throughout history, numerous techniques have been devised in an attempt to accelerate the healing process and to ensure the secure union of two bowel ends. However, despite modern suture technology and major advances in surgical and anaesthetic care, anastomotic dehiscence still complicates gastro-intestinal surgery and it would appear that our knowledge of the exact healing process is as yet incomplete.

The earliest description of the successful anastomosis of divided intestine dates from 1743. In his account of the "Bubonocoele Incarcerata", Heister describes Rhamdorius as having resected a strangulated hernia and achieving union of the two bowel ends by inserting one into the other in an intussuscepting technique (16). The only "suture" used was a piece of string to loosely hold the bowel ends together and to anchor the anastomosis to the mouth of the wound. Despite Rhamdorius's success, however, it was almost 150 years before intestinal suturing was attempted with any degree of regularity.

Prior to the end of the nineteenth century, it is apparent that the abdomen was very rarely opened. Observations of wound healing and attempts at intestinal suturing were largely restricted to hernias or occasionally extruded loops of bowel following penetrating injuries. So minimal were the chances of successful anastomotic

healing that when penetrating injuries were not accompanied by protrusion of the injured bowel loops, many patients were thought to have a better prognosis without operation than with (17). This was particularly the case with gunshot wounds where multiple bowel loops were frequently involved. Treatment of such injuries usually comprised the administration of large doses of opium in an attempt to arrest peristalsis in the hope that adhesions would form and procure healing (17). As one might anticipate, in the vast majority of cases this proved unsuccessful and the patient would succumb to sepsis secondary to generalised peritonitis.

The increasing confidence of surgeons during the nineteenth century with respect to their ability to achieve reliable intestinal wound healing owed much to the recognition of the basic principles necessary for successful intestinal suturing. This in turn stemmed from the clinical observations and the experimental work of several eminent surgeons of the era. Benjamin Travers recognised that it was possible to obtain primary intestinal wound healing by suturing and demonstrated this by successfully constructing end-to-end anastomoses in dogs (18). He emphasised the importance of careful suture placement in order to obtain accurate closure of the intestinal wound throughout its length, and believed that the actual suture material used was of secondary importance. In 1826 Antoine Lembert proposed that the key to succesful intestinal wound healing lay in achieving accurate serosa-to-serosa apposition (19). He advocated that an inverting anastomosis with serosa-to-serosa contact was fundamental to the union of two bowel ends. It had been observed that peri-anastomotic adhesion formation was more extensive when the contact was serosa-to-serosa than was the case with mucosa-to-mucosa apposition and Lembert assumed that these adhesions were essential for

adequate healing of the intestinal wound. Halstead later demonstrated that the true holding layer in any anastomosis was the submucosa and not the serosa and its associated adhesions (14). He emphasised the importance of incorporating the submucosa in every stitch and further suggested that care should be taken to avoid penetrating the bowel lumen in order to protect the suture tract from contamination. However, he still stressed the necessity for serosa-to-serosa contact and the avoidance of mucosa protruding through the suture line.

This doctrine of an inverting anastomosis with serosa-to-serosa apposition as being an essential pre-requisite for intestinal wound healing was to remain virtually unchallenged for more than a century. A two-layered suturing technique was usually recommended to achieve this (20). The inner layer, incorporating all coats, was designed to bring the bowel ends together and to secure haemostasis while the outer, or seromuscular layer, produced inversion and thus brought the serosal surfaces into contact. Such inversion carried with it the potential for anastomotic stenosis or obstruction. Halstead himself counselled against too much inversion (14) and this view was reiterated by others (21). Nevertheless, anastomotic stenosis remained a well recognised risk and this led to the development of new suture techniques in order to secure accurate serosa-to-serosa apposition of the bowel edges with at most only a minimal degree of inversion (22,23).

This traditional teaching of gastro-intestinal surgery was challenged in 1952 when Hertzler and Tuttle demonstrated that in experimental animals, everted anastomoses with mucosa-to-mucosa contact would heal satisfactorily (24). Although this was contrary to established surgical principles, great interest was immediately

aroused and other workers were stimulated to investigate this further. Getzen and his group (25) compared the healing of inverted and everted anastomoses throughout the small and large intestines of dogs and found no difference as judged by reticulum and collagen formation. However, the everted anastomoses were associated with a wider stoma, significantly less post-operative oedema, and greater strength in the immediate post-operative period. Initial clinical experiences with everting anastomoses were also favourable (26,27). However, Goligher and his colleagues carried out a prospective randomised controlled study of single layer everting suture versus two layer inverting suture for intra-peritoneal large bowel anastomoses and concluded that the former was inferior in terms of anastomotic integrity (28). Their study ceased prematurely when it was evident that a significantly higher proportion of patients in the everting group suffered anastomotic dehiscence and the everting suture technique was to gain little further support.

Around the same time period the first generation of modern surgical stapling instruments were emerging as an alternative means of constructing a variety of gastro-intestinal anastomoses. During their development and evaluation, experimental and clinical studies conclusively demonstrated that simple mucosa-to-mucosa healing of the stomach and duodenum could be reliably achieved using a simple linear stapling instrument which produced two staggered rows of stainless steel staples (29). Ravitch and his colleagues proceeded to demonstrate the successful construction and healing of stapled everted end-to-end intestinal anastomoses in dogs (30). Although with the modern instruments, the majority of stapled anastomoses can be constructed in the classical inverting manner with serosa-to-serosa contact, linear closures of, for example, the duodenal stump still

constitute an everting, mucosa-to-mucosa union. The increasing clinical use of stapling instruments in gastro-intestinal surgery over the past two decades has confirmed the reliability of this mucosa-to-mucosa anastomotic healing in man and so the long established doctrine of serosa-to-serosa contact as being essential for intestinal wound healing can no longer be considered to be absolute.

2.3 Mechanical Intestinal Wound Closure

The application of mechanical devices during the construction of gastro-intestinal anastomoses is not confined to comparatively recent times. From the earliest days of intestinal surgery until the present day a multitude of instruments have been described. Many of the earlier implements were devised simply as aids to conventional anastomotic techniques, while others were designed to totally replace manual suturing methods. This subject has recently been extensively reviewed by Steichen and Ravitch (31) and only a brief appraisal of the more important developments is described in this section.

The earliest device known to be designed primarily for the purposes of creating an anastomosis was presented by Felix-Nicholas Denans to a meeting of the Societe Royale de Medecine de Marseille on the 24th February, 1826 (32). His apparatus consisted of two short hollow metal cylinders or ferrules, one of which was inserted into the lumen of each of the two pieces of bowel to be anastomosed. The bowel ends were inverted over the outer edge of the ferrules and then brought together over a third longer hollow cylinder which acted as a stent across the anastomosis. Pressure between the three cylinders

was maintained by means of a complicated suture. Eventually pressure necrosis of the inverted bowel margins would lead to sloughing and all three rings would be passed in the faecal stream. Denans reported the successful construction of end-to-end small bowel anastomoses in dogs using this device but it is not known if he attempted his technique in clinical practice. A variety of other authors subsequently described modifications to Denans's device (32) but, despite the occasional report of their attempted use in man, they were largely confined to experiments on cadavers and laboratory animals.

In 1899, Senn described the use of plates made of bone in the construction of side-to-side enteral anastomoses (33). Each plate, decalcified in hydrochloric acid in order to render it totally absorbable, had a central opening which allowed for a lumen, and four small perforations equally spaced around the circumference of the plate. One plate was placed into each of the bowel loops to be anastomosed and the two plates then brought together by means of sutures passed through the perforations, further sutures usually being employed to reinforce the anastomosis. Abbe applied the same technique using plates made of heavy catgut rather than decalcified bone (34) and both his and Senn's plates rapidly achieved widespread recognition as a feasible method of intestinal anastomosis.

Their period of use, however, was short lived, owing to the introduction and instant success of the Murphy Button (35). By far the most successful implantable anastomotic device, this was initially described by its inventor, Dr. J.B. Murphy of Chicago for the construction of a cholecystoduodenostomy, although it was subsequently used for a wide range of anastomotic procedures. The button comprised two hollow metal mushrooms, the expanded ends of which were inserted into each of the bowel ends to be joined and anchored by

means of a purse string suture tied around the stem. The two stems telescoped into each other and compression between the two mushrooms was maintained by means of an internal spring. Luminal patency was ensured by the hollow nature of the expanded ends and stems of each half of the device. As with Denans's rings and ferrules, necrosis of the compressed bowel edges would eventually lead to the button being passed in the stool.

This device rapidly gained popularity throughout the world, probably because for the first time it allowed relatively unskilled surgeons to construct gastro-intestinal anastomoses with an acceptable success rate. Many of the great surgeons of the era also regarded it as the quickest and safest method of anastomotic construction such that the button and its various modifications were to remain in widespread clinical use well into the early part of the twentieth century (36).

2.4 Early Stapling Devices

Mechanical stapling devices owe their origins to the combined efforts of a surgeon, Humer Hultl, and a famous manufacturer of surgical instruments, Victor Fischer, both of whom worked in Budapest, Hungary. One of the leading surgeons of his time, Hultl was a strong believer in asepsis and was responsible for the introduction of face masks and sterile rubber gloves into Hungarian operating theatres. He was particularly concerned with minimising peritoneal contamination at laparotomy and took great care to isolate the operating field with laparotomy pads, a process he called the "wallpapering of the viscera" (37). It occurred to him that

contamination would be further reduced if an abdominal viscus was closed prior to its division and he challenged Fischer to design an instrument for this purpose. This he did and the Fischer/Hultl stapler was presented to the Second Annual Meeting of the Hungarian Surgical Society in May 1908 (38). This instrument comprised two large metal jaws, one of which housed the steel wire staples, the other being the anvil against which the staples were formed. Once these jaws were clamped onto a viscus, the staples were fired by the turning of a crank. Four parallel rows of staples were produced and the viscus could then be divided between the two middle rows without any spillage of intestinal contents.

Although an instant success, the Hultl instrument did have certain major disadvantages. It was rather heavy, weighing up to five kilograms, it took some two hours to assemble and load the staples, and it was expensive such that not all institutions could afford to purchase the device. In attempt to overcome some of these problems, an alternative instrument was developed by a young assistant surgeon, also working in Budapest, Adlar von Petz. His stapling device, weighing only one kilogram, was presented to the Eighth Annual Meeting of the Hungarian Surgical Society in September 1923 (39). Hultl himself is said to have pronounced the new instrument better and the Fischer/Hultl stapler was soon to cease production. The Von Petz instrument and its various modifications, however, were to remain the mainstay of surgical stapling for over thirty years.

2.5 Anastomotic Surgical Staplers

Neither the Hultl nor Von Petz intruments were intended for the permanent construction of a gastro-intestinal anastomosis. They were merely designed for temporary closure, particularly of the stomach during gastrectomy, and the anastomosis was always completed by hand. The first true anastomotic stapling instruments originated from the Scientific Research Institute for Experimental Surgical Apparatus in Moscow. Although the earliest instruments were for the construction of vascular anastomoses (40), early versions of the three principle anastomotic staplers currently available were developed; a linear stapler (41), a device for side-to-side gastro-intestinal anastomoses (42), and an instrument for inverting end-to-end circular anastomoses (43).

It was during a visit to the Soviet Union in 1958 that these stapling instruments were brought to the attention of an American surgeon, Dr. Mark M. Ravitch. He was immediately impressed by the concept of mechanical intestinal wound closure but recognised that the Soviet instruments had certain deficiencies which limited their clinical application. Most notably, for the most part the Russian instruments had to be partially dismantled after each firing and the staples loaded individually by hand, a factor which made multiple applications within the one operation a virtual impossibility. With Ravitch acting as an advisor, the first American stapling instruments were launched by the United States Surgical Corporation in 1967. These were both smaller and lighter than their Soviet counterparts and the staples were supplied in pre-loaded, sterilised, colour coded disposable cartridges, all factors which considerably facilitated their use.

Since their introduction, various modifications and design changes have occurred but there remains three principle anastomotic staplers; a linear stapler (TA series), an instrument for side-to-side gastro-intestinal anastomoses (GIA series), and a circular stapler (EEA series). The mode of action of all three instruments is essentially the same and comprises two stages. Firstly, as the instrument is closed, the tissues are approximated and fixed in position although the compression of the tissues is such that they are neither devitalised nor traumatised. The second stage consists of the driving of the staples through the immobilised tissues and their closure against the anvil of the instrument. It is interesting to note that the "B" shaped formation of staple closure has remained unchanged from the time of the original Hultl/Fischer stapler to the present day. The theoretical principle behind this chosen "B" configuration is that the approximation of tissues is sufficient to produce effective union of the bowel ends while the microvasculature is able to pass through the loops in the "B" and ensure a good blood supply to the healing anastomosis.

2.6 Surgical Stapling in Modern Gastro-Intestinal Surgery

The development of modern surgical stapling instruments has provided an alternative means of constructing the majority of gastro-intestinal anastomoses. Only anastomoses involving the biliary and pancreatic ducts are, as yet, not amenable to stapling techniques.

The linear staplers (GIA and TA series) may be used in combination to construct side-to-side, end-to-side, and functional end-to-end anastomoses of the stomach, small and large intestines (44,45). True end-to-end anastomoses by the technique of everted triangulation are also possible (45). The principle applications for the more complex circular staplers (EEA series) are the construction of inverting end-to-end colorectal (46,47) and oesophageal anastomoses (48,49) although the instrument can be used for end-to-end and end-to-side anastomoses throughout the gastro-intestinal tract (50,51,52). It also has a role in the transection of the oesophagus in patients with oesophageal varices (53,54,55).

Finally, stapling has to some extent, facilitated the construction of autologous organs such as ileal reservoirs and tube gastro-plasties and jejunal pouches for gastric replacement (56).

2.7 Previous Studies of Surgical Stapling

Coinciding with the emergence and increasing use of stapling instruments, the surgical literature has witnessed numerous, frequently anecdotal, reports of their potential applications. This section summarises the more important comparative studies and as is evident, retrospective reviews are more prominent than prospective trials. Linear staplers and the more recent circular staplers are considered separately.

2.7.1 Comparative Studies of Linear Stapling Instruments

i) Retrospective Comparisons

The reliability of surgical stapling in the construction of gastric anastomoses was emphasised by Gritsman in 1966 (57). Himself one of the pioneers of stapling in the U.S.S.R., he reported a collective series of 1663 stapled gastrectomies performed by a large number of surgeons using the Soviet instruments. The operative mortality for this series was 2.0% which Gristman compared with a mortality rate of 4.4% in a series of 52886 sutured gastrectomies gathered from the world literature. Other authors have similarly confirmed the reliable healing of anastomoses constructed using linear stapling instruments (29,58,59,60).

Chassin and his colleagues in New York writing in 1978 were the first to directly compare their own stapling experience with their results for traditional suturing methods (61). They reviewed a total of 812 gastro-intestinal procedures performed over the four year period 1973-1977 and reported no differences in the complication rates between suturing (3.0%) and stapling (2.8%) techniques. There then followed a number of similar retrospective reviews. Weil compared a series of sutured and stapled duodenal stump closures and gastric anastomoses and reported fewer anastomotic leaks and other complications in the stapled group (62). None of the differences, however, achieved statistical significance. In the U.K., Lowdon and his colleagues retrospectively compared the outcome of 182 patients who had undergone sutured upper gastro-intestinal anastomoses with 128 patients who had had stapled anastomoses (63). The overall complication rates were 21% and 16% for the sutured and stapled groups respectively. The authors report a significant reduction in the

clinical anastomotic leak rate associated with stapling (2% versus 7%; $p < 0.05$) which is accounted for by an excessive leak rate for sutured duodenal stump closure (12.1%).

ii) Prospective Comparisons

In 1973, Kabanov reported the findings of a prospective randomised comparison of the Russian staplers and manual suturing in 826 patients undergoing gastric surgery (64). To-date this remains the largest published prospective evaluation of surgical stapling. Kabanov found no significant differences in overall outcome between sutured and stapled groups although anastomotic leaks and septic complications tended to be slightly less frequent in the stapled group. However, post-operative anastomotic haemorrhage was more common in the stapled group.

A prospective randomised controlled trial of triangulated end-to-end stapled anastomoses at a variety of sites in the abdomen was reported by Reiling and his colleagues (65). Although no significant differences were observed with respect to operating time, complication rates, or length of hospital stay, the trial ceased prematurely at one hundred patients apparently because all the authors had convinced themselves, without scientific proof, that stapling was more time efficient.

More recently, a prospective randomised controlled trial of sutured versus stapled anastomoses in patients with gastro-intestinal tract cancer was published by Didolkar and his group from Baltimore (66). All the stapled anastomoses were of the functional end-to-end type. Patients were randomised to a sutured or a stapled anastomosis in the operating theatre after stratification for factors thought to have a major influence on anastomotic healing and integrity such as

obstruction, infection, and the anastomotic site. The authors found that stapling was associated with a significant reduction in the time taken to construct an anastomosis but there were no differences between the sutured and stapled groups with respect to total operating time, anastomotic leak rates, return of bowel function, infective complications or post-operative hospital stay.

2.7.2 Comparative Studies of Circular Stapling Instruments

i) Background and Retrospective Comparisons

The introduction of the circular stapler was met with considerable enthusiasm and further enhanced interest in surgical stapling. One aspect of surgery in which this new instrument was to gain an increasing role was in the construction of oesophageal anastomoses. However, despite there being numerous reports of the instruments application in this field with apparently acceptable complication rates (49,67,68,69,70,71), there is little published information comparing these results with conventional manual suturing techniques.

Much greater attention in the literature has been directed towards the role of circular stapling in the construction of colorectal anastomoses, and in particular those below the peritoneal reflection. The previous three decades have seen a shift away from the traditional combined abdomino-perineal resection for lesions of the mid-rectum (72) towards more restorative procedures. Such sphincter-saving operations comprise procedures such as abdomino-anal pullthrough (73,74), abdomino-transanal resection (75), abdomino-sacral resection (76,77), abdominotrans-sphincteric resection

(78) and low anterior resection (79,80). Most attention has been focused on the latter procedure (81). The trend in favour of low anterior resection for middle rectal cancer was well established prior to the introduction of circular stapling instruments as illustrated by data from St. Mark's Hospital, London. In this specialist centre the frequency with which restorative procedures were performed (mostly anterior resection) increased gradually from 16.9% of all rectal cancer operations for the 4 year period 1948-1952 to 41.1% for the period 1968-1972 (82).

One of the most immediate concerns of this change in surgical practice was patient safety in the per-operative period. It has since been conclusively demonstrated that the operative mortality associated with low anterior resection is comparable or lower than that associated with abdomino-perineal resection (83,84,85). Furthermore, anal sphincter function appears to be satisfactory following low anterior resection (86) and the quality of life following a restorative procedure is better than that following abdomino-perineal resection with its resultant permanent stoma (87). In terms of immediate outcome therefore, it is apparent that low anterior resection has more to offer the patient than does abdomino-perineal resection.

When the circular staplers became widely available there was undoubtedly an acceleration of this shift in the surgical management of rectal cancer throughout the surgical community (81,88). The instrument was immediately hailed as a device which could facilitate the construction of the low colorectal anastomosis and thus extend the range of low anterior resection to encompass most lesions of the middle rectum and some in the upper part of the lower rectum (88). Although some surgeons would disagree (89), it has been estimated that

the introduction of the circular stapler has resulted in an additional 15 of every 100 rectal cancer patients being spared a permanent colostomy (90). As a result, 60 to 70% of all patients presenting with a primary rectal cancer may be able to undergo a sphincter saving resection (88,90).

The risk of anastomotic dehiscence has been a major concern of all surgeons engaged in colorectal surgery, particularly with anastomoses involving the extra-peritoneal rectum. It was hoped that quite apart from technically facilitating the construction of low colorectal anastomoses the circular stapler would result in more reliable and secure anastomotic healing (47). Initial reports were favourable. Goligher and his colleagues (91) reported their experience with the Russian SPTU instrument in 62 consecutive anterior resections. Compared with their own historical controls, they found no difference in clinical leak rates between sutured and stapled groups. However, post-operative contrast enema studies revealed an astonishingly high radiological leak rate of 29% in patients with sutured anastomoses as compared with 6.5% in the stapled group. Adloff (92) in an uncontrolled non-randomised study, compared 26 stapled colorectal anastomoses with 25 single-layer hand sewn anastomoses. Two faecal fistulas developed in each group and in addition there were two intra-peritoneal anastomotic leaks in the sutured group as compared with none in the stapled group. Similarly, other authors have confirmed the reliable construction of low colorectal anastomoses using the circular stapler (93,94,95).

ii) Prospective Comparisons

Although a non-randomised comparison, Bolton and Britton, prospectively studied 30 patients undergoing anterior resection (96). Ten patients had a two layer sutured anastomosis with defunctioning colostomy, ten an EEA stapled anastomosis with caecostomy, and ten a stapled anastomosis with no faecal diversion. There were no differences in the incidence of complications between the three groups.

An attempt to scientifically evaluate the circular stapler in colorectal surgery was made by Beart and his group in the Mayo clinic (97). They randomised 70 patients undergoing anterior resection to either a two layer manually sutured or an EEA stapled anastomosis. Ten patients were excluded from study because the authors believed that the anastomosis would be too low to suture and these patients were electively stapled. There were no differences in clinical outcome between sutured and stapled groups. The time taken to construct the anastomosis was significantly less in the stapled group although no mention is made of the total operating time.

In the U.K., Brennan and his colleagues randomised 100 patients undergoing colonic or rectal resection to an SPTU stapled or a single layer sutured anastomosis and observed no significant differences between the two groups with respect to anastomotic leaks (98). However, in this study the incidence of minor wound infections and the length of post-operative hospital stay were significantly greater in the stapled group. Similarly, Everett and his colleagues in Cambridge (99) randomised and prospectively studied 100 patients undergoing elective left colonic or colorectal surgery, 50 to sutured and 50 to stapled anastomoses using the EEA instrument. Althougth 6 patients randomised to stapling were subsequently withdrawn and

electively sutured for a variety of technical and mechanical reasons, there were no significant differences between the sutured and stapled groups with respect to the incidence of clinical or radiological anastomotic leaks, operative mortality, or duration of hospital stay. Operating time was significantly shorter for the stapled group with a mean time saving of 17 minutes.

McGinn and his group in Southampton have recently been more critical of circular colorectal stapling (89). They studied 138 patients undergoing low anterior resection of whom 60 were randomised to a single layer sutured anastomosis and 58 to reconstruction by means of a circular stapler. The clinical leak rate was 3% in the sutured group as compared with 12% in the stapled group. Water soluble contrast enemas revealed an additional 7% incidence of asymptomatic radiological leaks in the sutured patients as compared with a radiological leak rate of 14% in the stapled group. Furthermore, they failed to demonstrate any time saving associated with stapling and there was no difference in the frequency with which abdomino-perineal resections were performed before and after the introduction of the stapling technique. However, it is fair to comment that the authors in this study were comparing their own very early experience with the stapler with their long established and very well practised manual suturing technique. It is well documented that there is a "learning curve" associated with any new surgical technique and in McGinn's study the incidence of anastomotic leaks and failures was certainly higher in the early part of the study. It could therefore be argued that it is doubtful if the results in this study represent a fair comparison of the two anastomotic techniques.

It is evident from the results of the above and numerous similar studies and comparisons that no clear message has emerged. It would appear that pro-stapling surgeons have produced results to suggest that stapling is superior to hand suturing while the anti-stapling, pro-suturing groups have claimed precisely the opposite. There remains no published prospective randomised controlled comparison of suturing and stapling at all potential anastomotic sites throughout the gastro-intestinal tract. Even studies performed in laboratory animals have failed to provide a clear cut answer. Bubrick demonstrated in dogs that EEA stapled anastomoses were associated with a highly significant reduction in radiological leak rate when compared with hand sewn anastomoses (100). Polglase, however, also using dogs, found no such difference in leak rates but demonstrated that mucosal healing was significantly delayed in animals receiving stapled anastomoses (101).

2.8 Summary

While there can be little doubt that surgical stapling has a role to play in gastro-intestinal surgery it's exact niche remains to be defined. The results available from the numerous retrospective reviews are varied and inconclusive and prospective studies are few and far between. On the other hand numerous, largely unsubstantiated, commercial claims have been made as to the theoretical benefits of stapling such as reduced tissue manipulation, less anastomotic oedema, and earlier return of gastro-intestinal function. As a result. there remains little rational basis to guide the surgeon as regards the role of stapling in his routine surgical

practice. In the interests of clinical science an independent prospective randomised controlled clinical trial is required comparing suturing and stapling throughout the gastro-intestinal tract. Only such a study can clarify the advantages and disadvantages of the two anastomotic techniques and more clearly define the relative roles of suturing and stapling techniques in modern surgical practice.

Chapter 3

Clinical Studies: Materials and Methods

3.1 Statement of Aims

As highlighted in Chapter 2, there is some debate as to the relative merits of suturing and stapling techniques in the construction of gastro-intestinal anastomoses. The clinical project described in this thesis was designed to address this controversy. It comprises a prospective randomised controlled comparison of surgical stapling and manual suturing in the construction of anastomoses at all sites amenable to either technique. Particular attention is directed as to the potential influence of the two anastomotic techniques on the following;

1. Operative Parameters

a) Anastomosis Time

b) Total Operating Time

c) Operative Complications

d) Design and Construction of Anastomoses

2. Post-Operative Outcome

a) Anastomotic Integrity

b) Post-Operative Sepsis

c) Return of Gastro-Intestinal Function

d) Operative Mortality

e) Hospital Stay

Although no formal comparison of costs has been carried out, the potential economic implications of surgical stapling are discussed in Chapter 4.

3.2 Patient Recruitment

At its inception on the 1st August, 1985, this study involved seven Consultant General Surgeons based in three surgical units. In March 1986, two further Consultant Surgeons from Raigmore Hospital in Inverness were recruited to the project. One of the surgical units involved is an Academic Department of Surgery of the University of Glasgow but functionally it behaves as a District General Surgical Unit for the West of Glasgow. The remaining three centres, Inverclyde Royal Hospital, Greenock, Crosshouse Hospital, Kilmarnock, and Raigmore Hospital Inverness are district general hospitals serving their respective population areas (figure 3.1). All the participating surgeons had some experience of surgical stapling prior to commencing the study but for the majority this experience was comparatively recent and limited to a few specific operative procedures.

Any patient within these participating surgical units undergoing any form of elective or emergency gastro-intestinal surgery was considered eligible for study. The requirments for randomisation were that the procedure involved the construction of an anastomosis which was amenable to either manual suturing or stapling techniques. This included all patients having gastro-intestinal resection, simple

Figure 3.1 Map of Scotland Illustrating the Location of the Four Surgical Units Participating in the Clinical Study

bypass of obstructing lesions, revisional gastric surgery, or reconstruction following a Hartmann's procedure. Linear closures such as pyloroplasty or closure of a gastrotomy were normally excluded from study by most surgeons although all closures of loop colostomy were included.

All patients under the care of the participating Consultants were studied, irrespective of whether the surgery was performed by the Consultant himself or by a member of his junior staff.

3.3 Study Design

The study design is illustrated diagrammatically in figure 3.2. All patients considered potential candidates for study were fully assessed prior to surgery and appropriate pre-operative data recorded. Randomisation to either a sutured or a stapled anastomosis occurred at laparotomy only once the surgeon was satisfied that either anastomotic techniques would be feasible and equally appropriate to the patient. If one technique was considered to confer a particular advantage to a patient then that technique was used and the patient followed up as part of a separate non-randomised category. In all cases various operative details were recorded and post-operatively patients were regularly assessed until such time as discharge or death.

3.4 Standardisation

In order to reduce the number of variables in the study the following parameters were standardised.

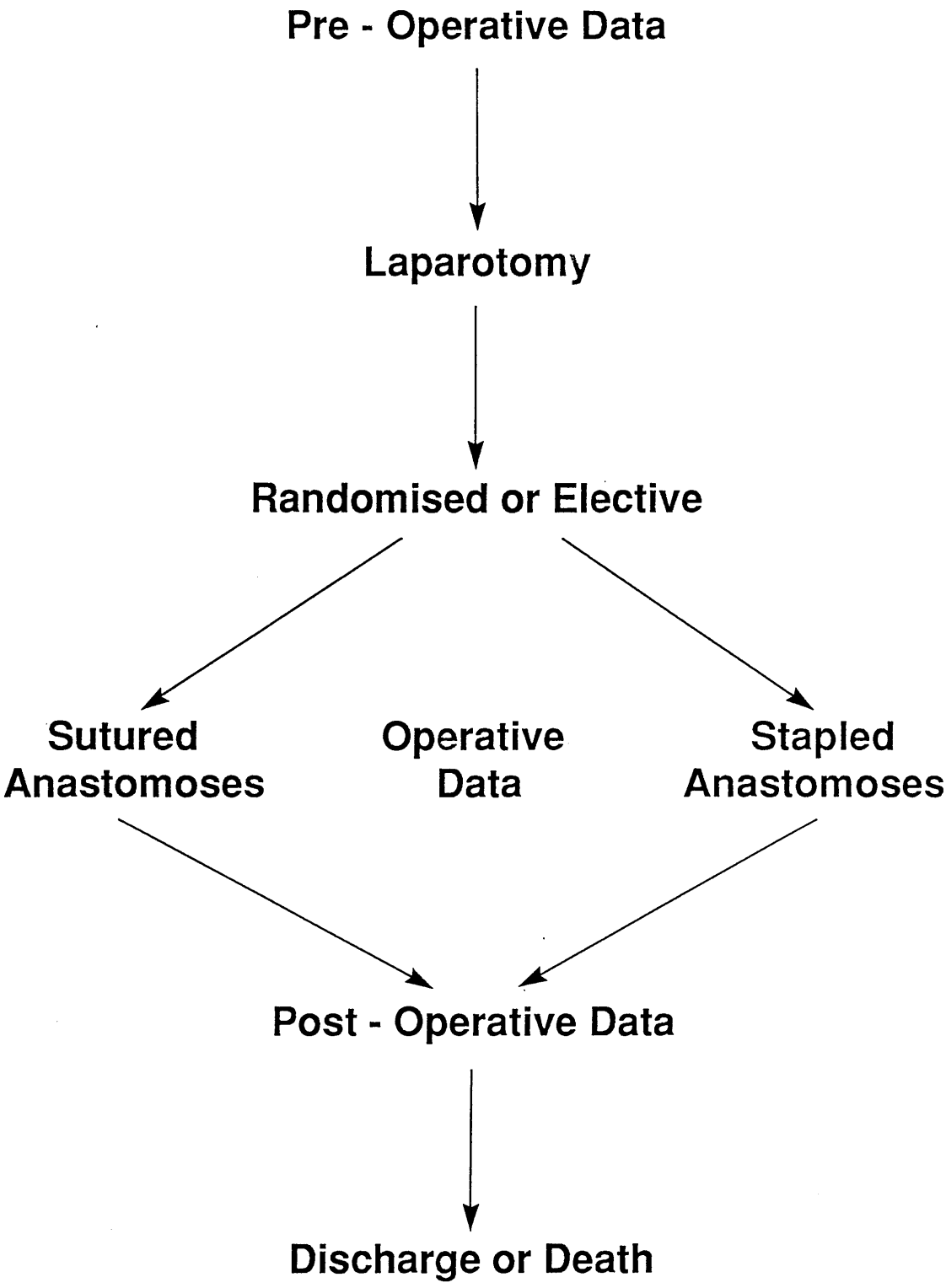


Figure 3:2

DESIGN OF CLINICAL STUDY

i) Pre-Operative Bowel Preparation

Patients undergoing elective large bowel surgery were limited to water, tea and a low residue liquid diet (Nutrauxil, KabiVitrum Ltd, Uxbridge, Middlesex) for 72 hours prior to surgery. In the absence of an obstructing lesion patients then received 500 mls of 10% mannitol solution orally the night before surgery which they were instructed to drink over a 10 to 15 minute period. Patients with obstructing large bowel lesions normally received bowel washouts. Any deviation from this standard form of bowel preparation was recorded.

ii) Per-Operative Anti-Microbial Prophylaxis

Patients undergoing upper gastro-intestinal procedures received a single intravenous injection of 1.5 grams of cefuroxime (Glaxo Laboratories, Greenford, Middlesex), administered at induction of anaesthesia. For large bowel surgery prophylaxis comprised three intravenous doses of 1 gram of cefotaxime (Roussel Laboratories Ltd., Uxbridge, Middlesex) and 500 mg of metronidazole (May and Baker Ltd., Dagenham, Essex). The first of these doses was administered at induction of anaesthesia, the second 6 hours later, and the final dose at 12 hours post surgery. As before, the reasons for any deviation from this protocol were recorded.

iii) Anastomotic Materials and Techniques

All stapled anastomoses were constructed using the GIA, TA, and EEA series Autosuture staplers (United States Surgical Corporation, Norwalk, Connecticut). Oesophago-gatric anastomoses were constructed end-to-end with the EEA circular stapling instrument. The GIA and TA series instruments were used for gastro-jejunal and cholecysto-jejunal

anastomoses in a side-to-side manner. Entero-enteric, ileo-colic, and colo-colic anastomoses were all constructed using the GIA and TA instruments to produce a functional end-to-end anastomosis (44). A small number of recto-sigmoid anastomoses were performed in the same manner but for the most part anastomoses involving the rectum were constructed end-to-end using the EEA circular stapling instrument. The technique for the construction of these EEA stapled colo-rectal anastomoses was not laid down in the protocol but was left to the discretion of the surgeon. In the majority of these cases the rectum was closed distal to the line of resection with a TA 55 instrument. The EEA stapler was then introduced transanally with the anvil removed and the central rod passed through a stab wound either through or immediately adjacent to the TA 55 staple line. This method, as originally described by Knight and Griffen (102) avoids the need for a distal purse string suture.

Similarly the study protocol dictated the materials to be used for the construction of sutured anastomoses. All sutured oesophageal, colo-colic, and colo-rectal anastomoses were constructed using a single layer of interrupted 2/0 braided polyamide ("Nurolon", Ethicon, Edinburgh). There was, however, a little variation in the construction of sutured gastric, small bowel, and ileo-colic anastomoses. Seven of the nine Consultant participants constructed these anastomoses in two layers, as originally agreed at the onset of the study. The inner layer comprised a continous suture of 2/0 polyglycolic acid ("Dexon Plus", Davis and Geck, Gosport, Hampshire) encompassing all layers whilst the outer, continous sero-muscular layer was constructed using 2/0 polyamide. The remaining two

Consultants who joined the project later preferred to use their well-practised single layer sero-submucosal suture of 2/0 polyamide for all procedures and no attempt was made to change this policy.

3.5 Randomisation

The patients of each participating Consultant Surgeon were randomised separately such that each surgeon acted as his own control. This was to eliminate the potential for any surgeon related bias which may have appeared following analysis of the results.

The method of randomisation comprised the opening of a sealed envelope in the operating theatre once the surgeon was satisfied that the procedure to be performed was suitable for randomisation. For the purposes of simplifying the block randomisation process, the surgical procedure was assigned to one of the following four categories and the appropriate envelope opened:-

i) Oesophageal:- any anastomosis where the oesophagus was involved.

ii) Gastric/Enteric:- any procedure involving an anastomosis of the stomach or small bowel but not of the oesophagus or large bowel.

iii) Colonic:- an anastomosis involving the ascending, transverse, descending or sigmoid colons but not the rectum.

iv) Colo-rectal:- any large bowel anastomosis involving the rectum.

In patients undergoing multiple anastomoses involving different areas of the gastro-intestinal tract only one randomisation was performed and the same anastomotic materials were employed throughout. In such cases the randomisation envelope opened was the one which related to the "highest" risk anastomosis. The assigned order of "risk" in descending order was oesophagus, rectum, colon, then gastro-enteric. For example, in a patient having both a colo-colic and a entero-enteric anastomosis constructed, a colonic randomisation envelope would be opened.

3.6 Assessment

The assessment of each patient in the study may be described with reference to the three categories of data recorded:-

i) Pre-operative data

The aim of pre-operative assessment was to allow the subsequent comparison of sutured and stapled groups with respect to pre-operative factors previously shown to have bearing on operative risk (103,104,105,106,107,108,109).

Data recorded included the simple anthropometric parameters height, weight, and the degree and time period of any weight loss. Routinely measured haematological and biochemical parameters were recorded and in addition the nutritional indices leucocyte ascorbic acid and serum transferrin were estimated.

ii) **Operative Data**

Note was made of the grade of surgical and anaesthetic staff and whether the operation was an elective, semi-elective, or emergency procedure. Elective operations were defined as pre-planned cases where sufficient time was available to allow full pre-operative assessment and preparation. Emergency procedures constituted acute surgical emergencies where surgery was performed on the day of admission immediately following initial resuscitation. The remainder comprised the semi-elective category. This consisted of patients admitted on an emergency basis and initially managed conservatively but in whom surgery was necessary within the subsequent seven days.

For each anastomosis, a detailed recording was made of its site in the gastro-intestinal tract, its mode of construction, and the time it took to construct. The anastomosis time was defined as the time taken from the end of dissection and mobilisation until a complete anastomosis had been achieved. This time period thus comprised all manoeuvres necessary to complete the anastomosis. For example, in stapled colorectal anastomoses this included the insertion of the purse string suture(s) as well as the introduction and firing of the stapler.

The total operating time was recorded as the time period elapsed from the commencement of the skin incision until skin suturing had been completed.

Any complications occurring during the course of the operation were recorded in detail, whether these were surgical or anaesthetic in nature. Surgical complications related to the construction of the anastomosis were by definition more frequent in the stapled group and

included technical problems with the instruments and operator induced errors such as failure to achieve complete resection margin rings with the EEA instrument.

The integrity of all colorectal anastomoses was tested intra-operatively by the following technique. The pelvis was filled with warm saline, a soft occlusion clamp placed across the bowel above the level of the anastomosis, and the rectum gently inflated by means of air injected through a Foley catheter introduced transanally. The emergence of air bubbles into the saline filled pelvis thus indicated an incomplete anastomosis and prompted attempts to repair the leak with further sutures.

Following anterior resection, the height of the anastomosis from the anal verge was estimated and recorded as was the use of a defunctioning stoma.

It has been recognised that neostigmine, used to reverse muscle relaxation when surgery is complete, may promote marked contractions of the smooth muscle of the bowel and therefore expose a newly created anastomosis to excessive strain (110). As the administration of neostigmine is therefore a potential risk factor for anastomotic leakage its usage by the anaesthetists was recorded.

iii) Post-Operative Data

This included an attempt to clinically assess anastomotic function, a comparison of anastomotic integrity, and a measure of other post-operative complications.

Although somewhat crude and open to criticism, the return of gastro-intestinal function following large bowel surgery was assessed by the post-operative day on which the patient first passed flatus and the day on which the first formed bowel motion was produced.

Similarly, the return of function following upper gastro-intestinal surgery was assessed by recording the first day on which the patient was able to tolerate an oral fluid intake of greater than one litre.

Anastomotic integrity was compared with respect to both clinical anastomotic leaks and asymptomatic radiological leaks. Clinically apparent anastomotic leaks comprised the following;

a) major anastomotic dehiscences resulting in generalised peritonitis proven at re-operation or at post-mortem.

b) the development of an entero-cutaneous fistula

c) localised peritonitis in combination with systemic signs of sepsis such as tachycardia, pyrexia and increased white blood cell count with or without radiographic evidence of anastomotic leakage

d) deep infective collections such as sub-phrenic or pelvic abscesses proven at operation, post-mortem, or radiologically to arise from an anastomotic leak.

Radiological studies using water soluble constrast media were performed between the 4th and 8th post-operative days only in patients in whom the anastomosis was readily accessible in order that accurate imaging could be carried without undue discomfort or risk to the patient. Such studies were therefore only carried out in patients with oesophageal, gastric, left colonic and rectal anastomoses. The dangers of perforating a healing low rectal anastomosis by the blind insertion of a rectal catheter for the purposes of instilling contrast medium are well recognised. In order to reduce this risk, all

patients with such anastomoses had a catheter placed in the rectum at the time of surgery through which the contrast medium could subsequently be injected by the radiologist. A medium to large bore Foley catheter was used and it was placed in the rectum such that its tip lay above the level of the anastomosis and secured in position with the balloon deflated. The placement of this catheter also served other purposes. Firstly it was used intra-operatively to test the completeness of a colorectal anastomosis as previously described. Secondly, it acted as a simople rectal drain potentially preventing the accumulation of faecal fluid, and sero-sanguinous exudates around the healing anastomosis.

Post-operative sepsis was numerically graded by means of a "sepsis score" as described by Elebute and Stoner (111). This scoring system takes account of localised sepsis (wound, peritoneal cavity, and respiratory tract), pyrexia, the effect of sepsis on renal and hepatic function, and the influence of sepsis on haematological and biochemical parameters. Wound infections were also recorded serparately for comparative purposes.

Any other post-operative complications such as cardio-respiratory failure and deep venous thrombosis were recorded and note was made of the requirement for blood transfusion. The post-operative day of discharge from hospital or of death was also recorded.

Additional information recorded, designed primarily to aid with long term follow up, included the diagnosis and, if appropriate, the stage, grade and fixity of a malignant lesion.

3.7 Collation of Data and Analysis

The completed patient information documents were collected from all participating centres and photocopied with the name and address of the patient covered. From that point onwards each patient was identified only by means of a study number and thus anonimity was preserved, thus complying with the Data Protection Act. The photocopied data sheets were then punched onto coded computer cards and all data analysis was performed on an ICL main frame computer using the BMDP software package (112).

3.8 Long Term Follow Up

Long term follow up is continuing for the majority of patients studied. No attempt has been made to alter the standard practice for review at surgical outpatient clinics. However, for patients who have had potentially curative resection of gastro-intestinal malignancy, the case records have been regularly recalled to detect cases of local recurrence. While it is accepted that this method of review is unlikely to accurately detect all cases of local recurrence more intensive follow up regimens were not possible outwith a research establishment, particularly in view of the large geographical areas served by the district general hospitals. Currently, however, there is a tendency in one of the centres (Western Infimary, Glasgow) to refer more patients for post-operative review colonoscopy and a prospective follow-up studied is being planned.

Chapter 4

Clinical Studies: Results

4.1 Introduction

At the time of writing, full computerised data is available for 682 consecutive patients entered into the study between 1st August, 1985 and 31st December, 1987. Six hundred (88.0%) were randomised at laparotomy to either sutured or stapled anastomoses and these patients constitute the "randomised" group. For the remaining 82 patients (12.0%), the surgeon chose to use one particular technique for various reasons and occasionally a combination of techniques was employed. These latter patients therefore form the "non-randomised" group. The results for the randomised and non-randomised groups will be presented separately in the forthcoming chapter.

4.2 Randomised Patients

This group comprised 600 patients undergoing gastro-intestinal surgery who were studied as per the protocol for the clinical study described in Chapter 3. A total of 792 anastomoses were constructed in these patients, 391 of which were sutured and 401 stapled. Because of the variable combinations of anastomotic procedures recorded, particularly in patients undergoing upper gastro-intestinal surgery, individual anastomosis times have been compared with respect to the total number of anastomoses performed rather than the total number of patients. The first part of this section therefore comprises a comparison of the anastomosis times for suturing and stapling techniques for single anastomoses constructed at each of the eleven anastomotic sites listed.

The remainder of the section then concentrates on results on a per patient basis. The patient cohort has been divided into two large subgroups, those undergoing upper gastro-intestinal surgery and those undergoing lower gastro-intestinal procedures. As was the case for the randomisation procedure, patients with multiple anastomoses were studied according to the "highest risk" anastomosis. For example, an appreciable number of the patients with small bowel anastomoses also had colonic anastomoses and these patients were therefore allocated to the lower gastro-intestinal subgroup.

4.2.1 Anastomosis Times

As described above, a total of 792 anastomoses were constructed in the 600 randomised patients studied. The distribution of this total by anastomotic site was as illustrated in the following tables. For each site, suturing and stapling techniques have been compared with respect to median anastomosis time and interquartile range. Results have been analysed statistically using the Mann-Whitney U Test and both p values and 95% confidence intervals are listed.

Site 1 - Oesophagus

Twenty one oesophageal anastomoses were constructed, 7 of which were oesophago-jejunal (3 sutured, 4 stapled) following total gastrectomy and the remainder oesophago-gastric. The anastomosis times are illustrated table 4.1

Table 4.1 Oesophageal Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (11)	50.00	38.00 - 55.00
Stapled (10)	23.00	20.00 - 33.75
95% confidence interval:	12.1 - 32.0;	p = 0.0014

Site 2. Gastric/Gastroduodenal

The majority of the anastomoses included under this category comprised simple linear closures of the divided stomach occuring during polya gastrectomy. However, the group also contained 3 Billroth I gastrectomies of which 1 was sutured and 2 stapled. Table 4.2 illustrates the median anastomosis times.

Table 4.2 Gastric/Gastroduodenal Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (21)	12.00	9.00 - 22.00
Stapled (23)	2.00	1.00 - 9.00
95% confidence interval:	6.0 - 14.0;	p < 0.001

Site 3 - Gastro-jejunal

A total of 125 gastro-jejunal anastomoses were constructed distributed between sutured and stapled groups as illustrated in table 4.3.

Table 4.3 **Gastro-jejunal Anastomosis Times**

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (49)	20.00	15.00 - 28.50
Stapled (76)	7.00	5.00 - 9.75
95% confidence interval:	10.0 - 15.0;	$p < 0.001$

Site 4 - Pyloroplasty

Although this was included as a category on the patient information document, most surgeons were reluctant to attempt a stapled pyloroplasty technique. As a result pyloroplasties as part of peptic ulcer surgery were not included in the study. However, 6 pyloroplasties performed in conjunction with oesophago-gastrectomy were randomised as follows. Because of the small group sizes, the ranges rather than the interquartile ranges are quoted (table 4.4).

Table 4.4 Pyloroplasty Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Range
Sutured (3)	5.00	3.00 - 14.00
Stapled (3)	8.00	4.00 - 10.00
95% confidence interval;	-7.0 - 10.0;	p = 1.00

Site 5 - Duodenal Stump Closure

The median anastomosis times for sutured and stapled duodenal stump closure are illustrated in table 4.5.

Table 4.5 Duodenal Stump Closure Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (23)	10.00	8.00 - 12.00
Stapled (33)	1.00	1.00 - 3.00
95% confidence interval:	6.0 - 10.0;	p < 0.001

Site 6 - Entero-enteric

Table 4.6 depicts the median anastomosis times for all the small bowel anastomoses constructed in the study.

Table 4.6 Entero-enteric Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (56)	15.00	10.25 - 20.00
Stapled (55)	6.00	4.00 - 10.00
95% confidence interval:	6.0 - 10.0;	$p < 0.001$

Site 7 - Ileo-colic

This group comprised anastomoses constructed following right hemicolectomy or for ileo-colic bypass. The anastomosis times for the sutured and stapled groups are illustrated in table 4.7.

Table 4.7 Ileo-colic Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (75)	18.00	12.00 - 26.00
Stapled (77)	7.00	4.00 - 10.00
95% confidence interval:	10.0 - 14.0;	$p < 0.001$

Site 8 - Colo-colic

This group comprised end-to-end, side-to-side, and end-to-side colo-colic anastomoses but did not include closure of loop colostomy. As before, the anastomosis times for the two groups are listed in table 4.8

Table 4.8 Colo-colic Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (48)	16.00	12.00 - 24.75
Stapled (44)	7.00	5.00 - 14.75
95% confidence interval:	6.0 - 11.0;	$p < 0.001$

Site 9 - Colo-rectal

This group included anastomoses constructed following anterior resection, sigmoid colectomy, and reversal of Hartmann's procedure. Table 4.9 depicts the median anastomosis times.

Table 4.9 Colo-rectal Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (71)	30.00	20.00 - 40.00
Stapled (57)	18.00	12.75 - 25.25
95% confidence interval:	7.0 - 16.0;	$p < 0.001$

Site 10 - Colostomy closure

The group comprised the closure of defunctioning loop colostomies, the anastomosis times for which are listed in table 4.10.

Table 4.10 Colostomy Closure Anastomosis Times

Anastomosis (n)	Median anastomosis time (minutes)	Interquartile range
Sutured (11)	14.00	12.00 - 17.00
Stapled (10)	5.00	3.75 - 7.75
95% confidence interval:	4.00 - 12.0;	$p = 0.0015$

Site 11 - Biliary-enteric

The anastomoses included in this group all comprised cholecysto-jejunostomy performed for the bypass of malignant obstruction of the common bile duct. As before, the median anastomosis times are listed in the following table 4.11;

Table 4.11 **Biliary-enteric Anastomosis Times**

Anastomosis (n)	**Median anastomosis time (minutes)**	**Interquartile range**
Sutured (23)	14.00	12.00 - 23.00
Stapled (13)	7.00	5.00 - 11.00
95% confidence interval:	4.0 - 13.0:	$p < 0.001$

4.2.2 Upper Gastro-Intestinal Surgery

This group comprised 216 randomised patients who underwent upper gastro-intestinal procedures. One hundred and three of these patients had sutured and 113 had stapled anastomoses. This section describes the characteristics of the sutured and stapled patient subgroups with respect to pre-operative, operative, and post-operative data.

a) Pre-operative Assessment

This involved a comparison of the sutured and stapled groups with respect to age, sex ratio, weight loss, diagnosis, and pre-operative haematological and biochemical data. The characteristics of the sutured and stapled groups are illustrated in table 4.12 and 4.13. Further subdivision into procedure related groups, for example gastric surgery or small bowel surgery, had no influence on the overall comparability of the sutured and stapled patients.

It is evident from the data presented in these two tables that the sutured and stapled groups were well matched pre-operatively. Furthermore, the two groups were found to be similar with respect to the proportion of patients suffering from malignant disease. Sixty two patients in the sutured group (60.2%) and 68 patients in the stapled group (61.3%) were found to have intra-abdominal malignancy.

Table 4.12

Upper GI Randomised Group: Anthropometric Data

	Sutured (103)	Stapled (113)
Sex Ratio (M:F)	53 M: 50 F	58 M: 55 F
Mean age (± SD)	64.3 ± 15.2	64.4 ± 15.4
Age Range (yrs)	20 - 90	19 - 96
No. with weight loss	54 (52.9%)	68 (61.3%)
Mean wt loss (kg) (± SD)	9.6 ± 5.4	9.1 ± 4.3

Table 4.13

Upper GI Randomised Group: Pre-operative Haematological and Biochemical Data (mean + SD)

	Sutured (103)	Stapled (113)
Haemoglobin (g/dl)	13.1 ± 1.9	12.9 ± 1.9
Albumin (g/l)	37.9 ± 5.5	37.1 ± 6.1
Transferrin (g/l)	2.5 ± 0.6	2.5 ± 0.7
Leucocyte Ascorbate (fmol/l)	1.2 ± 0.8	1.2 ± 1.0

b) Operative Data

Category of Operation

As illustrated in table 4.14, the sutured and stapled groups were similar with respect to the number of patients undergoing surgery electively, semi-electively, or as an emergency.

Table 4.14

Upper GI Randomised Group: Category of Surgery

	Sutured (103)	Stapled (113)
Elective	79 (76.7%)	91 (80.5%)
Semi-elective	10 (9.7%)	7 (6.2%)
Emergency	14 (13.6%)	15 (13.3%)

Grade of Surgical Staff

There were no major discrepancies between the sutured and stapled groups with respect to the grade of surgical staff (table 4.15). Although a Consultant was more frequently the first operator in the stapled group (69.0% vs 58.3%; $x^2 = 2.71$, $p = 0.010$), in total a Consultant was present in a similar proportion of cases in each group (80.6% of sutured and 84.9% of stapled cases).

Table 4.15

Upper GI Randomised Group: Grade of Surgeon

	Sutured (103)		Stapled (113)	
	Operator	Assistant	Operator	Assistant
Consultant	60 (58.3%)	23 (22.3%)	78 (69.0%)	18 (15.9%)
Senior Reg.	19 (18.4%)	11 (10.7%)	16 (14.2%)	20 (17.7%)
Registrar	18 (17.5%)	50 (48.5%)	18 (15.9%)	52 (46.0%)
SHO	6 (5.8%)	16 (15.5%)	1 (0.9%)	17 (15.0%)
none		3 (2.9%)		6 (5.3%)

Grade of Anaesthetic Staff

Similarly, there were no differences between the sutured and stapled groups with respect to the grade of the anaesthetist. A Consultant was present in 73.8% of sutured and 72.3% of stapled procedures with the remainder of cases being distributed between Senior Registrars and Registrars.

Total Anastomosis and Operating Times

As discussed, the previously listed anastomosis times (tables 4.1 - 4.11) were calculated on the basis of the total numbers of sutured and stapled anastomoses at each anastomotic site irrespective of the number of patients involved (792 anastomoses in 600 patients). In this section, total anastomosis and operating times for specific operative procedures in individual patients are compared. Some

patients necessarily had single anastomoses while others had various combinations at multiple sites. The total anastomosis time recorded for each patient comprises the total length of time taken to complete all anastomotic procedures. The total anastomosis times illustrated in table 4.16 therefore differ from the individual times for single anastomoses listed in tables 4.1 -4.11.

In order to compare the influence of the anastomotic techniques on the time taken for individual procedures, patients were allocated to one of five procedure groups as follows;

i) Oesophageal Surgery

This group was small, comprising only 21 patients of whom 11 had sutured and 10 stapled anastomoses. All of these patients had tumours of the lower oesophagus or proximal stomach. In 14 cases (8 sutured, 6 stapled) a proximal gastrectomy was carried out with oesophago-gastric reconstruction and in the remainder an oesophago-jejunal anastomosis was constructed following total gastrectomy.

ii) Gastro-enterostomy Group

Forty nine patients had a gastro-enterostomy constructed without resection or any other anastomotic procedure taking place. These comprised patients undergoing bypass of pyloric obstruction and truncal vagotomy and drainage.

iii) Gastric Resection Group

This group comprised patients undergoing partial gastrectomy. A total of 64 patients were assigned to this category, 26 sutured and 38 stapled. Sixty one patients had Billroth II (polya)

reconstruction of whom 15 underwent Roux polya gastrectomy. The remaining 3 patients had a Billroth I anastomosis. The proportion of patients undergoing each procedure was similar in the sutured and stapled groups.

iv) Biliary Bypass Surgery

This subgroup comprised 36 patients undergoing surgical bypass of malignant common bile duct obstruction. Twenty seven of these patients simultaneously had a gastro-enterostomy performed for actual or impending duodenal obstruction from pancreatic carcinoma.

v) Small Bowel Surgery

This subgroup comprised only those patients who were undergoing small bowel procedures without any other part of the gastro-intestinal tract being involved. As might be predicted a much higher proportion of cases in this category were carried out as an emergency (38%) and included strangulated hernias and adhesive obstructions. Similarly, the majority of this surgery was for benign disease (78%), the few malignant cases involving bypass of recurrent intra-abdominal tumour.

The uneven distribution of sutured and stapled cases according to the procedure groups can be explained with reference to the randomisation process. As will be recalled, randomisation in theatre was simplified to 4 categories; oseophageal, gastric/enteric, colonic, and colorectal. All but the oesophageal group therefore fall into the "gastric/enteric" category. Furthermore each participating surgeon randomised his own patients separately. When this is taken into account, the slight discrepancy in the number of sutured and stapled "gastric/enteric" patients is not unexpected.

Table 4.16

Upper GI Randomised Group: Total Anastomosis and Operating Times

Procedure (n)	Anastomosis Time (mean mins ± sem)	Operating Time (mean mins ± sem)
sutured (11)	64.7 ± 5.9	219.1 ± 13.9
Oesophageal		
stapled (10)	33.0 ± 4.4	210.3 ± 14.7
sutured (19)	19.2 ± 2.2	59.5 ± 4.7
Gastro-enterostomy		
stapled (31)	7.1 ± 0.7	56.3 ± 3.7
sutured (26)	31.8 ± 3.5	108.4 ± 7.2
Gastric resection		
stapled (38)	12.7 ± 0.8	90.9 ± 5.1
sutured (23)	29.0 ± 3.1	102.8 ± 15.1
Biliary bypass		
stapled (13)	15.4 ± 1.9	85.0 ± 6.2
sutured (24)	18.8 ± 1.0	110.4 ± 8.5
Small bowel		
stapled (21)	9.5 ± 1.3	96.1 ± 11.3

There was a reduction in total anastomosis time and operating time associated with stapling for all five procedures listed in table 4.16. Statistical testing comprised a two-way analysis of variance (112). This technique allows an overall comparison of sutured and stapled groups with an assessment of the contribution made by each procedure to any differences observed.

The overall reduction in total anastomosis time with stapling was highly statistically significant ($p < 0.001$). However, this difference between sutured and stapled groups varied according to the surgical procedure ($p < 0.001$). For example, as illustrated in table 4.16, stapling all the anastomoses involved in gastric resection saved a mean of 19.2 minutes compared with suturing whereas only 5.3 minutes were saved by stapling a small bowel resection.

Stapling also led to a significant reduction in operating time ($p = 0.046$). In this case, the difference between sutured and stapled patients was similar irrespective of the procedure performed ($p = 0.96$).

Surgical and Anaesthetic Complications

A total of 31 surgical complications were recorded (14.4%). Twenty six of these occured in the stapled group (23%) and five in the sutured group (4.9%) ($x^2 = 14.45$; $p < 0.001$).

Four patients in the stapled group required emergency re-operation within a few hours of surgery for haemorrhage. In each case, arterial bleeding was identified directly related to a GIA staple line (3 gastric, 1 biliary). A further 13 patients with stapled anastomoses required undersewing of staple lines at the time of surgery to arrest haemorrhage. The remaining 9 surgical

complications in the stapled group comprised 4 cases of incomplete closure of stab wounds with the TA 55 instrument requiring further sutures, 2 cases of misfiring of the stapler owing to incorrect insertion of the staple cartridge, 1 case of inadvertent stapling across a naso-gastric tube, 1 case of penetration of the small bowel wall with the forks of the GIA instrument, and one case of staple line disruption following excessive manipulation of the completed anastomosis.

The five surgical complications in the sutured group comprised one accidental partial division of the small bowel while dividing adhesions from previous surgery, one poorly vascularised small bowel anastomosis which required reconstruction, one "twisted" small bowel anastomosis also requiring reconstruction, one case of operative trauma to the spleen during truncal vagotomy and gastro-enterostomy, and finally one case where a defect in a small bowel anastomosis was noted intra-operatively and repaired.

Only one anaesthetic complication was recorded in the upper gastro-intestinal surgery group. This was in a patient undergoing sutured small bowel resection and anastomosis who developed marked cardiac instability with an irregular tachycardia during surgery.

Abdominal Drains

Abdominal drains were placed in a higher proportion of patients in the sutured group when compared with the stapled group (42.7% vs 6.5%) ($x^2 = 6.26$; $p = 0.012$). This is largely accounted for by differences in the biliary bypass and gastric resection groups but as the use of drains was left to the discretion of the surgeon the reasons for these differences are unclear.

Neostigmine

There were no differences between the sutured and stapled groups with respect to the number of patients receiving neostigmine at reversal of anaesthesia (82.5% of sutured group, 84.1% of stapled patients).

c) Post-Operative Results

Post-operative assessment was concerned with a comparison of sutured and stapled techniques with respect to the return of gastro-intestinal function, anastomotic integrity, septic and other complications, and overall outcome.

Return of GI Function

There were no differences between sutured and stapled groups with respect to the first post-operative day on which patients were able to tolerate an oral fluid intake of greater than 1 litre (5.5 $\pm$ 0.2 vs 5.0 $\pm$ 0.2 days, mean $\pm$ sem).

Anastomotic Integrity

i) Clinical Anastomotic Leaks

A total of 9 clinically apparent anastomotic leaks were recorded in the upper gastro-intestinal tract (4.2%). Seven of these occured in the stapled group (6.2%) and 2 in the sutured group (1.9%) (x^2 = 2.44; p = 0.118). These leaks can be summarised as follows;

Sutured

1. Duodenal stump fistula following surgery for benign disease which settled with conservative management.

2. Transient small bowel fistula following resection for Crohn's disease.

Stapled

1. Duodenal stump fistula following surgery for benign disease. The patient's condition settled with conservative management.

2. Duodenal stump leak following resection of locally advanced gastric carcinoma. The patient presented with recurrent sub-phrenic collections.

3. Duodenal stump leak following partial gastrectomy for gastric lymphoma. This patient developed peritonitis and required re-operation.

4. Duodenal stump leak following surgery for benign disease. This patient presented 4 weeks post-operatively with a large subphrenic collection which discharged into right pleural cavity.

5. Duodenal stump fistula following partial gastrectomy for carcinoma. Proven at re-operation.

6. Leak from gastro-enteric anastomosis following partial gastrectomy for carcinoma. Proven radiologically and required re-operation.

7. Transient small bowel fistula following small bowel resection for recurrent colonic carcinoma.

ii) Radiological Anastomotic Leaks

All patients with an oesophageal anastomosis had a water-soluble contrast swallow (gastrograffin) between days 4 and 8 post-operatively. In addition, 24 patients undergoing gastro-enterostomy alone (9 sutured, 15 stapled) and 34 undergoing partial gastrectomy (13 sutured, 24 stapled) were similarly studied.

Only one asymptomatic radiological leak was discovered. This was a small leak of contrast from the gastro-jejunal anastomosis of a patient who had undergone Billroth II gastrectomy for gastric carcinoma.

Infective Complications

i) Wound Infections

Bacteriologically proven wound infections were recorded in 14 patients (6.4%), 8 of which occurred in the sutured group (7.8%) and 6 in the stapled group (5.3%) (x^2= 0.54; p = 0.464). (table 4.17)

ii) Total Sepsis Score

As illustrated in table 4.17, there were no differences between the sutured and stapled groups with respect to the total calculated sepsis scores. Other individual post-operative complications were reported with equal frequency in the sutured and stapled groups.

Table 4.17

Upper GI Randomised Group: Post-operative Infective Complications

Procedure (n)	Wound Infections	Sepsis Score (mean ± sem)
sutured (11)	0	5.3 ± 1.3
Oesophageal		
stapled (10)	0	5.1 ± 0.9
sutured (19)	0	2.6 ± 0.4
Gastro-enterostomy		
stapled (31)	1	3.1 ± 0.5
sutured (26)	2	3.6 ± 0.6
Gastric resection		
stapled (38)	1	5.7 ± 0.9
sutured (23)	2	6.9 ± 0.7
Biliary bypass		
stapled (13)	1	6.8 ± 0.9
sutured (24)	4	5.2 ± 0.8
Small bowel		
stapled (21)	3	4.9 ± 0.9

Blood Tranfusion

Twenty three patients (22.3%) in the sutured group required to be transfused per-operatively or post-operatively as compared with 34 patients (30.1%) in the stapled group (x^2 = 1.67; p = 0.196). For those patients that were tranfused the mean number of units of blood required was similar in the sutured and stapled groups (3.18 $\pm$ 0.73 sem vs 3.75 $\pm$ 0.66).

Outcome

Twenty two patients died within 30 days of surgery, equating with a 30 day operative mortality of 10.2% (table 4.18). Eight deaths occurred in the sutured group (7.8%) and 14 in the stapled group (12.4%) (x^2 = 1.26; p = 0.262). Only 2 deaths were related to a clinically apparent anastomotic dehiscence, both of which occured in patients in the stapled group. The remaining deaths were all cardiorespiratory in causation.

For survivors, there were no differences between sutured and stapled groups with respect to the mean duration of post-operative hospital stay (table 4.18)

Table 4.18

Upper GI Randomised Group: Post-Operative Outcome

Procedure (n)	Deaths	Hospital Stay (mean days ± sem)
sutured (11)	1	14.7 ± 1.8
Oesophageal		
stapled (10)	0	13.2 ± 0.4
sutured (19)	0	10.6 ± 1.2
Gastro-enterostomy		
stapled (31)	2	10.7 ± 0.9
sutured (26)	0	12.1 ± 1.2
Gastric resection		
stapled (38)	6	14.6 ± 2.2
sutured (23)	5	15.2 ± 2.8
Biliary bypass		
stapled (13)	4	18.1 ± 3.4
sutured (24)	2	12.6 ± 1.8
Small bowel		
stapled (21)	2	14.8 ± 2.1

4.2.3 Lower Gastro-Intestinal Surgery

A total of 384 patients undergoing lower gastro-intestinal procedures were randomised, 198 to sutured and 186 to stapled anastomoses. This section is concerned with a comparison of these two groups.

a) Pre-Operative Assessment

The pre-operative characteristics of the sutured and stapled groups are summarised in tables 4.19 and 4.20. As was the case for upper gastro-intestinal surgery, it is evident that the sutured and stapled groups were highly comparable.

One hundred and fifty one of the patients in the sutured group (76.3%) received the standardised form of bowel preparation as compared with 135 patients (72.6%) in the stapled group. The remaining patients either received no pre-operative preparation (emergency cases) or had washouts (normally 500-1000ml soap suds enema) because of obstructing lesions.

Table 4.19

Lower GI Randomised Group: Anthropometric Data

	Sutured (198)	Stapled (186)
Sex Ratio (M:F)	81 M: 117 F	79 M: 107 F
Mean age (± SD)	64.7 ± 15.8	65.2 ± 14.2
Age Range (yrs)	10 - 92	18 - 94
No. with weight loss	83 (41.9%)	62 (33.3%)
Mean wt loss (kg) (± SD)	8.7 ± 4.8	8.9 ± 4.8

Table 4.20

Lower GI Randomised Group: Pre-operative Haematological and Biochemical Data (mean + SD)

	Sutured (198)	Stapled (186)
Haemoglobin (g/dl)	12.8 ± 1.8	13.0 ± 2.1
Albumin (g/l)	37.5 ± 5.4	37.9 ± 5.5
Transferrin (g/l)	2.7 ± 0.7	2.7 ± 0.7
Leucocyte Ascorbate (fmol/l)	1.2 ± 0.7	1.2 ± 0.8

Subsequent comparison of the sutured and stapled groups confirmed that a similar proportion of patients in each group had malignant disease. Furthermore, for those with colonic carcinoma, groups were comparable with respect to the extent of this as classified by the Astler-Coller modification of Dukes' staging (113) (table 4.21). Those classified as "others" comprised 2 patients with endometrial carcinoma invading the sigmoid colon, 1 with a carcinoid tumour of the caecum, 3 patients with lymphomas, and 15 patients with ovarian carcinoma.

Table 4.21

Lower GI Randomised Cases: Malignant Disease

Stage	Sutured (198)	Stapled (186)
A	2	4
B1	16	12
B2	57	62
C1	7	4
C2	36	30
D	14	13
Others	13	8
Total	**143 (73.2%)**	**133 (71.5%)**

b) Operative Data

Category of Operation

The proportions of patients in each group in whom surgery was carried out electively, semi-electively, or as an emergency were similar (table 4.22)

Table 4.22

Lower GI Randomised Group: Category of Operation

	Sutured (198)	Stapled (186)
Elective	157 (79.3%)	149 (80.1%)
Semi-elective	18 (9.1%)	14 (7.5%)
Emergency	23 (11.6%)	23 (12.4%)

Grade of Surgical Staff

Again there were no significant differences between the sutured and stapled groups with respect to the grade of operator and assistant (table 4.23). A Consultant Surgeon was in attendance during 77.7% of sutured and 80.7% of stapled procedures.

Table 4.23

Lower GI Randomised Group: Grade of Surgical Staff

	Sutured (198)		Stapled (186)	
	Operator	Assistant	Operator	Assistant
Consultant	105 (53.0%)	49 (24.7%)	108 (58.1%)	42 (22.6%)
Senior Reg	33 (16.7%)	22 (11.1%)	31 (16.7%)	19 (10.2%)
Registrar	57 (28.8%)	99 50.0%)	46 (24.7%)	91 (48.9%)
SHO	3 (1.5%)	22 (11.1%)	1 (0.5%)	20 (10.8%)
none		6 (3.0%)		14 (7.5%)

Grade of Anaesthetic Staff

A Consultant anaesthetist was present for 72.8% of sutured cases as compared with 74.5% of stapled operations. The majority of the remaining cases were anaesthetised by Registrars.

Total Anastomosis and Operating Times

Unlike surgery of the upper gastro-intestinal tract where variable combinations of anastomotic procedures were frequently encountered, colonic and colo-rectal operations for the most part involved a single anastomosis. However, 9 patients had two large bowel anastomoses, 26 had an additional small bowel anastomosis, and 7 had a simultaneous gastro-enterostomy. These were evenly distributed

between sutured and stapled groups and so total anastomosis and operating times have been compared as described for the upper gastro-intestinal group.

The four procedure groups which comprised lower gastro-intestinal surgery were as follows;

i) Ileo-colic Surgery

This group comprised a total of 147 patients who underwent right hemicolectomy (138) or ileo-colic bypass (9). Seventy two patients had sutured and 75 had stapled anastomoses.

ii) Colonic Surgery

A total of 90 patients had colo-colic anastomoses (46 sutured and 44 stapled). Sixteen of these patients underwent transverse colectomy (9 sutured, 7 stapled), 39 left hemicolectomy (22 sutured, 17 stapled), 30 limited sigmoid colectomy (13 sutured, 17 stapled), and 5 colo-colic bypass (2 sutured, 3 stapled) without resection.

iii) Colo-rectal Surgery

One hundred and twenty eight patients had an anterior resection, 71 with a sutured and 57 with a stapled anastomosis.

iii) Colostomy Closure

This group comprised patients who were undergoing closure of loop colostomy. Nineteen patients fell into this category of whom 9 were randomised to a sutured and 10 to a stapled anastomosis.

The mean total anastomosis and operating times are listed in table 4.24 (mean mins ± sem). Statistical analysis was again by means of a two-way analysis of variance (112).

Table 4.24

Lower GI Surgery: Total Anastomosis and Operating Times

Procedure (n)	Anastomosis Time (mean mins ± sem)	Operating Time (mean mins ± sem)
sutured (72)	24.4 ± 1.5	101.7 ± 3.9
Ileo-colic		
stapled (75)	8.6 ± 0.6	92.1 ± 4.0
sutured (46)	24.9 ± 2.3	115.0 ± 6.5
Colo-colic		
stapled (44)	10.9 ± 1.2	92.0 ± 5.0
sutured (71)	33.1 ± 1.7	139.8 ± 5.2
Colo-rectal		
stapled (57)	19.8 ± 1.4	124.2 ± 5.3
sutured (9)	16.2 ± 2.9	75.2 ± 12.4
Colostomy Closure		
stapled (10)	6.2 ± 1.2	54.5 ± 7.9

Stapling was associated with a highly significant reduction in the total anastomosis time ($p < 0.001$) and there was no evidence that this difference between sutured and stapled patients varied from procedure to procedure ($p = 0.24$). Similarly the overall difference between sutured and stapled groups with respect to operating time was highly significant ($p < 0.001$). Again there was no evidence that this reduction in operating time associated with stapling was dependent on the surgical procedure performed ($p = 0.78$).

Surgical and Anaesthetic Complications

Eight patients in the sutured group were recorded as having a surgical complication (4.0%) as compared with 39 patients in the stapled group (20.9%) ($x^2 = 25.58$; $p < 0.001$). Staple line bleeding was a much less frequently encountered problem in the large bowel and no patient required re-operation for anastomotic haemorrhage. Twelve patients, however, required under-running of a staple line at operation to arrest troublesome oozing. The remainder of the surgical complications in the stapled group largely comprised operator induced technical problems, the most common of which were failure to completely close stab wounds with the TA 55 instrument (11 cases), loose or incomplete purse string sutures (4 cases), and incorrect mounting of staple cartridges (3 cases).

Six of the surgical complications in the sutured group were directly related to the anastomosis. In two cases the completed anastomosis was deemed of doubtful vascularity and was subsequently reconstructed, in one the anastomosis was seen to be poorly orientated and was similarly reconstructed, in one there was gross peritoneal contamination owing to slippage of a bowel occlusion clamp, and in the

final case attempted repair of a defect in a colo-rectal anastomosis rendered the anastomosis ischaemic and necessitated its reconstruction.

Anaesthetic complications were much less frequent and were recorded in only 6 cases (1.6%), 5 of which were in the sutured and 1 in the stapled group. All of these were cardiovascular in nature.

Length of Rectal Stump

Although there appeared to be a tendency for stapled anastomoses to be constructed at a lower level in the rectum when compared with patients having sutured anastomoses, the difference between the groups was not statistically significant. The mean distance of the anastomosis from the anal verge for the sutured group was 11.1 cm $\pm$ 0.4 sem as compared with 10.3 cm $\pm$ 0.5 for the stapled group ($p = 0.28$; Mann-Whitney U Test). The characteristics of the two groups with respect to rectal stump length are summarised in table 4.25.

Table 4.25

Colo-rectal Anastomoses: Height of Anastomosis from Anus

Height of Anastomosis from Anal Margin (cms)	Number of Patients (%) Sutured	Stapled
< 5 cms	3 (4.2%)	4 (7.0%)
6 - 10 cms	28 (39.4%)	27 (47.4%)
> 10 cms	40 (56.3%)	26 (45.6%)

Defunctioning Stoma

Defunctioning stomas were performed in a total of 32 patients with colo-colic or colo-rectal anastomoses (14.4%). Twenty one of these patients had sutured anastomoses and 11 stapled anastomoses, these totals comprising 17.4% and 10.9% of the sutured and stapled colo-colic and colo-rectal groups respectively (x^2 = 2.76; p = 0.096).

Abdominal Drains

Peritoneal drains were placed in 114 patients in the sutured group (57.8%) and 106 patients (57.0%) in the stapled group (x^2 = 0.01; p = 0.908).

Neostigmine

Again there was no difference between sutured and stapled groups with respect to the use of neostigmine at reversal of anaesthesia. One hundred and fifty patients in the sutured group (75.8%) received the drug as compared with 143 patients with stapled anastomoses (76.9%).

c) Post-Operative Results

Return of GI Function

Sutured and stapled groups were comparable with respect to the first post-operative day on which flatus was passed and formed bowel motions were produced. The findings are summarised in table 4.26.

Table 4.26

Lower GI Randomised Group: Return of GI Function

	Flatus (mean day ± sem)	Formed Bowel Motion (mean day ± sem)
sutured (198)	4.2 ± 0.1	6.0 ± 0.1
stapled (186)	3.9 ± 0.1	5.6 ± 0.1
p value (Mann Whitney U Test)	**0.03**	**0.09**

Anastomotic Integrity

i) Clinical Anastomotic Leaks

Clinically apparent anastomotic leaks were recorded in a total of 20 patients (5.2%). Fourteen of these occured in the sutured group (7.1%) and 6 in the stapled group (3.2%) ($x^2 = 2.87$; $p = 0.090$). The distribution of these clinical anastomotic leaks according to anastomotic site was as illustrated in table 4.27.

Table 4.27

Lower GI Surgery: Clinical Anastomotic Leaks

Anastomotic Site	Sutured (198)	Stapled (186)
Ileo-colic	5 (6.9%)	1 (1.3%)
Colo-colic	3 (6.5%)	3 (6.8%)
Colo-rectal	6 (8.5%)	2 (3.5%)
Colostomy Closure	0	0
TOTALS	**14 (7.1%)**	**6 (3.2%)**

Emergency re-operation was required in 12 patients for peritonitis (7 sutured, 5 stapled). Six patients died as a result of their anastomotic dehiscence of which 4 were in the sutured group and 2 in the stapled group.

ii) Radiological Anastomotic Leaks

Water soluble contrast enemas were performed between days 4 and 8 post-operatively in a total of 175 patients with distal colo-colic (25 sutured, 34 stapled) or colo-rectal anastomoses (62 sutured, 54 stapled). This revealed an additional 15 asymptomatic radiological leaks of which 12 were in patients with sutured anastomoses (2 colo-colic, 10 colo-rectal) and 3 in patients in the stapled group (1 colo-colic, 2 colo-rectal), a difference which is statistically significant ($x^2 = 6.02$; $p = 0.014$)

Infective Complications

i) Wound Infections

Bacteriologically proven wound infections occurred in a total of 34 patients (8.9%). Although these were unevenly distributed between the sutured and stapled groups in terms of anastomotic site, in total there were equal numbers of infections in the two groups (table 4.2).

ii) Total Sepsis Score

The calculated total sepsis scores for sutured and stapled patients undergoing each procedure are illustrated in table 4.28. No significant differences between the two anastomotic techniques were demonstrated and groups were also similar with respect to the development of cardiovascular and respiratory complications.

Table 4.28

Lower GI Surgery: Post-Operative Infective Complications

Anastomosis (n)	Wound Infections	Sepsis Score (mean ± sem)
sutured (72)	2	3.78 ± 0.39
Ileo-colic		
stapled (75)	6	4.23 ± 0.35
sutured (46)	4	3.50 ± 0.39
Colo-colic		
stapled (44)	5	4.82 ± 0.75
sutured (71)	11	4.73 ± 0.40
Colo-rectal		
stapled (57)	5	3.83 ± 0.46
sutured (9)	0	1.33 ± 0.51
Colostomy Closure		
stapled (10)	1	2.70 ± 0.60

Blood Tranfusion

Fifty one patients in the sutured group (25.8%) received a per-operative or post-operative blood transfusion as compared with 55 patients in the stapled group (29.6%) ($x^2 = 0.76$; $p = 0.404$). For those patients requiring transfusion, the mean number of units of packed cells required was similar in the two groups (3.14 $\pm$ 0.31 sem vs 2.65 $\pm$ 0.28).

Immediate Post-Operative Outcome

Twenty patients died within 30 days of surgery, an operative mortality of 5.2%. Eleven of these deaths occured in the sutured group and 9 in the stapled group and their distribution according to surgical procedure is illustrated in table 4.29. Five deaths were directly attributable to anastomotic dehiscence (3 sutured, 2 stapled). One patient who had undergone sutured low anterior resection died of a ruptured aortic aneurysm on the second post-operative day while the remaining deaths were all cardiorespiratory in nature.

As with the upper gastro-intestinal group, there were no differences between the sutured and stapled patients with respect to the duration of post-operative hospital stay (table 4.29).

Table 4.29

Lower GI Randomised Group: Post-Operative Outcome

Procedure (n)	Deaths	Hospital Stay (mean days ± sem)
sutured (72)	5	12.3 ± 0.8
Ileo-colic		
stapled (75)	7	15.0 ± 1.7
sutured (46)	1	16.4 ± 1.6
Colo-colic		
stapled (44)	2	14.6 ± 2.1
sutured (71)	5	16.2 ± 1.1
Colo-rectal		
stapled (57)	0	14.7 ± 1.1
sutured (9)	0	7.7 ± 0.7
Colostomy Closure		
stapled (10)	0	9.8 ± 1.6

Long-Term Outcome

As previously pointed out, this project was designed primarily to compare the immediate clinical outcome associated with sutured and stapled gastro-intestinal anastomoses. The follow-up procedure did not allow an accurate determination of the incidence of long term complications. The following list of documented local recurrences and non-neoplastic anastomotic strictures is therefore not intended to represent the true incidence of these complications but merely serves to illustrate that there were no major discrepancies between the two groups.

i) Local Recurrences

Of the 230 patients (118 sutured, 112 stapled) who underwent resection of Dukes' A, B, or C large bowel cancers, a total of 11 developed proven local recurrence (4.8%). The methods of diagnosing recurrence were re-operation (4 cases), post-mortem (4 cases), endoscopic anastomotic biopsy (2 cases) and ultrasound scan (1 case). Six of these recurrences developed in patients who had a stapled anastomosis and five in patients with a sutured anastomosis. The six stapled cases comprised 4 colo-rectal and 2 ileo-colic anastomoses as compared with 3 colo-rectal and 2 ileo-colic cases in the sutured group. Nine of the 11 cases of local recurrence occurred in patients who had locally advanced carcinomas (Astler-Coller stage C2) at the time of initial surgery. Further "curative" surgery was possible in only one patient; a lady who developed pelvic recurrence following a sutured low anterior resection for rectal carcinoma and who subsequently had an abdomino-perineal resection.

ii) Anastomotic Strictures

Only 2 patients have developed symptomatic anastomotic strictures which have been non-neoplastic in nature. One of these occurred in a patient who had undergone emergency sigmoid colectomy for volvulus, a funtional end-to-end stapled anastomosis being constructed between the rectosigmoid and descending colons. This patient subsequently underwent re-operation with resection of the strictured anastomosis and has had no further problems since. The second stricture occurred in a patient who had undergone sutured low anterior resection for rectal carcinoma, the anastomosis being protected by a defunctioning tranverse colostomy. Repeated biopsies have shown no evidence of recurrent tumour and although the stricture has been dilated, the colostomy remains un-closed.

4.3 Non Randomised Patients

This group comprised a total of 82 patients in whom the surgeon chose to use a particular anastomotic technique. Forty five of these patients had their anastomoses electively stapled (54.9%), 28 were electively sutured (34.1%), and in 9 patients (11.0%) a combination of the two techniques was used. The surgical procedures which constituted this group are summarised in table 4.30.

Table 4.30

Non-Randomised Patients: Surgical Procedures

Procedure	Sutured	Stapled	Combination
Oesophagogastrectomy	3	3	3
Polya gastrectomy	1	3	1
Roux conversion	0	7	0
Roux Polya gastrectomy	0	4	0
Biliary bypass	1	4	1
Small bowel resection	5	2	1
Colonic surgery	8	8	2
Anterior resection	8	14	0
Colostomy closure	3	0	0
TOTALS	28	45	9

4.3.1 Reasons for Non-Randomisation

This can be described with reference to the list of surgical procedures illustrated in table 4.29.

1. Oesophago-gastrectomy

Nine patients underwent subtotal oesophago-gastrectomy of whom 3 had electively sutured anastomoses. Two of these patients had initially been randomised to stapling but were electively sutured when the oesophageal mucosa was seen to split on attempted insertion of the circular stapler. The remaining electively sutured case was one where the oesophagus was noted to be of particularly small calibre and the surgeon feared that it would split on insertion of the circular stapling instrument.

Three patients had electively stapled oesophago-gastric anastomoses. Two of these were constructed through the left chest at the level of the aortic arch and the surgeon felt that stapling was the only means of being able to achieve an anastomosis at this level. The reasons for the third electively stapled case are difficult to define and would appear to simply reflect the surgeon's preference.

The remaining 3 patients had a combination of anastomotic techniques employed. These patients were deemed unsuitable for randomisation because the oesophageal wall was extremely thin and fraible after dissection and was thought unsuitable for stapling. In each case the stomach was closed using a linear stapler (TA 90) and the oesophago-gastric anastomosis was completed by hand.

2. Polya Gastrectomy

Five patients in the non-randomised category underwent Billroth II or Polya partial gastrectomy. Three of these patients had electively stapled anastomoses, all because a shorter duration of operation was believed to be in each individual patient's best interests.

One patient with a locally advanced gastric carcinoma had electively sutured anastomoses. Resection was not thought possible but had to be carried out when the stomach was rendered ischaemic during assessment of the lesion. As the anastomosis was then constructed through friable tissue thought to be involved with tumour, the surgeon chose to use a suturing technique.

The remaining patient had a combination of techniques employed with the stomach and duodenum being closed with staples and the anastomosis completed by hand.

3. Roux Conversion/Roux Gastrectomy

Seven patients had Roux loop conversion of an existing gastro-enterostomy performed for biliary gastritis and 4 patients underwent Roux-en-Y reconstruction after a Polya gastrectomy. All but one of these cases were performed by one surgeon who has a special interest in this field and all were electively stapled because the surgeon was convinced of the benefit of the time saving when multiple anastomoses were involved.

4. Biliary Bypass

Four patients had electively stapled cholecysto-jejunostomy for malignant obstructive jaundice. All of these patients also had a prophylactic gastro-enterostomy for impending duodenal obstruction and stapling was chosen for speed of operation in these sick patients.

In two patients, cholecysto-jejunal anastomoses were electively sutured because the surgeons believed the grossly distended gallbladders to be too friable to permit stapling. In both cases the diagnosis was unresectable carcinoma of the head of the pancreas and in one patient a gastro-enterostomy was also performed.

5. Small Bowel Resection

Eight patients in the non-randomised category had small bowel procedures performed. Five of these had electively sutured anastomoses, 3 because the bowel was fixed owing to recurrent intra-abdominal tumour and could not be mobilised sufficiently to allow stapling, 1 because the bowel was obstructed and oedematous, and 1 following resection of ischaemic bowel.

Two patients had electively stapled small bowel resection for operative speed. In the final patient a combination of techniques was employed where the GIA instrument was used to anastomose the two bowel loops but the common entry wounds were sutured because of concern over the the possibility of narrowing the lumen by application of a linear stapler.

6. Colonic Bypass/Resection

Eight patients had electively sutured colonic anastomoses, 4 because of extremely oedematous bowel resulting from obstructing lesions. One patient had resection of an ischaemic sigmoid anastomotic stricture which had been stapled on the previous occasion and one had ileo-colic bypass of recurrent tumour obstructing the terminal ileum where sufficient mobility to allow stapling could not be achieved. The remaining electively sutured patient involved a localised sigmoid resection carried out under spinal anaesthetic through a small transverse incision where access was insufficient to allow stapling.

Eight patients undergoing colonic procedures had electively stapled anastomoses. In 6 cases the stapled technique was chosen because it was believed that reduced anaesthetic time would be of benefit to the patient. Three of these patients were undergoing bypass of unresectable recurrent intra-abdominal tumour. For the remaining 2 cases no particular reason for non-randomisation could be identified other than pressure to complete a busy operating list on time.

Two patients had a combination of techniques employed where the anastomosis was constructed using the GIA instrument but the common stab wounds made to allow introduction of the stapling instrument into the bowel lumen were sutured for fear of narrowing the anastomosis with a linear stapler.

7. Colo-rectal Surgery

There were a total of 22 patients with colo-rectal anastomoses in the non-randomised category. Eight of these patients had electively sutured anastomoses. In 3 cases this occurred because the lesion was

found to be at a lower level than had been anticipated pre-operatively with the result that the patient had not been placed in the Lloyd-Davies position necessary for the transanal introduction of the circular stapler. Three patients were randomised to have a stapled anastomosis but were subsequently electively sutured. In one case the circular stapling instrument could not be negotiated around the sacral hollow and in the second the proximal colon split during introduction of the 28mm sizing instrument. In the third patient an apparently satisfactory stapled anastomosis had been constructed at approximately 7 cms from the anal verge but when the integrity was tested intra-operatively a small posterior air leak was noticed. During attempted repair of this with interrupted sutures the anastomosis was rendered ischaemic and was then taken down and completed by hand. The two remaining electively sutured cases were performed unassisted by junior staff not fully familiar nor confident with the stapling instruments.

Fourteen patients had electively stapled colo-rectal anastomoses. All but one of these were cases of low anterior resection where the surgeon felt that because of the level of the resection and/or a narrow pelvis a hand-sewn anastomosis could not be safely performed. The one remaining case was a reversal of Hartmanns' where no reason for non-randomisation could be identified other than the surgeon's preference for that procedure.

8. Colostomy Closure

Three patients had electively sutured closure of transverse loop colostomy. The closure in all 3 cases was extra-peritoneal and mobility was considered insufficient to allow the application of a stapler.

4.3.2 Results

Because of the small group sizes and the heterogeneity of procedures carried out, no attempt has been made to compare the fine details of the sutured and stapled groups. The following section simply comprises an overall description of the major complications which occurred in the two groups.

There were no major differences between the non-randomised and randomised groups with respect to pre-operative or operative characteristics. In particular the number of patients undergoing emergency surgery and the grade of surgical staff were similar.

Anastomotic Integrity

a) Clinical Anastomotic Leaks

There were 7 clinically apparent anastomotic leaks (8.5%) which are summarised below. Four of these occurred in patients with sutured anastomoses, 2 in patients with stapled anastomoses, and one in a patient in whom a combination of techniques was employed. Three of these patients died as a direct consequence of anastomotic dehiscence.

Sutured Cases

1. Emergency resection of obstructing carcinoma of transverse colon. The patient was found to have a total anastomotic dehiscence at re-operation on day 5 and subsequently died.

2. Extra-peritoneal closure of tranverse colostomy. A faecal fistula became evident on the 6th post-operative day.

3. The development of a faecal fistula 30 days following reversal of a Hartmanns' procedure.

4. Extra-peritoneal closure of transverse colostomy followed by a faecal fistula becoming evident on the 3rd post-operative day.

Stapled Cases

1. Low anterior resection for carcinoma with the anastomosis at approximately 3cm from anal verge. The patient developed peritonitis on the third post-operative day and faecal fluid was noted in the pelvic drain. Re-operation was required with formation of a transverse loop colostomy.

2. Low anterior resection for carcinoma of rectum with anastomosis at 7cm from anal margin. This patient developed a faecal fistula on sixth post-operative day which resolved spontaneously on a low residue diet.

Combination Techniques

1. Resection of extensive carcinoma of splenic flexure which was invading the stomach. The colonic anastomosis was stapled and the gastric closure sutured. Faecal fluid was evident in abdominal drain on the 10th post-operative day. The patient died and no post-mortem was performed.

b) Radiological Anastomotic Leaks

A total of 5 asymptomatic radiological leaks were recorded. Three of these were patients who had undergone stapled low anterior resection for rectal carcinoma. One patient had a sutured anastomosis at the level of the pelvic brim following resection of a

previously stapled sigmoid anastomotic stricture. The final case was the patient where a sutured low colo-rectal anastomosis was constructed after the initial stapled anastomosis had been rendered ischaemic by attempted repair of a small air leak noticed during intra-operative testing of the anastomosis.

Operative Mortality

Ten patients died within 30 days of operation, an operative mortality of 12.2% for the non-randomised group. Four of these patients had electively sutured anastomoses, 3 had electively stapled anastomoses, and in 3 patients a combination of the two techniques had been used. In only 2 cases (1 sutured, 1 combination) could the cause of death be directly attributed to anastomotic leakage.

Other Complications

One patient who had undergone stapled Roux-en-Y loop conversion of a previous gastro-enterostomy for biliary gastritis required emergency re-exploration 4 hours post-operatively for intra-peritoneal haemorrhage. The source of the bleeding was identified as a small artery in the centre of the everted TA 55 staple line at the entero-enteric anastomosis. The haemorrhage was controlled by an under-running suture and the patient's post-operative recovery was unremarkable.

There were no differences between the sutured, stapled, and combination subgroups with respect to infective post-operative complications.

One patient developed an ischaemic stricture at the site of a functional end-to-end stapled anastomosis. This was a patient who had emergency sigmoid colectomy for volvulus and revisional surgery was later required.

Chapter 5

Clinical Studies: Discussion

5.1 Introduction

There can be little doubt that, on a global basis, mechanical stapling devices are being increasingly used for the construction of gastro-intestinal anastomoses. Although there is little published data in support of this, it is apparent from the author's discussions with the United States Surgical Corporation that at the present time they estimate their products to be employed for almost 25% of all anastomoses constructed in the USA. While the surgical community within the United Kingdom has tended to remain somewhat more conservative in its application of this new technology, manufacturers sales figures would suggest that the use of staplers is gradually becoming more widespread. This is true not only of procedures where staplers have frequently been regarded as possessing certain advantages over traditional suturing techniques, such as low anterior resection, but also of a variety of routinely constructed anastomoses throughout the gastro-intestinal tract.

Over the past two decades, the surgical press has witnessed a plethora of reports describing the use of the stapling instruments for a wide variety of procedures. Many of these publications have attempted to compare the results of stapling with conventional suturing methods but the majority of such studies have been retrospective, poorly controlled, and often anecdotal. Furthermore, they have varied widely in their conclusions and have failed to accurately define the relative roles of the two anastomotic techniques. Only a few controlled prospective studies have been performed and fewer still have involved more than a hundred or so patients. At the same time it has been widely claimed both by the manufacturers of the instruments and by the pro-stapling lobby that

staplers possess numerous, largely unproven advantages over hand suturing such as less tissue trauma, less blood loss, less anastomotic oedema and earlier return of gastro-intestinal function. The general reluctance of the pro-stapling lobby to perform scientifically controlled clinical trials is typified by the comments of Ravitch and Steichen, regarded by most as the father figures of modern surgical stapling in the Western World (114);

"Our own experience ..... has led to the conclusion that stapling is at least as reliable as manual suturing."

".....we have not felt justified in performing a prospective randomised comparison of stapling and manual suturing techniques..."

5.2 Study Design

The clinical project described in this thesis is the largest prospective randomised clinical comparison of stapling and suturing techniques which has been performed in the Western World. The participating surgeons all had some preliminary experience with stapling instruments prior to their involvment with the study but none could be classed as regular staple users. All the junior surgical staff attached to the participating Consultants contributed to the project and it must be pointed out that the number of junior surgeons involved was considerable given that the trial was carried out over a 30 month period and the junior staff change frequently. A further point worth emphasising is that the study was performed outwith specialist institutions for gastro-intestinal surgery. All the

participating surgical units function as district general surgical units for their respective catchment populations and no specific referral patterns are in operation for gastro-intestinal surgery.

There are, however, recognised pitfalls in interpreting the results of multicentre randomised controlled clinical trials (15). In order to ensure comparability between the various surgical units, standardisation of pre-operative bowel preparation, per-operative anti-microbial prophylaxis, stapling techniques and all anastomotic materials was agreed according to the study protocol. The only area of variability was in the technique of suturing anastomoses. Prior to commencing the study, the seven Consultants involved from the onset had agreed on a standardised suture technique, as described in Chapter 3, and these surgeons continued to use this technique throughout. The two surgeons who joined the project at a later date were familiar with a different suture technique (single-layer interrupted sero-submucosal) which they had been using throughout the gastrointestinal tract for many years. Naturally, they were reluctant to change their technique to one with which they were less familiar and so this variation was accepted. Perhaps this leaves the study design somewhat open to criticism but it was felt that to enforce a change in a long established suture technique would introduce a further variable into the study. In any case, although it may be possible to lay down a specific suture technique to be used, it seems unlikely that nine Consultant surgeons would suture in exactly the same manner and so to claim that the suture technique was standardised is probably unjustified.

All participants received instruction in the principles of surgical stapling from technical representatives of Autosuture Company, U.K., Ltd. This comprised laboratory workshops and in

theatre assistance. Whenever a surgeon was using an instrument or performing a particular procedure with staples for the first time, a representative was present in theatre to give technical advice. The author regularly visited all the participating surgical units, in each case spending time in theatre to ensure comparability of stapling techniques, the randomisation procedure, and the recording of times and complications. Finally, meetings between the participating surgeons were organised on a regular basis to discuss any problems with the running of the trial and such meetings also provided the opportunity to resolve any discrepancies in the recording of data.

The effect of the surgeon-related variable was well recognised before commencing the study (15) and it would therefore not have been unexpected if anastomotic dehiscence rates were found to vary markedly amongst the participants. In order to minimise the potential influence of this on the overall results of the trial, each participating surgeon's patients were randomised separately. As a result, each Consultant contributed approximately equal numbers of sutured and stapled patients to each procedure group and he thus acted as his own control.

The study was therefore designed to take account of as many of the potential pitfalls as possible. In the light of this and having involved a number of Consultant general surgeons and related junior staff in four general surgical units, it would seem reasonable to suggest that the findings of this project might more accurately reflect the results to be expected in general surgical practice rather than studies performed by a small number of surgeons either with a wide experience of one particular anastomotic technique or working in a specialist institution.

5.3 Immediate Clinical Results

a) Randomised Patients

The close similarity between the sutured and stapled groups with respect to pre-operative data, as described in the results section, emphasises the overall comparabiity of the two groups. There were therefore no obvious patient related factors which might account for any observed differences in outcome.

Anastomosis and Operating Times

The mean anastomosis times for single anastomoses listed on pages 73-79 illustrate that stapling can significantly reduce the time taken to construct a gastro-intestinal anastomosis. Where multiple anastomoses are involved this time saving may be considerable. For example, for patients undergoing partial gastrectomy, stapling saved a mean of 19.1 minutes in anastomosis time compared with manual suturing (table 4.16). These findings are in agreement with other authors (66,97).

Apart from Everett's study (99), this is the only prospective study which has demonstrated a significant reduction in operating time in association with stapled anastomoses. Although Didolkar and his colleagues did observe a significant reduction in anastomosis time, the reduction in operating time was insufficient to achieve statistical significance (66). Similarly, there was no difference in operating time in Reiling's study of triangulated stapled anastomoses (65). Beart and Kelly make no mention of operating time in their evaluation of the circular stapler (97) and in the prospective study

of colonic anastomoses reported by Brennan (98), anastomosis and operating times were not recorded. Finally, McGinn and his colleagues (89) stated that they achieved no time saving with stapled colorectal anastomoses.

The failure of many of these studies to demonstrate a difference in operating time may be related to the small numbers of patients involved. Didolkar's study included only 88 patients (66), Reiling's 100 patients (65), and McGinn's 118 patients (89), although it must be stated that Everett's study also involved only 94 patients (99). Total operating time is influenced by a large number of factors other than the length of time taken to construct an anastomosis. Such factors would include the physical characteristics of the patient, previous surgery and the presence or absence of adhesions, and the fixity or otherwise of the lesion to be resected. This wide variation in operating time is confirmed by the large standard errors listed in tables 4.16 and 4.24 in Chapter 4. Statistical testing is therefore likely only to yield significant results when numbers are sufficiently large as in the study described in this thesis.

Surgical Complications

Surgical complications were more frequently recorded in the stapled group. This is perhaps not surprising. Stapling an anastomosis is very much an "all or none" phenomenon. Once the instrument is locked in place and is being fired no adjustments can be made. On the other hand, while suturing an anastomosis slight amendments to position can be made by the placing of individual sutures. It is reassuring to note that the majority of the surgical complications in the stapled group occurred early in each individual surgeons experience with the stapling instruments and such

complications are now much less frequent. It has consistently been argued by the manufacturers that there is a "learning curve" associated with stapling and the results of this study would tend to confirm this. Once surgeons became more familiar with the stapling technique, operative complications became less frequent.

Of some concern was the frequency of major post-operative haemorrhage related to upper gastro-intestinal anastomotic staple lines. Five patients required re-operation within a few hours of initial surgery for significant blood loss. A much larger number of patients required under-running of the staple line to arrest overt bleeding at the time of operation. There were no major staple line haemorrhages in the lower gastro-intestinal tract.

This potential for staple line haemorrhage in gastric anastomoses has been previously recognised. Ravitch and Steichen report an incidence of major post-operative bleeding of 2.5% during their initial evaluation of stapling in gastric surgery (115). Approximately half of these patients required re-operation and in each case the source of haemorrhage was the GIA gastro-jejunal staple line. Similarly Rignault and his colleagues from France had 5 significant haemorrhages from GIA staple lines in a series of 158 stapled gastric operations (116). Four of the patients in this series required re-operation.

Staples are not intended to be completely haemostatic. The principle behind the B-shaped closure is that the small vessels are not occluded in the limbs of the B and remain patent to ensure a good blood supply to the healing anastomosis. The stomach is, of course, thicker and more vascular than most other gastro-intestinal tissue and so it is not surprising that oozing or frank bleeding from anastomotic staple lines is more commonly seen in gastric tissue. The risk is

greatest with the GIA staples which are manufactured from finer gauge wire than the TA series staples. In the light of our experiences, all the participating surgeons now take great care to inspect GIA and TA staple lines, particularly in the stomach and small bowel. Any bleeding point is immediately under-run and since adoption of this policy, no further patients have required re-operation for staple line haemorrhage. Diathermy is avoided because of the risk of conduction along the length of the staple line and resultant necrosis of the anastomosed tissue.

Anastomotic Integrity

i) Clinical Anastomotic Leaks

The overall clinical anastomotic leak rate for the 600 randomised patients in the project was 4.8% The incidence of leaks was surprisingly similar for upper (4.2%) and lower gastro-intestinal (5.2%) surgery.

The above figure for large bowel surgery is markedly lower than the anastomotic leak rate recorded in the Large Bowel Cancer Project (1). In this multicentre prospective study, Fielding and his colleagues reported an overall clinical anastomotic dehiscence rate of 13%, the incidence of leakage being significantly higher following anterior resection of the rectum (18.7%) than for intra-peritoneal colonic anastomoses (10.8%) (1). Other published series suggest a more typical clinical leak rate for colorectal surgery of approximately 8-10% for both suturing (28,117,118,119,) and stapling (98,120) techniques. One notable exception to this is Matheson's group in Aberdeen. Using a single layer, appositional, interrupted,

serosubmucosal manual suturing technique, they report only 3 clinical anastomotic leaks (1.5%) in a series of 204 patients undergoing elective large bowel surgery (121). All 3 of these leaks occurred in a group of 140 patients undergoing anterior resection, equating with a clinical leak rate of 2.1% for colorectal anastomoses. While these results are very impressive and frequently regarded as a "gold standard" against which other techniques are compared, it must be remembered that they represent the dedication of a single Consultant Surgeon with a special interest in this field of surgery. On balance, therefore, the results of the study described in this thesis stand up well in terms of the published literature, particularly an overall 3.2% clinical leak rate for stapled large bowel anastomoses.

A clinical leak rate of 4.2% for upper gastro-intestinal anastomoses is perhaps a little disappointing, although this figure is not dissimilar from published series (62,63,122). Six of the nine clinical leaks recorded were from duodenal stumps (1 sutured, 6 stapled) and this would appear to be a common site of leakage in the upper gastro-intestinal tract (122).

Although the overall incidence of clinical anastomotic dehiscence was very similar in the sutured and stapled groups, marked differences between the two techniques were observed in the upper and lower gastro-intestinal tracts (table 5.1)

Table 5.1

Summary of Clinical Anastomotic Leaks

	Sutured	Stapled	p value
Upper GI	2	7	0.12
Lower GI	14	6	0.09
TOTALS	**16 (5.3%)**	**13 (4.3%)**	**0.58**

The difference between the two techniques in the upper gastro-intestinal tract is accounted for by the number of stapled duodenal stump leaks. Of a total of 36 randomised stapled duodenal stumps, 5 leaked, an alarmingly high dehiscence rate of 13.9%. In one case leakage was possibly attributable to the use of diathermy to arrest staple line bleeding. Plain abdominal radiography on the third post-operative day revealed disruption of the centre of the staple line and at re-operation this area of the duodenal stump, corresponding to the area which had been cauterised, was necrotic. In the remaining 3 cases no cause could be identified and 2 of these patients were undergoing surgery for benign disease. All of these stapled duodenal stump leaks were recorded during the first 18 months of the study and since then many of the participating surgeons have been routinely inverting the stapled duodenal stump with interrupted sutures. No further leaks have been evident since adoption of this policy combined with conscious efforts to avoid the injudicious use of diathermy in close proximity to the stapled duodenal stump.

This high incidence of stapled duodenal stump leakage differs from the majority of published literature which suggest comparable leak rates for suturing and stapling techniques (123). In fact, Lowdon reported the exact opposite of our findings. He experienced a sutured duodenal stump leak of 12.1% against 1.9% for stapled closure (63). Most of his stapled duodenal stumps were inverted with chromic catgut sutures.

In contrast to the results for the upper gastro-intestinal tract, the experiences of this study were that in colonic and colorectal surgery, fewer clinical anastomotic leaks occurred in the stapled group. This difference was the result of the relatively high dehiscence rates for sutured ileo-colic and colorectal anastomoses. For the sutured and stapled groups as a whole, the difference in lower gastro-intestinal leak rates almost reached statistical significance ($p = 0.09$). These results are in contrast to the findings of McGinn and his colleagues. They reported a significantly higher incidence of dehiscence for stapled low colorectal anastomoses compared with their manual suturing technique (89). Most other authors have reported no difference in the incidence of clinically apparent large bowel anastomotic dehiscence for the two techniques (91,92,93,94,95,96,97,98,99) but it must be remembered that prospective studies are rare and those which have been reported have involved relatively small numbers of patients.

ii) Radiological Anastomotic Leaks

It had been intended that all readily accessible anastomoses be examined radiologically using water soluble media between the 4th and 8th post-operative days. In practice, for a variety of reasons, this

proved impossible to achieve. Only in the oesophageal and colorectal groups were the proportions of patients examined sufficiently high to allow valid comparisons to be made.

All the oesophageal anastomoses were examined radiologically but no contrast leaks were observed. Of the 62 sutured colorectal anastomoses examined radiologically, 10 asymptomatic contrast leaks were seen (16.1%) as compared with only 2 leaks among the 54 (3.7%) stapled anastomoses similarly examined, a difference which is statistically significant ($p = 0.03$). Adding these figures to the clinical anastomotic leaks results in a total of 16 sutured colorectal leaks (22.5%) and 4 stapled colorectal leaks (7.0%) ($p = 0.02$).

This high incidence of asymptomatic radiological leakage for sutured colorectal anastomoses is similar to Goligher's experience (91). He reported an astonishingly high contrast leak rate of 29% for such anastomoses as compared with only 6.5% for SPTU stapled anastomoses. The relevance of such asymptomatic leaks is uncertain although it seems reasonable to propose that they may give rise to peri-anastomotic sepsis and thus possibly predispose to significant anastomotic dehiscence or stenosis. Evidence to support this, however, is lacking.

For all anastomotic leaks, no correlation could be made with respect to the grade of the surgeon, the category of operation, or pre-operative patient characteristics.

Outcome

There were no differences between the sutured and stapled patients with respect to the incidence of infective post-operative complications, return of gastro-intestinal function, operative

mortality, hospital stay, or requirement for post-operative blood transfusion. In summary, the randomised study would suggest that the overall clinical results obtained with the two anastomotic techniques are comparable.

b) Non-Randomised Group

This heterogeneous group of 82 patients, amounting to some 12% of all patients studied, contains some of the most important data of this study. It is not so much the clinical results which are important but the reasons for non-randomisation.

From the data presented in Chapter 4, it is evident that there were two procedures in which surgeons frequently elected to use staples for the anastomosis. The first of these was the formation of Roux-en-Y loops, either as a revisional procedure following previous gastric surgery or in combination with a polya gastrectomy. All 11 of these operations were carried out by one surgeon who felt that the marked time saving by stapling these multiple anastomoses compared with suturing justified the expense of the equipment.

The second procedure which was frequently electively stapled was low anterior resection and these cases were contributed by all the participating surgeons. Fourteen such operations were performed and in each case the anastomosis was below the peritoneal reflection (mean 7.3cm $\pm$ 1.0 sem from anal verge). These 14 cases amount to 18.5% of all anastomoses constructed below the peritoneal reflection in the entire study. In each case, the recorded reason for non-randomisation was that the surgeon believed he was unable to or could not safely suture the anastomosis, either because of the low

level of the resection or for anatomical reasons such as a narrow pelvis which limited access. Without a circular stapler, the surgeons believed that these patients would require an abdomino-perineal resection.

There will always be debate as to the level at which an anastomosis can be sutured and below which it cannot. This level will depend not only on patient related factors but also on the skill and experience of the surgeon in low rectal surgery. Some surgeons with an interest in this field will argue that if an anastomosis can be stapled then it can also be sutured irrespective of its proximity to the anal verge (89). Other colorectal surgeons, however, are convinced that circular staplers allow restorative procedures to be performed at a lower level in the rectum (88,90,97). Rather then stating absolute levels, what is important for each individual surgeon is his own ability to safely construct a colorectal anastomosis and for the majority this can probably be done at a lower level with a stapler than it can by manual suturing.

With the exception of two patients in the oesophageal group who had anastomoses at the level of the aortic arch, there were no clear reasons for non-randomisation in the remainder of the electively stapled group. Human nature will inevitably come into a study such as this and it is probably fair to state that many of these cases were electively stapled simply to shorten the duration of surgery, for patient related reasons or otherwise.

Where obstructed and oedematous bowel was being anastomosed, most surgeons elected to use a suturing technique as they were uncertain about the use of staples in such conditions. In many of the other electively sutured cases, stapling would not have been possible.

Reasonable mobility is required to allow construction of a functional end-to-end stapled anastomosis and such mobility was often not possible where there was recurrent intra-abdominal tumour, or in extra-peritoneal closure of loop colostomy. The cases where the reason for electively suturing was given as unfamiliarity with the stapling equipment were all done by junior surgeons unassisted by Consultants.

5.4 Long-Term Outcome

The original aim of this study was to compare the immediate clinical outcome associated with the surgical stapling and manual suturing of gastro-intestinal anastomoses. At the onset of the project no specific attempt was made to accurately gauge the incidence of local recurrence. A number of reasons contributed to this decision. Firstly, the large geographical areas served by the district hospitals, particularly Inverclyde Hospital, Greenock, and Raigmore Hospital, Inverness, meant that many of the patients were primarily followed up at smaller local hospitals or by their General Practitioners and only referred back to the principal surgical unit if thought necessary. Secondly, outwith the academic unit in the Western Infirmary, neither the facilities nor the manpower were available to allow regular endoscopic examination of those patients who had undergone resection of distal colonic cancers. As previously discussed such a follow-up programme is essential along with full post mortem information if accurate local recurrence rates are to be determined. As a result, the only local recurrences which have been detected and are reported in Chapter 4 are simply those patients who

have presented to the review clinics with further symptoms or a palpable mass and have then been investigated and/or undergone re-operation.

Clearly this lack of information concerning recurrence rates must currently be regarded as a deficiency in this study. However, a prospective follow-up study is now in progress and will be reported at a later date. This is being coordinated by a full-time Surgical Research Fellow and has been restricted to those patients who have undergone resection of left colonic and rectal tumours in whom the anastomosis can be directly visualised by flexible lower gastro-intestinal endoscopy.

The same statements apply to the incidence of anastomotic strictures. Because of the lack of intensive follow-up the only strictures which have come to attention to date have been those which have been symptomatic and these are described in Chapter 4. The prospective follow-up study currently in progress should yield some information as regards the incidence of asymptomatic strictures although there may inevitably be some difficulty in attempting to grade such strictures

5.5 Economic Considerations

One of the most frequent criticisms of surgical stapling is the expense involved when compared with manual suturing. There can be no denying that staplers are vastly more expensive than conventional suture materials. At the present time each disposable linear stapler cartridge (GIA and TA series; Autosuture U.K. Ltd, Ascot, Berkshire) costs around £50 whereas the more complex disposable circular stapler

(curved EEA: Autosuture U.K. Ltd.) costs approximately £150. A single stapled small bowel, colonic, or gastrojejunal anastomosis therefore amounts to a minimum of £100 (1 GIA and 1 TA55) whereas a stapled anterior resection by the technique described by Knight and Griffen (102) involves approximately £200 in stapling costs. In marked contrast a box of twelve 2/0 polyamide sutures ("Nurolon", Ethicon) costs approximately £7.95 and a box of 36 coated polyglycolic acid sutures (Dexon Plus, Davis and Geck) approximately £46.05 (Pharmacy Department, Western Infirmary, Glasgow; October 1988).

The cost of materials used to construct the anastomosis has to be seen against the overall cost of surgical care. This is something which is extremely difficult to estimate in the context of the National Health Service in this country. Major complications requiring re-operation and prolongation of hospital stay would be regarded in the private sector as vastly increasing the cost of caring for that individual patient. However, to the Administrator of a Health Service Hospital, it is the overall cost of running the surgical service which is important rather than the individual patient cost. Under this set up, a lower incidence of complications might be regarded as increasing the total costs to the Health Service. A reduced duration of hospital stay would allow more patients to be admitted and to undergo surgery in any given time and this would undoubtedly incur greater total expense than having the same patients occupy the beds for a longer period of time. Greater efficiency is therefore not cheap in terms of overall cost although the cost per individual patient may be reduced.

To gauge the influence of anastomotic technique on the overall cost of surgical care, the private sector was approached. The following examples of hospital costing were kindly provided by AMI, Ross Hall Hospital, Glasgow in May, 1988.

Patient undergoing uncomplicated low anterior resection

Hospital Stay £130.00 per day x 18	=	£2340.00
haematological & biochemical assays	=	£ 63.50
pre-operative chest X-ray	=	£ 56.00
anaesthetic costs	=	£ 58.04
analgesia, per-operative antibiotics	=	£ 66.59
urinary catheter + drainage bags (3 days)	=	£ 8.94
intravenous fluids (including 2 units packed red cells)	=	£ 117.08
post-op gastrograffin enema	=	£ 87.00
Total excluding anastomotic materials	=	**£2797.15**

Sutured anastomosis: 2/0 nurolon x 10	=	£ 6.62
Stapled anastomosis: EEA 31 and TA 55	=	£ 200.00
Total cost if anastomosis sutured	=	£2803.77
Total cost if anastomosis stapled	=	£2997.15
Percentage increase in cost if stapled	=	6.9%

Anterior Resection Complicated by Anastomotic Dehiscence, Pelvic Sepsis and Requiring Re-Operation

Hospital Stay £130 per day x 39	=	£5070.00
haematological and biochemical assays	=	£ 296.00
pre-operative chest X-ray	=	£ 56.00
1st operation anaesthetic costs (anterior resection)	=	£ 58.04
2nd operation anaesthetic costs (defunctioning colostomy)	=	£ 33.11
urinary catheter and drainage for 18 days	=	£ 47.80
total intravenous fluids (including 5 units of packed red cells)	=	£ 185.35
total parenteral nutrition for 11 days	=	£ 980.00
gastrograffin enema x 2	=	£ 174.00
ultrasound abdomen and pelvis	=	£ 90.00
Total excluding anastomotic materials	=	**£6990.30**

Total cost if anastomosis sutured	=	£6996.92
Total cost if anastomosis stapled	=	£7190.30
Percentage increase in cost if stapled	=	2.8%

As is well illustrated above, the material costs involved in the construction of the anastomosis amount to only a very small proportion of the total cost of caring for a patient during their period of hospital stay. It is also evident that major complications such as anastomotic dehiscence markedly increase costs and so to simply compare the material costs of sutures and staples without regard to

clinical results is irrelevant to the overall expense of surgical care. If the results of the project described in this thesis were applied to the private sector, then the extra costs involved in stapling a large bowel anastomosis might be justified in terms of the apparent lower incidence of anastomotic leaks. The opposite would appear to be the tendency for upper gastro-intestinal surgery.

The reduction in the duration of operating time associated with stapling may also be intepreted differently in the Health Service and private hospitals. In the private sector, time costs money and so if the reduction in operating time means that an extra case can be placed on the theatre list then efficiency is improved and the revenue to the hospital is increased. Efficiency would also be improved in the Health Service hospital but again this may mean an increase in the total cost of running the service. Alternatively, if the reduced operating time simply meant more frequent or longer coffee breaks between cases, then the reduced operating time would be of no benefit.

One further point which must be taken into account in comparing costs is the frequency of abdomino-perineal resection. Figures from the Pharmacy Department of the Western Infirmary, Glasgow in October 1988 would suggest that the approximate per annum cost of providing colostomy bags for a patient who has undergone such a procedure would be around £900. As previously discussed, 14 patients in this study had electively stapled colorectal anastomoses because the surgeon felt that the anastomosis was too low to manually suture. Quite apart from the superior quality of life associated with restorative resection (87) the £200 cost of the staplers pales into insignificance when compared with the ongoing costs of supplying stoma bags after total rectal excision.

5.6 Summary

There can be little doubt that surgical stapling has a role to play in modern gastro-intestinal surgery although its exact niche remains to be defined. The current findings of the ongoing project described in this thesis would suggest that there is little difference in the overall clinical results associated with the two techniques in routine surgical practice, although there does appear to be a tendency towards fewer anastomotic leaks associated with stapling for colonic and colorectal surgery while the opposite appears to be the case for the upper gastro-intestinal tract. It seems likely, however, that anastomotic dehiscence will continue to complicate gastro-intestinal surgery irrespective of the anastomotic technique although it has recently been suggested that the intra-luminal protection of a newly created anastomosis by means of a bypass tube may markedly reduce the risk of leakage (2).

This study has conclusively demonstrated that stapling can significantly reduce operating time and if this is utilised correctly then there is the potential for improving efficiency. Undoubtedly, staples are more expensive than sutures but such material costs form only a small proportion of the total cost of running a surgical service. These increased costs should be considered against such factors as the reduced operating time and costs of providing colostomy bags after abdomino-perineal resection.

Finally, the long term fate of stapled anastomoses remains to be seen. To-date this study has not witnessed any excess of local recurrences or anastomotic strictures associated with stapling, although admittedly the method of follow-up is insufficient to detect

all cases. A prospective follow-up study is currently in progress utilising regular endoscopic examination and this will be reported at a later date.

PART III

EXPERIMENTAL STUDIES

SECTION 1

Background and Literature Review

Chapter 6

Local Recurrence of Colorectal Cancer

6.1 Introduction

Colorectal cancer remains the second most frequent cause of cancer related death in the Western World. Only carcinoma of the bronchus in the male and of the breast in the female are more prevalent. It is estimated that in the affluent societies of North Western Europe, North America, Australia, and New Zealand, approximately 1 in 20 of the population will develop large bowel cancer by the age of 75 years (124).

Surgery has a role in the management of all three of these major human malignancies, which account for over 50% of all cancer related deaths in Western countries (125). However, only a small proportion of patients with lung cancer are amenable to potentially curative resection, largely because of the typical late presentation and advanced stage of disease (126). Furthermore, for breast cancer, there is, as yet, little evidence that surgery significantly improves long term survival (127). In marked contrast, surgery has a major potentially curative role in the management of colorectal cancer. Approximately one half of all patients who undergo ostensibly curative resection of colorectal cancer may be expected to survive five years (83,128,129,130). After this time, about 80% of survivors appear safe from the threat of developing recurrent disease (131) although a small proportion (3-5%) will go on to develop a second primary tumour in the remaining large bowel (131,132).

At first site these figures for colorectal cancer may appear gratifying when compared with surgery for carcinoma of the breast and bronchus. However, it must be pointed out that despite improvements in operative and anaesthetic techniques, recent years have seen little

improvement in the long term prognosis for patients with colorectal cancer (133). Although Ohman did demonstrate a significant increase in the five year survival of patients presenting with potentially curative disease from 1950 to 1979, he found that this was largely due to a reduction in operative mortality (134). Other than the development of effective treatments for advanced disease what measures can therefore be taken to improve the prognosis?

In common with many other malignancies, the stage or extent of spread of large bowel cancer is a major determinant of outcome. It is recognised that little more than one half of all patients presenting with colorectal cancer have disease at a stage which is potentially curable (135,136,137,138). Although it seems probable that this figure could be increased by an effective population screening programme designed to detect disease at an earlier stage in its natural history, this remains to be proven.

For all patients who do undergo potentially curative surgery, attempts at improving the long term progosis must therefore focus on minimising the risk of developing recurrent disease. Recent work has suggested that adjuvant radiotherapy (139) or chemotherapy (140,141) may have a role here but clearly a full understanding of the patterns and mechanism of recurrence is required.

6.2 Recurrent Colorectal Cancer

Recurrence of colorectal cancer may be considered in terms of two distinct entities; distant or systemic metastases and local failure.

Distant Metastases

The development of distant metastases, most notably within the liver, is a frequent cause of death in patients with gastro-intestinal tract cancer. Approximately 15-20% of all patients coming to surgery with colorectal cancer already have overt metastatic liver disease (142,143,144,145). Autopsy series have revealed that around 50-80% of patients who die of colorectal cancer have hepatic metastases (146,147,148,149). Once established, the prognosis for patients with liver metastases is poor with median survival times of between 5 and 9 months (150,151,152,153).

The traditional theory for the development of liver metastases from a colorectal primary has been the embolisation of tumour cells along the portal venous drainage with resultant trapping of these cells in the hepatic sinusoids. Manipulation of the tumour at operation might encourage such embolisation (154,155,156), and experimental studies have demonstrated that anaesthetic (157) and surgical stress (158) may enhance the ability of these embolised cells to establish themselves within the liver. In order to reduce this risk of venous embolisation, Barnes advocated early ligation of the vascular pedicle prior to full mobilisation of the tumour bearing segment of bowel (159), a policy which subsequently gained popular support (154,160,161). Turnbull claimed, in an uncontrolled, non-randomised study, that this "no-touch isolation technique" offered a marked survival advantage over conventional dissection techniques

(161). However, he was comparing his results using this "no-touch" technique with other surgeons who continued to use conventional dissection techniques and it is apparent that he was operating on patients with less advanced disease and performing more extensive resections.

Nevertheless, there has recently been some objective evidence in support of the policy of early vascular ligation. Wiggers and his colleagues prospectively randomised 236 patients undergoing ostensibly curative resection of colorectal cancer to either conventional or "no-touch isolation" dissection techniques and found a slight but non-significant survival advantage over five years in favour of the "no-touch" technique (162). Twenty two patients in the conventional group developed liver metastases as compared with fourteen in the "no-touch" group and with the latter technique metastases tended to occur later.

Other authors have suggested that adjuvant cytotoxic portal vein perfusion may reduce the incidence of colorectal liver metastases (141,163). Taylor, in a prospective randomised study, reported that seven days of continuous portal vein infusion of 5-fluorouracil commencing at the time of surgery (1 gram per 24 hours administered via an umbilical vein catheter) led to fewer patients developing overt liver metastases and a significant overall survival advantage (141).

Recently, Finlay has proposed an alternative explanation for the metachronous development of liver metastases. He suggested that up to 30% of patients undergoing ostensibly curative resection of colorectal cancer may already have occult micrometastases within the liver (164). Because of the relative insensitivity of currently available diagnostic modalities, these patients are not readily identified. Finlay and his colleagues, however, infer that it is the

presence of these occult tumour deposits at the time of surgery which is the most important factor determining the later appearance of overt metastases and ultimate survival. Clearly early ligation of the vascular pedicle would have no influence on the subsequent development of overt liver metastases from such occult deposits and so it remains unclear as to whether portal vein tumour embolisation prior to or during surgery is the most important mechanism.

Local Recurrence

It is probably fair to state that the importance of local recurrence as a determinant of survival in patients with colorectal cancer has frequently been underestimated. Local recurrence may be considered a failure to surgically eradicate the lesion and so clearly the effect of surgical technique on local failure rates merits further investigation.

The remainder of this Chapter reviews the problem of local recurrence and considers the potential influence of anastomotic technique on the recurrence risk.

6.3 Incidence of Local Recurrence

The incidence of local recurrence is variously reported, ranging from 1.8% (165) to 38% (166) of cases in which surgery was thought to have been curative. This variation may partly reflect differences in defining what exactly constitutes local failure. For example, "anastomotic recurrence" or "suture-line recurrence" clearly represents a very localised form of tumour recurrence. Even then the

reported incidence is variable. Hardy described 16 anastomotic recurrences in a series of 1018 patients (1.6%) who had undergone colonic or rectal resection (167). In contrast, Beal and Cornell reported an anastomotic recurrence rate of 15% (168) whilst the incidence in most other series has tended to fall between these two extremes (169,170). However, the recognition of tumour at an anastomotic suture line is by no means indicative of purely "anastomotic recurrence". Labow (171) has proposed that in most cases "anastomotic" or "suture line" recurrence represents ingrowth from pelvic disease, a mechanism which had previously been recognised by others (167,172). Similarly, some cases of local recurrence may commence at the suture line and escape detection until they have penetrated the bowel wall and have presented with pelvic or peritoneal disease. A clear distinction between purely anastomotic recurrence and extra-mural recurrence is difficult to achieve and it is therefore better to consider them both as forming part of the whole spectrum of local recurrence.

For the purposes of this thesis, locally recurrent tumour is regarded as any form of recurrence falling into the category defined by Phillips, this being "convincing evidence of recurrence of cancer at the anastomosis, in the region of the anastomosis, in the abdominal wound, in the drain site or perineum, but <u>not</u> hepatic or peritoneal secondaries" (138). This definition therefore tends to encompass such terms as "anastomotic recurrence", "pelvic recurrence" and "regional recurrence" and its universal application might allow more accurate comparison of recurrence rates.

An illustration of the overall problem posed by local recurrence in general surgical practice was provided by the United Kingdom Large Bowel Cancer Project (138). This prospective audit of outcome

following large bowel cancer surgery involved a total of 94 Consultant surgeons distributed between specialist units and district general hospitals throughout the U.K., and a total of 2336 patients surviving curative surgery were studied. Local recurrence in the absence of distant tumour spread occurred in 14%. If this figure is applied to the United Kingdom as a whole, the true magnitude of the problem becomes manifest. Out of a total of approximately 27000 patients presenting as new cases of large bowel cancer each year (173), 50-60% may be expected to survive a curative surgical procedure (135,137, 138). If 14% of these patients were then to develop local tumour recurrence, this would imply that approximately 1900-2300 patients per annum in the United Kingdom may be expected to present with locally recurrent disease but with no evidence of tumour spread to distant organs.

The importance of local recurrence in terms of its deleterious effects on prognosis has been well documented. Cass and his colleagues retrospectively reviewed the case records of 280 patients who had undergone ostensibly curative resection of large bowel adenocarcinoma in the hospitals associated with the University of Florida over a 15 year period (174). They reported that 37% of all patients developed recurrent disease, of whom 60% presented with local recurrence alone in the absence of any distal spread. Fourteen percent had concomitant local recurrence and systemic spread and the remaining 26% had distant metastases alone. Likewise, Rao reported an overall recurrence rate of 38% in 204 patients following resection of tumours of the rectum, rectosigmoid or sigmoid colon (175). Forty per cent of those patients who developed recurrence had tumour confined to the pelvis or perineum, 28% had recurrent tumour within the abdomen (excluding the liver) either alone or in combination with

pelvic recurrence, 15% had concomitant local recurrence and distant metastases and only 17% had evidence of distant metastases alone. Most notably, in both these series, through five post-operative years local recurrence without evidence of distant metastases was the most common cause of death. Similarly, McDermott in a series of 1008 curative resections for primary rectal carcinoma reported a local failure rate of 11% in the absence of systemic spread, and combined local recurrence and distal metastases in a further 9% (176). Of all patients who died, 27% had evidence of local recurrence alone and a further 24% had both local recurrence and distant metastases.

Aggressive post-operative follow up regimes and autopsy series have revealed even higher local failure rates. In a group of 75 patients selected for single or multiple re-operations on the grounds of locally advanced rectal lesions at the time of original surgery, Gunderson and Sosin found recurrent disease in 52 (69%) (177). Forty eight patients (92%) had evidence of local recurrence with or without regional lymph node metastases and in 25 cases (48%) there was no evidence of systemic tumour spread. A total of 33 patients (44%) had recurrent tumour in the pelvic tissues either alone or in combination with other areas of failure. Shindo reviewed the post-mortem records of 1808 patients dying following curative excision of rectal cancer and discovered pelvic recurrence in 46% while in a clinical series he found that 60% of patients with recurrent rectal cancer had pelvic disease (149). Similarly, Taylor, in reporting an autopsy series of 125 patients, suggested that the cause of death was directly attributable to local failure in 75% of cases (178).

Although its exact incidence remains unclear, it is apparent that local recurrence is a major factor limiting survival in colorectal cancer. In general the post-mortem and "second look" laparotomy

studies as first suggested by Wangensteen (179) would suggest that most clinical series grossly under-estimate the true incidence of local failure. This view is shared by Gilbert and his colleagues (180). They studied the patterns of tumour recurrence in an autopsy series and compared their findings with a clinical series and found a statistically significant difference in the frequency with which recurrence was reported to be confined to a single site between the two groups (55% for the clinical series as compared with 27% for the autopsy group). Furthermore, local recurrence was more common than liver metastases in the autopsy group whereas the opposite was reported for the clinical series. By way of explanation for this variation between clinical and autopsy series, it has been suggested that local recurrence followed rapidly by the development of distant metastases may be a common sequence of events (177,181).

6.4 Characteristics and Prognosis of Local Recurrence

Local recurrence characteristically develops early, the majority of cases presenting within the first two post-operative years. In Cass's series (174), half of all local failures were detected during the first year and 80% were evident by the end of the second year post-operatively. Only 6% became manifest after 5 years post surgery. Polk and Spratt reported 65% of all local recurrences in the first two years and 88% by the end of the fourth year (182). Similarly, Tyndal retrospectively reviewed the records of 800 patients who had undergone curative resection of rectal carcinoma and found that 88% of all local recurrences had developed within two years of operation (183).

The diagnosis of local recurrence is generally regarded as carrying an abysmal prognosis. Further surgical resection offers the only real hope of cure but the reported "curative" re-resection rates of less than 15 per cent in most series serve to illustrate the fate of the vast majority of patients (182,184,185,186,187,188). In addition, the symptoms of locally recurrent disease are distressing and the quality of life consequently poor (189). Even for those patients who do undergo further "curative " surgery, the outlook has usually been regarded as unfavourable with five year survival rates of approximately 20-30% (190). Stulc reported median survival times of 5 months for patients who had no further surgery, 14 months for patients who had palliative resection of their recurrent tumour, and 23 months for patients who had further "curative" surgery (191).

Recently, however, it has been suggested that intensive post-operative follow-up may prove of benefit in that the earlier detection of local recurrence increases the resectability rate. Schiessel and his colleagues demonstrated in a prospective study that 41 per cent of all local recurrences could be diagnosed by carefully planned follow-up which included regular colonoscopy with a potentially curative re-resection rate of 40 per cent (192). Furthermore, Vassilopoulos has shown that complete re-resection of recurrent tumour diagnosed early may be associated with an acceptable long term prognosis, in his series a five year survival of almost 50 per cent with a median survival of 59 months (193).

6.5 Factors Associated with Local Recurrence

Site of Primary Tumour

The belief that extra-peritoneal rectal tumours are associated with a higher incidence of local recurrence when compared with intra-peritoneal colonic lesions has been long established. Gilchrist and David reported an incidence of 16.1% for patients with rectal cancers partly or completely below the peritoneal reflection as compared with 3.6% for patients with intra-peritoneal lesions (194). Furthermore, it has been demonstrated that within the rectum, the risk of local failure appeared to be inversely proportional to the height of the tumour from the anal verge. In 1953 Stearns and Binckley reported a series of 369 patients who had undergone abdomino-perineal resection of rectal cancer and found that the incidence of local recurrence was 30% where the lesion was 0-6cm from the anal margin, 20% for tumours between 6 and 11cm, and 14.5% for lesions above this level (195). Similar findings have since been been reported by other authors, both following restorative resection and total rectal excision (172,181,196,197,198).

More recently, however, Phillips and his colleagues involved in the Large Bowel Cancer Project found that the site of the primary tumour had no significant influence on the subsequent incidence of local recurrence (138). They reported local failure rates of 12.9% for the right colon, 19.3% for the splenic flexure, 12.0% for the left colon, and 13.9% for the rectum and rectosigmoid.

Nevertheless, most surgeons would still regard the risk of local failure being greatest when operating deep in the pelvis, although perhaps this may reflect anatomical and surgeon related factors rather than an effect of the tumour itself (199).

Tumour Stage and Grade

The stage of the primary tumour has a major influence on the subsequent risk of local recurrence. Many retrospective reviews have demonstrated that the more advanced the stage of the tumour at presentation, the higher the risk of local recurrence (175,200). In the U.K., the Large Bowel Cancer Project (138) has prospectively shown a significant association between Dukes' staging of the primary tumour and the subsequent development of local recurrence (A: 4%, B:13%, C:18%). Figures from the Massachusetts General Hospital for rectal cancer illustrate this point even more graphically. Using a modified Astler-Coller staging system (177), Rich and his colleagues report a linear increase in the pelvic recurrence rate from less than 8% for stage A and B1 to almost 67% for stage C3 (201).

Closely related to the Dukes's stage is the histological grade of the tumour (202,203). Whilst positive correlations between high grade tumours and increasing incidence of local recurrence have been reported (204), in the Large Bowel Cancer Project, correction for Dukes's stage rendered the histological grade of the lesion an insignificant variable (138).

Local Tumour Invasion and Tumour Fixity

Fixed tumours are associated with a higher incidence of local recurrence when compared with lesions which are freely mobile (138). Related to this, local tumour invasion has been shown to be of significant predictive value for local recurrence (205,206). In contrast, the actual physical dimensions of a tumour appear to bear no relationship to the risk of local failure (176,190,207,208).

Tumour Perforation and Obstruction

Although it is accepted that obstructed or perforated tumours are associated with a poorer prognosis (209), only Phillips and his colleagues have studied the influence of these variables on the subsequent incidence of local recurrence (138). Both were found to be associated with a significantly increased local recurrence rate.

Patient Age

In 1940 Dukes studied 1000 cases of rectal cancer and found that the lymphatic metastases were present in a significantly greater proportion of patients under the age of 40 as compared with older patients (202). Apparently in agreement with this, Ree reported significantly fewer local recurrences in patients over 60 years compared with those who presented with colorectal cancer under this age (210). Similarly, Malcolm found that the age of 60 years at presentation seemed to represent a watershed in terms of recurrence risk (211). In a review of 285 patients with complete follow-up information he reported that 38% of patients under 60 years developed local recurrence compared with only 24% of patients over this age.

Again, one of the the most valuable sources of information is the Large Bowel Cancer Project where the incidence of local recurrence was found to decrease in a linear manner with increasing age (138).

The Surgeon-Related Variable

The influence of the individual surgeon on outcome in clinical trials is well established (15). Fielding had previously reported this to be a major factor influencing anastomotic integrity following large bowel cancer surgery (1). Similarly, the individual Consultant Surgeon has been shown to be a major prognostic factor determining the

incidence of local recurrence. In the Large Bowel Cancer Project, the local recurrence rate for the 94 participating Consultant Surgeons varied from less than 5% to more than 20%, an effect which was independent of patient related factors (138).

6.6 Local Recurrence and Incomplete Primary Excision

The earliest attempts at rectal excision were made via a perineal approach. Using this technique, Miles reported a three year recurrence rate of 95% (72) because it was impossible to clear the "upward zone of spread". As a result, more radical techniques were developed including Miles's description of a combined abdomino-perineal approach (72,212).

It seems likely that a proportion of rectal cancers will recur because of incomplete primary clearance (213) although a recent review from St Mark's Hospital, London revealed no improvement in survival when radical resection was combined with extended abdomino-iliac lymphadenectomy (214). Most recently, the importance of adequate lateral clearance of rectal cancer has received attention. In 1986, Quirke and his colleagues in Leeds reported the results of a prospective study of 52 patients who had undergone surgery for rectal cancer (215). Serial transverse histological sections of the entire resection specimens were carefully examined by a single pathologist and evidence of tumour spread to the lateral resection margin was detected in 14 cases (27%). After a median follow-up period of 23 months, 85% of these patients with lateral spread had developed a proven pelvic recurrence as compared with a local recurrence rate of

3% for the group of patients in whom the lateral resection margin was clear of tumour, thus emphasising the prognostic significance of incomplete excision of the primary lesion.

Heald proposed that many locally recurrent rectal cancers could be explained by residual tumour being left in the lymphovascular tissues of the pelvis and carried out 113 consecutive anterior resections with total excision of the mesorectum (216). In 5 of these patients the mesorectum contained minute foci of tumour several centimetres distal to the macroscopic lower margin of the rectal tumour and in 2 cases this was the only evidence of lymphatic tumour spread. Employing this technique of mesorectal excision, Heald reports no local recurrences in 50 patients followed up for more than two years post-operatively and he proposes that the incidence of local recurrence of rectal cancer is related to the technique of pelvic dissection (216). There is some justification for his theory in that locally recurrent rectal tumour occurs most commonly posteriorly where the mesorectum lies (167). Similarly, local recurrence does not appear to be as great a problem following pull-through operations in which the mesorectum is likely to have been completely excised in order to permit the pull-through procedure (167). However, local recurrences do also occur following resection of intra-peritoneal colonic tumours (138) where there should be little difficulty in achieving wide resection margins and clearance of lymphatic tissues. Furthermore, local recurrences are reported after resection of Dukes' A carcinomas (138,217) and so clearly there must be other mechanisms to explain these recurrences.

As an alternative to extra-mural tumour spread, incomplete resection of a primary colo-rectal carcinoma may arise because of microscopic intramural tumour spread beyond the macroscopic edge of

the tumour. In 1913, Handley claimed that rectal cancer cells, travelling within the submucosal lymphatics, could frequently be detected within the rectal wall at considerable distances from the macroscopic lesion (218). Cole, writing in the same year, disagreed with this, and observed that in most cases intramural spread was very limited (219). Ever since then the debate has continued as to the extent and relative importance of intramural tumour spread (220,221, 222,223). However, because he was able to detect carcinoma cells at various distances up to four centimetres distal to macroscopic rectal cancers, Grinnell proposed that at least five centimetres of apparently normal bowel should be resected distal to a tumour in order to ensure complete clearance (223), a policy which was to gain popular support.

Williams and his colleagues have recently demonstrated that such a wide distal resection margin is very rarely necessary (224). They studied 50 abdomino-perineal resection specimens for evidence of distal intramural spread and found no such spread in 38 (76%), and spread of less than 1cm in 7 specimens (14%). In only 5 patients was there evidence of distal intramural spread greater than 1cm, all of whom had poorly differentiated Dukes' C carcinomas and were dead or dying from distal metastases within 3 years of surgery. Furthermore, they compared the outcome of patients following potentially curative anterior resection for rectal carcinoma and found that a distal resection margin of less than 5cm had no adverse effect on recurrence rates or on survival. Similarly, other authors have found no correlation between the margin of distal resection and the incidence of local recurrence (207,225,226). It would therefore appear that while microscopic intramural tumour spread may explain some cases of

local recurrence, mainly patients with the poorer prognosis locally advanced high grade tumours, it is unlikely to account for recurrences of less advanced growths.

6.7 Relationship of Local Recurrence to Surgical Procedure

As discussed in Chapter two, recent years have witnessed a gradual shift away from abdomino-perineal excision in favour of low anterior resection for middle rectal cancer. Many feared a concomitant increase in the incidence of local recurrence as most surgeons would probably regard a restorative procedure as a less radical clearance than total rectal excision. No prospective randomised controlled clinical trial of the two techniques has ever been carried out and so no objective information is available. Few valid conclusions can be drawn from the published literature because of the large number of variables involved in most comparisons (surgeon-related, patient-related, tumour-related), the reliance on historical controls, and frequent bias in favour of abdomino-perineal excision for the more advanced or anatomically difficult lesions with anterior resection being reserved for more favourable growths (82,207). However, with the exception of the Large Bowel Cancer Project (227) current evidence would suggest that when recurrence and survival are correlated with tumour stage, differentiation, and the extent of local spread, restorative resection can be regarded as being as curative as total rectal excision (201,208,228,229,230,231).

6.8 Stapled Anastomoses and Local Recurrence

Following the introduction of circular staplers and their use in low anterior resection, there were a number of reports of alarming rates of local recurrence. Hurst reported 11 early anastomotic recurrences following 34 stapled low anterior resections (232). All of these occurred in patients with locally advanced, lymph node positive lesions although the resection margins were histologically clear of tumour. Anderberg described a local recurrence rate of 24% in a series of 38 rectal cancer patients similarly managed (233). Bisgaard also warned of a potentially increased risk of local recurrence following low anterior resection with a stapled anastomosis (234).

Clearly they may be a number of valid reasons to explain this apparently higher than expected local recurrence rate following rectal stapling. Firstly, the inter-surgeon variation may have a role to play (138). It is clear that the specialist centres had already altered their practice in favour of restorative resection prior to the introduction of circular staplers (82). The greatest relative increase in stapled low anterior resection probably therefore occurred in general surgical units amongst surgeons who perform many fewer such procedures per year. It seems plausible to expect that the specialist centres' expertise in the techniques of radical pelvic dissection with preservation of the anal sphincter would be associated with a lower local recurrence rate. Secondly, it is almost without doubt that the increased enthusiasm for sphincter saving operations generated by stapling led to a number of patients with locally advanced, high grade tumours undergoing anterior resection when they might have been better served by total rectal excision. Thirdly,

anatomical factors resulting in difficult dissection or anatomosis, such as a narrow pelvis and/or a low tumour may encourage the use of the stapler and as previously discussed, it is such cases which tend to be associated with higher recurrence rates (172,181,195,196,197).

This latter point has recently been inferred by Rosen (235). In a retrospective review of 119 consecutive, potentially curative anterior resections he found that for middle rectal cancer the probability of recurrence was two and a half times greater with a stapled anastomosis than it was following a sutured anastomosis and that this difference could not be explained by differences in tumour stage or histological grade. No such differences were observed between the sutured and stapled groups with respect to recurrence of either upper or lower rectal cancer. Rosen therefore concluded that the anastomotic technique itself was unlikely to account for the differences observed for middle rectal cancer and proposed that the results might simply reflect patient selection.

As a result of these factors, some authors have counselled for careful selection of patients suitable for stapled low anterior resection and advised that this be restricted to more favourable lesions (233,234,236). However, Elliot has suggested that radical anterior resection can be as curative as abdomino-perineal resection for poorly differentiated carcinoma of the middle rectum (237). Similarly, Heald has proposed that with a policy of total excision of the mesorectum radical anterior resection with a stapled anastomosis can be regarded as an effective curative procedure for all grades of tumour throughout the length of the rectum but stressed the importance of specialisation if the best results are to be realised (238).

A variety of recent reviews have failed to confirm these initial fears of higher than expected recurrence rates following stapled anterior resection. Kennedy and his colleagues found that although use of the circular stapler was associated with a significant reduction in the number of patients with middle rectal cancer requiring abdomino-perineal excision, their local recurrence rates before and after introduction of this technique were similar (239). Odou in a review of his institution's management of rectal carcinoma before and after the introduction of' circular stapling demonstrated that although the frequency with which low anterior resection was performed increased from 42% to 62% of all procedures, the overall local recurrence rate was not significantly altered (240). Similarly, Williams, Durdey and Johnston compared their results of stapled low anterior resection with a historical control group who underwent radical abdomino-perineal resection and found that the corrected five year survival and recurrence rates for the two groups were similar (241). Gillen and Peel reported no significant increases in the rates of operative mortality, morbidity and local recurrence for stapled anterior resection compared with abdomino-perineal resection (242). Leff described similar findings (243). He retrospectively compared the local recurrence rates of sutured and stapled low anterior resection and found that these did not differ significantly despite the fact that in his series the stapler was being used to treat lower rectal lesions. Furthermore, Wolmark using data from two multicentre prospective randomised trials of adjuvant chemotherapy in colorectal cancer found no difference between patients receiving sutured or stapled anastomoses in terms of local recurrence rates or survival, although it must be remembered that the randomisation was not sutures versus staples but of various

radio- and chemotherapeutic regimens (244). Finally, Malmberg reported no increase in the incidence of local failure associated with circular colorectal stapling in Sweden (245).

6.9 Summary

It is evident that local recurrence is a major cause of morbidity and mortality following "curative" surgery for colorectal cancer. Until effective treatments are available for advanced disease, attempts to improve the prognosis of colorectal cancer must focus on minimising the risk of recurrence. Careful and detailed operative technique is essential to ensure complete primary clearance of the tumour. However, inadequate resection is unlikely to account for all cases of local failure and clearly there must be other responsible mechanisms.

As discussed, there are a number of reasons which may account for an apparently higher local recurrence rate associated with rectal stapling. Although many of these bear little direct relationship to the actual anastomotic technique it would nevertheless seem prudent to investigate the potential influence of anastomotic materials on the risks of local recurrence.

The following two sections summarise the evidence for two frequently proposed mechanisms of local recurrence. The related experimental work is addressed to an investigation of how these mechanisms may be influenced by the choice of anastomotic suture material.

SECTION 2

Metachronous Carcinogenesis and Local Recurrence

Chapter 6

Introduction

7.1 Introduction

Large bowel cancer is not infrequently a multifocal disease with synchronous or metachronous carcinomas being reported in 3 to 5 per cent of cases (132,246,247,248). The incidence of synchronous adenomatous polyps in colonic resection specimens is considerably higher and may approach 30% (249). Following resection of a colorectal carcinoma, the remaining colonic mucosa must therefore be considered as being susceptible to further neoplastic change.

7.2 Clinical Studies

Filipe has described abnormalities of the mucosal mucins in the apparently normal colonic mucosa of patients with colorectal cancer (251). This "field change" or "transitional mucosa" comprises a change from the normal sulphomucin predominant pattern to the abnormal sialomucin predominant, and similar changes have been identified in the colonic mucosa of experimental animals treated with carcinogens known to promote colorectal neoplasia (252).

Sunter studied biopsies taken from the anastomotic site of 28 patients following curative resection of colonic cancer (253). In 11 patients (39%) there were non specific inflammatory changes and in 7 cases there was "transitional" change of the mucosa which he regarded as potentially neoplastic. Similarly, Dawson and his colleagues have reported abnormally high sialomucin levels at at least one resection margin of large bowel cancer resection specimens (254).

There is no direct evidence to suggest that these alterations in the mucin patterns are pre-neoplastic. Alternatively, they may simply represent a non-specific response to chronic injury (255), or be a feature of immature colonic mucosa at a site of hyperplasia (256). However, there is accumulating evidence to suggest that they may be of predictive value in identifying patients at high risk of developing local recurrence. Dawson and his co-workers have recently reported the results of a prospective study of 358 patients in whom the presence of sialomucin at either resection margin was determined from the surgical specimen in the immediate post-operative period (257). A sialomucin predominant pattern was observed at one or other resection margin in almost 30% of patients and this proved to be of significant prognostic value, both for the development of local recurrence and for survival. Similar findings had previously been reported by the same group from a retrospective study (258).

7.3 Experimental Studies

Experimental studies have suggested that surgical trauma may act as a co-carcinogen. Rous and Kidd demonstrated that repeated injury could promote the reappearance of tar-induced rabbit ear tumours which had previously regressed (259). Similarly, Gottfried and his group reported the earlier appearance and more rapid progression of dibenzpyrene induced tumours in mice following repeated skin wounding or, more notably, repeated laparotomy (260).

The same applies to carcinogen induced experimental models of colorectal carcinogenesis. Pozharisski demonstrated that even the minimal trauma associated with a solitary implanted suture in the

caecum led to a clustering of colonic tumours around that site (261). Likewise, large bowel tumours in experimental animals have been shown to develop preferentially at the site of a colonic anastomosis, irrespective of whether carcinogen administration preceeds or follows surgery (262,263,264,265,266). Williamson's group have proposed that the adaptive hyperplasia of the mucosa which occurs at the anastomosis following colonic resection may be important in increasing the susceptibility to neoplastic change (263). Alternatively, Rubio has suggested that environmental carcinogens may promote cellular abberations in the colonic mucosa and that when these cells are stimulated to divide by the trauma of surgical resection, malignant transformation occurs (262).

The potential influence of surgical trauma on colorectal carcinogenesis has important implications for large bowel cancer surgery. If it is the case that different anastomotic techniques and indeed different suture materials are associated with variable degrees of trauma to the colonic mucosa then it is not inconceivable that these factors may have some bearing on the subsequent risk of local recurrence. To date, the only experimental study designed to investigate the influence of anastomotic materials and technique on colorectal carcinogenesis has been reported by Phillips (267). He performed transverse colotomies in Wag rats which were then re-sutured with interrupted sutures of either silk or stainless steel wire. After an interval period of two months, animals were subjected to a course of carcinogen injections consisting of dimethylhydrazine, 20mg/kg subcutaneously weekly for 28 consecutive weeks. Animals were sacrificed 32 weeks post-operatively when it was found that stainless steel sutures were associated with a significantly increased incidence of anastomotic tumours compared with the silk sutured group.

Phillips concluded that stainless steel sutures and, by implication, surgical stapling instruments, should not be used to construct an anastomosis following resection of large bowel cancer. However, quite apart from the general criticisms that rodent experimental results cannot be directly extrapolated to man, it can be argued that this experiment is not analogous to the human situation. Initiation of carcinogenesis did not commence until two months following surgery rather than the anastomosis being perfomed in bowel which was already predisposed to neoplastic change. Carcinogen was administered to animals which had a persistent source of inflammation in the colonic mucosa. Part of the experimental work to be described in this thesis has demonstrated that rodents are able to rapidly eliminate conventional suture materials from the bowel wall whereas steel sutures tend to persist much longer. Phillips similarly reported that 36% of steel sutures were still present at the time of animal sacrifice as compared with only 4% of silk sutures. It is therefore possible that the observed difference in tumour yield between silk and steel groups in this study merely reflects the number of sutures remaining and hence the degree of persistent inflammation at the time of carcinogen administration.

7.4 Summary

While there is no direct supportive evidence, it seems entirely plausible that a proportion of "local recurrences" may represent the development of a second primary tumour at or adjacent to the anastomotic site. Recurrence resulting from incomplete primary excision would be expected to occur early and so metachronous

carcinogenesis may be particularly applicable to the 20% or so of local recurrences which present after the second post-operative year. While the process of carcinogenesis is complex and not fully understood, it may be susceptible to local influences at the anastomotic site. The role of the anastomotic suture material therefore merits investigation.

Chapter 8

Experimental Colorectal Cancer and Dynamic Cell Population Kinetics

8.1 Experimental Colorectal Carcinogenesis

8.1.1 Introduction

Recent years have witnessed numerous experimental studies of colorectal carcinogenesis in laboratory animals. Inevitably there will be differences in the behaviour of spontaneously occurring gastro-intestinal tract cancer in man and chemically induced neoplasms in rodents such that the direct extrapolation of experimental results to man is subject to criticism. However, the accumulating experience with certain of these experimental models has suggested that they can be reliably employed to test certain hypotheses under carefully controlled laboratory conditions.

8.1.2 Carcinogens

A number of chemical agents have been shown to behave as fairly specific promoters of large bowel cancer in rodents, the most important of which are the hydrazine carcinogens. Most work has concentrated on three closely related compounds, namely 1,2 dimethylhydrazine, azoxymethane, and methylazoxymethanol. The chemical similarity is evident from the simplified degradative pathway illustrated in figure 8.1.

The carcinogenic activity of these compounds was discovered by Laqueur and his colleagues in 1963 while searching for a possible dietary explanation for the unusually high incidence of amyotrophic lateral sclerosis, a rare degenerative neurological condition, on the

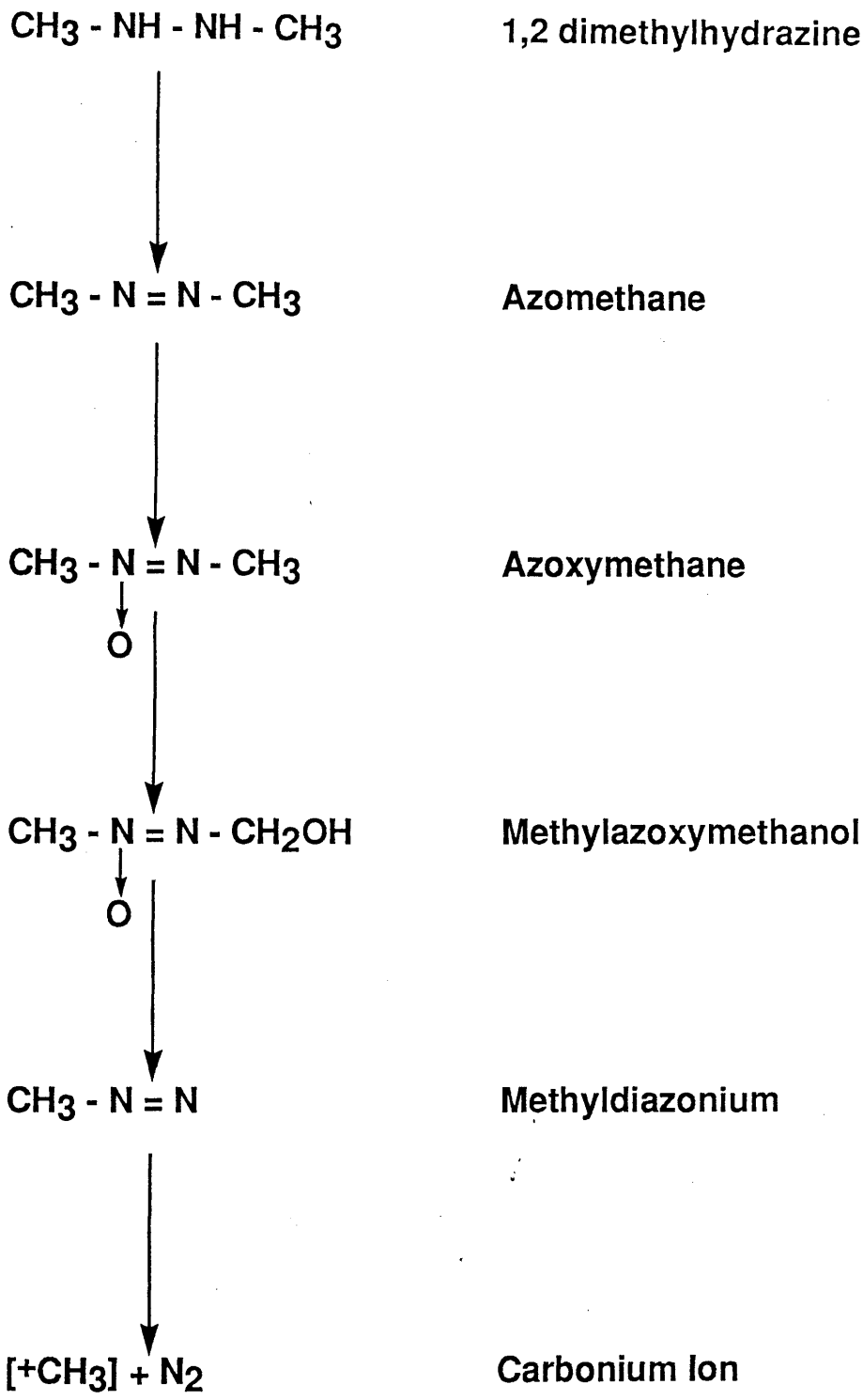


Figure 8:1

HYDRAZINE CARCINOGENS . METABOLIC PATHWAY

island of Guam (268). They observed that cycad flour, made from grinding the nuts of the tropical plant Cycas circinalis, was a major dietary component of natives of the island. It was known that animals which fed on the leaves of this plant developed paralysis and so Laqueur believed that this may be the cause of the human disease. However, when they fed the cycad flour to rats there was no resulting neurological damage but a small number of animals developed colonic adenocarcinomas (268). Prompted to investigate this unexpected observation further, Laqueur went on to demonstrate that the active carcinogen present in cycasin was the B glucoside of methylazoxymethanol (269).

Subsequently Miller suggested that azoxymethane, thought to be a precursor of methylaxozymethanol in the metabolic pathway, might possess similar carcinogenic activity (270). The carcinogenic role of 1,2 dimehylhydrazine was discovered by Druckrey (271) and it was shown that the properties of this compound were very similar to those of methylazoxymethanol. The same group of workers were able to demonstrate that a large proportion of an administered dose of 1,2 dimethylhydrazine is either exhaled as azomethane or is excreted in the urine as azoxymethane. A close biological relationship between these three carcinogens was therefore confirmed and it was proposed that 1,2 dimethylhydrazine was probably activated via azoxymethane to the proximate carcinogen methylazoxymethanol (271). Recently, the hydroxylation of azoxymethane to methylazoxymethanol has been confirmed both in vivo and by rat liver microsomes in vitro (272).

Methylazoxymethanol is chemically unstable and rapidly undergoes spontaneous degradation to formaldehyde and the unstable methyl diazonium ion (272). Further degradation of the latter agent leads to the generation of the methyl carbonium ion which is capable of

alkylating large molecules (272). It is this alkylation of nucleic acid residues which is probably responsible for the carcinogenic effect.

While this pathway of metabolic degradation explains the carcinogenicity of these hydrazine compounds, it does not explain their relative selectivity for the large bowel mucosa. One might expect their administration to result in tumour induction in a wide variety of body tissues but it is well established that 1,2 dimethylhydrazine and azoxymethane induced tumours occur predominantly in the descending colon. The tissue distribution of the carcinogen is unlikely to be responsible for this selectivity. Pozharisski and his group injected tritium labelled 1,2 dimethylhydrazine into rodents and noted extensive alkylation of epithelial cells along the entire length of the small and large bowel and also in the liver and kidney (273). Weisberger suggested that a combination of liver metabolism and bacterial enzymatic activity within the colon may account for the tumour distribution (274). His theory relies on the premise that the admininstered carcinogens are broken down in the liver and excreted in the bile as the chemically stable methylazoxymethanol glucuronide conjugate and that subsequent hydrolysis by bacterial B glucuronidase in the colonic lumen releases the active methylazoxymethanol. However, there is little evidence to support this hypothesis. It has been convincingly demonstrated that the parenteral administration of carcinogen regularly promotes large bowel tumours in segments of large bowel surgically isolated from the faecal stream (275,276). Furthermore, it has been shown that only approximately 1% of an administered dose of 1,2 dimethylhydrazine is excreted in the bile stream (277). It would therefore appear that sufficient carcinogen

can reach the colonic mucosa via the bloodstream to induce carcinogenesis and the relative selectivity of these hydrazine carcinogens for colonic mucosa remains largely unexplained.

Historically, 1,2 dimethylhydrazine has been more widely used than azoxymethane but the general characteristics of the two models are similar. However, on a molar basis azoxymethane is a more potent carcinogen and it possesses certain advantages which make it a more practical carcinogen to use, namely it is easier to dissolve in water and it is chemically more stable than 1,2 dimethylhydrazine (272).

8.1.3 Experimental Carcinogenesis

Both 1,2 dimethylhydrazine and azoxymethane are reliable inducers of colorectal tumours in rats, mice and hamsters. In all of these species the effective dose range of either carcinogen is a weekly parenteral injection of 10-20 mg per kg body weight for approximately 10-15 consecutive weeks (278). Almost all animals treated in this manner will subsequently develop large bowel tumours after a latency period of around 6 months. With increasing total carcinogen dosage, there tends to be an increase in tumour yield, a reduction in the latency period, and an increased frequency of extracolonic lesions (278). In azoxymethane treated animals, the dose of carcinogen also affects the distribution of colorectal tumours. High doses of greater than 15 mg/kg body weight per week leads to lesions predominantly in the distal colon while halving the dose produces mainly proximal lesions (279). The extracolonic manifestations of hydrazine carcinogen administration occur mainly in rats and range from benign abnormalities such as renal cysts and hepatic

haemangio-endotheliomas to malignant lesions such as adenocarcinomas arising in the duodenum and proximal jejunum and squamous carcinomas of the external ear (278).

A number of factors have been shown to influence hydrazine induced colorectal carcinogenesis in these animal models. Of particular importance is the effect of diet. Galloway (280) has recently confirmed previous reported findings that high fat diets increase tumour yield (281) while a high dietary fibre intake tends to have a protective effect (282). Diets high in fat and low in fibre have been shown to be associated with the greatest degree of tumour induction (280).

Increasing the load of bile salts presenting to the colonic lumen, either by surgically diverting the biliary stream (283) or by employing the bile salt binding agent cholestyramine (284) has been shown to increase hydrazine carcinogenicity.

Colonic bacterial flora may also influence chemical carcinogenesis. Laqueur's group demonstrated that cycasin was only effective as a promoter of large bowel neoplasia when administered orally. When injected parenterally, most of the compound was excreted unchanged in the urine (285). Laqueur had previously demonstrated that oral cycasin was ineffectual in germ-free rodents whereas methylazoxymethanol remained carcinogenic (269). From this he concluded that the hydrolysis of cycasin by intestinal bacteria was necessary to release methylazoxymethanol. With relevance to the commonly used carcinogens, Reddy at el found that in germ-free rats the incidence of azoxymethane induced large bowel tumours was increased whereas the opposite was the case for 1,2 dimethylhydrazine

(286). More recently, the oral administration of broad spectrum antibiotics has been shown to markedly reduce the carcinogenenic effect of 1,2 dimethlhydrazine (287).

Of particular importance with regard to the subject of this thesis is the relationship between non-specific colonic injury and chemical carcinogenesis. Various experimental studies have conclusively demonstrated that hydrazine carcinogen induced colorectal tumours occur with increased frequency around the site of colonic trauma or chronic irritation. Pozharisski demonstrated a significant increase in caecal tumour incidence in rats which previously had a non-absorbable suture inserted to construct a caecal diverticulum (261). The formation of a stoma was shown by Navarette to have a similar effect (288). An anastomotic suture line clearly represents a site of such chronic colonic irritation or injury. Consistent with this the anastomotic suture line has been shown to be a frequent site of tumour development in hydrazine carcinogen treated animals (263, 264,266).

8.1.4 Relevance to Colorectal Cancer in Man

A major criticism of all oncological research carried out in experimental animals is the relevance of results obtained to the human situation. However, many studies have suggested that there are certain similarities between chemically induced colorectal cancer in rodents and spontaneous colorectal cancer in man (278). Ross recently critically compared the features of human colorectal cancer and hydrazine induced (using 1,2 dimethylhydrazine, azoxymethane and methylazoxymethanol) large bowel cancer in male Fischer rats (289).

He was able to demonstrate marked similarities between the human and rodent tumours not only in general histological appearance but also with respect to the pathological features of invasive malignancy, the frequent occurrence of benign lesions in close proximity to malignant neoplasms, and the tendency for tumours to develop at sites of mucosal injury. Mucinous tumours, as is the case in man, were associated with a greater invasive tendency and although such lesions occured with greater frequency in the rodent compared with man, the site distribution of the non-mucinous lesions was similar. In terms of pathological differences, it had previously been suggested that hydrazine induced rodent carcinomas tend to be invasive from their earliest stages (278) whereas human colorectal cancers frequently arise in previously existing benign adenomas (290). These findings were supported by Ross (289) but more recently Galloway has presented good evidence of an adenoma/carcinoma sequence in azoxymethane induced rat tumours comparable with the human situation (291).

As previously mentioned, large doses of carcinogen tend to lead to a preponderance of lesions in the left rodent colon whereas smaller doses favour right sided colonic neoplasms. This may have some relevance to the human situation in that it is recognised that in high incidence areas human colon cancers are more frequently left sided as compared to low incidence areas where they are more frequently right sided (292). Similarly, the addition of selenium to the rodents diet can inhibit the carcinogenic effect of 1,2 dimethylhydrazine and methylazoxymethanol (293) and there are reports that human colorectal cancers are more common in areas of selenium deficiency (294). Finally, as discussed in Chapter seven, Filipe has identified changes in the predominant mucosal mucus pattern of carcinogen treated rodents comparable to those seen in patients with colorectal cancer (252).

It may be concluded therefore that azoxymethane or 1,2 dimethylhydrazine induced rat colorectal cancer is a reliable model of the human situation. Its reproducibility combined with the general similarity in histological and histochemical features makes it invaluable in the study of the promoters and protectors of colorectal carcinogenesis.

8.2 Dynamic Cell Population Kinetics

8.2.1 Introduction to Cell Kinetics

The process of neoplastic change implies a disturbance of the normally well controlled cellular kinetic equilibrium. Cellular proliferation and maturation in the normal colonic mucosa is well recognised to progress in an orderly manner. It is approximately the basal two thirds of the colorectal crypts which is regarded as the zone of cell division. Cells migrate from here through the upper part of the crypt, where maturation occurs, and then onto the luminal surface where they are ultimately shed. Under normal circumstances this balance between cellular proliferation and cell loss is delicately controlled and the dynamic equilibrium is maintained. Clearly, neoplastic change reflects a disturbance of the normal control mechanisms, the development of the macroscopic tumour being the manifestation of a prior disturbance of the kinetic equilibrium.

The assessment of cell kinetic parameters therefore forms an integral part of the study of carcinogenesis, although until relatively recently this area has received little attention.

8.2.2 Techniques of Assessing Cell Kinetics

A number of techniques may be used for assessing cell kinetic parameters in large bowel mucosa (295). The simplest but potentially the least accurate of the techniques of estimating kinetic activity is the determination of the mitotic index. This simply involves counting the proportion of cells in a given tissue sample which are in the mitosis phase of the cell cycle (296). The major disadvantage of this technique is that it is dependent on the proportion of the total cell cycle time taken up by mitosis. A change in the duration of the mitosis phase with no alteration in the length of the overall cell cycle would therefore tend to produce a misleading change in the calculated mitotic index (297). A similar disadvantage is possessed by the technique of "pulse" labelling of tissue with tritiated thymidine. In this method, the radiolabelled thymidine becomes incorporated into newly synthesised DNA and thus the labelling index calculated by autoradiography is dependent on the duration of the cell cycle occupied by the "S Phase".

Stathmokinetic analysis, as reviewed by Wright (298), has emerged as the most reproducible, inexpensive, and accurate technique for studying cell kinetics. This technique relies on the ability of certain drugs, namely the spindle poisons such as colchicine and the vinca alkaloids vincristine, vinblastine and vindesine, to arrest cell division during the metaphase of mitosis. Plotting the accumulation

of arrested metaphases against time from injection of the stathmokinetic agent allows calculation of cell production rates. When combined with microdissection of the colonic crypts as described by Clarke (299) this method allows a precise estimation of the rate of entry of cells into mitosis (crpyt cell production rate; CCPR) and the technique can be employed both *in vitro* and *in vivo*. This method is therefore independent of the cell cycle time or the growth fraction and it allows direct estimation of cell birth rate.

8.2.3 Studies of Cell Kinetics and Carcinogenesis

It is clear that neoplasia necessarily implies a disturbance of normal cellular kinetic equilibrium, the development of a macroscopic tumour reflecting an imbalance between cellular proliferation and cell loss. Cell kinetic studies are therefore of fundamental relevance to the process of carcinogenesis although the exact nature of the kinetic disturbances is unclear and somewhat controversial. This subject has recently been extensively reviewed by Galloway (291) and only a brief appraisal of some of the more salient points relevant to this thesis will be described here.

It is now generally accepted that carcinogenesis involves at least two phases (300). The first of these is the initiation phase and this is then followed by a more prolonged phase of promotion. The process of initiation occurs rapidly and comprises an alteration of the DNA of the affected cells which is probably irreversible. As long as the nuclear damage is not so severe that the cell dies, repeated cell division allows an abnormal clone to become established in the cell population.

It is apparent that the rate of cell proliferation will have an important influence on this initiation phase. Firstly, the more rapid the proliferation, the less time there is for DNA repair mechanisms to attempt to rectify the nuclear damage before cell division establishes the abnormal genetic material within the cell population. Secondly, rapid cell division will quickly increase the number of abnormal cells which are then able to proceed to the promotion phase of carcinogenesis.

There is considerable experimental evidence to support this hypothesis of high rates of cell proliferation enhancing initiation. One of the most important studies in this respect was carried out by Pozharhisski and his findings are particularly relevant to the experimental work to be described in this thesis (261). He devised a technique of implanting a purse string suture into the rat caecal wall in such a manner that it produced a diverticulum. He observed that the suture then invariably cut out within 7 to 10 days with the result that the diverticulum smoothed out. Almost all the mucosa had healed by 50 to 60 days post-operatively but the suture material persisted as a source of chronic injury which prevented complete regeneration. Using tritiated thymidine, Pozharisski demonstrated that following surgery there was an immediate increase in the labelling index of the caecal mucosa and this remained elevated until 40 to 50 days after operation. However, large numbers of labelled cells persisted around the residual suture material for much longer periods (100-150 days) suggesting continued cell division in response to the chronic irritation induced by the suture. The administration of 1,2 dimethylhydrazine to these animals within 3 to 7 days of surgery, that is during the phase of generalised epithelial proliferation at the site of injury, resulted in a significant increase in the incidence of

caecal tumours compared with their previous experience with the animal model. However, carcinogen administration 2 months after surgery was associated with a similarly high incidence of caecal tumours, the majority being located adjacent to the suture. This suggests that the localised but persistent proliferative activity as a result of the chronic injury induced by the suture was sufficient to enhance carcinogenesis. Similarly, other authors have demonstrated that increased cell proliferation rates at the time of the initiation phase tend to enhance tumour development (301,302,303).

In contrast to the general agreement regarding the kinetic processes involved during the initiation phase of carcinogenesis, there is controversy surrounding the mechanisms of promotion. The nature of the promotion phase itself is unclear. As already discussed, it is much more prolonged than initiation and may involve a number of steps. Whether promoting agents enhance or reduce cell proliferation is uncertain. The former had previously been suggested to be the case (304) but it must be pointed out that although malignant cells accumulate to form the macroscopic tumour, the rate of cell division in the tumour is frequently no higher than in the tissue from which the tumour arose (305). Recently, Galloway, in examining the effect of dietary manipulation on experimental colorectal carcinogenesis, found that the diet with the greatest promoting effect on carcinogenesis (high fat/low fibre) was associated with the slowest crypt cell production rate (291). Conversely, the diet associated with the lowest incidence of colorectal tumours (low fat/high fibre) led to the highest calculated crypt cell production rates. From this, Galloway proposed that a slow rate of cell proliferation during the promotional phase of carcinogenesis might allow initiated cells to more readily establish themselves within the cell population whereas

high rates of proliferation might be protective in that the initiated cells would be rapidly carried through the crypt and be lost from the luminal surface.

The exact nature of the promotion phase and the mechanism of action of promoting agents therefore remains unclear. It is possible that because of the prolonged and probably multistage process of promotion, enhanced proliferative activity at some stage might be promoting whereas reduced proliferative activity at other stages may allow the malignant cell population to establish their growth advantage. Clearly further investigation is required in attempt to clarify these processes.

Chapter 9

Experimental Work: Materials and Methods

An Investigation of the Influence of Anastomotic Suture Materials on Colorectal Carcinogenesis

9.1 Aims

The aim of this project was to assess the possible promoting or protecting effects of various anastomotic materials on colorectal carcinogenesis in an experimental animal model. The study design is described with reference to figure 9.1.

9.2 Experimental animals and management

The experimental animals used in this project were male Swiss Albino rats obtained from a well established colony within the University Department of Surgery of the Western Infirmary, Glasgow. Although technically this is not a recognised inbred strain, the colony had been entirely brother-sister mated for ten years such that the animals were incapable of rejecting a skin graft from other members of the colony.

Animals were housed in fours in cages made of moulded polypropylene with stainless steel mesh lids. These cages had stainless steel grid floors to minimise coprophagy and animal bedding consisted of wood shavings. All experimental groups were confined to one room in the animal research unit of the Department of Surgery. Within this unit temperature was thermostatically controlled and 12 hourly alternate light-dark cycles were ensured by means of time-clock operated artificial lighting.

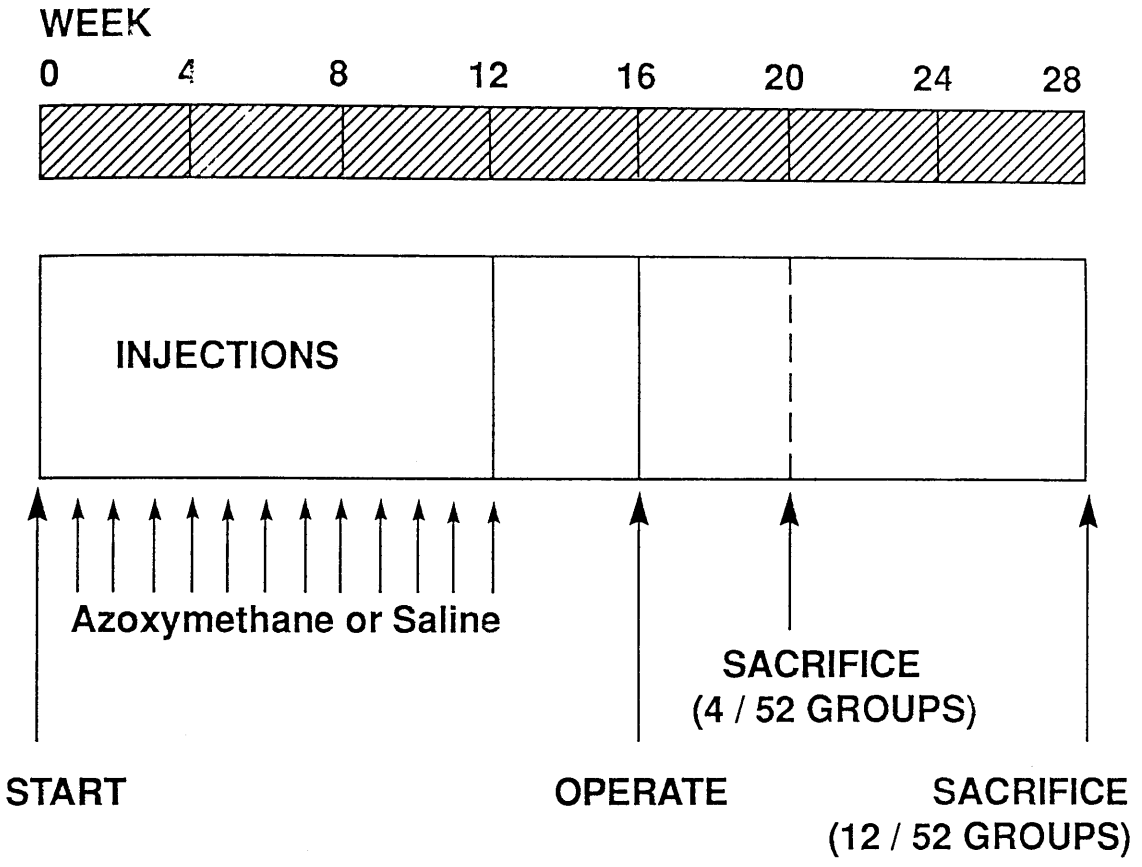


Figure 9:1

EXPERIMENTAL COLORECTAL CARCINOGENESIS

STUDY DESIGN

All experimental groups were allowed food and water ad libitum. Diet, standardised throughout, consisted of a high quality breeding formulation ("CRM", Biosure Ltd, Lavender Hill Manea, Cambridgeshire) and the amount of food consumed was recorded on a "per cage" basis. Animals were weighed weekly in order to chart changes in total body weight, each animal within a cage being identified by means of ear notching.

9.3 Carcinogen

The carcinogen used throughout this project was azoxymethane (AOM) which was obtained in 10 gram ampoules from Ash Stevens Inc., Detroit, Michigan, U.S.A. This was stored in a refrigerator at 4°C and diluted for injection with 0.9% saline. The carcinogen and carcinogen treated animals were handled only by authorised personnel employing full protective precautions as laid down by the University of Glasgow.

All carcinogen or control injections were carried out at 9.00 am. Each animal in a carcinogen treatment group received a weekly subcutaneous injection of azoxymethane in a dose of 10 mg per kilogram body weight for 12 consecutive weeks. The site of injection was the paravertebral region in all cases. The carcinogen was freshly diluted with saline on each day of injection and the concentration adjusted such that the injection volume was 1-2 mls. Control animals received injections of the same volume of 0.9% saline over the same time period. No anaesthesia was employed during the course of the injection procedure.

9.4 Operative Procedures

Four weeks following completion of the course of carcinogen or control injections, laparotomy was performed on all animals. Animals were fasted for solid food but allowed fluids liberally for 24 hrs prior to surgery. Ether anaesthesia was used in all cases, induction taking place in a bell type ether jar and maintenance being ensured by a "nose cone" containing an ether soaked cotton wool plug. All animals in each study group underwent laparotomy consecutively on the same day.

The abdomen was opened through a longitudinal midline incision and one of the following two surgical procedures was performed on a 2cm segment of the distal descending colon (figure 9.2);

i) The simple implantation of 8 interrupted transmural sutures, equally spaced along the anti-mesenteric border of the 2cm length of colon. Care was taken to avoid narrowing the colonic lumen and all knots were tied loosely on the serosal surface to avoid compressing the bowel and to minimise the trauma to the colonic wall. No intestinal wound was made in these animals

ii) The formation of a 2cm anti-mesenteric longitudinal colotomy along the same segment of colon which was immediately re-sutured with 8 interrupted transmural sutures as above.

Following completion of the surgical procedure the peritoneal cavity was filled with sterile 0.9% saline to ensure adequate fluid balance in the immediate post-operative period. The abdominal wound

was closed in two layers with continuous black silk suture and the animal then returned to the appropriate cage. Animals were allowed to resume drinking and eating ad libitum.

9.5 Anastomotic Materials Tested

It was decided to assess and compare the influence on carcinogenesis of 3 anastomotic materials commonly used in large bowel surgery as follows;

i) Polyamide ("Nurolon", Ethicon, Edinburgh, Scotland), a braided non-absorbable material.

ii) Coated Polyglycolic acid ("Dexon Plus", Davis and Geck, Gosport, Hampshire), a braided absorbable material.

iii) Monofilament stainless steel (obtained from Ethicon and identical to the material used in the manufacture of surgical staples).

Sutures of 5/0 gauge and atraumatic cutting needles were used throughout.

9.6 Sacrifice Time

Animals were sacrificed at two distinct time intervals, either 4 or 12 weeks post-operatively. It was known from previous experience with this animal model that both the total number of colonic tumours

and the number of animals developing such lesions would increase with the time from carcinogen administration. The 12 week sacrifice time for this experiment was chosen following earlier pilot experiments which demonstrated that by 14 to 15 weeks post-operatively a large proportion of the animals deteriorated rapidly and that sudden death was then common before planned sacrifice could be carried out. The reasons for the 4 week sacrifice time were, firstly to detect possible early neoplastic lesions in relation to certain suture materials and, secondly to more accurately assess cell population kinetics since the proliferative response to injury may only last 40 to 50 days (261).

9.7 Animal Groups

As previously discussed it was decided to compare the three anastomotic materials with respect to their influence on colorectal carcinogenesis, firstly where the sutures were simply implanted into the colonic wall and secondly where the same materials were used to repair a colotomy. Two distinct sacrifice times were chosen and for each category there were both carcinogen and saline treated animals. A total of 8 subgroups were therefore required per suture material as listed below.

In order to ensure that the equivalent animal groups for each suture material were comparable, as far as was possible the groups were run in parallel and were made up of a composite of several litters. This inevitably meant that group sizes were to some extent dictated by the animals's breeding pattern and litter sizes. The original aim was to achieve groups of approximately the following sizes for each material.

Group 1: Carcinogen/suture implantation/4 week sacrifice (8 animals).

Group 2: Carcinogen/suture implantation/12 week sacrifice (12 animals)

Group 3: Control/suture implantation/4 week sacrifice (6 animals).

Group 4: Control/suture implantation/12 week sacrifice (8 animals).

Group 5: Carcinogen/colotomy re-suture/4 week sacrifice (8 animals).

Group 6: Carcinogen/colotomy re-suture/12 week sacrifice (12 animals).

Group 7: Control/colotomy re-suture/4 week sacrifice (6 animals).

Group 8: Control/colotomy re-suture/12 week sacrifice (8 animals).

Sixty eight animals were therefore required per suture material. In addition it was planned to have 4 sham operated control groups corresponding to groups 1 to 4 above. A total animal requirement of 238 animals was therefore anticipated.

However, during the course of the experiment, differences between the various suture materials with respect to the number of carcinogen treated animals developing colonic tumours became apparent. At this stage it was decided to increase the numbers in these carcinogen treated groups in order to determine if these observations were consistent. At this stage animal numbers were adjusted to compensate for any excessive operative mortality in the first series of animal

groups. As before, the exact final group sizes were determined by animal availability and the capacity of the carcinogen room in the animal unit (Chapter 10).

9.8 Sacrifice Procedure

All animals used for stathmokinetic analysis received an intra-peritoneal injection of vincristine at 9 am on the morning of sacrifice. Individual animals within each group were then sequentially sacrificed over a three hour period at carefully defined time intervals of between 15 and 30 minutes depending on the size of the group concerned.

The procedure for sacrifice consisted of cervical dislocation carried out under ether anaesthesia. An immediate total colectomy was performed and the number of remaining sutures in the anastomotic zone was recorded. The colon was opened along the anti-mesenteric border throughout its entire length, pinned out on a cork board with the mucosal surface uppermost, and the number and distribution of all gross neoplastic lesions documented prior to any tissue sampling. A full post-mortem was carried out on the animal with particular attention being paid to the remainder of the gastro-intestinal tract, the liver, and the lungs.

9.9 Tissue Sampling

All macroscopically abnormal lesions recorded at autopsy were submitted for detailed histological analysis by an independent observer. In addition, a consistent area of the distal descending colon, corresponding to the site of suture implantation or colotomy re-suture, was examined microscopically irrespective of the macroscopic appearances. Finally, tissue samples were taken from the distal descending colon and from the rectum for stathmokinetic analysis.

The techniques for tissue sampling and preparation were as follows;

i) Histopathology

All tissue samples for histopathology were initially fixed in 10% formal saline at the time of autopsy. Further preparation took place within the Pathology Department of the Western Infirmary using standard techniques. Tissue dehydration and blocking in paraffin wax was carried out in a 22 hour cycle in a Histokine automatic tissue processing machine as follows. The dehydration sequence comprised one hour in 50% alcohol followed by a further hour in 80% alcohol, after which the sample was passed through three beakers each containing 8% phenol in methylated spirit over an eight hour period. Following immersion in two changes of absolute alcohol for 90 minutes each, the sample was placed in absolute alcohol and chloroform for 30 minutes followed by immersion in two separate solutions of xylol for 45 minutes each. The sample was then placed in two changes of melted

paraffin wax for 3 and 4 hours respectively before being finally embedded in fresh paraffin wax and mounted in the microtome chuck. Sections of 5 um were then cut and submitted for staining.

The procedure for tissue staining comprised a regressive haemalum and eosin technique. Each tissue section was firstly dewaxed in xylol and alcohol and then stained in haemalum which was differentiated in 1% acid alcohol. Following rinsing, the sample was transferred to eosin which was differentiated in 30% alcohol. Thereafter the tissue was submitted for further dehydration using alcohol followed by xylol prior to permanent mounting in D.P.X.

ii) Cell Population Kinetic Study

Two samples were submitted from each animal for stathmokinetic analysis. The first of these samples was taken from the "anastomotic" site in the distal descending colon and the second from the adjacent upper rectum. The reason for this was to allow assessment of the influence of the anastomosis on cell kinetic parameters. The two tissue samples were processed separately by the following technique.

After the dissection of the colon at post-mortem, the tissue samples for stathmokinetic analysis were immediately placed in Carnoys nuclear fixative in which they remained for 4 to 6 hours before being transferred to absolute alcohol for storage. Prior to staining, the tissue was rehydrated by successive immersion in 70%, 50%, and 30% alcohol each for a ten minute period. Following this, carefully controlled hydrolysis was performed by immersing the sample in normal hydrochloric acid at 60^{o}C for ten minutes after which the tissue

was transferred to Schiffs reagent and allowed to stain for one hour. After the completion of the staining procedure, the tissue could either be further processed as described below or it could be dehydrated and stored in 100% alcohol for processing at a later date.

The next step in tissue processing involved transferring the hydrated tissue onto a drop of 5% acetic acid on a microscope slide. Under the dissecting microscope the mucosa of the sample was carefully stripped away from the muscle, serosa and any attached connective tissue. Everything but the mucosa was discarded and the mucosa then microdissected using x10 magnification under the dissecting microscope in order to gently tease apart individual colonic crypts. The tissue sample was then resuspended in a drop of 5% acetic acid and a squashed preparation was obtained by compressing the microdissected crypts between the microscope slide and a cover slip.

Counting could be carried out at this stage but in practice a permanent slide preparation was made to allow counting to take place at a later, more convenient time. The permanent preparation was made by firstly soaking the squashed preparation in methylated spirit to allow the cover slip with the attached tissue sample to separate from the underlying microscope slide. The cover slip and attached tissue were then dipped in xylol and mounted on a fresh microscope slide. A drop of clear polyester resin was used to permanently bind the cover slip to the slide. During the hardening of this resin the slide was held in a purpose built clamp to prevent any conformational change of the squashed preparation.

This technique of tissue preparation and staining allowed the visualisation, under the microscope, of the individually dissected colonic crypts in which the arrested metaphases stain dark purple

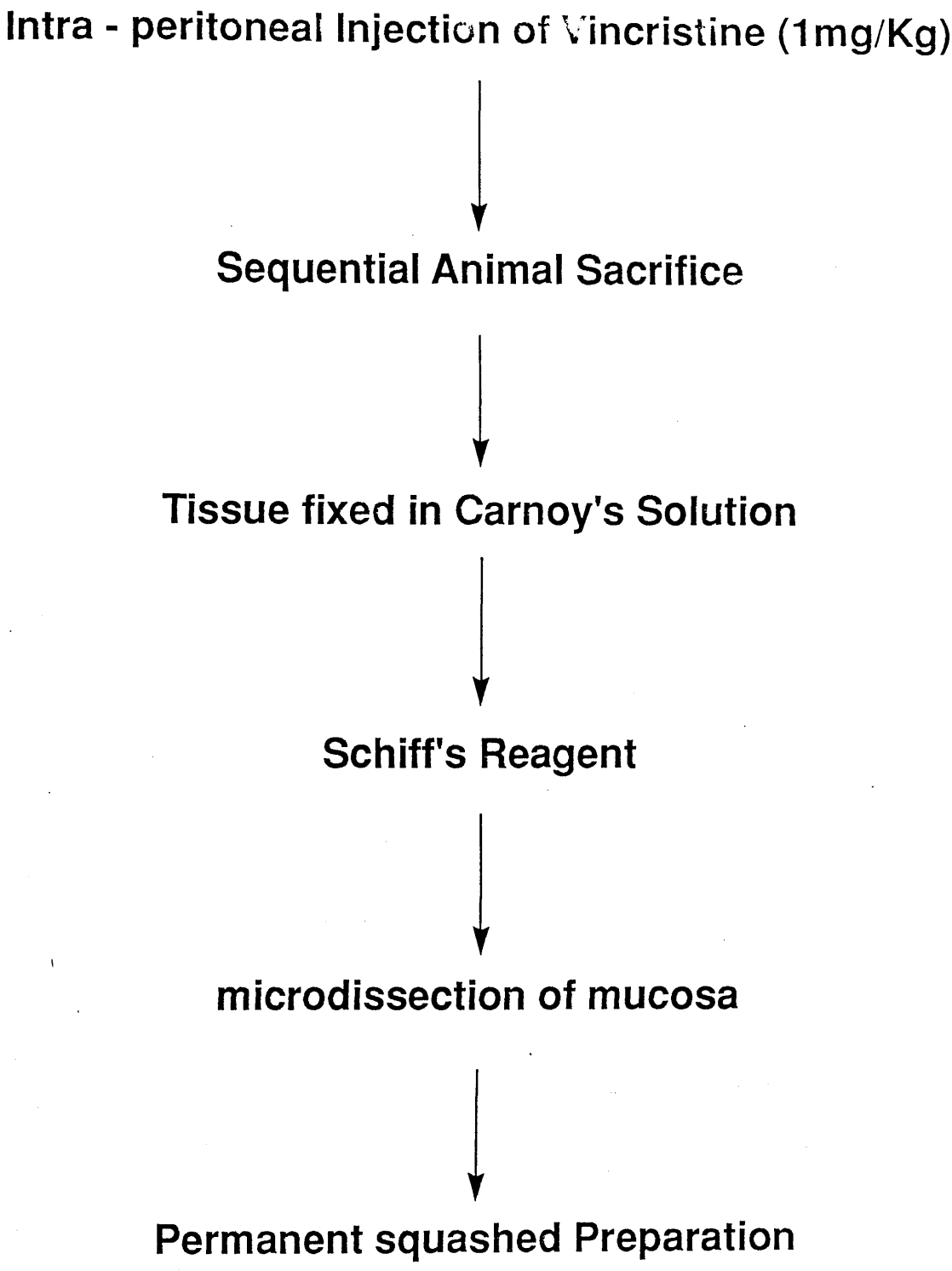


Figure 9:3

STATHMOKINETIC ANALYSIS : TISSUE PREPARATION

against the background colour of pink. For each tissue sample, the number of arrested metaphases in each of 10 whole colonic crypts were counted and recorded. All counting was carried out by the author, each slide being identified only by a code number at the time of counting. Following the completion of counting, the identity of each slide was revealed and the mean of the 10 whole crypt metaphase counts for each sample was plotted against time since injection of vincristine. Weighted linear regressional analysis was used to calculate the crypt cell production rates.

iii) Liquid Nitrogen

Tissue from the peri-anastomotic area from each animal was snap frozen in liquid nitrogen immediately following post-mortem and then transferred for storage at -70°C. This simply supplied a spare sample of tissue for each animal should any further analysis be required.

Chapter 10

Results: Animal Characteristics

10.1 Introduction

This Chapter is concerned with a description of the general characteristics of the groups of animals studied in the carcinogenesis experiment. The various animal groups for each of the 3 suture materials have been compared with respect to mean body weight and food intake. The mortality observed during the course of the experiment is described and finally a comparison is made between the 3 anastomotic materials with respect to the number of residual sutures remaining at the time of sacrifice.

10.2 Total Body Weight and Food Intake

The serial progressions of mean total body weight for all animal groups are illustrated in figures 10.1 - 10.8. Error bars have been deliberately omitted for reasons of clarity but the mean body weights and standard errors are listed numerically in Appendix 1.

It is evident from the data in Appendix 1 and from figures 10.1-10.8 that the various suture material groups were similar with respect to both mean total body weight and the progression of weight gain. In each case, the drop in body weight between weeks 16 and 17 represents the peri-operative period.

In addition, there were no major discrepancies betweem the suture material groups with respect to weekly food consumption as listed in Appendix 2.

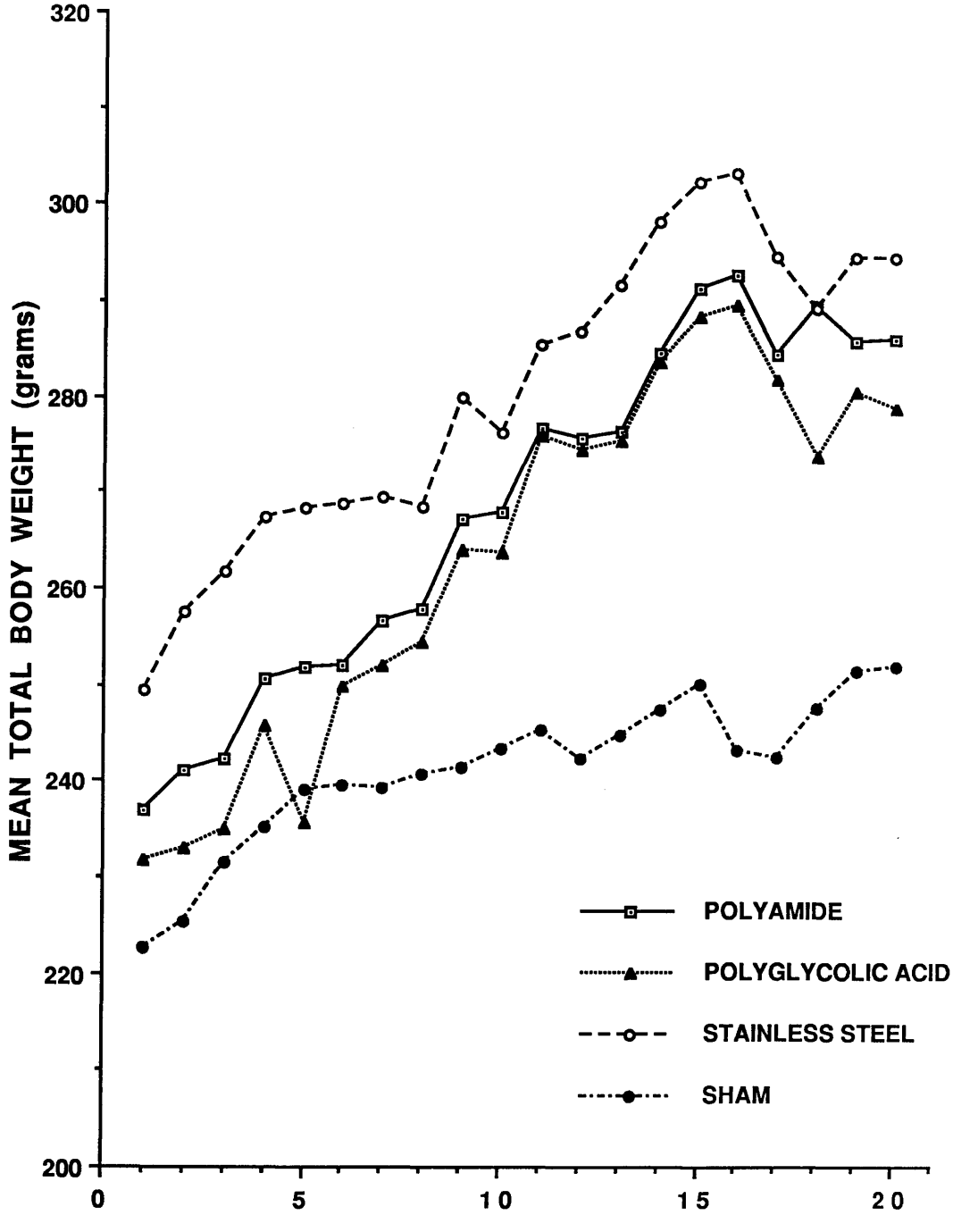


Figure 10.1

TOTAL ANIMAL BODY WEIGHT: AZOXYMETHANE\
IMPLANTATION OF SUTURES - 4 WEEK SACRIFICE

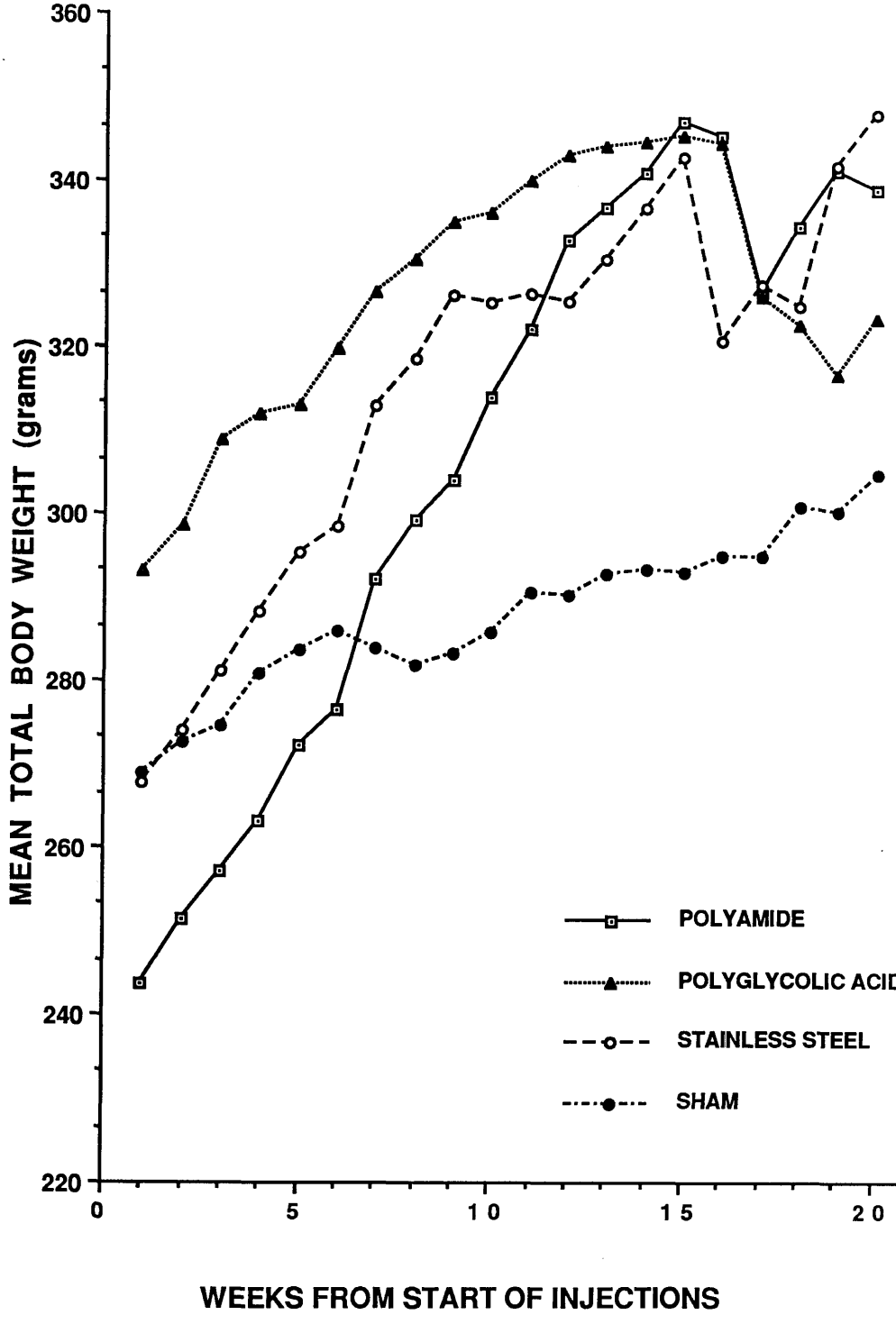


Figure 10.2

TOTAL ANIMAL BODY WEIGHT: SALINE (CONTROL)\
IMPLANTATION OF SUTURES - 4 WEEK SACRIFICE

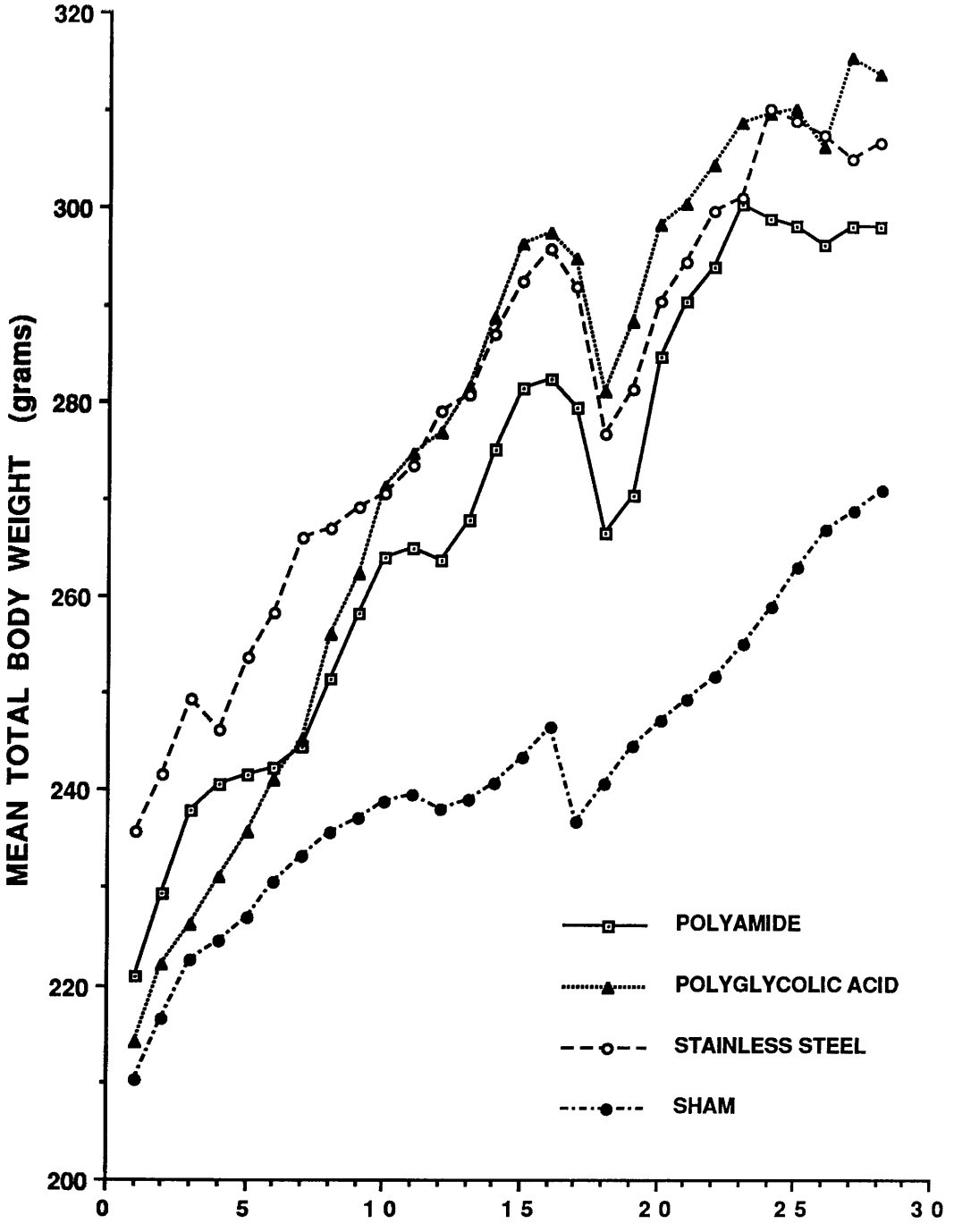


Figure 10.3

TOTAL ANIMAL BODY WEIGHT: AZOXYMETHANE\
IMPLANTATION OF SUTURES - 12 WEEK SACRIFICE

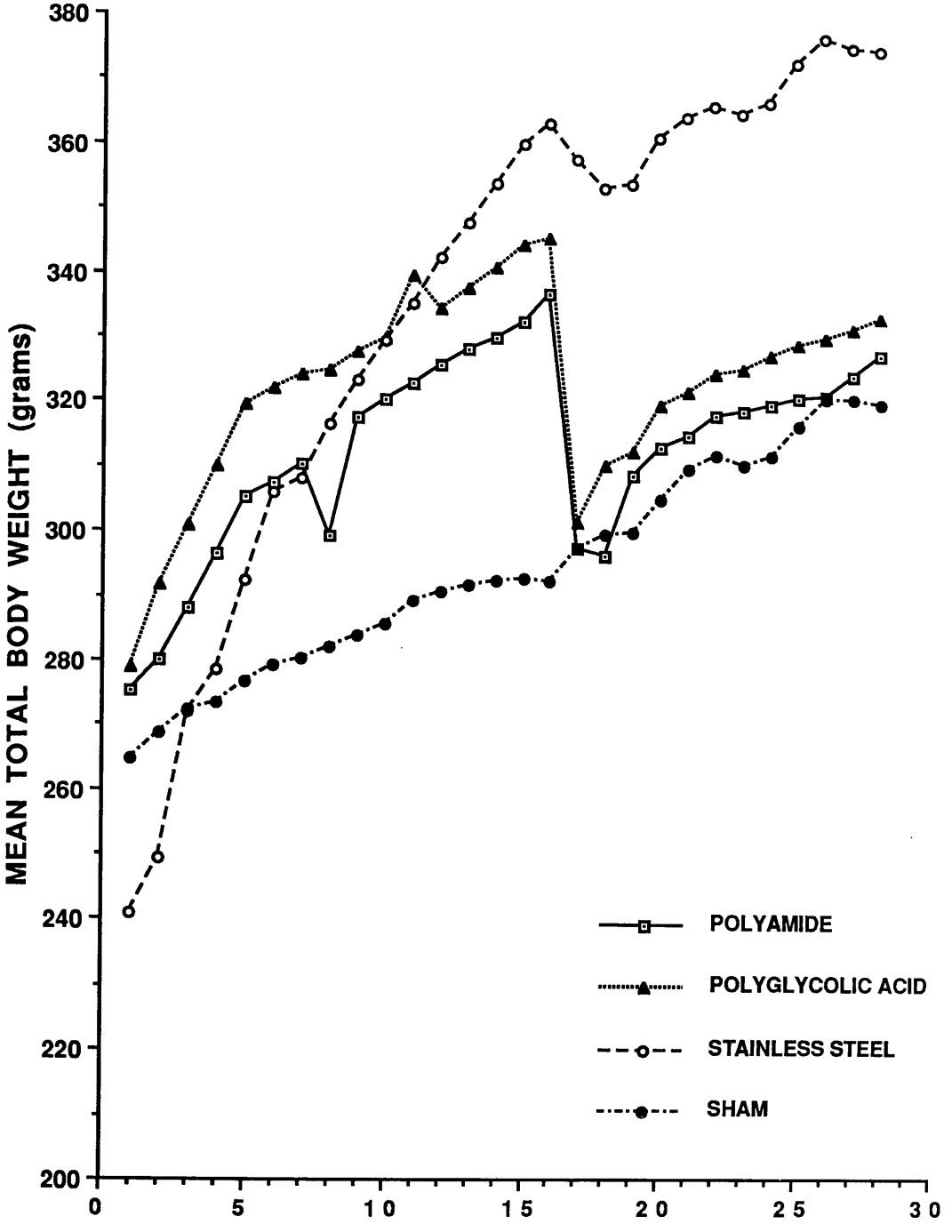


Figure 10.4

TOTAL ANIMAL BODY WEIGHT: SALINE (CONTROL)\
IMPLANTATION OF SUTURES - 12 WEEK SACRIFICE

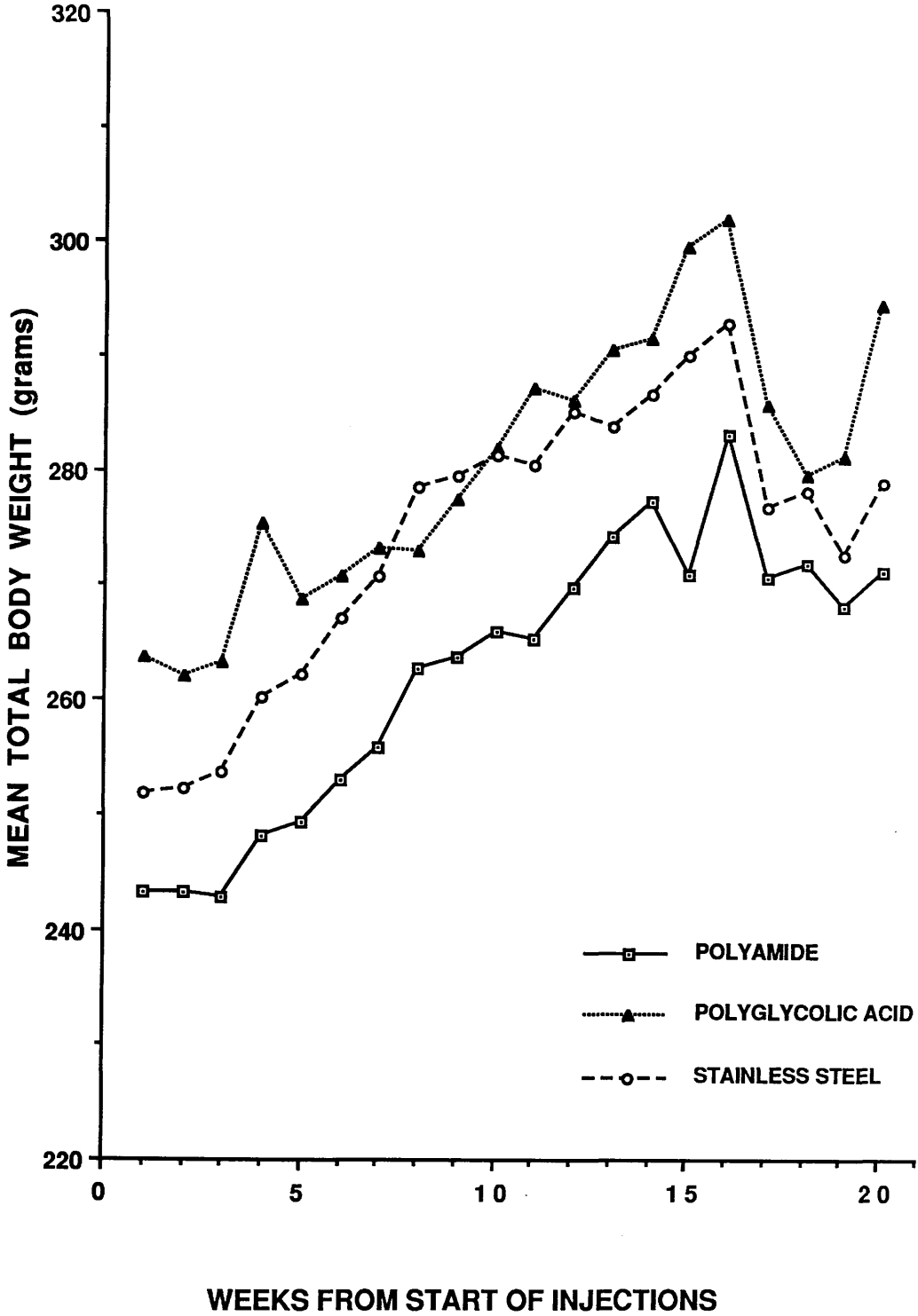


Figure 10.5

TOTAL ANIMAL BODY WEIGHT: AZOXYMETHANE\
COLOTOMY and RE-SUTURE - 4 WEEK SACRIFICE

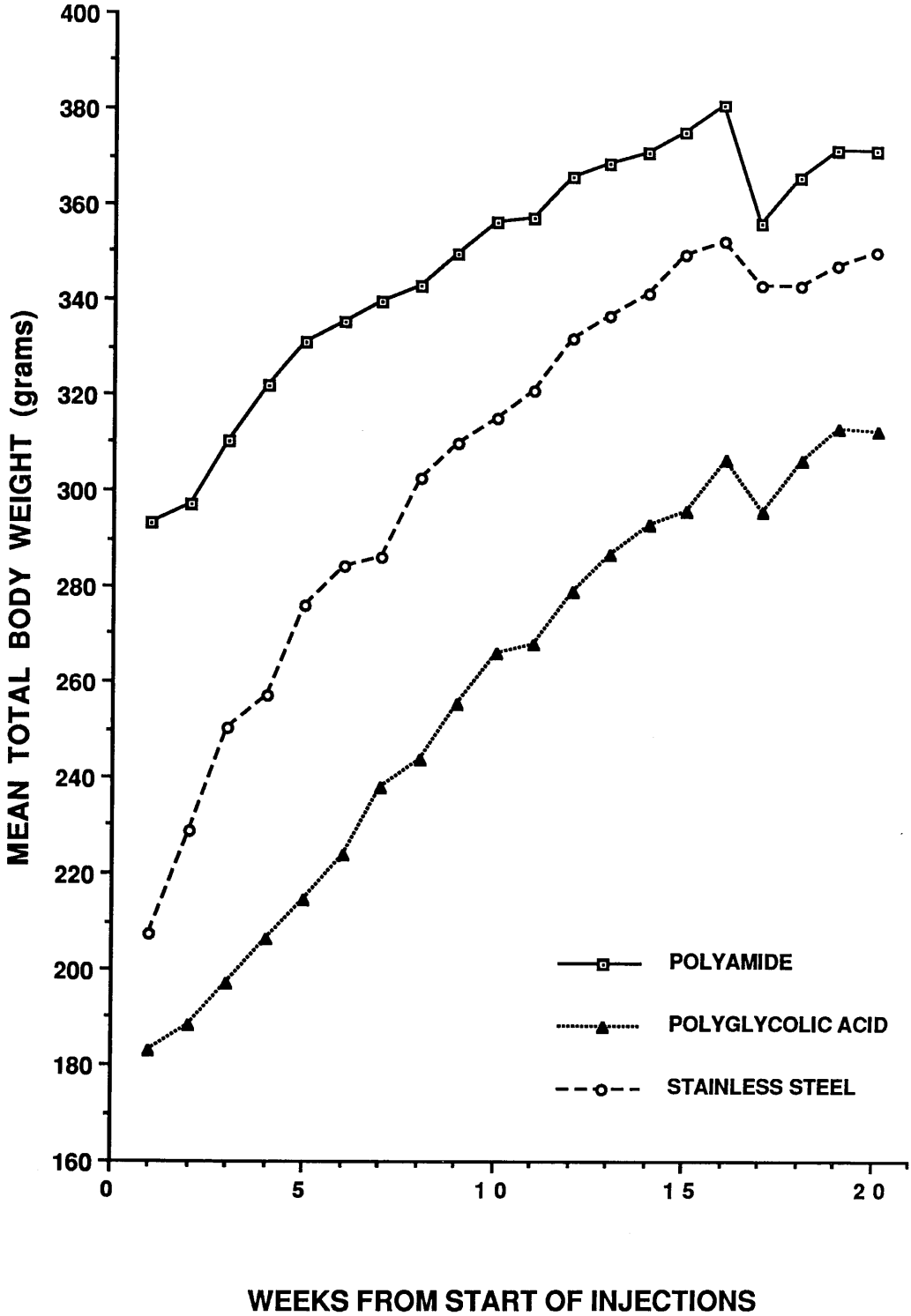


Figure 10.6

TOTAL ANIMAL BODY WEIGHT: SALINE (CONTROL)\
COLOTOMY and RE-SUTURE - 4 WEEK SACRIFICE

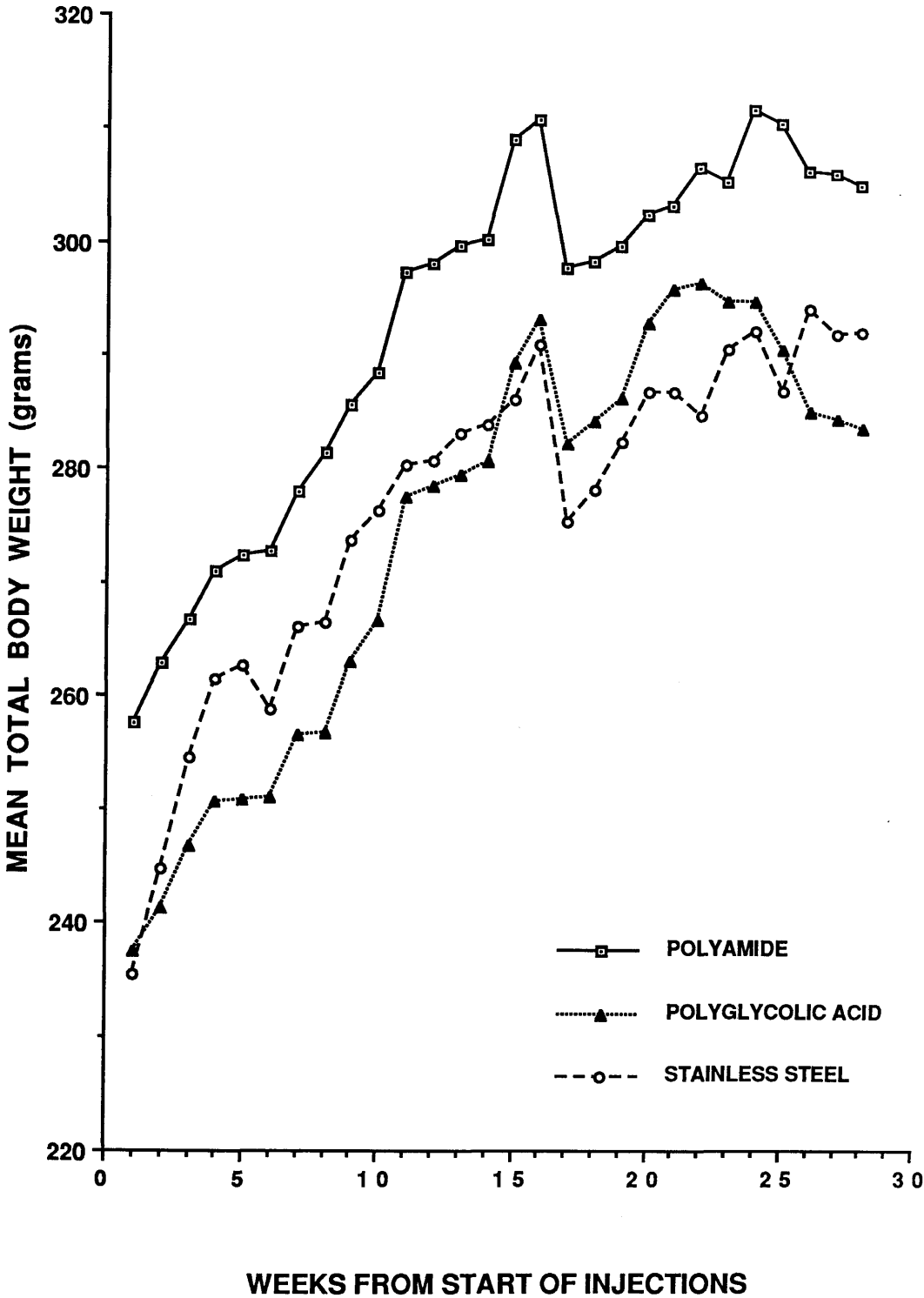


Figure 10.7

TOTAL ANIMAL BODY WEIGHT: AZOXYMETHANE\
COLOTOMY and RE-SUTURE - 12 WEEK SACRIFICE

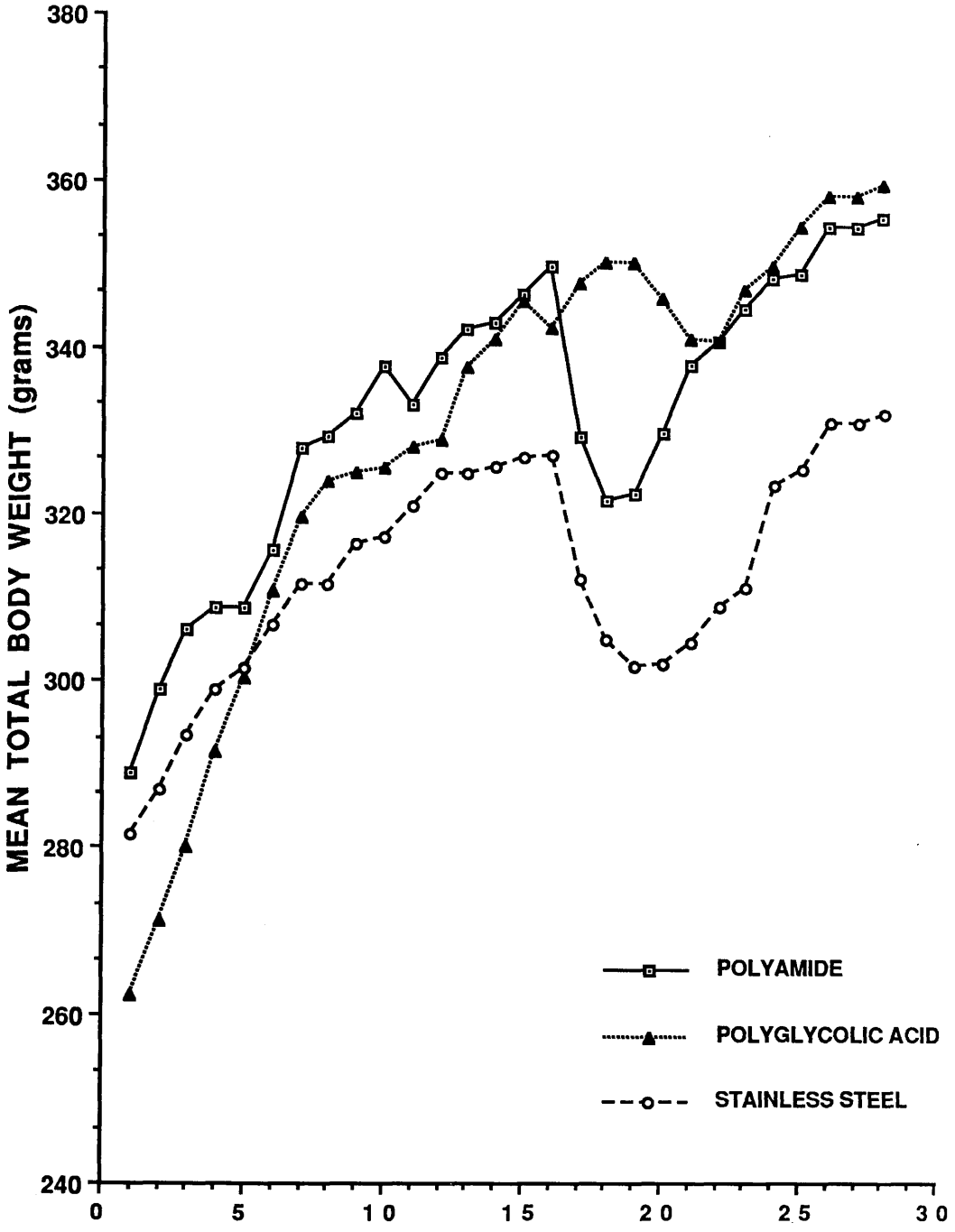


Figure 10.8

TOTAL ANIMAL BODY WEIGHT: SALINE (CONTROL)\
COLOTOMY and RE-SUTURE - 12 WEEK SACRIFICE

10.3 Mortality

A total of 326 animals were studied in this project, of which 239 received azoxymethane injections and 87 received injections of normal saline. Detailed autopsy data and histological reports are available for 301 animals (92.33%). Three hundred of these animals were electively sacrificed while the remaining rat was discovered within a very short time of its spontaneous death and an accurate post-mortem examination was possible. This latter animal had been carcinogen treated and had received an implant of steel sutures. Death occured 5 weeks post-operatively and autopsy revealed a large obstructing tumour mass of the distal stomach. There was no evidence of colorectal neoplasia.

Of the 25 premature deaths for which no pathological results are available, 23 occured in the carcinogen treated groups. These premature deaths can be described with respect to 3 distinct time periods;

i) Deaths During Injection Phase

Three animals died during the course of azoxymethane injections, presumably from toxicity. Cannibalism precluded accurate autopsy data as to the cause of death.

ii) Peri-operative Deaths

Twelve animals died in the peri-operative period, an operative mortality of 3.68%. Two deaths occured as a result of an overdose of ether. One of these was a control animal and was receiving an implant of steel sutures. The second animal to die in this manner had received azoxymethane and was undergoing re-suture of a colotomy

with stainless steel. The remaining 10 animals initially recovered from the anaesthetic but died within the first post-operative week without regaining their pre-operative appetite, weight or general well-being. All but one had received carcinogen. None of these 12 rats had had any gross gastro-intestinal abnormality noted at laparotomy.

Of the 10 animals which died following recovery from anaesthesia, 7 belonged to the stainless steel groups. One had post-mortem evidence of intra-peritoneal bleeding following re-suture of a colotomy while the remaining 6 were found to have developed obstruction of the distal descending colon. Five of these latter 6 animals had received simple implantation of steel sutures and it seems probable that the obstruction was the result of the loops of steel protruding into the colonic lumen and obstructing the passage of the solid, pellet-like rat stools.

The 3 remaining animals which died peri-operatively included one animal which had undergone re-suture of a colotomy with polyglycolic acid and at post-mortem was found to have bled from the anastomosis. The other 2 rats had received simple implantation of polyamide and polyglycolic acid sutures respectively and in neither was an obvious cause of death identified.

iii) Post-operative Deaths

Nine animals died after a variable post-operative interval. A combination of cannibalism and rapid autolysis precluded accurate post-mortem details and histological analysis being obtained. Two of these rats had received an implant of polyamide sutures, 2 an implant

of stainless steel sutures, 3 colotomy and re-suture with polyamide, 1 colotomy and re-suture with polyglycolic acid, and 1 colotomy and re-suture with stainless steel.

Others

One animal, belonging to the group planned for colotomy re-suture with stainless steel, was excluded from the experiment when at laparotomy it was found to already have an extensive tumour mass involving the upper caecal pole. This finding explained a 4 week period of weight loss and failure to thrive.

10.4 Animals Sacrificed Early

Because of the known problems of cannibalism and rapid autolysis it was decided that if any animal was losing weight and/or visibly unwell to the extent that it was thought unlikely to survive until the planned time of sacrifice, then sacrifice would be carried out at an earlier date. In practice this applied to a total of 26 carcinogen treated animals.

Twenty one of these animals were sacrificed together. They comprised the first groups of carcinogen treated animals which had undergone re-suture of a colotomy with polyamide (10 animals) or polyglycolic acid (11 animals) and which were planned for sacrifice 12 weeks post-operatively. By the ninth post-operative week, 2 of the animals in the polyamide group and one in the polyglycolic acid group had died and were lost to the study while many of the remaining animals in both groups were losing weight and were visibly unwell. A decision was therefore made to sacrifice these 2 groups at this point

in an attempt to avoid any further animal loss. By comparison, however, the steel group was relatively healthy and it was decided to postpone its sacrifice until the scheduled time of 12 weeks post-operatively.

The remaining 5 animals sacrificed early comprised individual cases which were thought unlikely to survive until the planned sacrifice date. Three animals belonged to the carcinogen treated group which had received an implant of polyglycolic acid sutures and was planned for sacrifice 12 weeks post-operatively. All 3 were noted to have developed tumours of the external ear and when sacrificed during the 10th post-operative week each was found to have colorectal neoplasia. Similar findings were recorded for 2 animals in the comparable polyamide group which were also sacrificed 10 weeks post-operatively.

10.5 Residual Sutures

At post-mortem the presence and number of remaining sutures were recorded. There were no apparent differences between the carcinogen and control groups with respect to this and so the equivalent carcinogen and control groups are considered together in the table 10.1 - 10.4. Statistical analysis was carried out using the Mann-Whitney U Test.

Table 10.1

Residual Sutures: Implantation of Sutures - 4 Week Sacrifice

Suture Material	Number of Animals	Sutures placed	Sutures recovered	No per animal (mean ± sem)
polyamide	19	152	98 (64.5%)	5.16 ± 0.61
polyglycolic acid	17	136	65 (47.8%)	3.82 ± 0.63
stainless steel	18	144	126 (87.5%)	7.00 ± 0.24

polyamide vs polyglycolic acid:	p = 0.087
polyamide vs steel:	p = 0.075
polyglycolic acid vs steel:	p = 0.001

Table 10.2

Residual Sutures: Implantation of Sutures - 12 Week Sacrifice

Suture Material	Number of Animals	Sutures placed	Sutures recovered	No per animal (mean ± sem)
polyamide	28	224	101 (45.1%)	3.61 ± 0.29
polyglycolic acid	29	232	8 (3.4%)	0.28 ± 0.09
stainless steel	29	232	172 (74.1%)	5.93 ± 0.20

polyamide vs polyglycolic acid:	p < 0.001
polyamide vs steel:	p < 0.001
polyglycolic acid vs steel:	p < 0.001

Table 10.3

Residual Sutures: Colotomy and Re-suture Groups - 4 Week Sacrifice

Suture Material	Number of Animals	Sutures placed	Sutures recovered	No per animal (mean ± sem)
polyamide	15	120	20 (16.7%)	1.33 ± 0.27
polyglycolic acid	16	128	41 (32.0%)	2.56 ± 0.33
stainless steel	16	128	45 (55.2%)	2.81 ± 0.36

polyamide vs polyglycolic acid:	$p = 0.012$
polyamide vs steel:	$p = 0.004$
polyglycolic acid vs steel:	$p = 0.867$

Table 10.4

Residual Sutures: Colotomy and Re-suture Groups - 12 Week Sacrifice

Suture Material	Number of Animals	Sutures placed	Sutures recovered	No per animal (mean ± sem)
polyamide	27	216	29 (13.4%)	1.07 ± 0.22
polyglycolic acid	28	224	1 (0.45%)	0.04 ± 0.04
stainless steel	25	200	55 (27.5%)	2.20 ± 0.20

polyamide vs polyglycolic acid:	$p < 0.001$
polyamide vs steel:	$p = 0.006$
polyglycolic acid vs steel:	$p < 0.001$

Chapter 11

Results: Pathology

11.1 Introduction

This Chapter comprises a description of the pathological findings in the carcinogenesis study. It consists of a listing of the gross morphology recorded at post-mortem examination and the results of the histological analyses.

Detailed autopsy data and histological reports are available for 302 of the total of 325 animals (92.9%) entered into the experiment. The results are listed according to operative procedure and sacrifice time. Azoxymethane treated rats and control (saline) animals are considered separately and the results are reported with reference to the three types of suture material studied. Detailed autopsy findings for each of the carcinogen treated animals are listed in Appendix 3.

For each subgroup of animals the pathological findings have been considered under three headings;

i) the pathology of the "peri-anastomotic" zone (distal descending colon).
ii) the pathology of the remainder of the large bowel
iii) any other abnormality noted at full autopsy

Under each of these three headings, the results are described with reference to the macroscopic pathology recorded at autopsy and the specific histological appearances of any abnormality present. In addition, for the peri-anastomotic site, where histological samples were taken irrespective of the macroscopic appearances, the histological findings are described under the category of microscopic pathology.

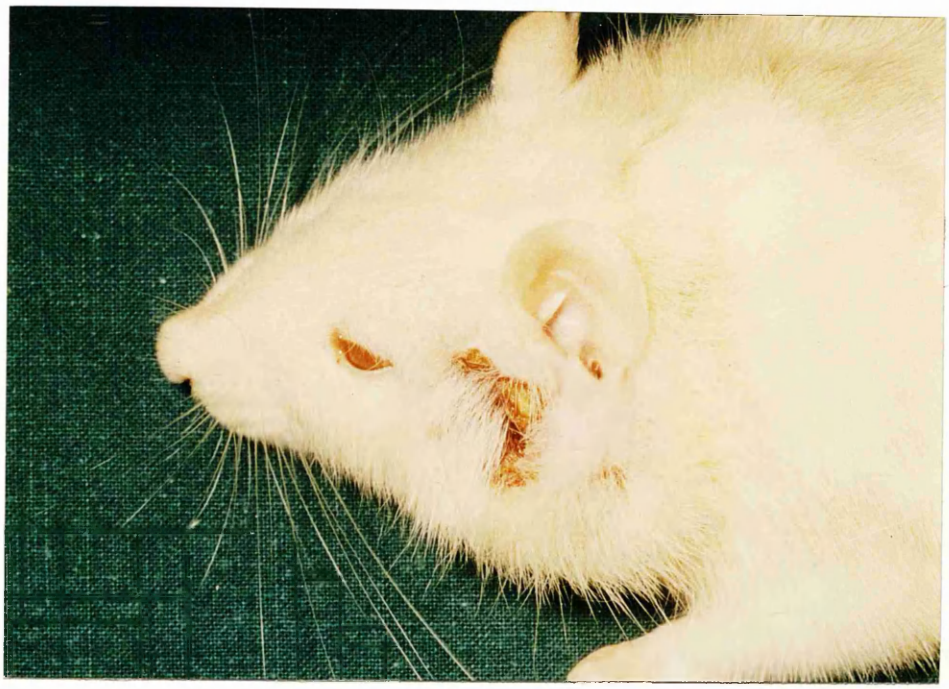


Figure 11.1 Squamous Carcinoma of the External Auditory Meatus

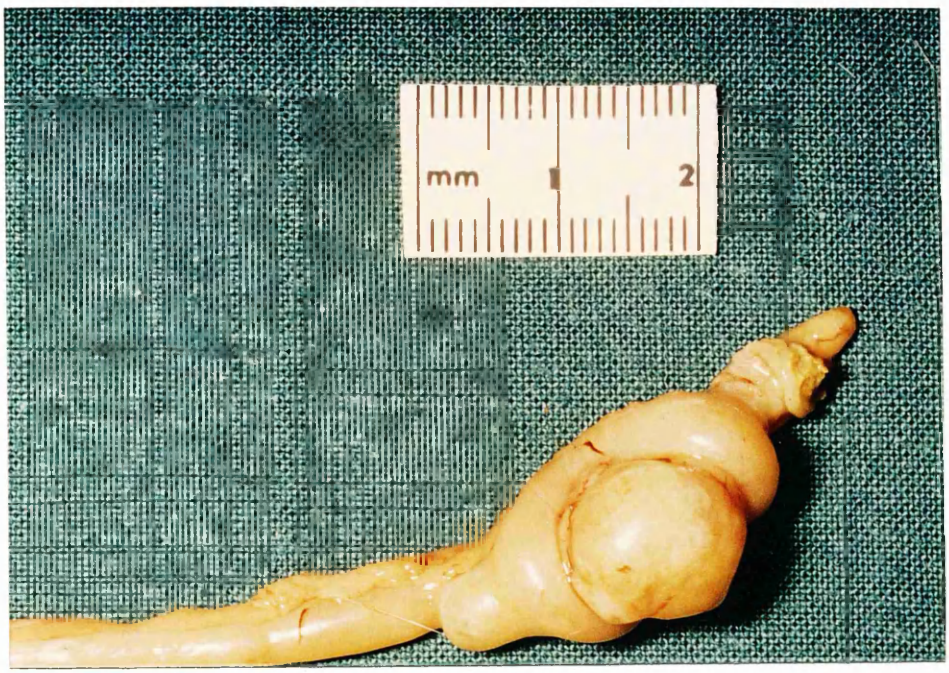


Figure 11.2 Intussuscepting Proximal Colonic Tumour

(well differentiated adenocarcinoma)

Figure 11.3 Large Polypoidal Tumour of the Distal Descending Colon (adenocarcinoma arising in tubulo-villous adenoma)

Figure 11.4 Ulcerating Malignant Tumour of Mid-Colon with Additional Benign Polyp

The findings are summarised in tables 11.1-11.8. In tables 11.1-11.4, the results are expressed as the number of animals developing large bowel tumours, both adjacent to and distant from the anastomotic site. In tables 11.5-11.8, the total numbers of large bowel tumours are listed.

11.2 Carcinogen Treated Animals

11.2.1 Implantation of Sutures - 4 week Sacrifice

a) Polyamide Suture

"Peri-anastomotic" Pathology

There were 13 animals in this subgroup and in only one rat was there a macroscopic abnormality at the site of suture implantation. The abnormality comprised 3 adjacent nodules each of 2mm diameter directly related to residual suture material. Histologically these consisted of dysplastic epithelium surrounded by infiltrating adenocarcinoma.

The histology of the 12 macroscopically normal rats revealed mixtures of acute and chronic inflammatory cell infiltrates with frequent granulomata surrounding residual suture material.

Pathology of the Remaining Large Bowel

The remainder of the large bowel was grossly normal in all 13 rats.

Other Pathology

The animal with carcinoma in relation to the sutures also had a large tumour mass surrounding the proximal small bowel. Histologically this was adenocarcinoma invading the serosa and muscle layers of the bowel wall. The mucosa was normal and so this was considered to represent spread from the colonic lesion.

No abnormality was detected in the remaining 12 animals.

b) Polyglycolic Acid Suture

"Peri-anastomotic" Pathology

Of this subgroup of 13 animals, 5 had macroscopic abnormalities at the site of suture implantation. Histologically 3 of these lesions were neoplastic but only 2 were considered primary neoplasms of the large bowel mucosa. In the remaining one animal the serosa was infiltrated by a small bowel adenocarcinoma but the mucosa was normal.

One animal had a 2mm polypoidal lesion adjacent to the sutures (tubulovillous adenoma), one had 2 sessile nodules each measuring 2mm (both granulomata), and one rat had evidence of serosal thickening around the sutures (infiltration by adenocarcinoma from small bowel primary, serosa intact). The abnormality in the remaining 2 rats comprised some thickening and heaping of the mucosa around the sutures (adenocarcinoma in one animal, granulomatous reaction in the other).

Microscopically, the remaining 8 animals showed varying degrees of inflammation surrounding the residual suture material with prominent foreign body granulomas.

Pathology of the Remaining Large Bowel

Five rats had macroscopically abnormal lesions at other sites in the large bowel, only one of which had an abnormality in the suture area. In 4 of these animals, the lesions were histologically neoplastic.

One animal had a 7mm ulcerating lesion in the caecum (adenocarcinoma), one a 3mm polyp in mid colon (adenocarcinoma), one a 2mm nodule in the mid colon (tubular adenoma), and one a 2mm sessile lesion in the proximal colon (adenocarcinoma). The non-neoplastic lesion comprised a 2mm nodule in the mid colon which histologically consisted of lymphoid aggregates.

Other Pathology

One animal had a large tumour mass involving the proximal small bowel (adenocarcinoma) and a further rat had a deposit of secondary adenocarcinoma in the liver from a caecal primary.

c) Stainless Steel Suture

"Peri-anastomotic" Pathology

There were 14 rats in this subgroup. In none was there any gross abnormality at the site of suture implantation.

Histologically the bowel of 2 animals was entirely normal. One rat had a small area of glandular hyperplasia around a suture tract while the remainder had variable degrees of inflammatory infiltrates with prominent foreign body granulomata in relation to the suture material.

Pathology of the Remaining Large Bowel

Single gross abnormalities were recorded in 3 rats. These comprised a 2mm nodule in the mid colon (adenocarcinoma), a 2mm polypoidal lesion in the mid colon (adenocarcinoma) and a 4mm polyp in the mid colon (tubulovillous adenoma).

Other Pathology

Two animals had small bowel tumours, one of which also had a large bowel lesion. One of these rats had a large obstructing lesion in the proximal ileum (adenocarcinoma), and the other had a 4mm nodular tumour in the duodenum (adenocarcinoma). No abnormalities were noted outwith the gastro-intestinal tract in any animal.

d) Sham Operated Group

This subgroup comprised 8 animals, none of which had any macroscopic abnormality recorded at post-mortem. Histologically, the bowel at the site equivalent to the suture implantation zone (distal descending colon) was entirely normal in each case.

11.2.2 Implantation of Sutures - 12 week Sacrifice

a) Polyamide Suture

"Peri-anastomotic" Pathology

This subgroup comprised 20 animals of which 14 had macroscopic mucosal abnormalities at the site of suture implantation. Five animals had 2 separate lesions and one had 3 lesions and so a total of 21 "peri-anastomotic tumours" were identified. Fifteen of these lesions occurring in 11 animals were histologically neoplastic comprising 9 adenomas and 6 adenocarcinomas, 3 of which appeared to be arising in tubulovillous adenomas. In addition, two rats had small foci of adenomatous change identified on histological sampling of a macroscopically normal suture area whilst a third animal had well differentiated adenocarcinoma with no corresponding macroscopic abnormality. A total of 14 animals in this group therefore had evidence of neoplastic change at the site of implanted suture material.

The histologically non-neoplastic mucosal abnormalities in the "peri-anastomotic" zone comprised 2 areas of mucosal heaping identified as lymphoid aggregates, one nodular lesion with similar histological appearances, one area of mucosal heaping around the sutures which represented a prominent foreign body giant cell reaction to the suture material, and one diverticulum. The one remaining lesion was a 2mm area of mucosal thickening related to the sutures but as a result of poor tissue fixation rapid autolysis had occurred and histology was not possible.

Pathology of the Remaining Large Bowel

A total of 19 macroscopic colonic mucosal abnormalities were recorded in 12 animals at sites distant from the area of suture implantation. Histologically, 16 of these lesions were primary neoplasms involving the mucosa (11 animals) of which 6 were adenomatous and 9 carcinomatous. One of the carcinomas appeared to be arising in a tubulovillous adenoma.

Nine of these rats also had histologically proven neoplasia at the "peri-anastomotic" site.

The 3 "non-neoplastic" macroscopic abnormalities included 2 cases where the mucosa was elevated by lymphoid aggregates only, although for one of these there was evidence of adenocarcinoma in the serosa representing spread from other lesions. The remaining abnormality was histologically malignant but it represented direct invasion of the proximal colon by a large gastric tumour mass.

Other Pathology

Seven animals had lesions of one or other external ear. In one rat the histological appearances were similar to those of a large sebaceous cyst and it was thought likely that this represented a necrotic squamous carcinoma. The remaining 6 lesions were proven squamous carcinomas and all 6 of these rats also had histologically proven colorectal neoplasia.

Two animals had large tumour masses involving the distal stomach (poorly differentiated adenocarcinoma with signet cells) and a third animal had an intussuscepting tumour of the mid small bowel (adenocarcinoma). One of the former animals had multiple peritoneal tumour deposits and blood stained ascites. Two animals had pale,

swollen, friable livers. The histological features in one were of a mild reactive hepatitis and in the other were of diffuse hepatocellular swelling with a chronic inflammatory infiltrate.

b) Polyglycolic Acid Suture

"Peri-anastomotic" Pathology

This subgroup comprised 21 animals of which 19 had macroscopic mucosal abnormalities at the site of suture implantation. Eight animals had 2 separate lesions and 3 rats had 3 lesions each such that a total of 33 "peri-anastomotic" abnormalities were recorded in this group. Twenty six of these abnormalities occurring in 17 animals were histologically neoplastic comprising 14 adenomas and 12 carcinomas.

The 7 histologically non-neoplastic lesions comprised one walled off abscess around a piece of suture, 1 inflammatory mass, 1 aggregation of lymphoid cells, and 4 areas of granulomatous reaction around residual suture material.

The remaining histology of the "peri-anastomotic" sites revealed no unsuspected neoplastic change. Microscopic examination demonstrated a variable degree of inflammatory cell infiltration around residual suture material with prominent foreign body granulomata.

Pathology of the Remaining Large Bowel

Eleven of the 21 animals had macroscopic large bowel "tumours" at sites distant from the area of suture implantation. A total of 19 lesions were recorded in these animals, all of which were

histologically neoplastic (11 adenomas, 8 carcinomas). All but two of these rats also had histologically proven neoplasms at the "peri-anastomotic" site.

Other Pathology

Five rats had tumours of the external ear (all histologically squamous carcinomas).

One animal had multiple peritoneal deposits of adenocarcinoma from a colonic primary tumour with associated blood stained ascitic fluid.

There were no gastric or small bowel tumours in this group nor did any animal have any macroscopic abnormality of the liver or lungs.

c) Stainless Steel Suture

"Peri-anastomotic" Pathology

There were 21 animals available for study in this subgroup. Three animals had macroscopic abnormalities of the mucosa in the area of suture implantation. One of these animals had 2 separate 2mm nodules adjacent to sutures (both adenomas), one had a 4mm nodule (adenoma) and a second smaller (2mm) nodule (histologically normal mucosa), and the third had an 8mm polypoidal lesion related to a piece of suture (adenoma). Histologically, one further rat had a small focus of superficial glandular dysplasia related to a suture tract but there was no evidence of neoplastic change.

Microscopic examination of the "peri-anastomotic" sites in the remaining animals revealed no unsuspected neoplasia. Three specimens were normal or had only mild serosal inflammation while the remainder

exhibited a variable degree and mixture of acute and chronic inflammation. One animal had marked thickening of the serosa surrounding the sutures. Histologically this was characterised by marked eosinophil inflammatory infiltrate.

Pathology of the Remaining Large Bowel

Gross large bowel mucosal lesions were detected in 13 animals in this group at sites distant from the area of suture implantation. A total of 21 lesions were identified of which 20 were histologically proven to be neoplastic (9 carcinomatous, 11 adenomatous). These neoplastic lesions were distributed between all 13 animals. No specific histological abnormality could be detected in the one remaining case.

Other Pathology

Three animals developed ear tumours, all of which were histologically squamous carcinomas.

One animal had a large upper abdominal tumour encasing the distal stomach and duodenum which histologically was a poorly differentiated signet cell carcinoma. The mucosa of both the stomach and duodenum was intact and the primary lesion was identified as a carcinoma of the proximal colon. The same animal had a pale friable liver but there was no specific microscopic abnormality. One further rat had a primary tumour of the distal stomach invading the duodenum which was histologically an adenocarcinoma. No abnormality was seen in the colon of this animal.

One rat had nodular deposits on both lungs although no gastro-intestinal tumour was identified. Histologically these represented a lymphoid inflammatory infiltrate with marked

peri-bronchial "cuffing". Similar, although less prominent macroscopic changes were seen in the lungs of a second animal but on this occasion there was no histological abnormality.

One animal had evidence of altered blood within the lumen of the small bowel but no source for this could be identified.

d) Sham Operated Group

"Peri-anastomotic" Pathology

There were 15 animals in this subgroup. None of these had any macroscopic abnormality in the distal descending colon, the area equivalent to the site of suture implantation in the other groups. Microscopically, the appearances were of normal large bowel mucosa in each case.

Pathology of the Remaining Large Bowel

Single large bowel lesions were identified in each of 3 rats at sites distant from the distal descending colon. One animal had a 2mm nodule in the mid-colon (adenoma), one had a 3mm nodule at the same site (adenocarcinoma) and the third had a 3mm polypoidal lesion in the mid-colon (adenocarcinoma).

Other Pathology

Three animals had altered blood in the small bowel. Despite careful and extensive autopsy no source for this could be found. Otherwise there were no abnormal post-mortem findings.

11.2.3 Colotomy and Re-suture - 4 Week Sacrifice

a) Polyamide Suture

"Peri-anastomotic" Pathology

Of the 11 animals in this subgroup, only one had single macroscopic abnormalities detectable at the peri-anastomotic site. This comprised a 2mm raised plaque which histologically was a moderately dysplastic tubular adenoma.

Microscopy revealed normal bowel at the anastomosis in 4 animals while the remainder had mixed inflammatory cell infiltrates and prominent granulomata in relation to residual suture material.

Pathology of the Remaining Large Bowel

Three animals had single lesions more proximally in the colon. Two of these tumours were histologically adenomas and one was a poorly differentiated adenocarcinoma.

Other Pathology

No other autopsy abnormalities were recorded in this subgroup.

b) Polyglycolic Acid Suture

"Peri-anastomotic" Pathology

Of the 12 animals in this subgroup, 6 had single macroscopic peri-anastomotic lesions. Five of these abnormalities were

histologically proven neoplasms comprising 4 adenomas and one adenocarcinoma. The non-neoplastic lesion was a 6mm raised plaque related to the sutures which histologically consisted of prominent granulomata with lymphoid aggregates.

Microscopic examination of the anastomotic site of the macroscopically normal animals revealed an acute inflammatory infiltrate in two animals and foreign body granulomatous reactions in the remainder.

Pathology of the Remaining Large Bowel

Six animals exhibited abnormalities of the colonic mucosa at sites distant from the anastomosis. Two of these animals had no peri-anastomotic abnormality. A total of 9 lesions were seen of which 7 were histologically primary neoplasms of the colonic mucosa. These comprised 4 adenomas and 3 adenocarcinomas and these were distributed amongst the 6 animals. Two of the carcinomas appeared to be arising from adenomatous lesions. Of the other two macroscopic abnormalities, one consisted of deposits of adenocarcinoma in the serosa of the caecum from a primary lesion more distal in the colon but the caecal mucosa was intact. The remaining lesion was an aggregation of lymphoid cells.

Other Pathology

No autopsy abnormalities were recorded in the upper gastro-intestinal tract, liver, lungs, or external ear.

c) Stainless Steel Suture

"Peri-anastomotic" Pathology

There were 12 animals in this subgroup, none of which had any macroscopic abnormality related to the anastomosis. Microscopically, however, one animal was found to have a small focus of adenocarcinoma adjacent to a suture tract.

Histological examination of the anastomotic site in the remaining 11 animals revealed normal bowel in 6 cases. One animal had marked submucosal scarring and the rest had a variable inflammatory cell infiltrate.

Pathology of the Remaining Large Bowel

Two animals had single tumours at other sites in the large bowel and one rat had 3 separate tumours and so a total of 5 lesions were recorded. Histologically all 5 lesions were neoplastic comprising 3 adenomas and 2 carcinomas.

Other Pathology

No other post-mortem abnormalities were recorded.

11.2.4 Colotomy and Re-suture - 12 Week Sacrifice

a) Polyamide Suture

"Peri-anastomotic" Pathology

This subgroup consisted of 19 animals, 11 of which had macroscopic lesions at the site of the anastomosis. Eight of these animals had single lesions, 2 animals had 2 distinct lesions and one rat had 3 tumours and so a total of 15 macroscopic peri-anastomotic tumours were recorded. All of these lesions were histologically neoplastic comprising 5 adenocarcinomas and 10 adenomas. One of the carcinomas appeared to be arising in a tubular adenoma.

Microscopic examination of the peri-anastomotic mucosa of the remaining animals revealed an unsuspected focus of adenocarcinoma arising in an area of diffuse adenomatous change in one animal. In addition, a second rat had diffuse adenomatous change surrounding the sutures with marked dysplasia. A total of 13 animals in this subgroup were therefore considered as exhibiting neoplastic features in relation to the anastomosis.

The microscopic pathology of the peri-anastomotic site in the remaining 5 animals revealed normal features in one rat while the rest had inflammatory cell infiltrates with foreign body granulomata in relation to residual suture material.

Pathology of the Remaining Large Bowel

Eleven rats had a total of 16 macroscopically abnormal lesions recorded at large bowel sites distant from the anastomosis. Thirteen of these lesions distributed between 9 animals were histologically

neoplastic, comprising 7 adenomas and 6 adenocarcinomas. Two of the carcinomas were arising from additional adenomas. All 9 animals also had histologically proven neoplasia at the anastomotic site.

The 3 non-neoplastic lesions comprised 2 cases where no histological abnormality could be detected and one area of prominent lymphoid aggregates.

Other Pathology

Three animals developed external ear tumours, all of which were squamous carcinomas. One of these animals had no evidence of gastro-intestinal neoplasia.

One animal had a large obstructing tumour of the duodenum (adenocarcinoma) in the absence of neoplasia elsewhere while a second animal had a small duodenal lesion (adenocarcinoma) in association with large bowel tumours.

b) Polyglycolic Acid Suture

"Peri-anastomotic" Pathology

There were 20 rats available for study in this subgroup. Sixteen of these animals had abnormal peri-anastomotic lesions. A total of 26 lesions were documented, single lesions occurring in 8 animals, 2 lesions in 6 animals, and 3 separate lesions in 2 rats. Twenty three of these 26 lesions were histologically proven primary neoplasms. These were distributed between 14 animals and comprised 10 adenomas and 13 adenocarcinomas. Four of the carcinomas were arising from adenomatous lesions. Microscopically, one animal had a separate small focus of adenomatous change related to a piece of

suture material and so a total of 24 peri-anastomotic neoplastic lesions were recorded. One of the abnormalities labelled as non-neoplastic consisted of infiltration of the serosa by poorly differentiated adenocarcinoma but microscopically the mucosa of the bowel was normal. The remaining 2 non-neoplastic abnormalities comprised aggregations of lymphoid cells.

Microscopic examination of the peri-anastomotic bowel of the 4 rats with no macroscopic abnormality revealed normal histology in one animal and mixed inflammatory infiltrates in the remainder.

Pathology of the Remaining Large Bowel

Ten animals had macroscopic large bowel lesions at sites distant from the anastomosis. Eight of these rats also had histologically proven neoplasms of the anastomotic site. A total of 14 lesions were recorded of which 13, distributed between 9 animals, were histologically neoplastic. These 13 tumours comprised 7 adenomas and 6 adenocarcinomas, 2 of the latter arising in additional tubulo-villous adenomas. No histological abnormality could be identified to correspond with the remaining macroscopic abnormality.

Other Pathology

Three animals developed squamous carcinomas of the external ear, one of which had no evidence of gastro-intestinal neoplasia. This latter animal also had a cystic lesion in the liver which histologically was lined with biliary epithelium and termed a biliary hamartoma. One animal had a large tumour mass involving the distal stomach and duodenum and histologically this was necrotic adenocarcinoma. This animal also had primary tumours of the

anastomotic site and remaining large bowel. One further animal had a solid mass of intra-peritoneal tumour from a primary lesion in the mid-colon.

d) Stainless Steel Suture

"Peri-anastomotic" Pathology

There were 18 animals in this subgroup. Six animals exhibited a total of 7 macroscopic lesions in relation to the anastomosis of which 4 lesions in 3 animals were histologically neoplastic (2 adenomas, 2 adenocarcinomas). The remaining 3 animals comprised one rat in which no histological abnormality could be identified, one animal in which a 2mm nodule consisted of an aggregation of lymphoid cells, and one rat in which the histology showed some glandular dysplasia but no evidence of neoplastic change.

Microscopic examination of the anastomotic area of the remaining animals, however, revealed 2 unsuspected neoplasms. One rat had a small focus of dysplastic adenomatous change and a second animal had a small villous adenoma. In total, therefore, 5 animals in this subgroup had neoplasia at the site of the anastomosis.

One further animal had a focus of minor metaplastic change at the anastomosis, one had lymphoid aggregates, and 4 had normal histology at the anastomosis. The remainder exhibited a variable inflammatory infiltrate with occasional foreign body granulomata.

Pathology of the Remaining Large Bowel

Eleven animals in this group had abnormal lesions elsewhere in the large bowel. A total of 14 lesions were documented of which 13 distributed between all 11 animals were histologically neoplastic. Of these 13 neoplasms, 7 were adenomatous and 6 carcinomatous. The one lesion which was not a primary neoplasm consisted of invasion of the proximal colon from the serosal surface by adenocarcinoma. The source of this was from a primary lesion in the adjacent large bowel which had extended into the pancreas and was then re-invading the bowel from there.

Other Pathology

Three animals developed tumours of the external ear, 2 of which were squamous carcinomas while the third had the histological appearances of a sebaceous cyst.

One animal had metastatic deposits of poorly differentiated adenocarcinoma throughout the peritoneal cavity and in the liver and lungs. The primary lesion was in the proximal colon. A second animal had multiple peritoneal deposits from a similar primary tumour.

One rat had evidence of upper gastro-intestinal bleeding in that the distal small bowel and colon contained altered blood. No lesions could be found to explain this.

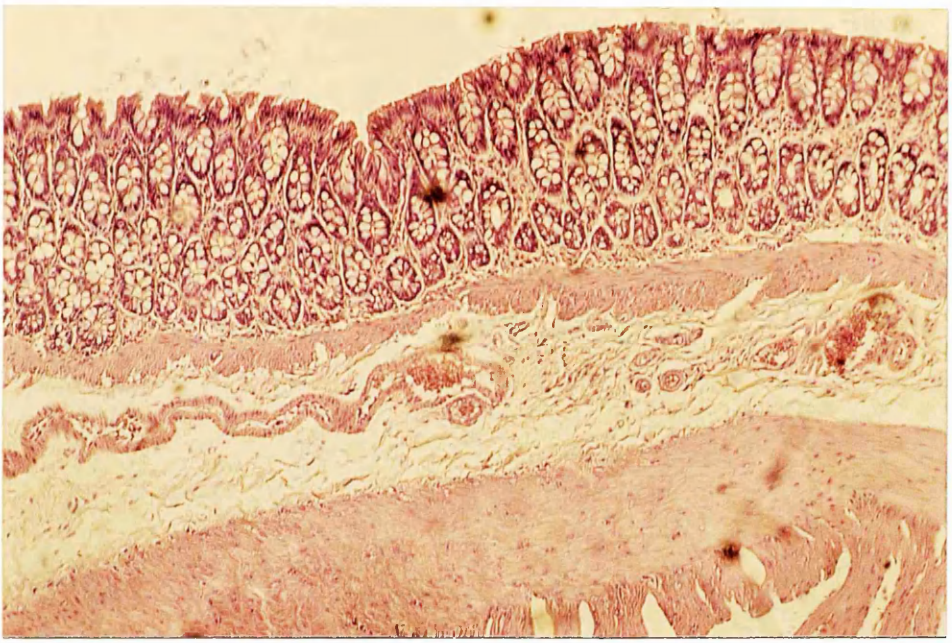


Figure 11.5 Normal Rat Colon

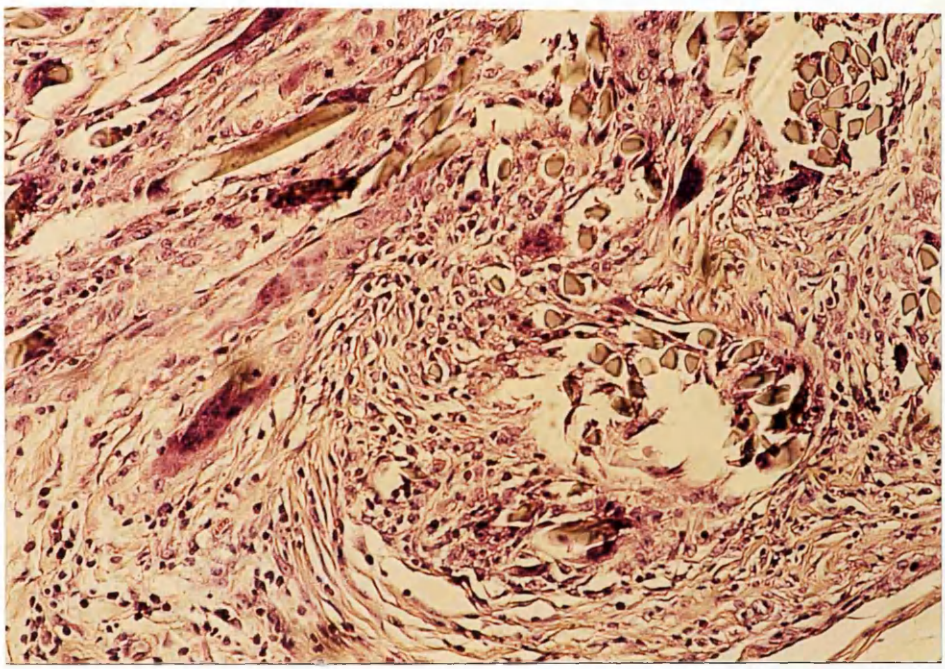


Figure 11.6 Granulomatous Reaction in Relation to Residual Polyamide Suture Material (x400)

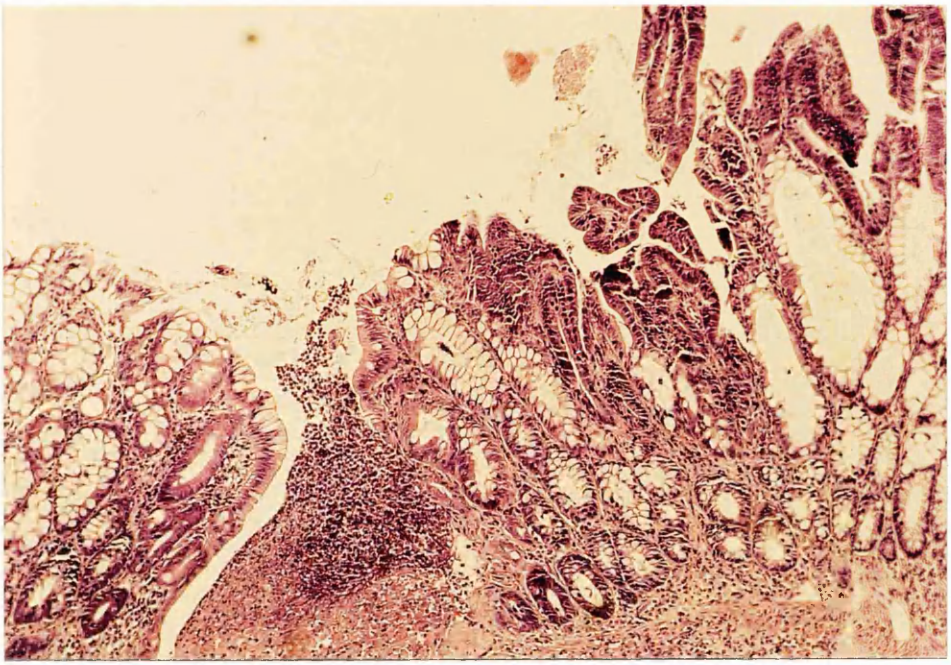


Figure 11.7 Adenomatous Focus Adjacent to a Stainless Steel Suture Tract (x100)

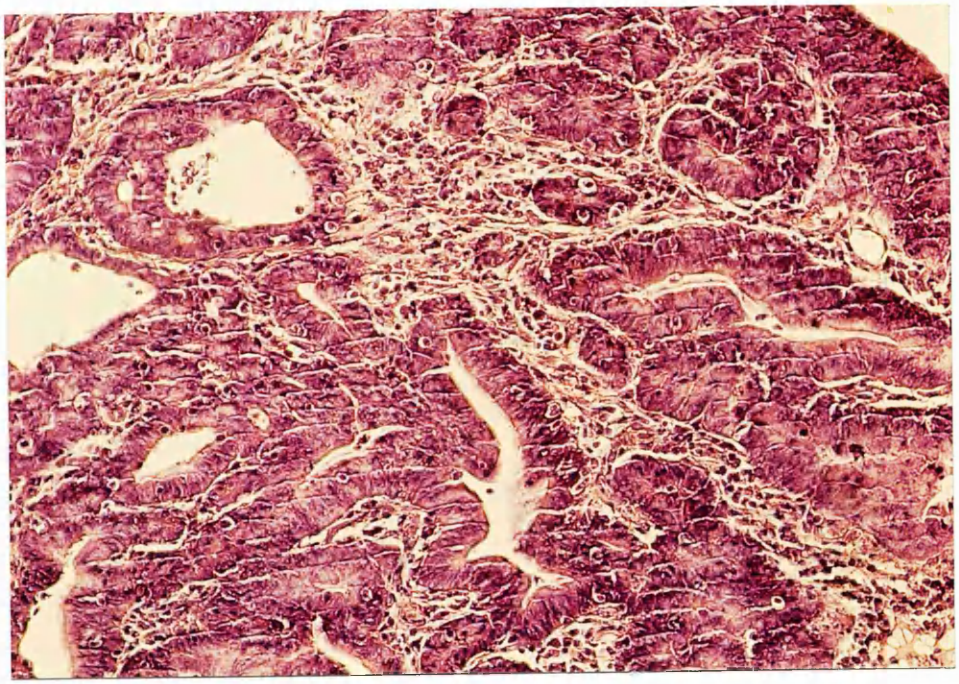


Figure 11.8 Moderately Dysplastic Tubulo-Villous Adenoma (x200)

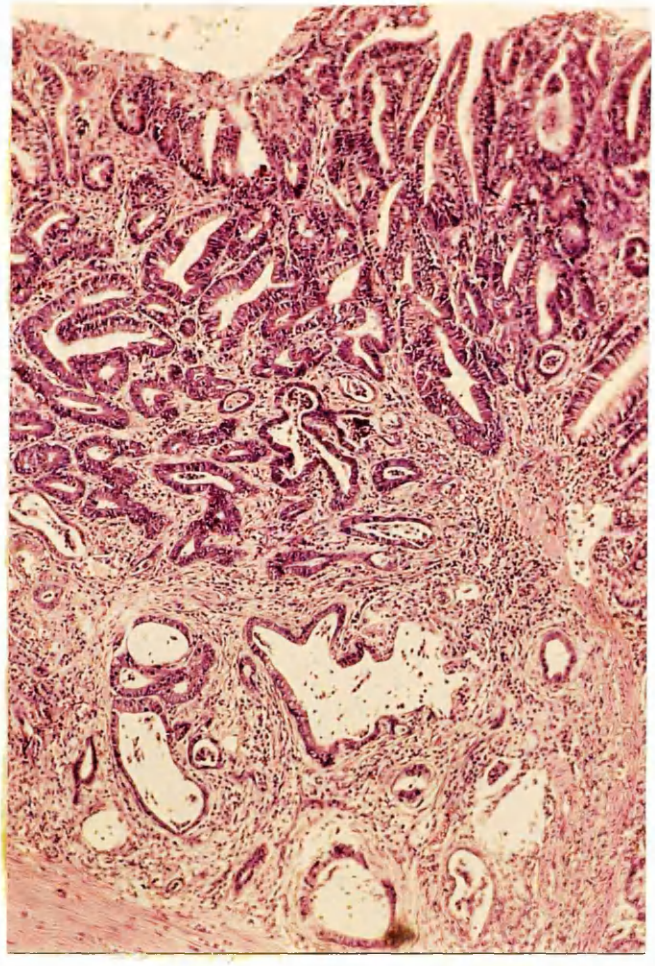


Figure 11.9 Well Differentiated Adenocarcinoma arising from a Tubular Adenoma in the Proximal Colon (x100)

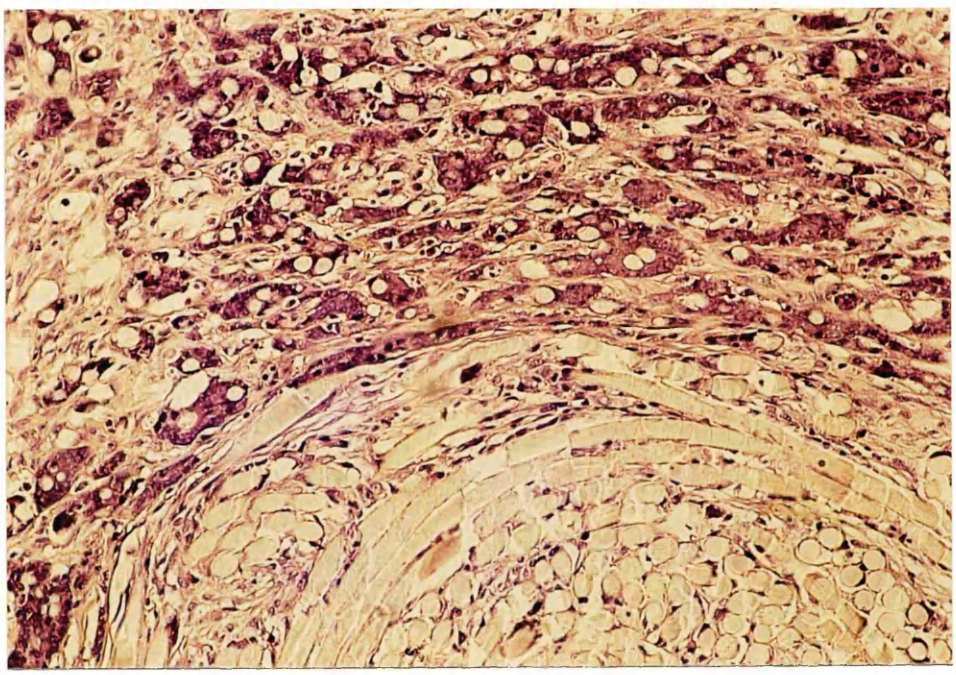


Figure 11.10 Poorly Differentiated Adenocarcinoma Surrounding Polyglycolic Acid Sutures (x400)

11.3 Control (Saline Treated) Animals

None of the saline treated control animals developed any form of large bowel neoplastic lesion nor did they exhibit any abnormalities at other sites in the gastro-intestinal tract. The only macroscopic abnormalites recorded were occasional nodular heaping of the mucosa at the site of penetration of the bowel wall by the sutures and a variable degree of serosal thickening in the same area. There did not appear to be any correlation between the type of suture material and the number of animals exhibiting these features. Histologically, the majority of the nodular lesions comprised aggregations of lymphoid cells surrounding the suture material. In one animal similar appearances were explained by a small walled-off abscess and occasionally no corresponding histological abnormality was seen. The serosal thickening was a manifestation of localised inflammation promoted by the suture materials.

All 3 types of suture material were associated with inflammatory changes. Variable types of inflammatory cell infiltrate were seen ranging from acute to chronic inflammation. A frequent observation was the accumulation of foreign body granulomata around the suture tracts. These changes were seen irrespective of the type of suture material involved. Unfortunately it proved impossible to grade the degree of inflammatory reaction associated with each material for a variety of reasons. Firstly, in order to prevent damage to the microtome used to section the tissue, residual suture material, and most importantly stainless steel, had to be removed prior to tissue processing. This inevitably led to distortion of the tissue architecture surrounding the suture tract. Secondly, as discussed above, a wide range of inflammatory reactions were seen for each type

of material and no consistent pattern emerged. The only suture material related observation which could be made was that submucosal scarring occurred with a much higher frequency and with increased severity in the stainless steel groups. This was particularly noticable in the animals sacrificed at 12 weeks and frequently rendered the stripping of the mucosa from the submucosa as required in the preparation of the tissue for stathmokinetic analysis extremely difficult.

Azoxymethane Treated Groups: Animals with Large Bowel Tumours

Table 11.1

Implantation of Sutures - 4 Week Sacrifice

Suture Material (n)		Peri-anastomotic	Remaining Colon
polyamide	(13)	1	0
polyglycolic acid	(13)	2	4
stainless steel	(14)	0	3
sham	(8)	0	0

Table 11.2

Implantation of Sutures - 12 Week Sacrifice

Suture Material (n)		Peri-anastomotic	Remaining Colon
polyamide	(20)	14	11
polyglycolic acid	(21)	17	11
stainless steel	(21)	3	11
sham	(15)	0	3

Azoxymethane Treated Groups: Animals with Large Bowel Tumours

Table 11.3

Colotomy and Re-Suture - 4 Week Sacrifice

Suture Material (n)	Peri-anastomotic	Remaining Colon
polyamide (11)	1	3
polyglycolic acid (12)	5	6
stainless steel (12)	1	3

Table 11.4

Colotomy and Re-Suture - 12 Week Sacrifice

Suture Material (n)	Peri-anastomotic	Remaining Colon
polyamide (19)	13	9
polyglycolic acid (20)	14	9
stainless steel (18)	5	11

Azoxymethane Treated Groups: Total Numbers of Large Bowel Tumours

Table 11.5

Implantation of Sutures - 4 Week Sacrifice

Suture Material (n)		Peri-anastomotic	Remaining Colon
polyamide	(13)	1	0
polyglycolic acid	(13)	2	4
stainless steel	(14)	0	3
sham	(8)	0	0

Table 11.6

Implantation of Sutures - 12 Week Sacrifice

Suture Material (n)		Peri-anastomotic	Remaining Colon
polyamide	(20)	18	16
polyglycolic acid	(21)	26	19
stainless steel	(21)	4	20
sham	(15)	0	3

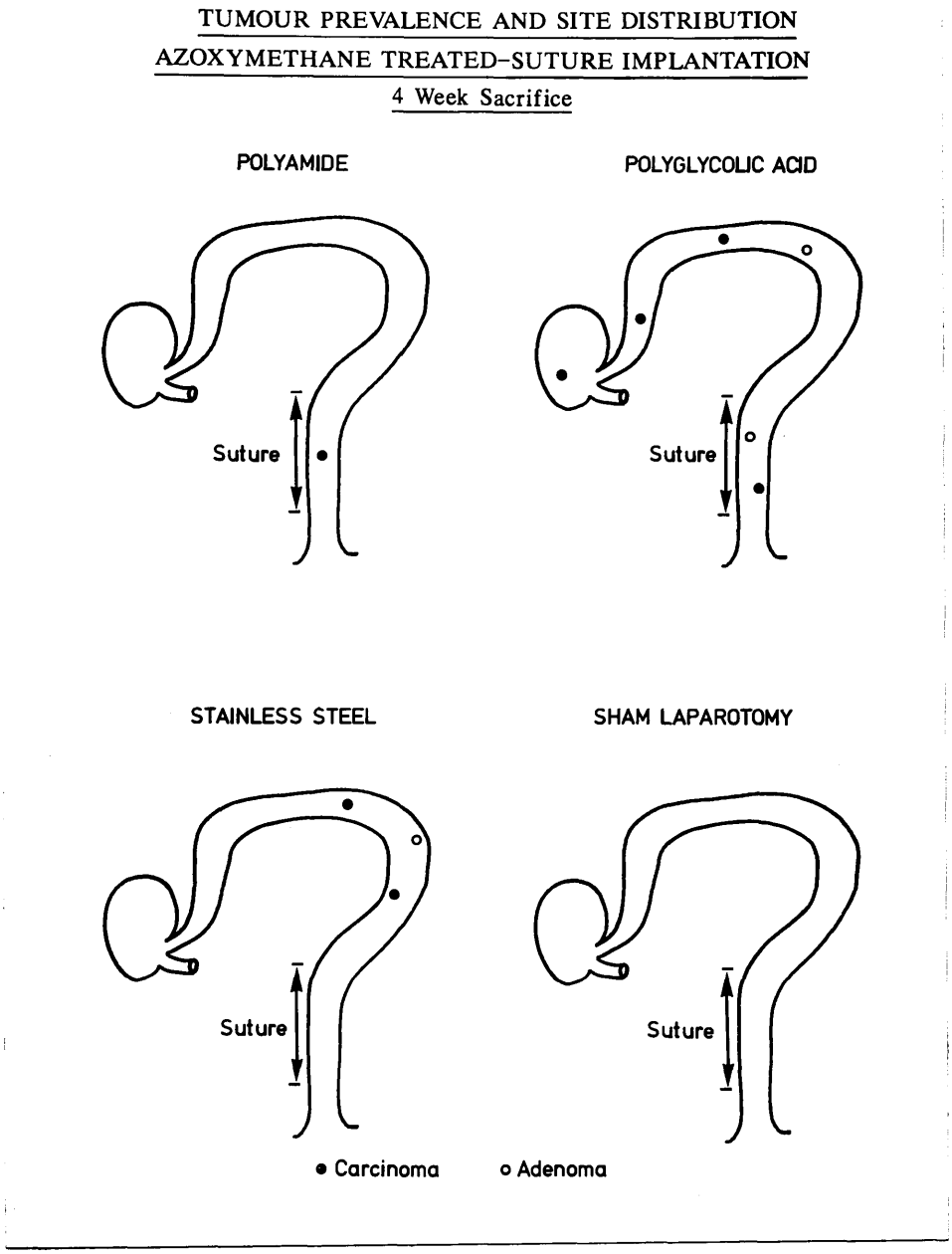


Figure 11.11 Large Bowel Tumour Prevalence and Site Distribution Implantation of Sutures: 4 Week Sacrifice

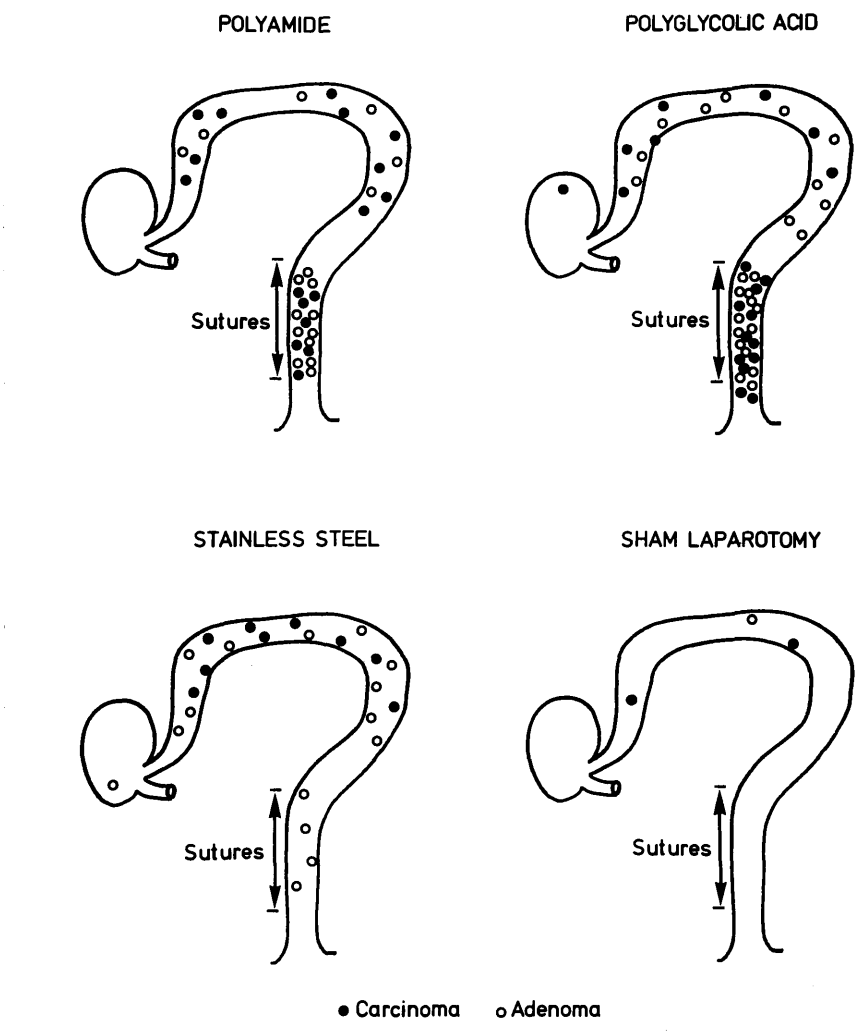


Figure 11.12 Large Bowel Tumour Prevalence and Site Distribution Implantation of Sutures: 12 Week Sacrifice

Azoxymethane Treated Groups: Total Numbers of Large Bowel Tumours

Table 11.7

Colotomy and Re-Suture - 4 Week Sacrifice

Suture Material (n)	Peri-anastomotic	Remaining Colon
polyamide (11)	1	3
polyglycolic acid (12)	5	8
stainless steel (12)	1	5

Table 11.8

Colotomy and Re-Suture - 12 Week Sacrifice

Suture Material (n)	Peri-anastomotic	Remaining Colon
polyamide (19)	17	13
polyglycolic acid (20)	24	13
stainless steel (18)	6	13

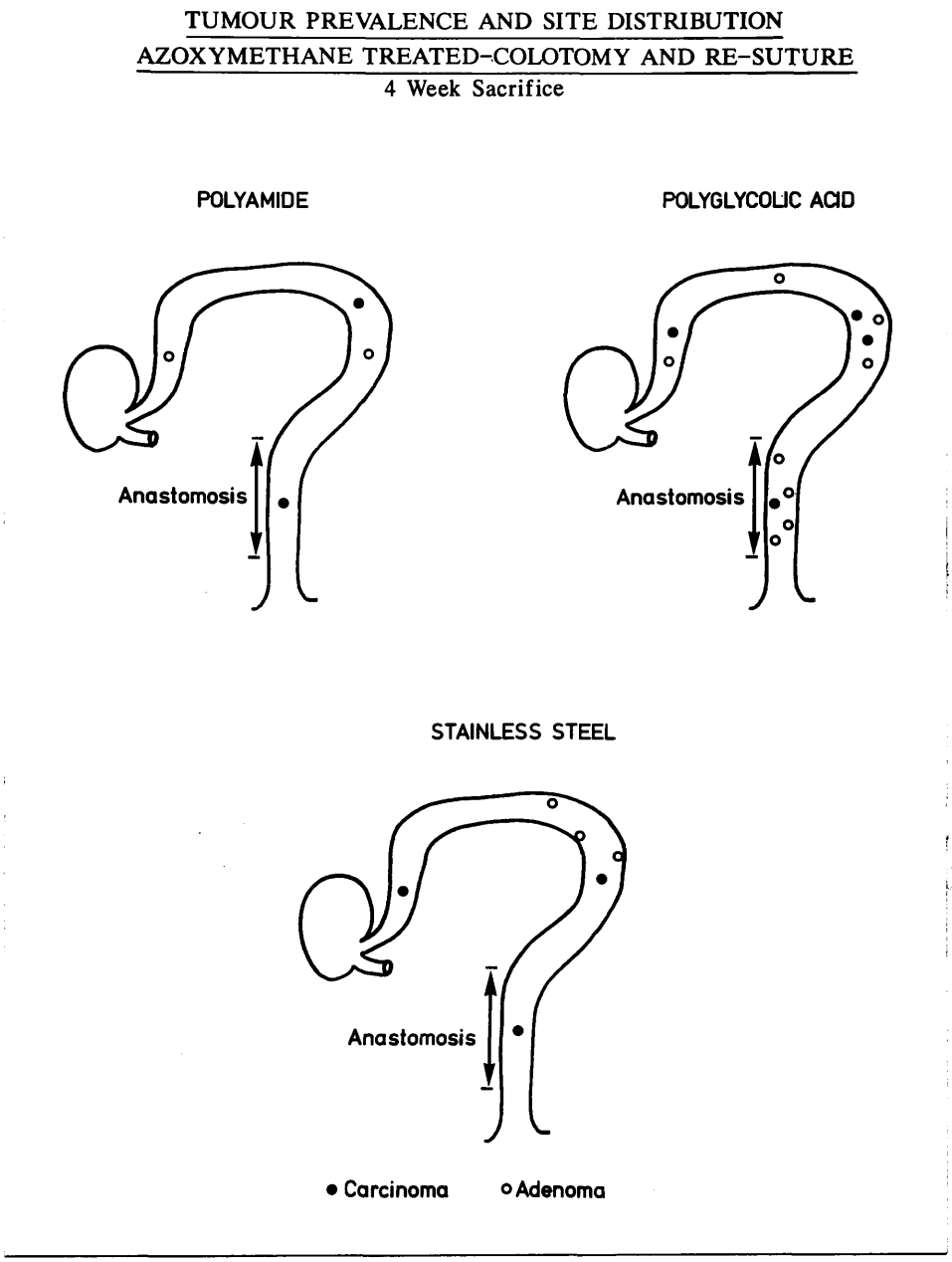


Figure 11.13 Large Bowel Tumour Prevalence and Site Distribution Colotomy and Re-Suture - 4 Week Sacrifice

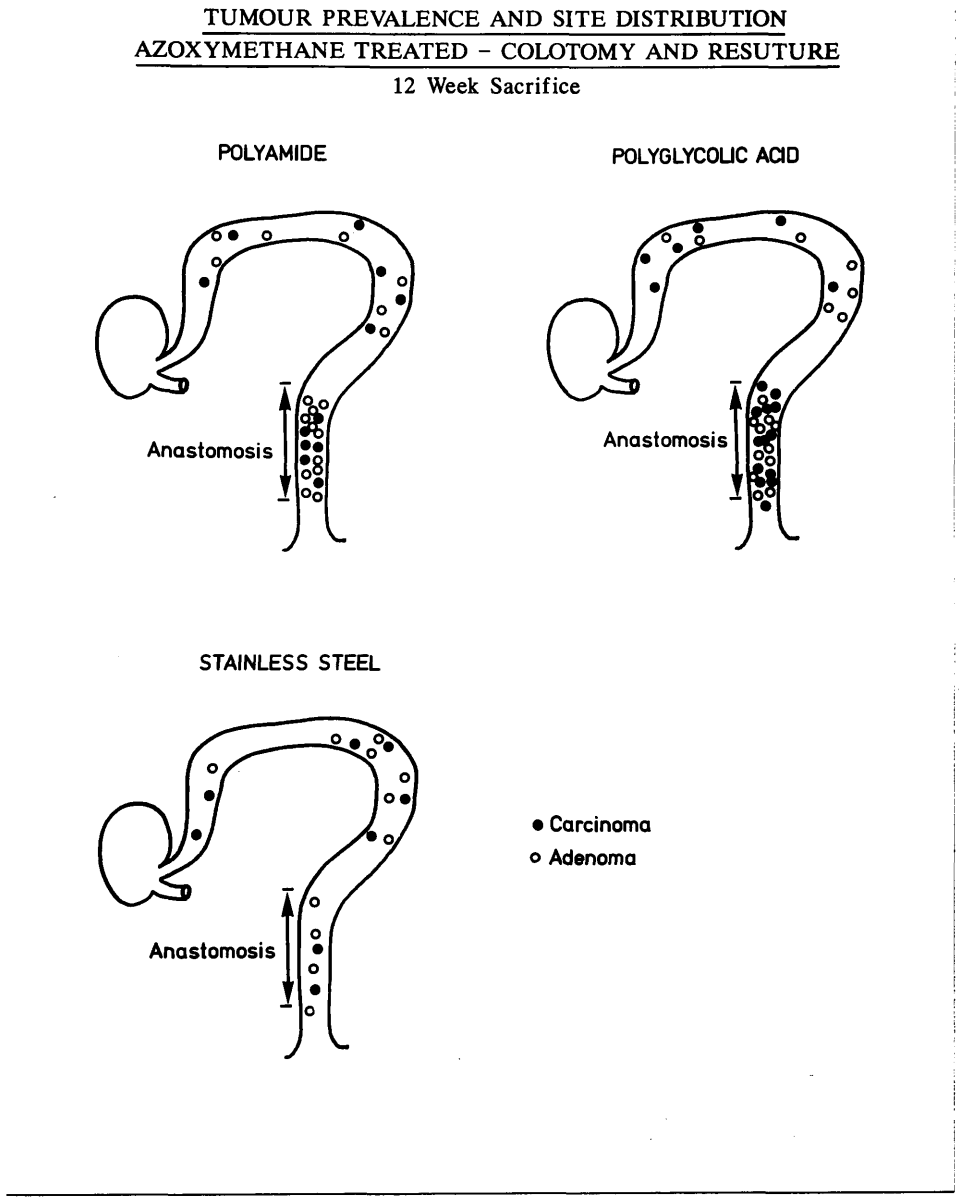


Figure 11.14 Large Bowel Tumour Prevalence and Site Distribution Colotomy and Re-Suture - 12 Week Sacrifice

11.4 Statistical Analysis: Carcinogen Treated Groups

The various suture material groups were compared statistically with respect to both the number of animals developing large bowel tumours and the total number of lesions recorded. The former comparison was analysed by Fishers Exact Test while the Mann-Whitney U Test was used to compare total numbers of lesions.

No significant differences emerged from analysis of the groups of animals sacrificed 4 weeks post-operatively. As can be gauged from the figures listed in tables 11.1-11.8, for the groups sacrificed 12 weeks following surgery, the three suture materials were similar with respect to the number of animals developing large bowel tumours distant from the "anastomotic" site and the total number of such lesions recorded. Interestingly, however, fewer of the sham operated animals developed such lesions and this resulted in a smaller total number of lesions recorded in this group ($p < 0.05$). It is possible that this simply reflects the lesser degree of operative trauma in these animals compared with the suture material groups. Otherwise the significance of this observation is uncertain.

More relevant to the subject of this thesis were the differences between the three anastomotic materials with respect to the development of "peri-anastomotic" large bowel tumours. The results of the statistical analyses are listed below. Irrespective of whether the sutures were simply implanted into the colonic wall or used to repair a colotomy, it is evident that stainless steel sutures were consistently associated with significantly fewer "peri-anastomotic" tumours when compared with either of the other two materials tested. These findings are discussed in Chapter 13.

Azoxymethane Treated Groups - 12 Week Sacrifice

1. Number of animals developing peri-anastomotic tumours

(Fishers Exact Test)

a) Implantation of Sutures

sham < polyamide	$p < 0.001$	
sham < polyglycolic acid	$p < 0.001$	
sham vs stainless steel	$p = 0.186$	N.S.
stainless steel < polyamide	$p < 0.001$	
stainless steel < polyglycolic acid	$p < 0.001$	
polyamide vs polyglycolic acid	$p = 0.326$	N.S.

b) Colotomy and Re-Suture

stainless steel < polyamide	$p = 0.015$	
stainless steel < polyglycolic acid	$p = 0.011$	
polyamide vs polyglycolic acid	$p = 0.590$	N.S.

2. **Total number of peri-anastomotic tumours** (Mann-Whitney U Test)

a) **Implantation of Sutures**

sham < polyamide	$p < 0.001$	
sham < polyglycolic acid	$p < 0.001$	
sham vs stainless steel	$p = 0.127$	N.S.
stainless steel < polyamide	$p < 0.001$	
stainless steel < polyglycolic acid	$p < 0.001$	
polyamide vs polyglycolic acid	$p = 0.248$	N.S.

b) **Colotomy and Re-Suture**

stainless steel < polyamide	$p = 0.031$	
stainless steel < polyglycolic acid	$p = 0.008$	
polyamide vs polyglycolic acid	$p = 0.365$	N.S.

Chapter 12

Results: Dynamic Cell Population Kinetics

12.1 Introduction

This Chapter describes the results of the stathmokinetic assessment of crypt cell production rates (CCPR) in the colorectal mucosa. The CCPR values, in the units cells/crypt/hour, have been derived from the whole crypt metaphase counts using weighted linear regressional analysis. The detailed whole crypt metaphase counts are listed in Appendix 4

The first part of this Chapter is concerned with the listing of the crypt cell production rates for each sacrifice time and operative procedure. The results of recounting the six groups are included for comparison and this illustrates the reproducibility of the technique. At the end of the Chapter an attempt is made to interpret the results in terms of the influences of carcinogen and the type of suture material.

12.2 Implantation of Sutures - 4 Week Sacrifice

Table 12.1 illustrates the calculated crypt cell production rates (CCPR) for the groups of azoxymethane treated animals which received an implant of sutures and were sacrificed 4 weeks post-operatively. Table 12.2 depicts the comparable data for the saline treated control animals. In each case comparative values are illustrated for the areas of suture implantation and for the rectum

Table 12.1

CCPR: Azoxymethane/Implantation of Sutures/4 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	27.18 ± 1.68	17.28 ± 0.90
polyglycolic acid	21.12 ± 1.56	17.28 ± 2.34
stainless steel	17.34 ± 0.84	12.60 ± 0.84
sham	17.52 ± 2.22	13.56 ± 2.04

Table 12.2

CCPR: Saline/Implantation of Sutures/4 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	10.92 ± 0.36	8.28 ± 0.36
polyglycolic acid	18.90 ± 4.08	13.32 ± 2.64
stainless steel	11.64 ± 1.62	9.84 ± 1.92
sham	14.70 ± 0.48	18.36 ± 3.84

Considerable variability can be seen to exist. In general it appears that the administration of azoxymethane was associated with an increase in the CCPR both in the area of suture implantation and in the rectum. The only exception to this is the sham operated group where the CCPR in the rectal mucosa was higher in the saline treated animals. For both azoxymethane and saline treated rats the CCPR was greater in the area of suture implantation than it was in the rectum. Comparing the equivalent areas of bowel for the carcinogen treated sham operated animals reveals the same observation but the opposite is seen in the saline treated shams where the CCPR was higher in the rectum than it was in the distal descending colon.

There does not appear to be any clear cut influence by the type of suture material. The highest CCPR was associated with polyamide sutures in azoxymethane treated animals and yet the same sutures yielded the lowest CCPR in the saline treated controls.

12.3 Implantation of Sutures - 12 Week Sacrifice

Animals in this group received an implant of sutures and were sacrificed 12 weeks post-operatively. The crypt cell production rates for the axozymethane and saline treated rats are listed in table 12.3 and 12.4 respectively.

Table 12.3

CCPR: Azoxymethane/Implantation of Sutures/12 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	23.52 ± 1.14	14.10 ± 0.90
polyglycolic acid	22.68 ± 2.46	12.36 ± 1.26
stainless steel	19.62 ± 1.98	17.94 ± 1.08
sham	14.34 ± 1.14	12.00 ± 0.78

Table 12.4

CCPR: Saline/Implantation of Sutures/4 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	10.74 ± 1.32	8.94 ± 2.10
polyglycolic acid	12.72 ± 0.54	10.38 ± 2.10
stainless steel	14.46 ± 1.44	15.42 ± 1.26
sham	18.54 ± 0.90	17.34 ± 1.08

The overall pattern is similar to that observed in the animals sacrificed at 4 weeks post-operatively. Again the CCPRs were higher in the azoxymethane treated animals, particularly in the zone of suture implantation. As was the case for the 4 week sacrifice group the highest CCPR was associated with polyamide sutures in carcinogen treated animals. However, no distinct effect related to the presence or type of suture material could be identified. In the saline treated control groups, the highest CCPR was recorded in the sham operated group.

12.4 Colotomy and Re-Suture - 4 Week Sacrifice

Animals included in this category were those who had re-suture of the longitudinal colotomy with one of the three suture materials under test and were then sacrificed 4 weeks post-operatively. The CCPRs for the azoxymethane and saline treated subgroups are illustrated in tables 12.5 and 12.6 respectively.

Table 12.5

CCPR: Azoxymethane/Colotomy and Re-Suture/4 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	26.58 ± 1.14	25.86 ± 0.72
polyglycolic acid	26.70 ± 0.96	22.50 ± 0.78
stainless steel	24.24 ± 1.62	18.60 ± 1.86

Table 12.6

CCPR: Saline/Colotomy and Re-Suture/4 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	10.80 ± 2.22	11.70 ± 1.92
polyglycolic acid	18.54 ± 5.16	16.62 ± 3.96
stainless steel	13.98 ± 1.68	13.92 ± 0.60

As before there was considerable variablity and it is difficult to identify any clear cut effect other then an increase in CCPR associated with the administration of azoxymethane.

12.5 Colotomy and Re-Suture - 12 Week Sacrifice

This final subgroup comprises the animals which had re-suture of a colotomy and were then sacrificed 12 weeks post-operatively. The calculated crypt cell production rates for the carcinogen and control animals are listed in tables 12.7 and 12.8 respectively where the layout is the same as before.

The pattern here is similar to that seen in the other groups. Crypt cell production rates were higher in the azoxymethane treated animals compared with the saline treated controls and for all subgroups, the CCPR was higher in the peri-anastomotic zone than it was in the rectum. There was no clear effect related to the type of anastomotic suture material.

Table 12.7

CCPR: Azoxymethane/Colotomy and Re-Suture/12 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	23.76 ± 2.16	17.34 ± 2.64
polyglycolic acid	30.24 ± 0.90	15.60 ± 1.44
stainless steel	22.32 ± 2.88	19.62 ± 1.80

Table 12.8

CCPR: Saline/Colotomy and Re-Suture/4 Week Sacrifice

(mean cells/crypt/hr + sem)

Suture Material	Suture Area	Rectum
polyamide	20.10 ± 1.20	15.06 ± 2.04
polyglycolic acid	16.62 ± 1.38	9.96 ± 0.48
stainless steel	16.32 ± 2.46	13.68 ± 1.56

Figure 12.1 Squashed Crypt Preparation Illustrating the Arrested Metaphases in a Complete Colonic Crypt

Table 12.9

CCPR: Reproducibility Study: Results

Group	Original count	Recount	% Variation
A	27.18 ± 1.68	26.04 ± 1.26	4.2%
B	22.68 ± 2.46	19.74 ± 2.04	12.9%
C	8.94 ± 2.10	9.72 ± 1.32	8.7%
D	22.50 ± 0.78	21.84 ± 1.68	3.1%
E	24.24 ± 1.62	24.18 ± 1.74	0.2%
F	23.76 ± 2.16	25.38 ± 2.40	6.8%
Mean Variation			**5.9%**

12.7 Interpretation of Results

Owing to the large number of variables in this project (carcinogen administration, suture material, operative procedure, sacrifice time, site of sample) and resultant multiple comparisons, detailed formal statistical analysis proved to be impossible. It is evident from the figures presented in tables 12.1-8 that the only constant observation was an increase in the calculated crypt cell production rate in association with the administration of azoxymethane. The effect of the type of suture material appeared to

be highly variable with no consistent pattern emerging. The same applied to the nature of the surgical procedure and to the site of tissue sampling.

Although the technique appears to be highly reproducible, few conclusions can therefore be drawn from these kinetic studies. In particular, they provide no explanation for the variable incidence of peri-anastomotic large bowel tumours presented in Chapter 11. The inference of these findings is discussed in Chapter 13.

Chapter 13

Discussion

13.1 Introduction

Although the Albino Swiss rat is not a commonly used laboratory animal, previous experimentation within the University Department of Surgery, Western Infirmary, Glasgow had found that the incidence of azoxymethane induced colonic tumours and the histological features of these lesions were similar to reported findings with other rat strains (291). The experiments carried out for this thesis serve to confirm these findings. The administration of azoxymethane was found to reliably induce large bowel tumours, with little effect on other tissues, and with an acceptably low incidence of animal loss from acute toxicity.

13.2 Histological Appearances

The value of the hydrazine induced rodent model of colorectal carcinogenesis is enhanced by the extreme rarity of spontaneous colorectal neoplasms in laboratory rodents (278). In this study, none of the saline treated control animals exhibited any form of colorectal neoplasia. For the azoxymethane treated groups, a wide range of neoplastic lesions was identified. Some of these were invisible macroscopically and were labelled as adenomatous foci. The characteristic microscopic appearances of these lesions were of atypical hyperchromatic cells involving several adjacent crypts. It must be remembered, however, that it was only the area of suture implantation/colotomy re-suture in the distal descending colon which

was examined histologically in the absence of any macroscopic abnormality and so this study cannot comment on the frequency or distribution of such adenomatous foci throughout the large bowel.

Macroscopic abnormalities varied considerably in their gross appearances from raised mucosal plaques through small pedunculated lesions to large ulcerating, obstructing tumours. In many cases it proved difficult to determine from the macroscopic appearances whether the lesion was benign or malignant. Histologically, benign lesions comprised tubular and tubulovillous adenomas. Isolated villous adenomas were rarely identified. Various degrees of dysplasia were seen ranging from very mildly dysplastic changes to severely dysplastic "carcinoma-in-situ" appearances. Not infrequently, areas of malignant change were seen within adenomatous lesions thus providing evidence for an adenoma-carcinoma sequence. A variety of malignant lesions were observed ranging from well differentiated adenocarcinomas to poorly differentiated lesions. In these latter cases, a characteristic feature was the frequent occurrence of signet ring cells. The degree of invasiveness was also variable. In the most advanced cases there was wide dissemination of poorly differentiated adenocarcinoma throughout the peritoneal cavity. However, as has been previously documented (278) the incidence of liver metastases in this animal model was low.

The extra-colonic manifestations comprised tumours of the proximal small bowel and stomach and squamous carcinomas of the external ear. Both of these are well recognised features of hydrazine induced rodent carcinogenesis (278). Of interest were the four rats found to have altered blood within the small bowel lumen at autopsy for which no source could be found despite extensive searching. Similarly, in the case of one animal with a macroscopic

ear tumour, no definite histological evidence of malignancy could be detected and the appearances were similar to those of a sebaceous cyst. It seems reasonable to speculate that this may have represented a necrotic squamous carcinoma.

13.3 Colonic Trauma: Influence on Carcinogenesis

Non specific colonic trauma has been shown experimentally to promote the development of hydrazine induced large bowel tumours (261). Similarly, various authors have demonstrated that azoxymethane or 1,2 dimethylhydrazine induced large bowel tumours in rodents tend to cluster around a colonic anastomosis (262,263,264,265, 266,267). These observations may be relevant to colorectal cancer surgery in man.

Metachronous carcinomas are known to occur in approximately 2-3% of patients who have undergone resection of colorectal cancer (132,247) and this risk is doubled if synchronous adenomas are present at the time of primary resection (306). These figures would almost certainly be higher were patients to live longer. Colorectal cancer predominantly affects an elderly population and metachronous carinomas take on average approximately 13.5 years to develop (306). Morson has suggested that the cumulative risk of a second carcinoma may reach 5% at 25 years (306). Furthermore, approximately 20% of local recurrences do develop more than two years post-operatively and it seems unlikely that either incomplete primary resection or the surgical implantation of exfoliated malignant cells could account for these late recurrences. These facts combined with clinical evidence of "field change" in the apparently normal colonic mucosa of patients

with colorectal cancer (251,253,254), and experimental evidence of continuing proliferative instability at the site of a colonic anastomosis (266), would tend to suggest that a proportion of "local recurrences" may represent the development of a metachronous carcinoma adjacent to the anastomotic site.

Controversy remains as to the curative potential of sphincter saving procedures versus total rectal excision for rectal carcinoma. Most evidence would now favour the belief that there is little to chose between the two operations in terms of cure rates (201,208,228, 229,230,231). Nevertheless, the largest prospective study, the U.K. Large Bowel Cancer Project reported that the incidence of local recurrence was significantly higher following anterior resection than it was after abdomino-perineal resection and that this difference could not be explained by differences in tumour stage or differentiation or by distal resection margin (227). While there may be many patient or surgeon related variables which might contribute to this difference, it may also be interpreted as suggesting that the presence of an anastomosis increases the risk of local recurrence. There is certainly evidence to suggest that chronic irritation promotes large bowel carcinogenesis in man. The strong association between longstanding ulcerative colitis (307) and to a lesser extent Crohn's disease (308) and the development of colorectal cancer serve as examples. There is little doubt that a large bowel anastomosis, and in particular persisting suture material, will act as a focus of inflammation and irritation and this may therefore have some promoting influence on colonic carcinogenesis.

The carcinogenesis study described in this thesis was directed towards comparing the influence of three anastomotic suture materials on colorectal carcinogenesis in the azoxymethane induced rodent model.

Monofilament stainless steel was used as a model of surgical stapling in the light of some recent suggestions that stapled anterior resection may be associated with an increased incidence of local recurrence (232,233,234). The remaining two materials (polyamide and polyglycolic acid) were chosen as examples of anastomotic sutures commonly used in intestinal surgery. Inevitably, it would have been desirable to evaluate a wider range of materials but the number of animal groups was restricted by the capacity of the animal research unit.

13.4 Tumour Induction: Implantation of Sutures vs Sham Operated Controls

As described in Chapter 11, in this study the transmural implantation of polyamide or polyglycolic acid sutures into the distal descending colon following a course of azoxymethane injections led to significant increases in the number of animals developing large bowel tumours in that vicinity when compared with sham operated controls ($p < 0.01$; Fishers Exact Test). There were no differences, however, between the group receiving an implant of stainless steel sutures and the sham operated group with respect to the number of animals exhibiting "peri-anastomotic" tumours. Whether this represents some protective feature of the stainless steel or whether it simply reflects the lack of a promoting influence is unclear from the current experimental work. The sham operated animals simply underwent laparotomy with the distal descending colon being handled with instruments for five minutes. Perhaps a better and more

accurate sham operated control would have been to pass a needle without any attached suture material through the bowel wall such that the initial trauma to the bowel wall would have been similar in the sham operated and suture material groups. Such a control group might have been able to discriminate between the promoting or protective effects of the sutures alone as the only difference between the various groups and the control group would be the persistence of suture material.

13.5 Tumour Induction: Implantation of Sutures vs Colotomy and Re-suture.

Surgical injury has been shown to act as a non-specific co-carcinogen for chemically induced carcinogenesis (259,260). The process of mobilising, resecting, and anastomosing large bowel necessarily involves a variable degree of trauma to the colon depending on such factors as tumour fixity and site, the skill and experience of the surgeon, and anatomical considerations. It might therefore be anticipated that the greater the surgical trauma, the greater the risk of subsequent tumour development.

The design of this study allowed this hypothesis to be tested experimentally. For all three suture materials studied, there were no differences in the incidence of "peri-anastomotic" tumours between the groups of animals which had simple implantation of sutures and those which had re-suture of a colotomy. It would appear that in this animal model, the mere presence and type of suture material was

the most important factor determining tumour induction and that the additional surgical trauma associated with the formation and re-suture of an intestinal wound had no synergistic promoting effect.

If this mechanism were to apply to colorectal carcinogenesis in man then these findings could have major implications for large bowel cancer surgery. They would suggest that the surgeons choice of anastomotic suture material might be an important surgical factor influencing the risk of developing a metachronous peri-anastomotic carcinoma.

13.6 *Tumour Induction: Colorectal Carcinogenesis Distant from the "Anastomosis"*

There were no differences between the three suture materials with respect to the number of animals developing large bowel tumours at sites distant from the "peri-anastomotic" region in the distal descending colon. This serves to illustrate the comparability of the animal groups with respect to carcinogen dosage and effect. These findings therefore imply that the variable incidence of "peri-anastomotic" tumours was the result of local promoting or protecting influences induced by the presence of the different suture materials rather than any difference in the susceptibility of the animal groups to colorectal neoplasia.

13.7 The Variable Influence of Sutures on Colorectal Carcinogenesis: Mechanism of action.

While significant differences were observed between the three suture materials with respect to the number of animals developing peri-anastomotic large bowel tumours, the mechanism of this variable influence is unclear. Cell kinetic studies failed to provide the answer. There were no consistent differences between the various suture materials with respect to calculated crypt cell production rates in either the animals sacrificed at four or twelve weeks post-operatively. The only apparently consistent finding was of an increase in cell birth rate associated with carcinogen administration compared to saline treated controls. It may be that the critical time period at which the suture material might influence cell kinetics has been missed in this study. However, tritiated thymidine labelling studies carried out by Pozharisski revealed that the proliferative response to mucosal injury in the rat colon lasts for at least forty to fifty days (261). Similarly, Roe and his colleagues have recently provided evidence of persisting high rates of cell proliferation three months after the construction of an experimental large bowel anastomosis (266). If major kinetic differences were induced by the three materials tested in this thesis, then one would have expected to detect them, especially in the groups of animals sacrificed four weeks post-operatively. It is possible, particularly in the suture implantation groups, that the proliferative response was confined to a very small area immediately surrounding the suture material. While care was taken in sectioning the bowel for stathmokinetic analysis to include the tissue adjacent to residual sutures, it is possible that such a localised area of proliferation

could be missed. This would be most likely to be the case with polyglycolic acid, particularly at 12 weeks sacrifice when almost all of the material had been absorbed.

Nevertheless, it would seem important in future work to determine the immediate influence of the implantation of suture materials on cell kinetic parameters, perhaps by sacrificing animals at various shorter time intervals post-operatively.

13.8 Significance of Results in Terms of Previous Work

The findings of this project seem to be the opposite of those recently reported by Phillips and Cook (267). Using a 1,2 dimethylhydrazine/Wag rat model they found that the use of stainless steel wire sutures to repair a transverse colotomy in the descending colon of Wag rats resulted in a significantly higher incidence of peri-anastomotic tumours when compared with a similarly treated group re-sutured with silk sutures. However, the two studies are not directly analogous. Phillips subjected entirely normal rats to laparotomy and colonic trauma and only after a post-operative period of eight weeks did he commence the series of carcinogen injections. In his study there was a potential source of chronic irritation in the form of residual suture material present in the colon prior to the initiation of carcinogenesis.

Phillips's study therefore has strong similarities to the work of Pozharisski. Pozharisski demonstrated that in general the proliferative response of the colonic mucosa to injury (in this case a colotomy) lasts no more than 40 to 50 days but that a persistent source of irritation such as residual suture material produced a

localised area of enhanced mitotic activity over a much longer period (261). Recently Roe has provided evidence of proliferative instability in the vicinity of an experimental colonic anastomosis for up to three morths following surgical injury (266). Enhanced proliferative activity during the initiation phase of carcinogenesis has been previously recognised as a promoter of tumour development (283,301,302,303). In the light of Roe's findings (266), when Phillip's administered the initiating influence (the carcinogen), persisting enhanced proliferative activity was likely to be present at the site of the colotomy repair. Furthermore, Pozharisski's work had previously shown that the degree of chronic irritation incited by the presence of residual suture material in the colonic wall was sufficient to significantly enhance tumour induction.

Phillips reports that at the time of sacrifice, 36% of steel sutures were still present but only 4% of silk sutures were left. He further comments that scarring at the site of the anastomosis was greatest in the animals sutured with steel wire and this was most marked in the presence of residual suture material. Although there would undoubtedly be a greater number of sutures persisting at the time of carcinogen administration than there were at sacrifice, it seems likely that the number of steel sutures persisting would be greater than the number of silk sutures. It could therefore be postulated that the results Phillips observed were simply a manifestation of the number rather than the type of residual sutures, and the resultant degree of proliferative activity during the initiation phase of carcinogenesis. Certainly in the study described in this thesis it was observed that of the two non-absorbable

materials, stainless steel sutures persisted in the bowel wall in greater numbers and for much longer periods than did polyamide sutures.

The experiments in this thesis differ fundamentally from Phillips' work in that surgery was performed in the post-initiation phase of tumour induction. Sutures were placed in the colon during the prolonged, and probable multistage, process of promotion. In many respects this appears much more analogous to the human situation. As discussed in Chapter 7 large bowel cancer cannot be regarded as a unifocal disease and the remaining colon following resection is susceptible to further neoplastic change. In other words, the colonic mucosa in patients undergoing large bowel cancer surgery may already be initiated and exposure to suitable promoting factors may lead to the development of a macroscopic carcinoma after a variable period of time.

As pointed out in Chapter 8, the kinetic mechanisms operating during the promotion phase are unclear and are a matter of some debate. It is possible that reduced proliferative activity at some stage during promotion may allow the abnormal population of transformed malignant cells to establish their growth advantage and equally enhanced proliferation at other times may promote tumour development (291).

Unfortunately the present series of experiments cannot shed any light on this matter as all operative procedures were carried out within a fixed time period of carcinogen administration. Futhermore, no significant differences were observed between the different suture materials with respect to crypt cell production rates although the incidence of tumours was markedly different.

13.9 Scope for Future Work

The experimental work described in this thesis has demonstrated that in this experimental animal model of colorectal carcinogenesis, the type of anastomotic suture material does appear to have a significant influence on local tumour development at a large bowel anastomosis. Comparing the results with the sham operated control groups would suggest that this represents a variable promotion of carcinogenesis by the different materials but the mechanism of this action is unclear. Further investigation is required in an attempt to clarify the mechanism of promotion and to compare a further variety of commonly used suture materials.

At first impressions, the results presented in this thesis would appear to call into question the conclusions drawn by Phillips. However, the two experiments differ fundamentally with respect to the timing of surgical injury in relation to carcinogen administration. In addition, different animal strains and suture materials were tested. Further work is required to resolve the controversy and this would require a single investigator repeating both experiments with a standardised carcinogen, animal strain, operative procedures, and suture materials.

SECTION 3

Implantation Metastasis and Local Recurrence of Colorectal Cancer

Chapter 14

Introduction

14.1 History

Perhaps the most frequently cited cause of local recurrence has been the dissemination of viable malignant cells at the time of surgery. This mechanism was first proposed by Gerster in 1885 (309) and shortly thereafter reiterated by Lack (310) who proposed that local recurrences were the result of cancer cells being spread by contaminated instruments or by the surgeons hands. This hypothesis is supported by reports of recurrent tumour developing in the abdominal scar after resection of a colonic carcinoma (311,312), tumour developing in the skin puncture wound following paracentesis for malignant ascites (312,313), and anecdotal accounts of tumour developing on raw mucosal surfaces distal to colonic lesions such as haemorrhoidectomy wounds and anal fissures and fistulae (314,315). Sir Charles Ryall considered cancer to be a spreading infective process and drew attention to the importance of minimising the spread of the "infection" (312,316). His theory was that malignant cells could implant on all freshly cut tissues and he was able to demonstrate free cancer cells under his finger nails and on the blade of his scalpel after surgery for both colonic and breast tumours.

14.2 Clinical Evidence

With the recognition of the problem of local recurrence of colorectal cancer it became a widely adopted belief that exfoliated malignant cells were likely to be responsible (317,318,319). Further evidence supporting this mechanism was provided by McGrew who performed Papanicolaou stains of smears taken from the lumen of 50

specimens of large bowel carcinoma (320). She found malignant cells to be present at 42% of proximal and 65% of distal resection margins at average distances of 21cm and 10cm respectively from the macroscopic edge of the tumour, the percentage of positive smears being inversely proportional to the distance from the tumour. Similarly, Rygick demonstrated apparently viable cells in washings taken from surgical instruments and from surgeons' hands (321). Pomeranz studied a series of smears taken from the serosal surface of 20 colonic carcinomas and found malignant cells to be present in 2 cases (322). He proposed that the dissemination of these cells from the serosal surface at operation would explain peritoneal and abdominal wound recurrences.

14.3 Viability of Exfoliated Cells

The presence of intra-luminal exfoliated tumour cells in patients with large bowel cancer thus became a well recognised phenomenon to the extent that it has been proposed that the detection of such cells may be of value in the diagnosis of this disease (323,324). The viability of these desquamated cells, and hence their ability to give rise to recurrent tumour was subsequently questioned by Rosenberg (325,326). Although he was able to demonstrate the presence of large numbers of exfoliated colorectal cancer cells both by *in vivo* colonic lavage and by *ex vivo* manipulation of surgical resection specimens in Hartmann's solution, Rosenberg found that the cells he isolated were unable to exclude the supravital dye, trypan blue. Only in tumour homogenate cell suspensions could he demonstrate the presence of *viable* cells. He therefore concluded that although exfoliated

malignant cells were likely to be present in the operative field during large bowel cancer surgery, these cells were probably non viable and therefore incapable of giving rise to recurrent tumour growth.

More recent evidence, however, suggests that this is not the case. Umpleby and his colleagues in Bristol performed pre-operative colonic lavage with Hartmann's solution in 19 patients with colonic cancer and found malignant cells to be present in the washings in 14 cases with a median viability of 92% (327). The resection margins of surgical resection specimens were also irrigated and malignant cells with a median percentage viability of 70% were recovered from 57% of proximal and from 84% of distal resection margins. The number of cells obtained was inversely proportional to the distance from the macroscopic tumour edge to the resection margin. The same group have since proceeded to demonstrate that exfoliated malignant cells isolated in this manner are capable of proliferating in immune deprived mice (328). Similarly, Skipper reported the in vitro monolayer growth of tumour cells obtained from luminal washings, luminal mucus specimens, mesorectum specimens, and in one case from the serosal surface of patients undergoing surgery for colorectal cancer (329). All colonies stained positive for epithelial markers and for carcino-embryonic antigen.

14.4 Preventative Measures

Considerable circumstantial evidence implicating exfoliated malignant cells as the cause of local recurrence has therefore been accumulated. Goligher, in reviewing the St Mark's Hospital

experience of local recurrence of carcinoma of the rectum and rectosigmoid proposed that approximately half of the observed recurrences could be explained by the development of a second primary tumour or by incomplete excision of high grade anaplastic lesions (319). The only plausible explanation for the remaining half, he believed, was the implantation of viable exfoliated cancer cells. Perhaps the most convincing evidence supporting this mechanism has come from reports of a reduction in the incidence of local recurrence associated with the introduction of specific measures designed to reduce the likelihood of malignant cell implantation (330,331). Such preventative measures include the placing of occlusive ligatures around the bowel both proximal and distal to the tumour (332,333,334). McGrew and her colleagues demonstrated that this might form an effective barrier to the intra-luminal spread of free carcinoma cells in that smears taken from the bowel lumen both proximal and distal to the ligatures were invariably negative whereas similar smears taken from the isolated segment were positive for tumour cells (320).

As an alternative to, or in combination with such ligatures it has been suggested the proximal and distal bowel ends should be irrigated with cytotoxic agents prior to their anastomosis (330,331). The original chemotherapeutic agent advocated for this latter role was mercury bichloride (80) but a variety of agents have since been employed with variable reported efficacy (335).

There has been general agreement that malignant cells appear to be incapable of implanting on intact colonic mucosa (332,336). Yu and Cohn confirmed this experimentally in rabbits (337) but demonstrated that cells would readily implant and grow on serosal and peritoneal surfaces. They therefore emphasised the importance of

avoiding spillage of cells at surgery and reiterated the belief of others that closed anastomotic techniques may reduce the incidence of implantation metastasis (338,339).

14.5 The Role of Suture Materials

It has been proposed that the suture material used to construct the anastomosis following large bowel cancer resection may have a role in local recurrence. McGrew suggested that contamination of the suture with free intra-luminal tumour cells and the subsequent implantation of these cells into the colonic wall during the passage of the suture was the probable mechanism of local recurrence (320). Since then it had been established that bacteria can adhere to various forms of suture material (340) and it seems likely that tumour cells might be able to do likewise.

Experimental studies have added support to this theory of suture implantation. Haverback and Smith demonstrated that suture materials, when drawn through a solid mast cell tumour, were able to transport sufficient malignant cells to give rise to tumour growth in syngeneic mice (341). Gubareff and Suntzeff similarly demonstrated the transfer of a highly malignant mouse rhabomyosarcoma by suture material and found that pre-treatment of the suture material with iodine solution effectively decreased the tumour transplantation (342). Similarly, Cohn and his colleagues were able to demonstrate that the use of iodised catgut was of value in reducing the incidence of suture implantation of the Brown-Pearce tumour in rabbits (343).

Anastomotic sutures may, however, have a second potential role to play in implantation metastasis in that they may act as a nidus for implanted tumour cells within the colonic wall, allowing the cells to multiply and establish their growth advantage. With relevance to this theory, O'Dwyer has recently demonstrated that tumour cells variably adhere to different suture materials (344). He quantified the tumour cell adherence to a variety of sutures and demonstrated that their in vitro adherence characteristics correlated well with the ability of these materials to give rise to implantation metastases in syngeneic rodents.

14.6 Summary

There is therefore considerable clinical and experimental evidence to support the theory that local recurrence of colorectal cancer may be due to the surgical implantation of viable exfoliated malignant cells. It is also evident that anastomotic sutures may have a role in this mechanism of implantation metastasis. The experimental work described in this section was designed to investigate the variable adherence of tumour cells to different types of suture material.

Chapter 15

An Investigation of the Potential Role of Anastomotic Sutures in Implantation Metastasis

15.1 Introduction

The studies to be described in this section were designed to investigate the potential role of anastomotic suture materials in the implantation of exfoliated viable malignant cells. The aims were as follows:

1. To quantify the ability of different suture materials to entrap viable tumour cells *in vivo* and to transfer such cells from the colonic lumen.

2. To assess the tendency for sutures to act as a nidus for implanted malignant cells by comparing the degree with which tumour cells were able to firmly adhere to various suture materials.

3. To develop an animal model of implantation metastasis.

4. To further assess the conditions necessary for the implantation of malignant cells to take place and result in tumour growth.

5. To assess the influence of various anastomotic materials on implantation metastasis in the experimental animal model.

15.2 Experimental Materials

15.2.1 Tumour Cell Line

Because it was proposed to carry out both *in vitro* experiments and studies in live animals it was necessary to obtain a cell line which was syngeneic with an available strain of experimental animal. Unfortunately, no cell line was available for the Albino Swiss rat used in the carcinogenesis experiments as this animal is not a recognised inbred strain. Furthermore, at the time of commencing the study there was no known colonic adenocarcinoma cell line for the readily available experimental animals within the University of Glasgow. However, other workers within the Department of Surgery of the Western Infirmary had some experience with a metastasising rat mammary carcinoma cell line. While it is accepted that this tumour cell line is breast and not colonic in origin, it is an adenocarcinoma and it was thought suitable for the purposes of the experimental studies proposed.

This tumour cell line was the Mtln3 clone of the spontaneously metastatic rat mammary adenocarcinoma 13762NF. The cells were donated to the Department by the originators of the clone, Drs Neri and Nicolson of the MD Anderson Hospital, Houston, Texas, U.S.A. (345).

15.2.2 Cell Culture Materials

Cells were cultured in either 75 cm^3 or 150 cm^3 tissue culture flasks (Nunc/Intermed, Kamstrup, Denmark) depending on the number of cells required for each experiment. The culture medium consisted of equal parts of Hams' F10 and Dulbeccos's Modified Eagles Medium (F10/DMEM) with 10% added foetal calf serum (FCS) (all obtained from Flow Laboratories Ltd., Rickmansworth, Hertfordshire). No antibiotics were added to the medium. Cultures were incubated at 37°C in equilibrium with 2% carbon dioxide in air in a CO_2 gas incubator

In the experiments involving the use of radio-isotopes, the cells were washed after labelling using RPMI 1640 medium (Flow Laboratories) to which 10% FCS had also been added.

15.2.3 Experimental Animals

The animals used throughout this project were male Fischer F344 inbred rats obtained from Olac Limited, Bicester, U.K. Animals were aged twelve weeks at the start of each experiment and they were housed in fours in polypropylene cages with stainless steel mesh lids. Food and water were freely available and their diet consisted of the high quality breeding formulation ("CRM", Biosure Ltd, Lavender Hill Manea, Cambridgeshire) as for the carcinogenesis study described in Chapter 9. No measures were taken to avoid coprophagy in this experiment.

Animals were fasted for solid food for 24 hours prior to all experiments but were allowed water ad libitum. For most of the *in vivo* tumour growth studies, the caecum was used for tumour cell

implantation as it was easily brought out of the peritoneal cavity, isolated with gauze swabs, and the risk of peritoneal contamination with Mtln3 cells minimised.

15.2.4 Anastomotic Materials

The following materials were studied in all experiments, 5/0 gauge being used throughout;

1. Polyamide ("Nurolon", Ethicon, Edinburgh, Scotland), braided, non-absorbable.

2. Coated Polyglycolic Acid ("Dexon Plus", Davis and Geck, Gosport, Hampshire), braided absorbable.

3. Stainless Steel (Ethicon), monofilament, non-absorbable.

4. Polypropylene ("Prolene", Ethicon), monofilament, non-absorbable.

15.2.5 Radioisotopes

The isotope used for all labelling experiments was chromium 51 (^{51}Cr) obtained in the form of sodium chromate ($Na_2{}^{51}CrO_4$) in minimal volume from the Regional Radio-Nuclide Dispensary within the Western Infirmary, Glasgow. This isotope is a gamma emitter and has a half life of 27.7 days. Full precautions for the handling of radioactive materials were taken, as

laid down by the Radiation Protection Officers of the University of Glasgow and Greater Glasgow Health Board. All radioisotope experiments were carried out in one designated laboratory

15.2.6 Measurement of Radioactivity

The radioactivity of all samples was measured in a ("Compugamma", LKB/Pharmacia, Milton Keynes) gamma counter. Radioactive counts were acquired over a three minute period and the mean number of counts per minute calculated. On each occasion, the radioactivity of a standard labelled cell suspension was measured and used to calculate the number of cells represented by the radioactivity of each sample of suture material. Similarly, the background radioactive count was recorded on each occasion and corrected for in all calculations of cell numbers.

15.3 Cell Culture Methods

15.3.1 Preparation of Fresh Culture

All cell culture was carried out under a laminar flow hood using sterile materials.

Fresh stocks of the Mtln3 clone were stored in liquid nitrogen in individual vials each containing approximately one million cells suspended in 1ml of culture medium. Preparation of a fresh cell

culture involved firstly thawing the frozen cells by placing the sealed vial in water at 37°C. The cell suspension was then withdrawn using a sterile micro-pipette tip and placed in a 75 cm^3 tissue culture flask. A total of 24 mls of the F10/DMEM/FCS culture medium was added very slowly over a five minute period to minimise heat shock to the cells and the flask placed in the CO_2 incubator. Twenty four hours later the medium was changed after which the culture was replaced in the incubator until near-confluent cultures were obtained, normally after a period of four to five days.

15.3.2 Cell Passage

Once near-confluence was observed, the culture was ready for passage. All culture medium was drawn from the tissue culture flask by pipette, and the cells removed from the base of the flask by treatment with Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) followed by ten minutes incubation in 0.2% trypsin solution (Gibco, Paisley, Scotland). Ten mls (an excess) of F10/DMEM/FCS were then added to neutralise the trypsin activity, after which the cells were washed three times in this medium by centrifugation at 300g for 5 minutes and resuspension of the cell pellet. Following the final resuspension, the cells were counted and the dilution adjusted. Cell viability was ascertained by trypan blue exclusion and suspensions with over 95% viability were obtained. Each batch of cells was passaged a maximum of six times before being discarded to minimise problems with phenotypic drift (346).

15.3.3 Radioactive Labelling

With the exception of the *in vivo* tumour growth studies, cells were radiolabelled with ^{51}Cr in order to quantify the number of tumour cells adhering to the sutures. The process for cell labelling was as follows.

After counting of cell numbers and assessment of viability, a suspension of 10^7 viable tumour cells per ml of culture medium (F10/DMEM/FCS) was prepared. The radiochromium was then added to the cell suspension in a dose of 10MBQ ^{51}Cr in minimal volume per 10 million tumour cells. Labelling was carried out by incubation of this tumour cell/^{51}Cr suspension for 90 minutes in a thermostatically controlled water bath at $37^{0}C$ with constant agitation. Once incubation was complete, the tumour cells were washed four times in RPMI 1640 medium containing 10% FCS (Flow laboratories) prior to counting of cell numbers and further assessment of viability. Any suspension with a percentage viability of less than 90% at this stage was discarded and the procedure abandoned. Finally, the dilution was adjusted to produce a suspension of 10^7 viable labelled cells per ml of culture medium.

15.4 Experimental Studies

15.4.1

The "In-Vivo" Entrapment and Transfer of Tumour Cells by Sutures

This study was designed to compare the four suture materials with respect to their ability to entrap and transfer free tumour cells from the colonic lumen. The experimental method can be described with reference to figure 15.1.

Twelve week old fasted male F344 rats were used throughout. Laparotomy was performed through a long midline incision and all residual faecal material was milked out of the caecum into the distal colon. Animals were then sacrificed and immediately transferred to the radio-isotope laboratory. An occlusive clamp was placed to isolate the upper caecal pole into the lumen of which 0.1mls of the radiolabelled tumour cell suspension (10^6 cells) was injected via a 24 gauge needle. A single 10cm length of each of the four types of suture material in turn was slowly passed transmurally through this isolated, tumour cell containing segment. Atraumatic needles and sutures of 5/0 gauge were used throughout. Immediately following its passage through the caecum, each length of suture was placed in the gamma counter for measurement of radioactivity.

Animals were used in groups of four or eight, and the order in which the four types of suture were passed through the caecum was pre-determined by a "Latin Square" distribution which ensured that each suture was placed in each rank order once in every group of 4 animals (or twice in every group of 8).

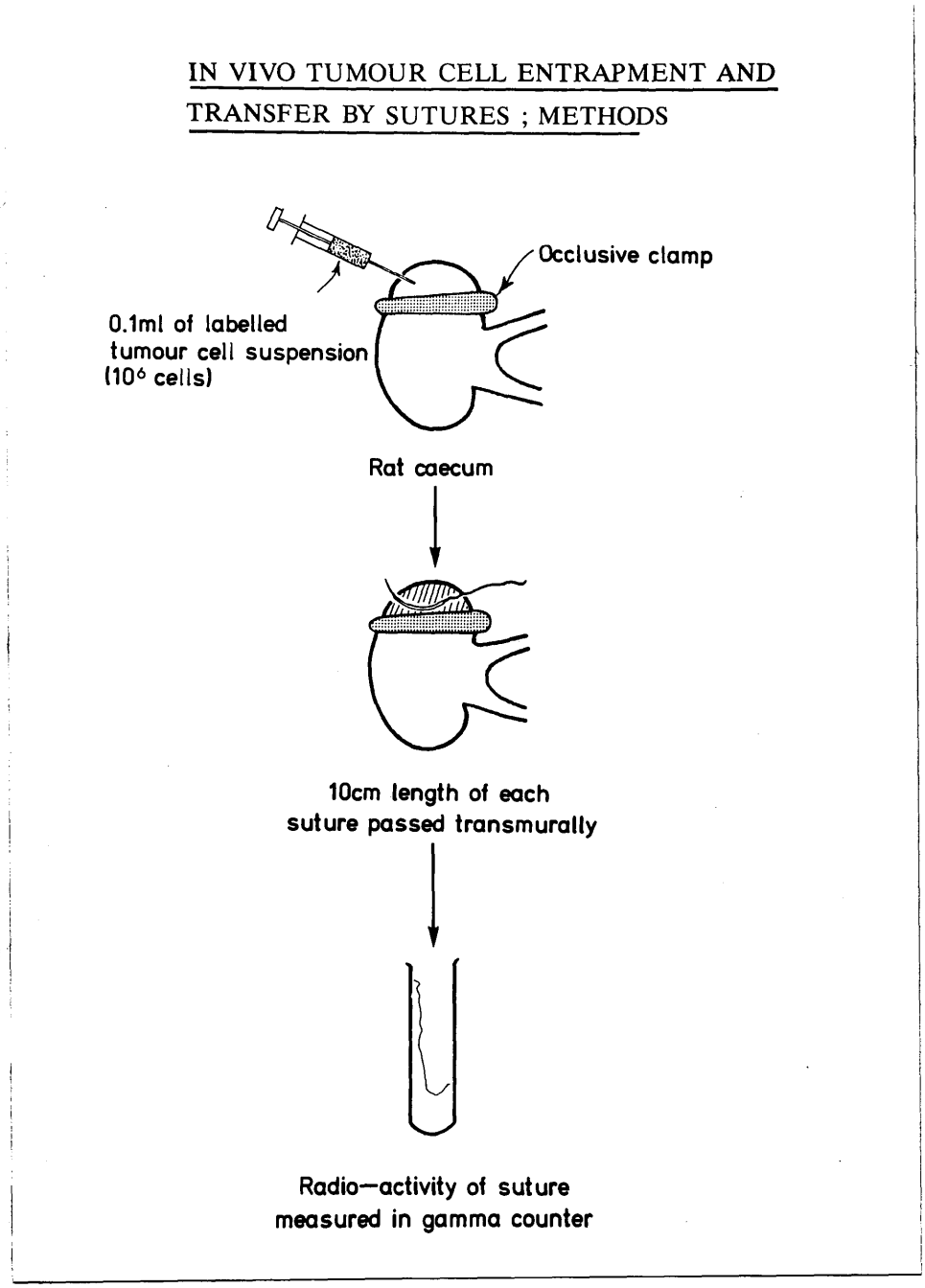


Figure 15.1 "In Vivo" Tumour Cell Entrapment and Transfer by Sutures: Experimental Methods

The entire experiment was carried out four times, twice with 4 animals and twice with 8 animals. For each assay, the radioactivity of a standard radiolabelled suspension of Mtln3 cells (10^6 cells) was measured in order to allow calculation of the number of tumour cells adherent to the sutures following correction for background activity.

15.4.2

Suture Materials as a Nidus for Implanted Tumour Cells

Anastomotic suture materials may act as a nidus for implanted malignant cells within the colonic wall. The aim of this experiment was to assess the propensity for the anastomotic materials under test to fulfil such a role. An *in vitro* technique was used to compare the degree with which the tumour cells were able to firmly adhere to the four sutures.

Aliquots of 10 million radiolabelled tumour cells, each suspended in 4 mls of RPMI1640 + 10% FCS medium, were placed in four centrifuge tubes, one corresponding to each of the suture materials under test. Each tube received three separate 10 cm lengths of the appropriate suture and the suspensions then incubated for 90 minutes at 37°C in the water bath with constant agitation. Viability of the Mtln3 suspensions was assessed by trypan blue exclusion immediately before and after incubation with the sutures and the percentage of viable cells was found not to change significantly over the 90 minute period. Following incubation, each length of suture was carefully removed with fine forceps and thoroughly washed in sterile, PBS in order to remove

all cells which were not firmly adherent to the suture. This manoeuvre was standardised by placing a beaker containing PBS on a constant speed magnetic stirrer to produce a moving stream of PBS in which each length of suture was held for 15 seconds at a standardised distance from the centre. Thereafter radioactivity of the suture lengths was measured in the gamma counter as before. The procedure was repeated four times in total and, as in the previous experiment, the radioactivity of standard labelled tumour cell suspensions (10^6 cells) was used to calculate the numbers of cells adhered.

15.4.3 Controls

The above two procedures were also carried out using a cell free solution of $Na^{51}CrO_4$ in culture medium instead of the radiolabelled tumour cell suspensions. The purpose of this exercise was to determine if any differences observed between the various suture materials reflected a difference in the number of tumour cells adhered or whether it simply represented the attachment of non-cell bound chromium to the suture. The source of this cell-free chromium solution was the supernatant obtained from the first centrifugation of the Mtln3 suspensions following radio-isotope labelling and this contained an excess of chromium 51.

15.5 In Vivo Tumour Growth Studies

15.5.1 The Development of an Animal Model of Implantation Metastasis

A series of pilot studies was performed in an attempt to develop an animal model of implantation metastasis which could subsequently be employed to assess the influence of the various anastomotic materials. The Mtln3 tumour cell line and male Fischer F344 rats were used throughout. These preliminary experiments were designed firstly to determine if the tumour would grow in the peritoneal cavity and in the colon, and to ascertain the optimum tumour cell inoculum and sacrifice time which would result in reliable but controlled tumour growth. Secondly, the aim was to investigate the conditions neccesary for free intra-luminal malignant cells to implant and result in colonic tumour growth.

All operative procedures were carried out using the animal caecum. This was partly because the upper pole of the caecum could be easily resected or have sutures implanted through it without risk of intestinal obstruction which was occasionally encountered when more distal large bowel sites were used. More importantly, however, the caecum was chosen for the ease with which it was brought out of the body cavity and isolated from other intra-peritoneal structures. This meant that suspensions of tumour cells could be instilled into the caecal lumen and the anastomosis constructed without risk of peritoneal contamination.

For all experiments the rats were fasted for 48 hours prior to surgery. Laparotomy was performed through a long mid-line abdominal incision, the caecum delivered through the wound and placed on a gauze

swab soaked in 0.9% saline. Further swabs were used to cover the abdominal incision and to shield the peritoneum and other viscera. Post-operatively, the abdomen was closed with continuous black silk suture in two layers and animals were allowed to resume food and water ad libitum.

Experiment 1: To determine the pattern of growth of Mtln3 cells at a colonic anastomosis

All residual faecal material within the caecum was manually milked into the distal colon and an atraumatic occlusion clamp was placed across the viscus to isolate the upper caecal pole. Suspensions of viable Mtln3 tumour cells in F10/DMEM + 10% FCS culture medium were injected intra-luminally into the upper caecal pole via a 24 gauge needle. Inoculums ranging from 10^3 Mtln3 cells to 10^6 cells were tested and the injection volume was maintained constant at 0.2 mls. Groups of 6 animals per dose of tumour cells were used on each occasion and the procedure was carried out 3 times in total.

The tip of the upper caecal pole was resected in such a manner that a 1 cm linear defect in the caecal wall was produced. This defect was then closed with 5 interrupted transmural 5/0 sutures, polyamide (nurolon) being used throughout. All knots were tied on the serosal surface. The occlusive bowel clamp was then removed and the serosal surface of the caecum thoroughly lavaged with 0.9% saline in an attempt to wash off any spillage of tumour cells prior to replacing the viscus within the peritoneal cavity.

Sacrifice time was initially chosen arbitrarily as the 21st post-operative day but this was later reduced to 18 days as many of the animals receiving the larger inoculums of tumour cells were markedly unwell by this time and were found to have extensive intra-peritoneal tumour at post-mortem. In arriving at a suitable sacrifice time, an attempt was made to estimate the extent and rate of tumour growth by injecting a second inoculum of the same dose of Mtln3 cells subcutaneously into the left shoulder pad of each rat simultaneous with abdominal procedure. However, it was immediately apparent that the tumour growth was much more rapid and aggressive within the peritoneal cavity than it was in the subcutaneous tissues and thus the growth of the subcutaneous tumour bore little reflection to the extent of intraperitoneal tumour.

Results:

In summary, this pilot experiment demonstrated that Mtln3 tumour cell line behaves in an extremely agressive manner within the peritoneal cavity. An inoculum of either 10^5 or 10^6 cells rapidly resulted in extensive spread of tumour from the caecum to the peritoneum in every animal and this was associated with blood stained ascites. Smaller inoculums (10^3 or 10^4 cells) produced more controlled tumour growth. The most ideal results were obtained using an inoculum of 10^3 cells. Animals receiving this dose of Mtln3 cells reliably developed a localised anastomotic tumour when sacrificed at 18 days. Accordingly, this inoculum was chosen for some of the later experimental work to be described.

Experiment 2: To determine the conditions neccesary for Mtln3 cells to implant and result in tumour growth

Having established that the Mtln3 tumour cell line was apparently able to readily implant and grow on exposed peritoneal surfaces and at a colonic anastomosis, the aim of the next series of pilot studies was to determine the exact pre-requisites necessary for this implantation to occur.

A total of 36 Fischer F344 rats were used. All underwent laparotomy in the manner previously described with the upper caecal pole being isolated by means of an occlusion clamp. As before suspensions of Mtln3 cells were injected intra-luminally into the isolated segment of caecum using a 24 gauge needle. Twelve rats received an inoculum of 10^6 cells, twelve received 10^5 cells and the remaining twelve received 10^4 cells. No part of the caecum was resected but for each tumour cell inoculum, six of the animals had 5 interrupted transmural sutures of 5/0 polyamide inserted into the tumour cell containing upper caecal pole. The remaining six animals in each group had no procedure performed other than the injection of tumour cells and therefore acted as controls.

Results:

Of the total of 18 control rats which received only the intra-luminal injection of Mtln3 cells, only one had macroscopic evidence of tumour growth when sacrificed 18 days post-operatively. This occurred in an animal which received an inoculum of 10^6 Mtln3 cells. One further rat in the 10^6 cell injection group

had microscopic tumour growth confirmed histologically but the remaining 16 animals were both macroscopically and microscopically free of tumour.

For the rats receiving transmural implantation of sutures, an inoculum of 10^6 intra-luminal Mtln3 cells resulted in a similar picture to that seen in the previous experiment with the larger tumour cell doses. All 6 rats had extensive intra-peritoneal tumour growth with the caecum encased in a solid tumour mass. An inoculum of 10^5 Mtln3 cells also produced macroscopic tumour growth in all 6 animals, in 4 cases there was extensive spread throughout the peritoneal cavity whilst in the remaining 2 rats the tumour growth was localised to the site of suture insertion in the caecal wall. Five of the 6 rats which received an intra-luminal injection of 10^4 Mtln3 cells prior to the insertion of the sutures through the caecal wall developed macroscopic, histologically proven, tumour well localised to the site of penetration of the caecal wall by the sutures. The remaining rat in this group showed evidence of microcopic tumour growth only.

Conclusions

The findings in these preliminary experiments firstly tend to support previous reports that free malignant cells will readily implant and grow on peritoneal surfaces. Dividing the bowel and allowing such cells to escape from the colonic lumen resulted in extensive tumour growth involving both the anastomosis and all exposed peritoneal surfaces. Secondly, it appeared from the results of the second experiment that the Mtln3 malignant cells were incapable of implanting and growing on normal, undamaged colonic mucosa but

whenever the mucosa was breached by the passage of a suture in the presence of Mtln3 cells, tumour growth tended to occur. It was, however, unclear from these preliminary observations as to whether this was the result of cells being dragged through the wall to the serosal surface by the passage of the suture material or whether it simply represented tumour cells implanting and growing on the exposed submucosa.

15.5.2 The Influence of Suture Type on the Growth of Implanted Tumour Cells at a Colonic Anastomosis

This experiment essentially comprised a repeat of the first pilot experiment described above. Suspensions of 10^3 Mtln3 carcinoma cells in 0.2 mls of F10/DMEM + 10% FCS medium were injected intra-luminally into the isolated upper caecal pole of anaesthetised 12 week old male Fischer F344 rats via a 24 gauge needle. The tip of the caecal pole, including the injection site, was resected to leave a 1cm longitudinal caecotomy which was immediately repaired with 5 interrupted transmural sutures of one of the four suture materials under test. Throughout the procedure, care was taken to avoid contamination of the peritoneum with tumour cells by isolating the caecum with saline soaked swabs and the repaired viscus was thoroughly lavaged prior to returning it to the peritoneal cavity. Post-operatively, the animals were maintained in cages of four in standardised conditions until sacrifice 18 days later. At that time a full autopsy was performed, the presence or absence of macroscopic

tumour recorded, and the caecum submitted for independent histological analysis. Eight rats were used for each of the four anastomotic materials, a total of 32 animals therefore being required.

15.5.3 The Influence of the Type of Suture Material Alone on Tumour Cell Implantation

A total of 32 fasted male F344 rats underwent laparotomy under deep ether anaesthesia. The upper caecal pole was isolated in the manner previously described and into the lumen of this, 10^4 viable Mtln3 cells in 0.2 mls of F10/DMEM/FCS medium were introduced via a 24 gauge needle. No part of the bowel was resected and no caecotomy was performed. In each animal, five interrupted transmural sutures of one of the four materials under test were inserted into the upper caecal pole, the bowel clamp across the caecum removed, the viscus washed with 0.9% saline and returned to the peritoneal cavity. Eight rats were used per suture material. As before, sacrifice was on the 18th post-operative day at which time the distribution of any macroscopic tumour was recorded and the caecum submitted for histological analysis.

The procedure was later repeated using a tumour cell inoculum of 10^3 cells. Twenty four animals, 4 per suture material were used on this occasion.

15.5.4 The Implantation of Mtln3 Cells at Intact Colonic Anastomoses

The aim of this experiment was to investigate if the Mtln3 carcinoma cells could implant and give rise to tumour growth at the site of a previously constructed and seemingly intact colonic anastomosis. A total of 30 F344 fasted rats were used for this experiment comprising 5 groups each of 6 animals. Four of these groups corresponded to the anastomotic materials under test whilst the fifth acted as a sham operated control. Laparotomy was performed on all rats under deep ether anaesthesia. In each case a plastic cannula was inserted transanally and advanced proximally such that the tip lay in the descending colon. The cannula used was a "butterfly" intravenous infusion device from which the needle had been removed (Abbott Ltd, Sligo, Republic of Ireland). The animals in 4 of the groups (24 rats) then had a 1 cm longitudinal colotomy fashioned along the anti-mesenteric wall of the distal descending colon at a point distal to the tip of the cannula. The colotomy was then repaired with 5 interrupted full-thickness sutures of the anastomotic material appropriate to the group. The remaining 6 animals (control group) underwent sham laparotomy only, no colotomy being made.

A small volume of 0.9% saline was injected through the cannula to assess the integrity of the anastomosis and further sutures were inserted if necessary. Once a water-tight anastomosis had been achieved, a suspension of 10^4 viable Mtln3 cells in 0.2 mls of F10/DMEM/FCS culture medium was injected through the cannula under low pressure. The cannula was then removed and the abdominal incision was closed with 2 layers of continuous silk suture.

Sacrifice was on the the 18th post-operative day at which time the distribution of macroscopic tumour was assessed and the distal colon submitted for histological examination.

15.5.5 The Adherence of Tumour Cells To Sutures In Vivo

This experiment was carried out in conjunction with the experimental *in vitro* adhesion assay described in the previous section. Each of the 4 types of suture material were incubated for 90 minutes in a suspension consisting of 10^7 viable Mtln3 carcinoma cells in 4ml of culture medium. After this incubation period, the sutures were washed in the manner previously described to remove any non-adherent tumour cells. Five full-thickness interrupted sutures of one type of suture were then inserted into the upper caecal pole of anaesthetised F344 rats and all knots tied loosely on the serosal surface. Eight rats were used per suture material, a total of 32 rats therefore being required.

Sacrifice in this experiment was planned for day 21 in view of the smaller size of the tumour cell inoculums likely to be involved. The experiment was later repeated using a further 24 rats but on this latter occasion groups were only sacrificed when the animals were visibly unwell of when two or more members of the group had died.

Chapter 16

Experimental Results: Anastomotic Suture Materials and Implantation Metastasis

16.1 Introduction

This Chapter describes the results of the studies which were designed with the aim of investigating the potential role of anastomotic suture materials in implantation metastasis. Section 16.2 deals with the experimental studies where the aim was to quantify the adherence of tumour cells to the anastomotic materials under test. The results of the *in vivo* tumour growth studies are described in section 16.3.

16.2 Experimental Studies

16.2.1 The "In Vivo" Entrapment and Transfer of Tumour Cells by Sutures

The results of this experiment can be described with reference to table 16.1(a) and Appendix 5.

The radioactivity levels of the labelled aliquots of one million cells were found to differ widely on the 4 occasions the assay was repeated, as is illustrated in Appendix 5. This was thought to be a function of the batch of Mtln3 cells used on each occasion and was possibly related to the number of times the cells had been passaged prior to their experimental use. The number of tumour cells transferred by each individual suture type also varied considerably.

Again it was thought that variations in the age of the tumour cells and the number of times they had been passaged in culture might have affected their adherence characteristics.

The cumulative results of this experiment are illustrated in table 16.1(a). The results of the statistical comparisons using the Mann-Whitney U Test and both 95% confidence intervals and probability values are listed in table 16.1(b). It is evident that the two braided suture materials, and in particular polyglycolic acid, entrapped and transferred significantly greater numbers of free tumour cells from the caecal lumen than did either monofilament stainless steel or monofilament polypropylene ($p < 0.001$). However, there were also differences between the two braided materials and between the two monofilaments. Polyglycolic acid suture was associated with a significantly greater number of transferred Mtln3 cells than was polyamide ($p < 0.0001$). Similarly, the number of cells transferred by polypropylene was significantly greater than the number transferred by steel ($p < 0.05$).

16.2.2 The In Vitro Adherence of Tumour Cells To Sutures

As for the previous study, the full experimental data are listed in Appendix 5. Compared with the previous experiment, a similar degree of variation was evident with respect to both the labelling of the Mtln3 cells and the calculated number of tumour cells adhering to the suture materials.

The summarised results and statistical analyses are illustrated in tables 16.2(a) and 16.2(b) respectively. Significant differences were again observed between the braided and monofilament materials

($p<0.001$; Kruskal-Wallis Test). The Mtln3 tumour cells adhered in significantly greater numbers to polyamide and polyglycolic acid sutures than they did to either stainless steel or polypropylene ($p < 0.001$; Mann-Whitney U Test). However, in this experiment polyglycolic acid and polyamide could not be separated statistically nor could steel and polypropylene.

Table 16.1(a)

Number of Mtln3 Carcinoma Cells Transferred "In Vivo"

Suture Material	Median no. Cells Transferred	Range
1. polyamide	4226	1348 - 15175
2. polyglycolic acid	13987	4671 - 30616
3. stainless steel	545	141 - 3371
4. polypropylene	1197	59 - 3434

Table 16.1(b)

Mann-Whitney Confidence Intervals and Tests

Comparison	95% Confidence Interval	p value
1 vs 2	5659 - 13305	< 0.001
1 vs 3	2519 - 4560	< 0.001
1 vs 4	1900 - 4053	< 0.001
2 vs 3	9924 - 17661	< 0.001
2 vs 4	9331 - 17088	< 0.001
3 vs 4	45 - 1011	0.035

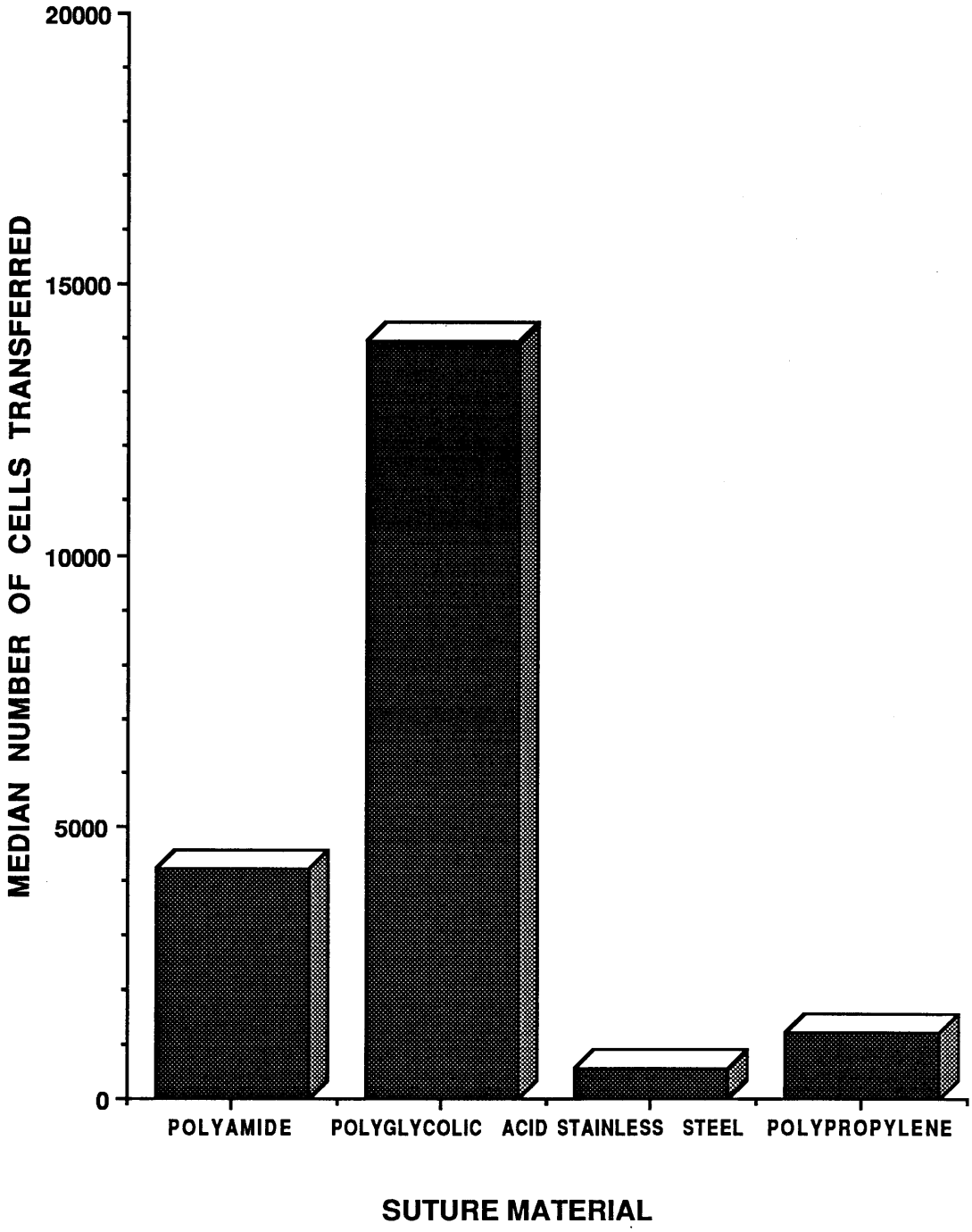


Figure 16.1

"IN VIVO" TUMOUR CELL ENTRAPMENT AND TRANSFER BY SUTURES: RESULTS

Table 16.2(a)

Number of Adherent Mtln3 Carcinoma Cells In Vitro

Suture Material	Median no. Adherent Cells	Range
1. polyamide	2911	1003 - 10125
2. polyglycolic acid	7082	2342 - 27095
3. stainless steel	282	172 - 861
4. polypropylene	155	56 - 662

Table 16.2(b)

Mann-Whitney Confidence Intervals and Tests

Comparison	95% Confidence Interval	p value
1 vs 2	265 - 11719	0.046 *
1 vs 3	1433 - 7172	< 0.001
1 vs 4	1544 - 7302	< 0.001
2 vs 3	3691 - 14410	< 0.001
2 vs 4	3646 - 14540	< 0.001
3 vs 4	-27 - 221	0.069 *

* values not considered significant

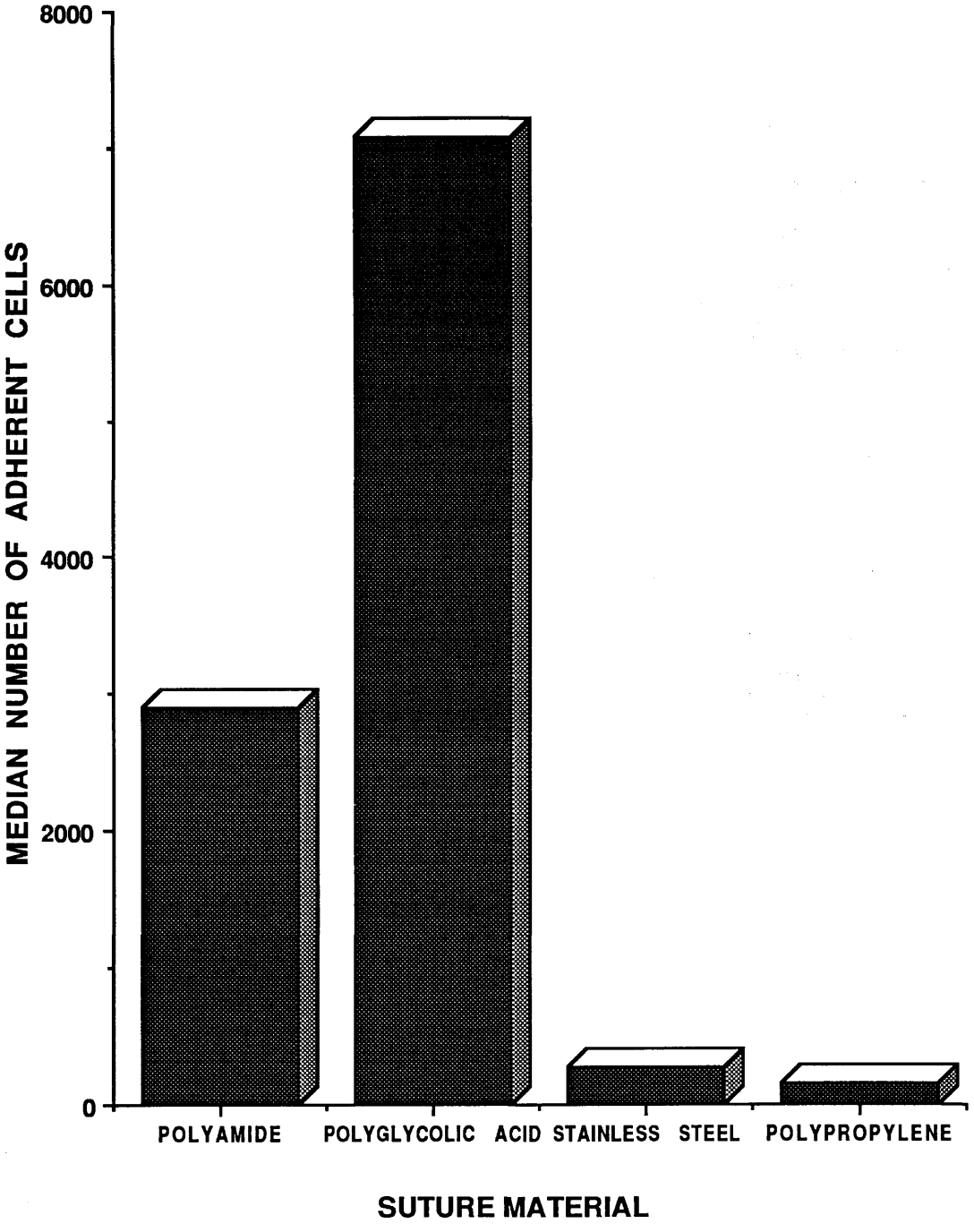


Figure 16.2

IN VITRO TUMOUR CELL ADHERENCE TO SUTURES

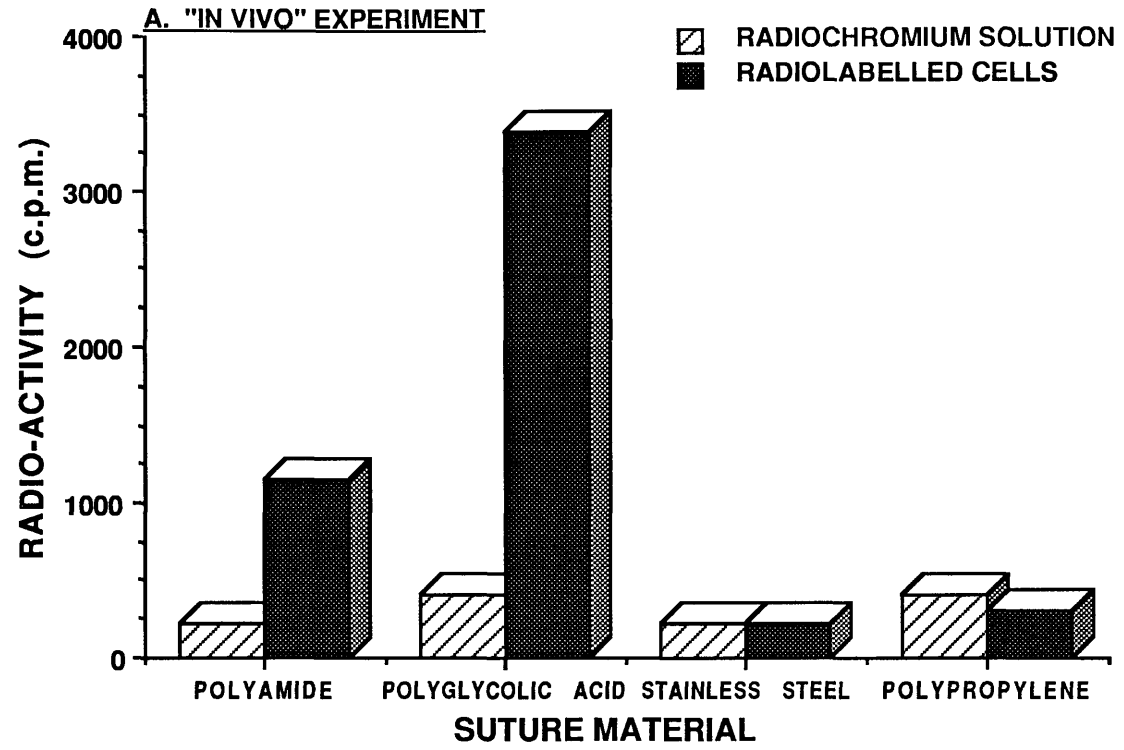
16.2.3 Controls: Results Using Free ^{51}Cr

There were no significant differences between the various suture materials with respect to the measured radioactivity levels when either of the experiments were performed using a solution of free radiochromium in culture medium. The results are expressed in table 16.3 as medians and ranges and illustrated graphically in figure 16.3. For comparison, the radioactivity of the sutures recorded when the experiments were carried out using the labelled cell suspension are also shown in figure 16.3.

Table 16.3

Radioactivity levels Using Solution of Free ^{51}Cr

Suture Material	Counts/min "in vivo" study	Counts/min in vitro study
polyamide	223 (171-365)	197 (136-504)
polyglycolic acid	403 (179-490)	284 (131-739)
stainless steel	220 (130-452)	159 (114-337)
polypropylene	399 (155-698)	144 (97-243)



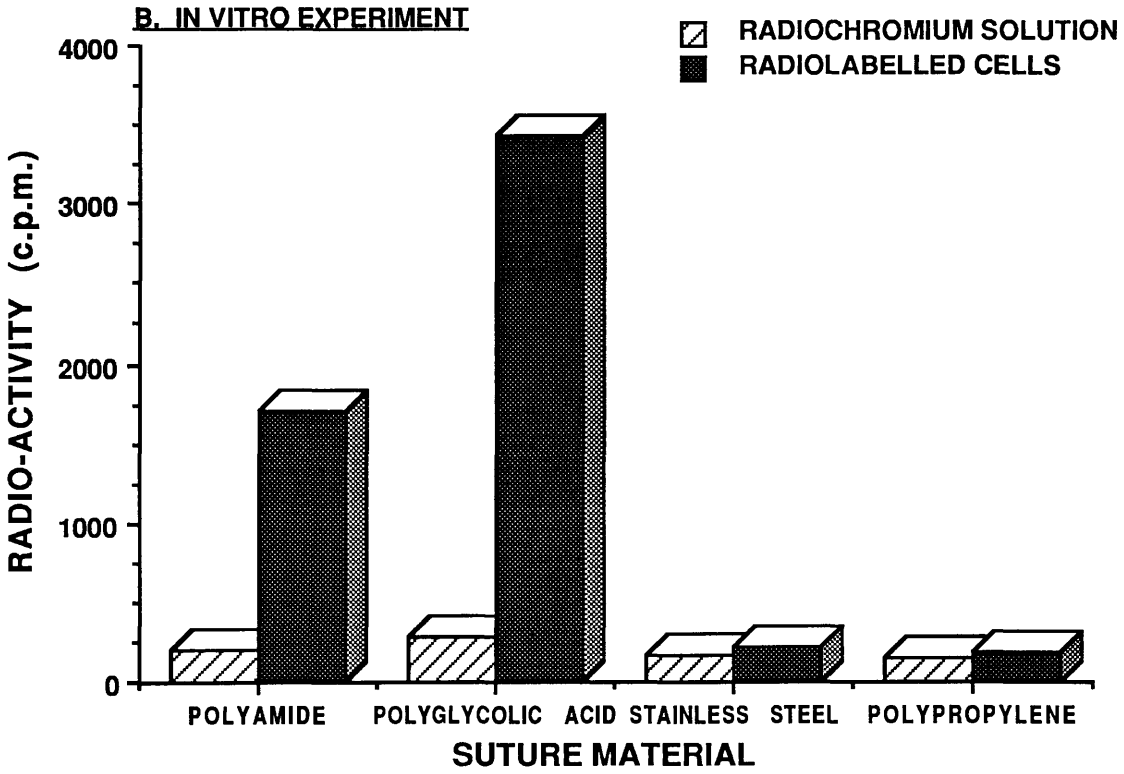


Figure 16.3 CONTROLS: RESULTS USING FREE RADIOCHROMIUM SOLUTION

16.3 In Vivo Tumour Growth Studies

This section comprises the results of the animal studies performed using Fischer F344 rats and the Mtln3 adenocarcinoma cell line. The autopsy findings of each individual animal are detailed in Appendix 6.

16.3.1 The Influence of the Type of Suture Material on the Growth of Implanted Tumour at a Colonic Anastomosis

These results are concerned with the groups of animals which had Mtln3 tumour cells injected into the caecal lumen, the upper caecal pole resected and the intestinal wound closed with interrupted sutures.

Of the thirty two animals entered into this experiment, 29 survived until sacrifice on day 18. One animal died immediately post-operatively, presumably from an overdose of ether. The second animal was found dead on the third post-operative morning. Post-mortem revealed that the cause of death had been intra-peritoneal bleeding. The third animal appeared extremely pale and listless on the 3rd post-operative day and had marked abdominal distension. The animal was sacrificed at this time and was found to have marked intra-peritoneal bleeding from the caecal anastomosis.

All 29 surviving animals had evidence of macroscopic tumour growth at sacrifice. Histology confirmed that these macroscopic lesions were all invasive adenocarcinomas with marked cystic degeneration. The tumours varied in size from small nodular lesions localised to the suture line to extensive tumour masses invading

adjacent viscera. However, there did not appear to be any correlation between the extent of tumour growth and the type of suture material. Ten animals (3 polyamide, 3 polyglycolic acid, 2 steel, 2 polypropylene) had disseminated intra-peritoneal tumour deposits with blood stained ascitic fluid.

16.3.2 The Influence of Suture Type on the Implantation of Tumour Cells

This section describes the results of the experiments where tumour cells were injected into the caecal lumen and transmural sutures implanted through the caecal wall without an intestinal wound being made.

All 32 animals receiving an inoculum of 10^4 Mtln3 cells survived until sacrifice on day 18. All had gross evidence of tumour growth involving the upper caecal pole and histology confirmed that this was adenocarcinoma. Sixteen of the animals had multiple peritoneal tumour seedlings. The type of suture material did not appear to have any influence on either the size of the primary tumour or the number of animals developing peritoneal deposits.

A similar result was obtained when the experiment was repeated using an inoculum of 10^3 tumour cells. On this occasion 23 of the total of 24 animals survived to sacrifice. The one death was a rat in the polyamide group which died intra-operatively from an overdose of ether.

At post-mortem all 5 of the surviving polyamide rats had macroscopic tumour growth as compared with all 6 of the polyglycolic acid group, 4 of the 6 steel animals, and 3 of the six polypropylene

Figure 16.4 Early Caecal Tumours Surrounding Implanted Polyamide Sutures

animals. Of the 5 macroscopically normal animals, microscopy revealed that 3 had small foci of tumour growth in relation to the sutures (1 steel, 1 polypropylene). Only 1 steel animal and 1 polypropylene animal were therefore totally tumour free.

16.3.3 The Implantation of Mtln3 Tumour Cells at Intact Colonic Anastomoses

All 30 animals used in this experiment survived laparotomy and remained alive until sacrifice on the 18th post-operative day.

None of the six animals in the control group, where the tumour cells were injected intra-luminally but no intestinal wound was made, had either macroscopic or microscopic evidence of tumour growth. In marked contrast, all of the animals which had re-suture of a colotomy performed before intra-luminal injection of tumour cells, developed large bowel tumours, irrespective of the type of suture material which had been used to repair the colotomy. For all suture groups, a frequent finding was extensive tumour dissemination throughout the peritoneal cavity.

16.3.4 The Transplantation of Tumour Cells by Suture Materials

This experiment was concerned with an assessment of the ability of the suture materials to transplant tumour cells into the bowel of syngeneic animals in sufficient numbers to result in tumour growth. All 32 animals used for the initial phase of the experiment survived until the time of sacrifice on the 21st post-operative day.

Nineteen animals had macroscopic evidence of tumour growth at autopsy and in all cases this was confirmed microscopically. No histological abnormalities were seen in the 13 macroscopically normal animals apart from a variable acute inflammatory infiltrate surrounding the sutures. The distribution of the animals developing tumours by suture material is illustrated in table 16.4

These findings therefore tend to support the results of the second experimental study where it was demonstrated that the Mtln3 tumour cells adhered in significantly greater numbers to the braided sutures than they did to the monofilament sutures.

Table 16.4

Mtln3 Cell Transfer by Sutures: Animals Developing Caecal Tumours

Suture Material	No. of Animals	No. with caecal tumours
polyamide	8	7
polyglycolic acid	8	8
stainless steel	8	3
polypropylene	8	1

polyamide v polypropylene	$p < 0.01$
polyamide v stainless steel	N.S.
polyglycolic acid v stainless steel	$p < 0.05$
polyglycolic acid v polypropylene	$p < 0.001$

Fisher's Exact Test

However, the highly malignant nature of the Mtln3 tumour cell line was emphasised when the experiment was repeated with a further 24 animals, 6 per suture material. The only change in the procedure was that the animals were not sacrificed until they were visibly unwell or when two or more members of a group had died. At sacrifice only 2 animals were found to be entirely tumour free, one of which was in the steel group and the other in the polypropylene group. The remaining 22 animals all had macroscopic evidence of intra-abdominal tumour growth, although the length of time from surgery until the animals became visibly unwell appeared to correlate with the type of suture material. Two of the polyglycolic acid animals died on day 26 and the group was therefore sacrificed on that day. The polyamide animals were also visibly unwell by this time and were sacrificed. The steel group was sacrificed on day 33 when 2 animals were cachectic whereas the polypropylene group did not reach this stage until day 36.

Chapter 17

Discussion

17.1 Introduction

Current evidence suggests that viable exfoliated malignant cells may be present in the operative field during colorectal cancer surgery (327). The surgical implantation of these cells may therefore be responsible for local recurrence following apparently curative resection, a process frequently termed implantation metastasis. Perhaps the most convincing clinical evidence in support of this mechanism is the reported reduction in the incidence of local recurrence following the introduction of measures designed to kill or limit the spread of these free intra-luminal tumour cells (330,331).

Experimental work has suggested that suture materials used to construct the anastomosis may have a role to play in this mechanism of implantation metastasis. As outlined in Chapter 14, the implantation of tumour cells by sutures has been described in animal models (341,342) and it has been demonstrated that the use of iodised sutures effectively decreases the incidence of tumour development (343).

The experimental and in vivo tumour growth studies carried out for this thesis were designed to investigate the differential adherence of carcinoma cells to various suture materials, and the potential influence of these materials on implantation metastasis.

17.2 Experimental Studies

a) The "In Vivo" Tumour Cell Entrapment and Transfer by Sutures

This first experimental study demonstrated the ability of all 4 suture materials to entrap free intra-luminal Mtln3 carcinoma cells and to transfer these cells from the caecal lumen. This implies that

all 4 materials could implant viable tumour cells into the colonic wall or onto the serosal surface of the bowel. The number of tumour cells associated with each type of suture material was seen to vary considerably each time the experiment was repeated but the pattern was consistent. The two braided materials, and in particular polyglycolic acid, transferred significantly greater numbers of intra-luminal Mtln3 cells than did either monofilament stainless steel or monofilament polypropylene ($p < 0.001$). It is possible that these findings may be partially explained by the difference in surface area between the braided and monofilament materials. However, the significant differences between polyamide and polyglycolic acid and between steel and polypropylene with respect to the numbers of Mtln3 cells entrapped and transferred would suggest that that the composition of the suture material is important in addition to its physical characteristics. Clearly further assessment of a variety of difference anastomotic materials is required and in such future work it would be useful to compare braided and monofilament versions of the same material to determine the respective roles of the surface area versus the composition of a suture.

b) <u>In Vitro Tumour Cell Adhesion to Sutures</u>

The aim of this experiment was to assess the propensity of various suture materials to act as a nidus for Mtln3 carcinoma cells. Washing the sutures in phosphate buffered saline following their 90 minute incubation with the radiolabelled Mtln3 cell suspension was designed to remove any tumour cells which were not firmly adherent to

the sutures. The persistent viablity of the Mtln3 cells following the 90 minute incubation period was later confirmed by the in vivo tumour growth studies.

As for the preceding experiment, considerable variability was noted on the four occasions the assay was repeated. Nevertheless, the pattern of cellular adhesion to the 4 types of suture material was consistent. The tumour cells adhered in significantly greater numbers to the two braided materials than they did to the monofilaments ($p < 0.001$). In this experiment, however, no distinction could be made between polyamide and polyglycolic acid nor between stainless steel and polypropylene. This might imply that the differences recorded reflected the larger surface area of the braided compared with the monofilament materials rather than any influence of the composition of the suture material. Further experimentation using a variety of different materials is merited. Ideally braided and monofilament versions of the same material are required to assess the relative importance of the surface area of the suture.

This study has close similarities to work previously reported by O'Dwyer and his colleagues (344). They quantified the in vitro adhesion of two tumour cell lines (W 163, a hydrazine induced colon cancer, and SPK, a spontaneously occurring renal cell carcinoma, both in the Wistar/Furth rat) to five types of suture material. Three centimetre lengths of 5/0 gauge sutures were used throughout. Adherence of both the cell lines was consistently greatest to the braided materials silk and polyglactin 910 ("Vicryl", Ethicon). Significant differences, however, were observed between the 3 monofilament materials tested (polypropylene, chromic catgut, and

nylon). Significantly fewer cells adhered to polypropylene than to either chromic catgut or nylon implying that the composition of a suture material did have a significant influence on cellular adhesion.

17.3 In Vivo Tumour Growth Studies

The animal studies illustrated the highly malignant nature of the Mtln3 tumour cell line used for these experiments. Even relatively small tumour cell inoculums led to rapid dissemination of tumour throughout the peritoneal cavity and this made all the experiments difficult to control. As a result, few valid conclusions can be drawn.

The experiments did confirm previous reports that tumour cells appear incapable of implanting and growing on intact colonic mucosa (337). When the Mtln3 cells were simply injected into the colonic lumen without any mucosal injury taking place, tumour growth very rarely occurred. However, whenever the mucosa was breached, either by the creation of an anastomosis or by the simple passage of a suture, tumour growth was consistently observed. In these experiments there was no apparent influence on tumour growth attributable to the type of suture material. For the first experiment in particular, this is perhaps not surprising as the tumour cell suspensions were injected into the caecal lumen, a caecotomy fashioned, and the tumour cells then allowed to come into direct contact with the divided bowel wall and exposed serosal surface, both fertile soils for tumour cell implantation.

The simple transmural passage of a suture through the tumour cell filled caecum was designed to reflect the procedure in the first experimental study where the entrapment and transfer of free intra-luminal tumour cells by sutures was quantified. Despite the differences between the various suture materials recorded in the experimental study, the type of suture material had no apparent influence on in vivo tumour growth. There may be several explanations for this. Firstly, as previously discussed, the Mtln3 tumour behaves in an extremely aggressive and highly malignant manner in the bowel. As a result, even the small number of cells entrapped by the monofilament sutures during their passage through the caecal lumen may be sufficient to give rise to tumour growth. The rapid growth and short doubling time of the tumour would then make differences between the various materials difficult to discern. Secondly, entrapment of cells by the sutures may not be important. The penetration of the mucosa by the suture may simply expose the underlying submucosa on which the Mtln3 cells are able to implant and proliferate. Such mucosal damage is likely to be a simple function of the needle used rather than the type of suture material. Alternatively, it is possible that intra-luminal tumour cells can filter along the suture tract into the colonic wall where again the influencing factor would the simple presence of a suture rather than its composition.

The third experiment was designed to investigate the implantation of intra-luminal malignant cells at intact colonic anastomoses. The results suggest that the Mtln3 cells were able to establish a growth advantage at apparently water tight anastomoses and give rise to tumour involving all surfaces, including the peritoneal aspect of the bowel. Similar findings in this respect have also recently been

reported by O'Dwyer (347). Tumour growth in this experiment also appeared to be independent of the type of suture material used to repair the intestinal wound. The exact mechanism responsible for these findings has not been established by the current work. The control animals again demonstrated that the Mtln3 cells were incapable of implanting and giving rise to tumour growth on intact colonic mucosa. It is inevitable, however, that there will be damaged mucosa and possibly exposed submucosa on the luminal aspect of a colonic anastomosis on which the tumour cells could implant. Proliferation of the tumour cells might then result in macroscopic tumour breaking through the line of the anastomosis to reach the serosal surface. Alternatively, it is possible that the tumour cells are capable of seeping along suture tracts or directly through the apparently intact anastomosis into the substance of the colonic wall and onto the serosal surface.

The final *in vivo* tumour growth experiment was designed in association with the *in vitro* study of tumour cell adhesion to the 4 types of suture material. This experiment served to illustrate the persistent viability of the Mtln3 cells following 90 minutes incubation with the lengths of suture material. Furthermore, the differences seen in tumour growth correlated precisely with the rank order in which the *in vitro* study placed the adhesion of tumour cells to the 4 types of suture material. However, the observation that macroscopic tumour would eventually become evident in almost all animals again illustrated the highly malignant nature of the Mtln3 tumour cell line in that even the very small inoculums associated with the monofilament materials were sufficient to lead to tumour growth.

17.4 Summary

In summary, the experimental studies have demonstrated that suture materials vary in their ability to entrap free intra-luminal cancer cells. Similarly, it was demonstrated that tumour cells will differentially adhere to various sutures and this was confirmed by *in vivo* tumour growth studies. It may therefore be concluded that the type of anastomotic suture material may have some bearing on the risk of implantation metastasis after apparently curative surgery for large bowel cancer.

The animal model of implantation metastasis proved to be a little disappointing, partly because of the aggressive nature of the Mtln3 adenocarcinoma cell line and partly owing to the flimsy nature of the rodent colonic wall which meant that whenever it was breached by the passage of a suture the tumour cell suspension tended to leak through to the serosal surface. As a result, no differences were observed between the various suture materials with respect to the number of animals developing tumours or the extent of tumour growth, although the model was able to confirm previously reported findings as regards the necessary conditions for tumour cell implantation.

Further work is required to clarify the mechanisms of tumour cell implantation. In particular, it remains unclear as to whether the intra-luminal tumour cells are entrapped by the sutures and dragged into the colonic wall, whether they filter along the suture tract, or whether they simply implant on the damaged mucosa and submucosa at the site of suture penetration.

A less aggressive colonic adenocarcinoma cell line is currently being obtained for these further experiments. It is also planned to repeat the experimental studies with this second tumour cell line as

it is possible that different cells will vary in their adherence characteristics.

PART IV

Chapter 18

Summary and Conclusions

This Chapter comprises a series of statements which summarise the main points highlighted in this thesis. The basis for these has been derived both from the literature, which has been reviewed, and from the results of the original research carried out for this thesis.

1. Dehiscence of a gastro-intestinal anastomosis is an important post-operative complication with potentially disastrous consequences for the patient.

2. Throughout history, numerous techniques have been devised in an attempt to achieve reliable intestinal wound healing.

3. Traditional surgical teaching has advocated a manual suturing anastomotic technique, with emphasis on the strength of the submucosal layer and on serosa-to-serosa apposition.

4. Recent years have witnessed the emergence of surgical stapling as an alternative to conventional suturing methods.

5. The use of such stapling techniques is increasing, particularly in the field of colorectal surgery where circular stapling may facilitate low anterior resection.

6. Numerous advantages of the stapling technique have been claimed, both commercially and by sections of the surgical community, but most of these claims have been scientifically unfounded.

7. Although the safety of stapled anastomoses has been confirmed by numerous retrospective reviews, few attempts have been made to critically compare manual suturing and surgical stapling techniques.

8. A multicentre prospective randomised controlled trial was designed to address the controversy surrounding the clinical implications of surgical stapling. This study forms the largest such series in the Western World.

9. The results of this study have demonstrated that surgical stapling requires close attention to detail during anastomotic construction to minimise the risk of operator induced errors.

10. Stapling was associated with a significant reduction in anastomosis time and in total operating time when compared with manual suturing methods.

11. The overall incidence of clinically apparent anastomotic dehiscence was similar in the sutured and stapled groups. However, the distribution of these anastomotic leaks throughout the gastro-intestinal tract differed in the two groups.

12. In upper gastro-intestinal surgery, stapling was associated with a high incidence of leakage from duodenal stump closure (13.9%). The reasons for this are not entirely clear but all of these failures occurred early in the course of the study.

13. In colonic and colorectal surgery, stapled anastomoses clinically dehisced less frequently than did sutured anastomoses ($p = 0.09$).

14. For distal colonic and colorectal anastomoses, stapling was associated with a significantly lower incidence of asymptomatic radiological leaks compared with manual suturing.

15. The frequent elective use of the circular stapler in colorectal surgery has suggested that stapling may, for many surgeons, extend the range of low anterior resection to encompass lower rectal lesions and thus reduce the requirement for combined abdomino-perineal resection.

16. Staple line haemorrhage is an appreciable risk with gastric anastomoses and care must be taken to inspect the staple line and under-run any bleeding points at the time of surgery.

17. The incidence of infective and other post-operative complications was similar with suturing and stapling techniques.

18. There was no apparent excessive incidence of local tumour recurrence or of symptomatic anastomotic strictures in either group.

19. Staples are considerably more expensive than surgical sutures. The true cost of surgical care is difficult to estimate but the cost of anastomotic materials is likely to amount to only a small fraction of the total expense of patient care.

20. Colorectal cancer is a common disease. At the present time it is the second most frequent cause of cancer related death in the Western World and the prognosis following "curative" surgery has not changed significantly in recent years.

21. The incidence of local tumour recurrence is a major factor limiting improvements in long term survival.

22. There has recently been some concern regarding a possible association between stapled colorectal anastomoses and an increased incidence of local recurrence. This could reflect anatomical or tumour related factors or it may represent some unknown influence of the anastomotic technique.

23. Local recurrence may result from the implantation of viable exfoliated malignant cells at the time of surgery (implantation metastasis) or it may represent the development of a second primary (metachronous) carcinoma. There is potential for both these suggested mechanisms to be influenced by the type of anastomotic suture material.

24. Previous investigators have demonstrated that hydrazine induced rodent colorectal carcinogenesis forms an acceptable model of the human disease to test experimental hypotheses under controlled laboratory conditions.

25. Using such a model, an experiment was designed to assess the possible promoting or protecting influences of three commonly used anastomotic suture materials, including stainless steel as a model of surgical stapling, on large bowel tumour induction.

26. When sutures were implanted into the distal descending colon of albino Swiss rats in the post-initiation phase of tumour induction, the type of suture material had a significant influence on local tumour development. There were no differences between the sham operated group and the animals receiving steel sutures but compared with both of these, polyamide and polyglycolic acid sutures were associated with a significant promotion of carcinogenesis.

27. The addition of an intestinal wound (a colotomy) had no additional promoting influence on carcinogenesis, the incidence of tumours being the same as for the groups having simple implantation of sutures.

28. The type of anastomotic suture material had no influence on the incidence of large bowel tumours distant from the "peri-anastomotic" area.

29. The mechanism responsible for this differential promotion of carcinogenesis in relation to implanted suture material is unclear. Crypt cell production rates were similar in all suture material and sham operated groups.

30. These results are the opposite to a similar experiment reported recently. There were, however, important differences in the timing of the surgical injury (promotion) in relation to carcinogen administration (initiation) such that the two studies cannot be directly compared.

31. Experimental studies were designed to assess the potential role of various suture materials in implantation metastasis.

32. Braided sutures (polyamide and polyglycolic acid) were able to entrap and transfer greater numbers of free intra-luminal tumour cells compared with the monofilament materials (stainless steel and polypropylene) tested. There were, however, differences between the individual braided and monofilament materials suggesting that the composition of a suture material is important in addition to its physical characteristics.

33. Braided sutures have a greater propensity to act as a nidus for implanted tumour cells in that the cells adhered in significantly greater quantities to them than they did to the monofilament materials.

34. An animal model of implantation metastasis was developed but this proved to be of limited use in the investigation of the role of suture materials.

35. The previously reported observation that tumour cells will not implant on intact colonic mucosa was confirmed.

36. All types of suture material tested were able to adhere sufficient numbers of tumour cells to result in tumour growth when implanted into syngeneic animals. The rapidity of tumour growth appeared to correlate with the degree of adherence to the sutures calculated in the in vitro experimental study (polyamide and polyglycolic acid > steel and polypropylene)

Future Work

1. The clinical project is ongoing with the aim of recruiting a total in excess of 1000 randomised patients.

2. A prospective follow-up study is currently in progress to accurately assess the incidence of local recurrence of colorectal cancer in the sutured and stapled groups. It is important to determine if the findings of the above animal and experimental studies have implications for clinical practice.

3. Carcinogenesis is a complex multistage process involving initiation and promotion. Knowledge regarding the timing of surgical injury and the association between cell kinetics and other promoting or protecting influences is incomplete and requires further investigation.

4. The studies relevant to implantation metastasis require to be repeated with at least one other tumour cell line as different cells may vary in their adherence characteristics.

5. Investigation with a wider range of suture materials is also required and it would be particularly important to test braided and monofilament versions of the same material to determine the relative roles of the physical characteristics and the composition of the suture.

6. In many respects, this thesis has provided an overview of the clinical aspects of surgical stapling, the potential for anastomotic materials to influence experimental colorectal carcinogenesis, and the variable adherence of tumour cells to different materials. It has demonstrated the scope for further investigation to clarify all three areas.

CLINICAL AND EXPERIMENTAL STUDIES OF GASTRO-INTESTINAL ANASTOMOTIC TECHNIQUES

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APPENDIX 1

Experimental Colorectal Carcinogenesis:

Total Animal Body Weight

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	236.8 ± 7.5	231.7 ± 6.8	249.5 ± 11.6
2	241.0 ± 7.5	232.9 ± 5.3	257.5 ± 11.2
3	242.4 ± 7.5	234.8 ± 5.6	261.6 ± 11.3
4	250.6 ± 6.6	245.8 ± 4.3	267.3 ± 9.7
5	251.9 ± 6.3	235.6 ± 7.8	268.4 ± 9.0
6	252.2 ± 5.8	249.9 ± 3.2	268.9 ± 9.2
7	256.7 ± 6.7	252.2 ± 3.7	269.7 ± 9.2
8	258.0 ± 7.4	254.7 ± 4.3	268.6 ± 10.7
9	267.3 ± 7.4	264.3 ± 4.0	280.2 ± 8.6
10	268.2 ± 7.3	263.9 ± 4.0	276.4 ± 8.0
11	276.9 ± 7.9	276.2 ± 4.3	285.9 ± 8.4
12	275.9 ± 8.7	274.8 ± 4.9	287.4 ± 10.1
13	276.6 ± 8.4	275.7 ± 5.6	292.3 ± 9.3
14	285.8 ± 8.2	284.2 ± 5.6	298.9 ± 9.5
15	292.0 ± 8.8	288.9 ± 6.0	303.1 ± 10.2
16	293.4 ± 9.1	290.2 ± 6.3	304.1 ± 10.5
17	285.0 ± 7.7	282.4 ± 4.6	295.3 ± 8.2
18	280.3 ± 7.6	274.2 ± 4.7	289.9 ± 9.2
19	286.6 ± 8.3	281.2 ± 5.5	295.4 ± 10.0
20	286.9 ± 8.6	279.5 ± 7.8	295.3 ± 10.2

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	221.0 ± 5.2	214.0 ± 6.8	235.6 ± 5.8
2	229.3 ± 6.1	222.1 ± 5.4	241.5 ± 5.8
3	237.9 ± 8.9	226.2 ± 4.2	249.3 ± 6.1
4	240.5 ± 8.2	231.1 ± 4.7	246.1 ± 6.1
5	241.5 ± 7.7	235.6 ± 4.6	253.7 ± 6.2
6	242.2 ± 5.8	241.0 ± 4.7	258.3 ± 6.2
7	244.5 ± 7.0	245.1 ± 4.0	266.1 ± 6.3
8	251.4 ± 7.5	256.0 ± 4.3	267.1 ± 5.8
9	258.2 ± 7.3	262.5 ± 4.4	269.3 ± 5.7
10	264.2 ± 8.0	271.5 ± 5.1	270.8 ± 5.6
11	265.0 ± 8.0	274.7 ± 5.2	273.6 ± 5.3
12	264.0 ± 8.1	276.9 ± 5.5	279.2 ± 5.3
13	268.0 ± 8.3	281.6 ± 6.1	280.9 ± 5.0
14	275.2 ± 8.2	288.8 ± 5.8	287.2 ± 5.2
15	281.5 ± 8.8	296.6 ± 5.4	292.9 ± 5.1
16	282.5 ± 8.2	297.9 ± 5.2	296.2 ± 5.1
17	279.7 ± 8.1	295.2 ± 5.6	292.2 ± 5.5
18	266.7 ± 8.0	281.3 ± 5.0	277.1 ± 5.8
19	270.8 ± 8.9	288.7 ± 4.7	281.6 ± 5.3
20	285.1 ± 7.7	298.8 ± 5.4	290.8 ± 5.6
21	290.8 ± 8.0	301.1 ± 5.6	295.0 ± 6.5
22	294.5 ± 8.3	305.3 ± 6.2	300.4 ± 6.2
23	301.0 ± 8.6	309.5 ± 6.6	301.7 ± 6.6
24	299.7 ± 8.6	310.5 ± 7.1	311.0 ± 5.3
25	298.8 ± 9.1	311.0 ± 7.3	309.7 ± 6.3
26	297.0 ± 9.0	307.1 ± 8.7	308.3 ± 6.3
27	298.9 ± 8.5	316.3 ± 8.3	305.9 ± 6.5
28	297.4 ± 9.3	314.7 ± 9.4	307.6 ± 7.0

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	243.3 ± 4.6	263.7 ± 3.0	251.9 ± 4.7
2	243.3 ± 5.4	262.0 ± 5.5	252.3 ± 6.0
3	242.8 ± 6.2	263.4 ± 5.4	253.6 ± 7.9
4	248.1 ± 6.5	275.5 ± 9.4	260.3 ± 7.6
5	249.4 ± 6.2	268.8 ± 5.2	262.4 ± 7.1
6	253.0 ± 6.1	271.0 ± 5.1	267.3 ± 6.8
7	256.0 ± 6.4	273.4 ± 5.4	271.0 ± 6.9
8	263.0 ± 6.9	273.2 ± 5.3	278.8 ± 6.4
9	264.0 ± 6.8	277.8 ± 5.9	279.8 ± 6.6
10	266.3 ± 7.4	282.3 ± 6.5	281.7 ± 6.6
11	265.6 ± 7.3	287.5 ± 6.7	280.7 ± 6.5
12	270.1 ± 8.9	286.6 ± 6.8	285.6 ± 7.3
13	274.6 ± 8.1	291.1 ± 7.3	284.3 ± 6.9
14	277.6 ± 8.6	292.0 ± 7.4	287.2 ± 6.9
15	281.4 ± 8.7	300.3 ± 8.2	290.6 ± 6.8
16	283.6 ± 8.0	302.7 ± 7.8	293.3 ± 7.1
17	271.2 ± 7.8	286.3 ± 7.5	277.2 ± 8.4
18	272.3 ± 7.5	280.2 ± 9.8	278.7 ± 8.6
19	268.7 ± 7.1	281.8 ± 9.9	279.1 ± 8.7
20	271.8 ± 7.3	295.3 ± 8.2	279.6 ± 8.4

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	257.5 ± 3.8	237.5 ± 6.8	235.5 ± 9.6
2	262.8 ± 3.9	241.3 ± 6.1	244.7 ± 9.9
3	266.6 ± 4.1	246.8 ± 5.9	254.5 ± 9.6
4	270.9 ± 3.8	250.6 ± 5.6	261.4 ± 8.7
5	272.3 ± 3.8	250.8 ± 5.4	262.5 ± 8.6
6	272.8 ± 3.8	251.1 ± 5.3	258.7 ± 8.7
7	278.1 ± 3.8	256.5 ± 5.6	266.0 ± 9.1
8	281.5 ± 3.9	256.8 ± 5.2	266.5 ± 9.0
9	285.7 ± 3.9	263.1 ± 5.4	273.7 ± 8.1
10	288.5 ± 4.0	266.6 ± 5.9	276.3 ± 8.2
11	297.5 ± 3.6	277.6 ± 5.7	280.5 ± 7.3
12	298.3 ± 3.8	278.6 ± 5.7	280.8 ± 7.4
13	299.9 ± 3.8	279.6 ± 5.7	283.2 ± 7.6
14	300.6 ± 3.9	280.8 ± 5.7	284.0 ± 7.5
15	309.4 ± 4.6	289.6 ± 6.8	286.4 ± 7.7
16	311.2 ± 4.4	293.4 ± 6.6	291.2 ± 7.8
17	298.0 ± 4.4	282.5 ± 6.3	275.7 ± 7.8
18	298.8 ± 4.1	284.5 ± 5.7	278.4 ± 7.7
19	300.2 ± 4.3	286.5 ± 6.5	282.6 ± 7.9
20	302.9 ± 5.4	293.3 ± 6.2	287.2 ± 8.2
21	303.8 ± 6.4	296.2 ± 6.2	287.1 ± 8.0
22	307.3 ± 5.0	296.9 ± 6.3	285.2 ± 8.0
23	306.1 ± 5.7	295.3 ± 6.2	291.0 ± 8.2
24	312.5 ± 4.2	295.2 ± 6.3	292.6 ± 8.5
25	311.2 ± 4.4	291.0 ± 7.0	287.3 ± 8.8
26	307.1 ± 7.0	285.6 ± 10.5	294.6 ± 9.5
27	306.8 ± 7.9	284.9 ± 10.3	292.4 ± 9.7
28	305.9 ± 8.0	284.1 ± 10.6	292.6 ± 9.8

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	243.7 ± 11.1	293.2 ± 14.3	267.8 ± 9.8
2	251.5 ± 11.8	298.5 ± 15.2	274.0 ± 11.1
3	257.0 ± 11.9	308.8 ± 14.8	281.2 ± 11.6
4	263.2 ± 12.3	312.0 ± 15.3	288.3 ± 10.6
5	272.2 ± 12.7	313.0 ± 15.5	295.2 ± 12.0
6	276.5 ± 12.7	320.0 ± 16.5	298.5 ± 12.0
7	292.3 ± 11.5	327.0 ± 18.5	313.2 ± 8.2
8	299.3 ± 11.7	331.0 ± 19.9	319.0 ± 12.1
9	304.2 ± 11.1	335.3 ± 20.1	326.5 ± 10.6
10	314.2 ± 11.3	336.5 ± 22.5	325.8 ± 9.5
11	322.5 ± 11.1	340.5 ± 23.3	326.8 ± 9.5
12	333.3 ± 10.4	343.7 ± 23.6	326.0 ± 11.4
13	337.5 ± 9.8	344.8 ± 23.6	331.2 ± 10.8
14	341.7 ± 9.4	345.5 ± 23.4	337.3 ± 10.6
15	348.0 ± 9.3	346.3 ± 23.6	343.7 ± 10.4
16	346.2 ± 10.1	345.5 ± 23.9	321.4 ± 12.2
17	327.3 ± 10.2	327.0 ± 24.7	328.2 ± 13.9
18	335.4 ± 9.9	323.3 ± 23.8	325.8 ± 12.9
19	342.3 ± 9.3	317.3 ± 23.3	343.0 ± 6.9
20	340.0 ± 10.0	324.3 ± 20.5	349.0 ± 7.5

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	275.2 ± 9.1	279.0 ± 10.5	240.8 ± 7.4
2	280.0 ± 8.7	291.6 ± 9.4	249.3 ± 6.4
3	288.1 ± 8.7	300.8 ± 9.8	272.0 ± 7.0
4	296.4 ± 8.9	310.0 ± 10.1	278.6 ± 7.6
5	305.0 ± 8.6	319.5 ± 10.4	292.3 ± 6.7
6	307.5 ± 8.8	322.1 ± 11.3	305.8 ± 6.6
7	310.3 ± 8.6	324.0 ± 10.5	308.0 ± 8.2
8	299.3 ± 11.7	324.9 ± 10.6	316.6 ± 6.4
9	317.6 ± 9.0	327.8 ± 10.7	323.5 ± 6.0
10	320.5 ± 8.3	329.8 ± 11.1	329.6 ± 5.8
11	323.0 ± 8.1	339.7 ± 13.8	335.6 ± 5.9
12	325.8 ± 8.4	334.9 ± 11.2	342.8 ± 7.3
13	328.6 ± 8.3	338.1 ± 12.0	348.4 ± 5.8
14	330.5 ± 8.2	341.2 ± 12.8	354.3 ± 5.4
15	333.0 ± 8.6	344.9 ± 13.1	360.5 ± 5.1
16	337.4 ± 8.9	346.2 ± 11.7	363.8 ± 4.9
17	298.0 ± 6.3	301.7 ± 10.1	358.4 ± 5.1
18	296.8 ± 6.5	310.7 ± 9.8	353.9 ± 4.8
19	309.1 ± 5.0	312.7 ± 8.5	354.8 ± 4.9
20	313.4 ± 6.3	320.1 ± 11.6	362.0 ± 6.5
21	315.5 ± 6.9	322.4 ± 11.0	365.5 ± 6.3
22	318.6 ± 7.5	325.3 ± 10.0	367.1 ± 6.8
23	319.5 ± 7.4	326.1 ± 9.9	366.1 ± 7.1
24	320.4 ± 7.5	328.1 ± 10.2	367.8 ± 6.1
25	321.4 ± 7.5	330.1 ± 10.5	374.3 ± 6.6
26	322.0 ± 7.7	330.9 ± 10.6	378.3 ± 6.6
27	325.4 ± 7.5	332.6 ± 10.1	376.6 ± 6.9
28	328.6 ± 7.4	334.4 ± 9.6	376.5 ± 7.0

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	293.0 ± 10.5	182.8 ± 2.5	207.5 ± 2.5
2	297.2 ± 11.8	188.3 ± 4.0	229.0 ± 4.0
3	310.0 ± 11.9	197.0 ± 4.5	250.3 ± 5.5
4	322.0 ± 12.9	206.5 ± 6.3	257.0 ± 5.4
5	331.2 ± 13.7	214.8 ± 7.2	276.3 ± 5.0
6	335.7 ± 13.9	224.0 ± 8.4	284.5 ± 4.7
7	340.0 ± 13.3	238.0 ± 10.0	286.3 ± 5.3
8	343.2 ± 13.1	244.0 ± 10.8	303.0 ± 8.3
9	350.0 ± 14.3	255.5 ± 13.6	310.0 ± 8.8
10	357.0 ± 14.4	266.5 ± 16.0	315.8 ± 9.4
11	358.0 ± 14.7	268.5 ± 15.4	321.5 ± 9.9
12	366.7 ± 12.6	279.3 ± 18.1	332.8 ± 9.5
13	369.8 ± 12.5	287.8 ± 19.4	337.8 ± 9.6
14	372.2 ± 12.1	293.8 ± 21.4	342.5 ± 10.6
15	376.5 ± 11.4	296.5 ± 21.8	350.5 ± 9.8
16	382.5 ± 13.2	307.3 ± 19.9	353.5 ± 8.9
17	357.8 ± 9.4	296.7 ± 19.2	344.3 ± 11.0
18	367.2 ± 11.3	307.3 ± 23.0	344.5 ± 10.1
19	373.0 ± 10.2	314.3 ± 22.4	349.0 ± 10.2
20	373.0 ± 10.1	313.8 ± 22.7	351.5 ± 9.6

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	289.0 ± 3.0	262.4 ± 4.5	281.6 ± 8.2
2	299.0 ± 4.0	271.3 ± 5.0	287.0 ± 8.0
3	306.1 ± 3.6	280.1 ± 5.4	293.4 ± 8.3
4	308.8 ± 3.6	291.5 ± 5.6	299.1 ± 8.2
5	308.6 ± 3.8	300.4 ± 5.2	301.6 ± 8.9
6	315.5 ± 4.7	310.8 ± 5.5	306.7 ± 9.7
7	327.9 ± 4.5	319.6 ± 6.2	311.6 ± 8.8
8	329.4 ± 5.0	323.9 ± 6.7	311.7 ± 11.3
9	332.1 ± 4.8	325.0 ± 4.1	316.4 ± 12.2
10	338.0 ± 5.0	325.5 ± 6.5	317.4 ± 13.3
11	333.4 ± 6.1	328.3 ± 6.7	321.0 ± 14.0
12	339.0 ± 6.2	328.9 ± 5.1	324.9 ± 14.6
13	342.4 ± 6.2	337.9 ± 5.0	325.0 ± 14.5
14	343.3 ± 6.4	341.3 ± 5.0	325.9 ± 14.6
15	346.9 ± 6.1	345.9 ± 5.5	326.9 ± 14.8
16	350.3 ± 6.1	342.9 ± 5.0	327.3 ± 14.6
17	329.5 ± 6.2	348.3 ± 4.6	312.3 ± 15.0
18	322.0 ± 6.9	350.8 ± 4.3	305.3 ± 14.5
19	322.6 ± 7.4	350.9 ± 4.6	302.1 ± 14.2
20	330.3 ± 7.1	346.5 ± 4.5	302.4 ± 13.4
21	338.4 ± 7.7	341.6 ± 4.9	305.0 ± 13.8
22	341.4 ± 7.9	341.4 ± 5.2	309.3 ± 14.3
23	345.4 ± 8.1	347.6 ± 5.8	311.6 ± 14.3
24	349.1 ± 7.9	350.4 ± 7.4	324.0 ± 14.3
25	349.6 ± 7.8	355.3 ± 7.4	325.9 ± 15.0
26	355.3 ± 7.7	359.0 ± 7.9	331.6 ± 14.7
27	355.5 ± 7.7	359.1 ± 7.8	331.6 ± 14.8
28	356.4 ± 7.4	360.5 ± 8.0	332.7 ± 14.7

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Operative Procedure: Sham laparotomy

Sacrifice Time: 4 weeks

Week	Azoxymethane Treated	Saline Treated
1	222.5 ± 4.5	268.8 ± 6.4
2	227.4 ± 5.2	274.5 ± 6.2
3	231.4 ± 5.8	278.5 ± 6.8
4	235.1 ± 6.2	280.8 ± 7.8
5	239.1 ± 7.2	283.8 ± 7.4
6	239.6 ± 7.1	286.0 ± 7.0
7	239.4 ± 6.9	284.0 ± 7.1
8	240.8 ± 7.0	282.0 ± 7.1
9	241.5 ± 7.0	283.5 ± 7.6
10	243.5 ± 7.0	286.0 ± 7.5
11	245.6 ± 7.2	291.0 ± 6.3
12	242.6 ± 7.0	290.5 ± 7.9
13	245.0 ± 7.8	293.0 ± 8.4
14	247.6 ± 8.0	293.8 ± 8.9
15	250.4 ± 8.3	293.5 ± 9.3
16	253.6 ± 7.9	295.5 ± 9.3
17	242.7 ± 7.3	295.3 ± 9.9
18	248.0 ± 6.8	301.3 ± 10.8
19	252.0 ± 6.4	300.8 ± 9.6
20	252.4 ± 6.5	305.5 ± 10.2

Appendix 1 - Total Animal Body Weight

(mean grams ± sem)

Operative Procedure: Sham Laparotomy

Sacrifice Time: 12 weeks

Week	Azoxymethane Treated	Saline Treated
1	210.1 ± 7.2	264.8 ± 2.0
2	216.6 ± 7.5	268.8 ± 1.5
3	222.6 ± 8.0	272.4 ± 1.8
4	224.6 ± 7.9	273.5 ± 2.2
5	227.0 ± 7.5	276.6 ± 2.4
6	230.5 ± 8.4	279.1 ± 2.3
7	233.3 ± 9.2	280.5 ± 2.0
8	235.8 ± 9.3	282.1 ± 2.0
9	237.1 ± 9.2	284.0 ± 2.5
10	238.8 ± 9.1	285.8 ± 2.5
11	239.7 ± 9.0	289.3 ± 3.3
12	238.1 ± 9.4	291.0 ± 3.4
13	239.1 ± 9.3	292.0 ± 3.7
14	240.1 ± 9.1	292.6 ± 4.0
15	243.6 ± 8.9	293.0 ± 4.2
16	246.5 ± 8.8	297.5 ± 3.9
17	237.0 ± 8.5	293.3 ± 4.0
18	240.7 ± 8.6	299.9 ± 4.2
19	244.6 ± 8.6	300.3 ± 4.3
20	247.3 ± 8.3	305.6 ± 4.0
21	249.5 ± 8.3	310.4 ± 3.4
22	251.9 ± 8.2	312.3 ± 3.3
23	255.4 ± 8.3	310.9 ± 3.1
24	259.2 ± 8.0	312.5 ± 3.2
25	263.4 ± 7.4	317.1 ± 3.3
26	267.1 ± 7.4	321.4 ± 3.1
27	269.3 ± 7.3	321.5 ± 3.5
28	271.5 ± 6.9	321.0 ± 3.5

APPENDIX 2

Experimental Colorectal Carcinogenesis:

Animal Food Intake

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	72.0 ± 0.8	72.9 ± 0.5	73.4 ± 0.6
2	73.5 ± 0.5	74.9 ± 0.8	74.6 ± 0.7
3	75.5 ± 0.5	76.2 ± 1.1	75.1 ± 0.9
4	71.7 ± 2.1	69.2 ± 1.9	73.4 ± 2.0
5	71.7 ± 1.0	67.4 ± 3.6	69.1 ± 2.0
6	74.6 ± 1.4	70.6 ± 3.2	73.1 ± 2.1
7	77.2 ± 1.8	69.9 ± 3.1	75.1 ± 3.1
8	81.5 ± 2.3	70.8 ± 2.8	78.9 ± 3.3
9	80.0 ± 2.2	69.2 ± 3.0	73.2 ± 2.4
10	77.4 ± 1.0	70.3 ± 2.3	72.6 ± 1.7
11	75.1 ± 0.8	68.6 ± 1.9	70.0 ± 1.5
12	75.7 ± 1.1	69.2 ± 1.9	70.6 ± 1.8
13	79.5 ± 0.9	73.4 ± 1.5	74.3 ± 1.6
14	77.2 ± 1.4	70.5 ± 2.3	70.3 ± 2.1
15	78.0 ± 1.2	74.0 ± 1.6	75.1 ± 1.4
16	73.2 ± 1.0	69.2 ± 1.9	72.0 ± 1.1
17	42.5 ± 3.2	49.9 ± 2.5	39.6 ± 4.3
18	67.7 ± 0.8	65.1 ± 1.7	68.6 ± 0.9
19	67.4 ± 1.3	67.2 ± 1.1	74.6 ± 1.1
20	69.0 ± 1.1	68.8 ± 1.4	68.9 ± 2.3

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	75.2 ± 0.9	71.7 ± 0.7	64.5 ± 1.1
2	80.5 ± 0.2	73.2 ± 1.1	71.0 ± 0.9
3	73.4 ± 1.9	76.3 ± 1.9	70.7 ± 1.1
4	72.6 ± 1.1	76.7 ± 1.8	74.5 ± 1.0
5	75.8 ± 0.9	76.3 ± 1.5	77.0 ± 1.5
6	74.6 ± 1.5	77.8 ± 1.0	81.0 ± 1.4
7	81.1 ± 1.1	80.7 ± 1.0	77.7 ± 1.4
8	82.6 ± 0.7	79.2 ± 0.7	72.3 ± 1.6
9	84.5 ± 0.5	83.7 ± 1.1	73.2 ± 1.7
10	82.9 ± 1.0	78.9 ± 1.2	76.2 ± 1.2
11	82.0 ± 0.6	77.3 ± 1.5	79.2 ± 1.0
12	79.3 ± 0.8	76.6 ± 0.8	76.5 ± 0.9
13	81.6 ± 0.7	75.4 ± 1.0	78.5 ± 0.9
14	83.1 ± 0.4	75.7 ± 0.8	79.7 ± 0.7
15	81.6 ± 0.5	80.3 ± 0.7	85.2 ± 1.7
16	64.7 ± 3.5	66.9 ± 2.0	43.8 ± 0.6
17	57.8 ± 2.4	62.4 ± 2.0	49.5 ± 1.6
18	58.9 ± 1.6	61.6 ± 1.3	57.6 ± 1.7
19	62.9 ± 1.7	64.0 ± 1.3	56.6 ± 0.9
20	67.1 ± 1.9	67.2 ± 1.4	55.8 ± 0.7
21	67.6 ± 1.6	68.0 ± 0.8	59.0 ± 1.1
22	67.3 ± 1.1	69.7 ± 1.1	57.2 ± 0.5
23	68.5 ± 0.6	68.8 ± 1.4	58.3 ± 0.5
24	66.0 ± 0.9	65.9 ± 1.4	58.9 ± 0.6
25	64.0 ± 1.1	62.7 ± 1.4	51.8 ± 0.8
26	62.1 ± 1.7	61.8 ± 1.0	46.5 ± 1.0
27	61.3 ± 2.0	62.4 ± 1.0	47.8 ± 0.9
28	59.9 ± 2.1	58.4 ± 1.9	48.5 ± 0.9

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	66.3 ± 0.9	62.0 ± 0.4	63.3 ± 0.6
2	66.7 ± 0.3	64.3 ± 0.8	66.3 ± 1.2
3	66.3 ± 0.5	65.0 ± 0.2	68.3 ± 1.6
4	66.0 ± 0.9	72.0 ± 0.2	70.7 ± 1.0
5	68.3 ± 0.1	71.3 ± 0.5	71.7 ± 1.5
6	67.0 ± 1.0	73.7 ± 0.6	71.0 ± 1.0
7	70.3 ± 1.4	72.3 ± 0.8	71.0 ± 0.4
8	73.0 ± 0.9	68.7 ± 1.5	65.0 ± 2.3
9	74.0 ± 0.2	68.0 ± 1.7	69.7 ± 2.1
10	71.7 ± 0.1	67.0 ± 1.3	62.0 ± 2.2
11	68.7 ± 1.5	70.3 ± 0.5	61.0 ± 0.4
12	63.7 ± 1.6	68.9 ± 1.0	60.3 ± 1.3
13	63.3 ± 0.6	71.0 ± 0.8	64.0 ± 1.3
14	65.0 ± 1.1	70.7 ± 0.7	66.3 ± 1.2
15	67.0 ± 1.1	68.6 ± 1.2	65.3 ± 0.9
16	63.3 ± 1.2	69.2 ± 0.9	67.4 ± 0.5
17	55.3 ± 1.9	51.9 ± 2.3	51.6 ± 1.1
18	65.2 ± 0.8	53.5 ± 2.0	56.7 ± 3.6
19	62.3 ± 0.4	56.3 ± 4.4	58.2 ± 3.6
20	61.5 ± 1.1	54.5 ± 2.7	54.6 ± 1.2

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	66.7 ± 0.6	70.9 ± 0.9	66.9 ± 1.1
2	68.4 ± 0.8	73.8 ± 2.4	67.3 ± 1.2
3	68.4 ± 1.3	75.8 ± 2.6	62.1 ± 1.4
4	66.9 ± 2.5	76.7 ± 2.8	60.6 ± 2.5
5	69.5 ± 0.7	76.7 ± 2.6	61.6 ± 1.3
6	67.6 ± 0.5	77.1 ± 2.1	64.9 ± 2.0
7	67.8 ± 1.4	70.1 ± 2.9	63.9 ± 0.5
8	70.6 ± 1.3	81.3 ± 3.0	67.7 ± 1.2
9	71.6 ± 0.9	78.9 ± 2.0	68.5 ± 1.0
10	72.4 ± 0.9	79.6 ± 2.6	66.4 ± 1.5
11	66.5 ± 0.8	77.1 ± 1.5	60.9 ± 0.9
12	66.2 ± 1.9	69.6 ± 1.1	60.7 ± 0.5
13	69.1 ± 1.3	64.7 ± 2.6	61.7 ± 0.7
14	73.8 ± 1.3	70.7 ± 2.5	61.1 ± 0.5
15	73.6 ± 1.1	70.7 ± 2.4	61.3 ± 0.8
16	68.6 ± 1.1	66.0 ± 2.1	60.9 ± 0.8
17	59.9 ± 1.8	61.2 ± 3.1	45.6 ± 1.4
18	71.1 ± 1.2	64.7 ± 1.9	49.2 ± 1.8
19	71.6 ± 1.4	66.9 ± 2.5	47.3 ± 1.6
20	63.5 ± 1.8	64.0 ± 2.0	51.4 ± 1.1
21	65.1 ± 1.8	64.9 ± 2.1	53.2 ± 0.7
22	63.3 ± 2.0	61.1 ± 2.5	49.5 ± 0.6
23	62.3 ± 2.2	61.1 ± 2.6	52.9 ± 1.5
24	61.7 ± 2.2	61.5 ± 2.4	51.6 ± 0.8
25	58.2 ± 2.7	59.4 ± 3.0	49.8 ± 1.3
26	68.4 ± 2.8	69.3 ± 0.8	51.2 ± 0.5
27	69.1 ± 1.5	66.4 ± 0.3	54.1 ± 0.6
28	65.8 ± 1.9	67.5 ± 2.0	53.6 ± 0.6

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week) *

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	74.5	77.0	81.1
2	77.0	77.5	79.4
3	71.0	82.2	84.3
4	83.0	84.0	91.0
5	89.0	89.0	94.0
6	88.0	92.0	97.0
7	91.0	96.0	101.5
8	89.0	98.0	106.0
9	92.0	104.5	113.5
10	93.0	116.0	125.0
11	91.0	110.0	110.5
12	94.0	115.0	128.0
13	91.0	120.0	134.0
14	90.0	120.5	131.0
15	81.0	125.0	133.5
16	75.0	115.6	121.5
17	49.0	70.5	65.0
18	53.3	70.5	85.0
19	68.0	71.0	69.0
20	72.0	71.0	58.0

* Owing to the small group sizes and the fact that food intake was calculated on a "per cage" basis, no statistical calculations were possible and so only the mean food intake per animal is listed.

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	81.6 ± 0.9	76.4 ± 0.4	68.2 ± 0.7
2	79.9 ± 1.9	82.2 ± 0.2	66.5 ± 0.5
3	76.5 ± 1.5	84.2 ± 0.5	72.0 ± 0.4
4	93.2 ± 2.8	85.0 ± 0.2	75.0 ± 0.7
5	91.0 ± 3.9	91.0 ± 0.4	78.0 ± 0.0
6	101.4 ± 5.1	90.0 ± 0.7	82.5 ± 0.2
7	84.0 ± 5.2	98.0 ± 0.0	84.0 ± 0.4
8	78.4 ± 2.4	102.0 ± 0.7	84.0 ± 0.4
9	78.1 ± 1.7	101.0 ± 0.4	85.0 ± 2.3
10	75.6 ± 2.2	115.0 ± 0.2	85.0 ± 0.7
11	72.9 ± 2.2	115.5 ± 1.9	87.0 ± 0.4
12	67.3 ± 2.1	116.0 ± 0.7	87.5 ± 0.2
13	77.0 ± 2.6	116.0 ± 0.2	79.0 ± 1.1
14	78.6 ± 2.8	117.0 ± 1.4	82.0 ± 0.7
15	81.4 ± 0.6	118.0 ± 0.7	83.0 ± 1.8
16	91.0 ± 3.9	104.7 ± 0.7	82.5 ± 1.4
17	73.8 ± 0.9	76.0 ± 2.5	43.5 ± 1.6
18	91.4 ± 5.1	63.0 ± 1.4	49.0 ± 2.5
19	84.0 ± 5.2	67.0 ± 2.1	50.0 ± 0.4
20	68.4 ± 2.4	72.0 ± 2.1	52.2 ± 0.3
21	68.1 ± 1.7	74.0 ± 1.8	58.5 ± 0.9
22	65.9 ± 2.2	58.5 ± 1.6	60.0 ± 1.1
23	62.9 ± 2.2	65.5 ± 1:6	59.0 ± 1.8
24	67.3 ± 2.1	66.5 ± 1.6	61.5 ± 2.3
25	67.0 ± 2.6	68.5 ± 1.2	66.5 ± 1.9
26	68.6 ± 2.8	73.0 ± 0.4	66.5 ± 2.3
27	71.4 ± 1.1	75.5 ± 0.2	60.0 ± 0.2
28	73.9 ± 0.9	74.0 ± 0.4	63.0 ± 1.8

Appendix 2 - Animal Food Intake
(mean grams diet/animal/week) *

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	64.0	68.0	64.0
2	68.0	60.0	62.0
3	62.0	82.0	78.0
4	72.0	94.0	82.0
5	74.0	98.0	96.0
6	82.0	102.0	102.0
7	86.0	106.0	98.0
8	92.0	112.0	106.0
9	98.0	118.0	110.0
10	106.0	124.0	104.0
11	108.0	120.0	118.0
12	118.0	126.0	122.0
13	122.0	128.0	118.0
14	126.0	134.0	126.0
15	128.0	138.0	132.0
16	132.0	142.0	112.0
17	76.0	86.0	78.0
18	82.0	98.0	82.0
19	88.0	102.0	102.0
20	92.0	92.0	88.0

* Owing to the small group sizes and the fact that food intake was calculated on a "per cage" basis, no statistical calculations were possible and so only the mean food intake per animal is listed.

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Week	Polyamide	Polyglycolic acid	Stainless Steel
1	60.6 ± 0.6	65.0 ± 1.0	62.4 ± 0.4
2	62.5 ± 0.2	65.5 ± 0.2	61.4 ± 0.2
3	63.5 ± 0.2	67.5 ± 0.2	63.3 ± 0.2
4	65.0 ± 0.0	75.0 ± 0.4	64.3 ± 0.7
5	72.5 ± 0.2	66.5 ± 1.6	63.4 ± 1.5
6	76.0 ± 0.0	70.0 ± 1.4	64.4 ± 1.2
7	78.5 ± 0.2	77.0 ± 0.4	66.9 ± 0.8
8	78.0 ± 0.7	75.0 ± 0.4	67.3 ± 1.5
9	68.0 ± 0.7	77.5 ± 0.2	69.0 ± 1.4
10	71.5 ± 0.9	79.0 ± 0.0	70.1 ± 1.1
11	62.5 ± 0.5	78.5 ± 0.9	70.7 ± 0.7
12	60.5 ± 2.7	79.5 ± 0.9	72.6 ± 0.7
13	63.0 ± 2.1	80.0 ± 1.8	71.0 ± 0.9
14	65.5 ± 2.3	68.5 ± 0.9	68.9 ± 1.4
15	67.5 ± 2.3	65.5 ± 1.2	68.7 ± 0.9
16	66.5 ± 1.2	67.5 ± 0.9	61.3 ± 0.8
17	49.0 ± 1.4	48.0 ± 0.2	39.0 ± 1.1
18	51.0 ± 1.4	40.0 ± 0.4	41.3 ± 0.6
19	52.0 ± 0.7	41.5 ± 0.2	43.7 ± 2.1
20	48.5 ± 0.9	45.0 ± 0.2	44.2 ± 0.2
21	46.5 ± 0.9	48.5 ± 0.2	44.9 ± 0.4
22	45.0 ± 0.4	50.0 ± 0.4	48.7 ± 0.7
23	48.0 ± 0.4	50.5 ± 0.5	50.7 ± 0.5
24	49.0 ± 0.2	54.5 ± 2.3	53.7 ± 0.7
25	50.0 ± 0.4	54.0 ± 2.8	51.7 ± 0.7
26	52.0 ± 0.4	56.0 ± 2.5	54.7 ± 0.7
27	53.0 ± 0.4	58.5 ± 2.7	51.4 ± 0.2
28	49.0 ± 0.4	52.0 ± 2.1	45.8 ± 0.8

Appendix 2 - Animal Food Intake
(mean grams diet/animal/week) *

Operative Procedure: Sham Laparotomy

Sacrifice Time: 4 weeks

Week	Azoxymethane Treated	Saline Treated
1		
2	63.0	70.0
3	68.0	76.0
4	72.0	80.0
5	76.0	92.0
6	72.0	86.0
7	76.0	78.0
8	77.0	78.0
9	83.0	80.0
10	85.0	84.0
11	80.0	86.0
12	72.0	80.0
13	72.0	78.0
14	78.0	82.0
15	77.0	92.0
16	79.0	88.0
17	72.0	92.0
18	76.0	82.0
19	69.0	84.0
20	77.0	86.0

* Owing to the small group sizes and the calculation of food intake on a "per cage" basis, no statistical analysis was carried out and so only mean animal food intakes are listed.

Appendix 2 - Animal Food Intake

(mean grams diet/animal/week ± sem)

Operative Procedure: Sham Laparotomy

Sacrifice Time: 12 weeks

Week	Azoxymethane Treated	Saline Treated
1	63.5 ± 0.9	69.3 ± 0.7
2	62.0 ± 1.1	70.0 ± 0.7
3	67.5 ± 1.1	81.0 ± 0.4
4	72.5 ± 1.3	83.0 ± 0.4
5	66.0 ± 0.2	90.0 ± 0.7
6	73.5 ± 0.7	92.0 ± 0.7
7	72.0 ± 0.9	88.0 ± 1.8
8	71.5 ± 0.9	93.0 ± 1.8
9	72.5 ± 1.1	85.0 ± 0.4
10	73.5 ± 1.0	81.0 ± 0.4
11	73.5 ± 2.0	85.0 ± 0.8
12	66.9 ± 1.5	78.0 ± 0.2
13	64.8 ± 1.2	71.0 ± 0.4
14	66.8 ± 0.6	75.0 ± 0.4
15	68.0 ± 1.3	71.0 ± 1.1
16	64.5 ± 1.2	75.0 ± 1.1
17	59.6 ± 1.5	83.0 ± 1.8
18	61.5 ± 1.2	88.0 ± 1.4
19	64.0 ± 0.7	88.0 ± 0.2
20	73.5 ± 0.8	84.0 ± 0.7
21	74.8 ± 1.1	88.0 ± 0.7
22	71.9 ± 0.6	82.0 ± 0.0
23	73.5 ± 0.9	77.0 ± 0.4
24	72.8 ± 0.9	82.0 ± 0.0
25	72.7 ± 0.8	73.0 ± 0.4
26	70.0 ± 0.4	77.0 ± 0.4
27	66.8 ± 0.6	69.0 ± 1.1
28	64.9 ± 0.3	69.0 ± 1.1

APPENDIX 3

Experimental Colorectal Carcinogenesis:

Gross and Microscopic Pathology

Appendix 3 - Gross and Microscopic Pathology

Suture Material: Polyamide

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) no abnormality	a) granulomatous reaction around residual sutures
2	a) no abnormality	a) granulomata around sutures
3	a) solid tumour mass around jejunum b) 3 adjacent 2mm nodules suture area	a) poorly differentiated adenocarcinoma ? from colon b) all dysplastic surrounded by poorly differentiated adenocarcinoma
4	a) no abnormality	a) foreign body giant cell reaction around sutures
5	a) no abnormality	a) mixed giant cell reaction and polymorph infiltrate around sutures
6	a) normal	a) acute inflammation suture area
7	a) normal	a) acute inflammation suture area
8	a) normal	a) focal inflammation around sutures
9	a) normal	a) focal inflammatory infiltrate around sutures
10	a) no abnormality	a) chronic inflammatory infiltrate
11	a) no abnormality	a) focal inflammation
12	a) normal	a) chronic scarring around suture
13	a) normal	a) foreign body granuloma in relation to suture

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyglycolic Acid

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) Normal mucosa but some thickening of serosa surrounding sutures	a) Florid granulomatous reaction around residual suture.
2	a) Two 2mm nodules around sutures	a) Granulomatous reaction with local acute inflammation
3	a) Large solid tumour mass in jejunum b) 3mm polypoidal tumour descending colon c) Mucosal thickening in suture area	a) Poorly differentiated adenocarcinoma b) well differentiated adenocarcinoma c) infiltration by poorly differentiated adeno-carcinoma ? spread from jejunum. Mucosa intact
4	a) Normal	a) granulomata around sutures
5	a) Thickening and mucosal heaping around sutures b) deposit on liver	a) poorly differentiated adenocarcinoma with surrounding granulomata b) adenocarcinoma
6	a) 2mm nodule mid-colon b) suture area normal	a) lymphoid aggregates b) granulomata around sutures
7	a) Normal	a) acute inflammation and granulomata around sutures
8	a) large solid tumour lower pole of caecum b) single liver nodule c) suture area normal	a) poorly differentiated adenocarcinoma b) adenocarcinoma c) focal granulomatous reaction to sutures
9	a) normal	a) suture granuloma in serosa
10	a) 2mm nodule descending colon b) suture area normal	a) small tubular adenoma b) chronic inflammatory infiltrate around sutures

11	a) 2mm sessile lesion proximal colon	a) poorly differentiated adenocarcinoma with signet ring cells
	b) suture area normal	b) foreign body granuloma surrounding suture
12	a) normal	a) foreign body granuloma surrounding residual suture
13	a) 2mm polypoidal tumour in suture area	a) moderately dysplastic tubulovillous adenoma

Appendix 3: Gross and Microscopic Pathology

Suture Material: Stainless Steel

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) normal	a) granulomata at site of suture implantation
2	a) normal	a) granulomatous reaction around sutures
3	a) 2mm nodular lesion descending colon b) suture area normal	a) moderately differentiated adenocarcinoma b) diffuse sub-mucosal inflammatory reaction around sutures
4	a) normal	a) granulomata around sutures
5	a) obstructing tumour proximal ileum a) suture area normal	a) poorly differentiated adenocarcinoma b) granulomata around sutures
6	a) normal	a) severe acute inflammation around sutures
7	a) 4mm nodular tumour in duodenum b) 2mm polyp proximal descending colon c) suture area normal	a) moderately differentiated adenocarcinoma b) moderately differentiated adenocarcinoma c) granulomata around sutures
8	a) normal	a) small area glandular hyperplasia around suture tract
9	a) 4mm polypoidal tumour descending colon b) suture area normal	a) severely dysplastic tubulovillous adenoma b) serosal foreign body granuloma in suture area
10	a) normal	a) normal histology
11	a) normal	a) normal histology
12	a) normal	a) foreign body granuloma in serosa of suture area
13	a) normal	a) serosal granulomata in suture area
14	a) normal	a) granulomata in suture area

Appendix 3: Gross and Microscopic Pathology

Suture Material: None

Operative Procedure: Sham Laparotomy

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	normal	normal
2	normal	normal
3	normal	normal
4	normal	normal
5	normal	normal
6	normal	normal
7	normal	normal

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyamide

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) 2mm sessile lesion suture area	a) moderately dysplastic tubular adenoma
2	a) tumour left ear b) mucosal heaping around suture	a) squamous carcinoma b) lymphoid aggregates
3	a) normal	a) acute inflammation around sutures, lymphoid aggregates
4	a) 3mm nodule proximal colon b) 1mm mucosal heaping proximal colon c) 2mm sessile lesion mid-colon d) 3mm polyp mid-colon e) 2mm nodule suture area f) 2mm polyp suture area g) intussuscepting lesion mid-ileum	a) poorly differentiated adenocarcinoma b) lymphoid aggregates c) adenomatous focus d) poorly differentiated adenocarcinoma e) dysplastic tubular adenoma f) infiltrating adenocarcinoma arising in dysplastic tubular adenoma g) well differentiated adenocarcinoma
5	a) 3mm polypoidal lesion suture area b) 2mm nodule suture area	a) poorly differentiated carcinoma with signet cells b) tubulo-villous adenoma
6	a) 4mm polyp proximal colon b) 2mm nodule mid-colon c) 3mm nodule suture area	a) moderately dysplastic tubulovillous adenoma b) moderately dysplastic tubular adenoma c) poorly differentiated adenocarcinoma arising in tubulovillous adenoma
7	a) 3mm nodule proximal colon b) 3mm nodule mid-colon c) 2mm raised lesion mid-colon d) 3mm plaque suture area e) 2mm nodule suture area	a) tubulovillous adenoma b) poorly differentiated adenocarcinoma extending into a lymph node c) early adenocarcinoma arising in adenoma d) adenomatous focus e) lymphoid aggregates

8	a) 2mm polyp suture area	a) well differentiated adenocarcinoma
	b) mucosal heaping suture area	b) diverticulum
9	a) large obstructing tumour mass distal stomach	a) poorly differentiated adenocarcinoma with signet cells
	b) tumour proximal colon	b) invasion from gastric lesion
	c) pale friable liver	c) diffuse hepato-cellular swelling with inflammatory infiltrate
	d) multiple tumour deposits studding peritoneum	d) poorly differentiated adenocarcinoma
	e) mucosal heaping around sutures	e) lymphoid aggregates
	e) blood stained ascites	
10	a) 2mm nodule mid colon	a) tubulo-villous adenoma
	b) 2mm polyp suture area	b) tubular adenoma
	c) 2mm nodule suture area	c) tubular adenoma
	d) mucosal heaping suture area	d) foreign body giant cell reaction around sutures
11	a) ulcerating right ear tumour	a) squamous carcinoma
	b) large gastric tumour mass	b) poorly differentiated adenocarcinoma
	c) 2mm thickening suture area	c) tissue autolysed, histology not possible
	d) swollen friable liver	d) mild reactive hepatitis
12	a) 3mm polypoidal lesion mid colon	a) moderately differentiated adenocarcinoma
	b) suture area normal	b) foreign body granulomatous reaction
13	a) 3mm polypoidal lesion mid-colon	a) focal adenomatous change
	b) 5mm polyp suture area	b) severly dysplastic tubulovillous adenoma
	c) 7mm polypoidal lesion suture area	c) moderately differentiated adenocarcinoma
14	a) minimal thickening around sutures	a) severely dysplastic tubulovillous adenoma
15	a) ulcerating left ear tumour	a) squamous carcinoma
	b) 5mm sessile plaque proximal colon	b) moderately differentiated adenocarcinoma
	c) 6mm polypoidal tumour suture area	c) moderately dysplastic tubulovillous adenoma

16	a) ulcerating left ear tumour	a) squamous carcinoma
	b) 4mm polypoidal lesion mid-colon	b) moderately differentiated adenocarcinoma
	c) suture area normal	c) focus of adenomatous change
17	a) ulcerated right ear tumour	a) squamous carcinoma
	b) 4mm polypoidal tumour mid-colon	b) moderately differentiated adenocarcinoma
18	a) small right ear tumour	a) "sebaceous" cyst
	b) 2mm sessile lesion mid-colon	b) moderately differentiated adenocarcinoma
	c) 3mm nodule mid-colon	c) adenocarcinoma in serosa mucosa raised only by lymphoid aggregates
	d) 4mm polypoidal tumour suture area	d) moderately differentiated adenocarcinoma arising in a tubulovillous adenoma
19	a) ulcerated tumour left ear	a) squamous carcinoma
	b) 4mm polypoidal lesion descending colon	b) moderately differentiated adenocarcinoma
	c) suture area normal	c) well differentiated adeno-carcinoma suture area
20	a) normal	a) small focus adenomatous change surrounding suture material

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyglycolic Acid

Operative Procedure: Suture Implantation

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Micoscopic Pathology
1	a) 2mm nodule proximal descending colon b) 2mm nodule suture area	a) moderately differentiated adenocarcinoma b) poorly differentiated adenocarcinoma
2	a) 3mm nodule proximal descending colon b) 2mm nodule around piece of suture	a) moderately differentiated adenocarcinoma b) granulomatous
3	a) 3mm nodule mid-descending colon b) 2mm nodule suture area c) deposits on diaphragm d) blood stained ascites	a) moderately differentiated adenocarcinoma b) moderately differentiated adenocarcinoma c) adenocarcinoma
4	a) 3mm nodule suture area	a) severely dysplastic tubular adenoma
5	a) 3mm nodule suture area b) 2mm nodule suture area	a) well differentiated adenocarcinoma b) suture material with granulomatous reaction
6	a) normal	a) granulomata around sutures
7	a) 2mm nodule suture area b) 2mm nodule suture area	a) moderately dysplastic tubullo-villous adenoma b) inflammatory
8	a) 2mm nodule proximal descending colon b) 2mm nodule suture area	a) polypoidal tubulovillous adenoma b) walled off abscess
9	a) 4mm bleeding nodule proximal colon b) 2mm nodule suture area	a) polypoidal tubulovillous adenoma b) mildly dysplastic tubular adenoma
10	a) 10mm lesion upper caecal pole b) 2mm nodule suture area	a) poorly differentiated adenocarcinoma with signet ring cells b) well differentiated adenocarcinoma

	c) 4mm nodule suture area	c) well differentiated adenocarcinoma
	d) 2mm nodule suture area	d) moderately dysplastic tubulovillous adenoma
11	a) 2mm nodule suture area	a) moderately differentiated adenocarcinoma
	b) 2mm nodule suture area	b) moderately dysplastic tubular adenoma
	c) 3mm nodule suture area	c) well differentiated adenocarcinoma
12	a) ulcerated left ear tumour	a) squamous carcinoma
	b) 6mm intussuscepting tumour suture area	b) mildly dyplastic tubulovillous adenoma
	c) 3mm nodule suture area	c) moderately differentiated adenocarcinoma
13	a) small tumour left ear	a) squamous carcinoma
	b) 1mm nodule proximal colon	b) moderately differentiated adenocarcinoma
	c) 4mm nodule proximal colon	c) moderately dysplastic tubulovillous adenoma
	d) 3mm polyp descending colon	d) moderately dyplastic tubulovillous adenoma
	e) 3mm nodule suture area	e) poorly differentiated adenocarcinoma
	f) 5mm polyp suture area	f) moderately dysplastic tubulovillous adenoma
14	a) 3mm polyp proximal colon	a) moderately dysplastic tubulovillous adenoma
	b) 3mm poly mid-colon	b) moderately dysplastic tubulovillous adenoma
	c) 8mm intussuscepting tumour in suture area with embedded suture material	c) moderately dysplastic tubulovillous adenoma
	d) separate 1mm nodule in suture area	d) foreign body granulomata
15	a) 3mm nodule proximal colon	a) severely dysplastic tubulovillous adenoma
	b) 2mm nodule proximal colon	b) moderately differentiated adenocarcinoma
	c) 8mm intussuscepting polypoidal lesion in mid-colon	c) severely dysplastic tubulovillous adenoma
	d) separate 7mm polyp adjacent to (c)	d) severely dysplastic tubulovillous adenoma
	e) 2mm nodule suture area	e) moderately differentiated adenocarcinoma
	f) three 3mm nodules suture area	f) moderately differentiated adenocarcinoma
16	a) no abnormality	a) serosal granulomata in suture area

17	a) ulcerated right ear tumour	a) squamous carcinoma
	b) 5mm polpoidal lesion in suture area	b) moderately dysplastic tubulovillous adenoma
18	a) 2mm nodule suture area	a) mildly dysplastic tubular adenoma
	b) 2mm area of mucosal heaping around sutures	b) lymphoid aggregates
19	a) 2mm nodule proximal colon	a) moderately differentiated adenocarcinoma
	b) 4mm polyp mid-colon	b) moderately dyplastic tubulovillous adenoma
	c) 6mm polypoidal lesion suture area	c) moderately dyplastic tubulovillous adenoma
	d) 7mm polyp suture area	d) moderately dyplastic tubulovillous adenoma
	e) 2mm area of mucosal thickening around sutures	e) foreign body granuloma around sutures
20	a) ulcerated left ear tumour	a) squamous carcinoma
	b) 4mm polyp suture area	b) moderately differentiated adenocarcinoma
	c) 4mm nodule suture area	c) severely dysplastic tubulovillous adenoma
21	a) large right ear tumour	a) squamous carcinoma
	b) 5mm polypoidal tumour in proximal colon	b) poorly differentiated adenocarcinoma
	c) 2mm nodule mid-colon	c) moderately dysplastic tubulovillous adenoma
	c) 3mm polyp suture area	d) severely dysplastic tubulovillous adenoma

Appendix 3: Gross and Microscopic Pathology

Suture Material: Stainless Steel

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) normal	a) mucosal ulceration with crypt abscesses suture area
2	a) 2mm nodule proximal colon	a) poorly differentiated adenocarcinoma with signet ring cells
	b) suture area normal	b) acute mucosal inflammation focus of superficial glandular dysplasia
3	a) 2mm nodule mid-colon	a) moderately dysplastic tubular adenoma
	b) lungs pale, mottled	b) lymphoid inflammatory infiltrate
	c) suture area normal	c) normal histology
4	a) ulcerated left ear tumour	a) squamous carcinoma
	b) obstructing tumour mass proximal colon	b) poorly differentiated adenocarcinoma
	c) suture area normal	c) fine histological detail obscured by poor tissue fixation. No obvious neoplastic tissue
5	a) 2mm nodule proximal colon	a) poorly differentiated signet cell adenocarcinoma
	b) 2mm polyp mid-colon	b) moderately dysplastic tubular adenoma
	c) 2mm plaque mid-colon	c) normal histology
	d) suture area normal	d) mixed inflammatory infiltrate
6	a) 2mm nodular lesion proximal colon	a) poorly differentiated adenocarcinoma
	b) 2mm nodules suture area	b) moderately dysplastic tubular adenoma
	c) separate 2mm nodule in suture area	c) moderately dysplastic tubular adenoma
7	a) ulcerated right ear tumour	a) squamous carcinoma
	b) 2mm nodule proximal colon	b) moderately dysplastic tubular adenoma
	c) 3mm polyp mid-colon	c) moderately dysplastic tubulovillous adenoma

	d) 5mm bleeding polyp mid-colon	d) poorly differentiated adenocarcinoma arising from tubulovillous adenoma
	e) 2mm nodule suture area	e) normal histologically
	f) 4mm nodule suture area	f) severely dysplastic tubular adenoma
8	a) 3mm polyp proximal colon	a) moderately dysplastic tubular adenoma
	b) 2mm nodule mid colon	b) moderately dysplastic tubular adenoma
	c) 2mm nodule mid colon	c) moderately dysplastic tubular adenoma
	d) 4mm polyp mid-colon	d) moderately dysplastic tubular adenoma
	e) 8mm polypoidal lesion in suture area	e) moderately dysplastic tubulovillous adenoma
9	a) normal	a) granulomata in serosa of suture area
10	a) 3mm area of mucosal heaping lower caecal pole	a) severely dysplastic tubulovillous adenoma
	b) 2mm nodule mid-colon	b) severely dysplastic tubulovillous adenoma
	c) suture area normal	c) foreign body granuloma in serosa
11	a) ulcerated left ear tumour	a) squamous carcinoma
	b) suture area normal	b) serosal inflammation in suture area
	c) blood in small bowel, no tumour found	
12	a) large tumour mass upper abdomen. Primary site uncertain	a) poorly differentiated adenocarcinoma with signet cells
	b) 3mm nodule proximal colon	b) poorly differentiated adenocarcinoma
	c) suture area normal	c) mild chronic inflammation adenocarcinoma in serosa. no mucosal abnormality
	d) pale shrunken liver	d) normal histology
13	a) normal	a) mild serosal inflammation suture area
14	a) 2mm nodular lesion mid-colon	a) moderately differentiated adenocarcinoma
	b) suture area normal	b) small dysplastic focus in suture area
15	a) 2mm nodule proximal colon	a) moderately dysplastic tubular adenoma
	b) suture area normal	b) serosal granulomata in suture area

16	a) ? deposits in lungs b) otherwise normal	a) normal histology b) serosal inflammation in "peri-anastomotic" area
17	a) normal	a) mild serosal inflammation surrounding sutures
18	a) normal	a) normal histology
19	a) 2mm plaque proximal colon b) marked serosal thickening surrounding sutures Mucosa normal	a) severely dysplastic tubular adenoma b) marked submucosal eosinophil infiltrate around sutures. No neoplastic tissue
20	a) obstructing tumour mass distal stomach b) suture area normal	a) poorly differentiated adenocarcinoma b) serosal foreign body giant cell reaction
21	a) 2mm area of mucosal heaping mid-colon b) suture area normal	a) small focus of adenomatous change b) tiny area of tubular dysplasia

Appendix 3: Gross and Microscopic Pathology

Suture Material: None

Operative Procedure: Sham Laparotomy

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) normal appearance	a) normal bowel
2	a) blood in small bowel, no mucosal abnormality b) suture area normal	b) normal histology
3	a) blood in small bowel, no mucosal abnormality b) suture area normal	b) normal bowel
4	a) normal	a) normal
5	a) 2mm nodule mid-colon b) suture area normal	a) moderately dysplastic tubular adenoma b) normal histology
6	a) normal	a) normal bowel
7	a) 3mm nodule in mid-colon b) suture area normal	a) poorly differentiated adenocarcinoma with signet ring cells b) normal histology
8	a) normal	a) normal bowel
9	a) normal	a) normal bowel
10	a) 3mm polyp proximal colon b) suture area normal	a) moderately differentiated adenocarcinoma b) normal histology
11	a) normal	a) normal bowel
12	a) blood in small bowel, no mucosal lesion b) colo-rectum normal	b) normal histology
13	a) normal	a) normal bowel
14	a) normal	a) normal histology
15	a) normal	a) normal bowel

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyamide

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) normal	a) mild mixed inflammatory infiltrate at anastomosis
2	a) normal	a) normal
3	a) 3mm nodule anastomotic area	a) moderately dysplastic tubulo-villous adenoma
4	a) 2mm raised plaque proximal colon b) anastomotic area normal	a) mildly dysplastic tubular adenoma b) focal granulomatous reaction around sutures
5	a) normal	a) normal
6	a) 4mm polypoidal bleeding lesion mid-colon b) anastomotic area normal	a) poorly differentiated adenocarcinoma b) normal
7	a) 2mm polyp mid-colon b) anastomotic area normal	a) moderately dysplastic tubular adenoma b) normal
8	a) normal	a) granulomata serosal surface
9	a) normal	a) foreign body granulomata on serosal surface
10	a) normal	a) lymphoid aggregates in submucosa of suture area
11	a) normal	a) foreign body granuloma around sutures

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyglycolic Acid

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) 2mm nodule proximal colon b) 4mm bleeding polyp mid-colon c) anastomotic area normal	a) mildly dysplastic tubular adenoma b) well differentiated adenocarcinoma arising in tubulovillous adenoma c) acute inflammatory infiltrate
2	a) normal	a) granulomata surrounding suture material
3	a) 2mm raised plaque in mid-colon b) 5mm bleeding nodular lesion suture area directly related to suture material	a) moderately dysplastic tubulovillous adenoma b) moderately dysplastic tubular adenoma with acute inflammatory infiltrate
4	a) 3mm nodular lesion mid-colon b) suture area normal	a) moderately differentiated adenocarcinoma b) mild acute inflammation suture area
5	a) 2mm mucosal heaping mid-colon b) 3mm polyp mid-colon c) 4mm polypoidal lesion in anastomotic area	a) lymphoid aggregates b) mildly dysplastic tubular adenoma c) moderately differentiated adenocarcinoma
6	a) 2mm nodule mid-colon b) 4mm polyp anastomotic area	a) moderately dysplastic tubular adenoma b) moderately dysplastic tubular adenoma
7	a) obstructing 8mm lesion suture area	a) severely dysplastic tubulovillous adenoma
8	a) 3mm raised plaque in caecum b) 2mm sessile plaque proximal colon	a) deposits of secondary poorly differentiated adenocarcinoma. No mucosal primary. b) poorly differentiated adenocarcinoma arising from a moderately dysplastic tubular adenoma

	c) 5mm bleeding polypoidal lesion anastomotic area	c) moderately dysplastic tubulovillous adenoma
9	a) 6mm raised plaque in anastomotic area	a) granulomata with lymphoid aggregates
10	a) normal	a) granulomatous reaction surrounding sutures
11	a) normal	a) foreign body granuloma serosal surface suture area
12	a) normal	a) granulomata serosal surface suture area

Appendix 3: Gross and Microscopic Pathology

Suture Material: Stainless Steel

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) normal	a) normal
2	a) 3mm nodule mid-colon	a) mildly dysplastic adenomatous focus
	b) suture area normal	b) normal
3	a) 3mm polyp mid-colon	a) moderately differentiated adenocarcinoma
	b) suture area normal	b) normal
4	a) normal	a) normal
5	a) normal	a) granulomatous reaction around sutures
6	a) 3mm nodule proximal colon	a) poorly differentiated adenocarcinoma
	b) 2mm nodule mid-colon	b) moderately dysplastic tubulovillous adenoma
	c) 3mm raised lesion mid-colon	c) moderately dysplastic tubulovillous adenoma
	d) suture area normal	d) normal
7	a) normal	a) acute inflammation around sutures
8	a) normal	a) submucosal scarring
10	a) normal	a) lymphoid aggregates in submucosa suture area
11	a) normal	a) normal bowel
12	a) normal	a) well differentiated adeno-carcinoma suture area

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyamide

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) 3mm bleeding lesion peri-anastomotic area	a) well differentiated adenocarcinoma
2	a) ulcerated ear tumour b) obstructing tumour mass in duodenum c) colon normal	a) squamous carcinoma b) moderately diffentiated adenocarcinoma c) foreign body granulomatous reaction to suture material
3	a) 5mm polyp mid-colon b) 2mm nodule anastomotic area	a) moderately dysplastic tubular adenoma b) poorly differentiated adenocarcinoma
4	a) 5mm sessile tumour in duodenum b) 7mm polyp proximal colon c) 6mm peri-anastomotic polypoidal lesion	a) moderately differentiated adenocarcinoma b) early well differentiated adenocarcinoma arising in a tubulo-villous adenoma c) moderately differentiated adenocarcinoma arising in a tubular adenoma
5	a) 6mm ulcerated lesion mid-colon b) anastomotic area macroscopically normal	a) moderately dysplastic tubular adenoma b) early moderately differentiated adeno-carcinoma in area of diffuse adenomatous change
6	a) 4mm polyp anastomotic area b) 2mm polypoidal peri-anastomotic lesion c) 3mm polypoidal peri-anastomotic lesion	a) moderately dysplastic tubular adenoma b) moderately dysplastic tubular adenoma c) moderately dysplastic tubular adenoma
7	a) 3mm raised area proximal colon b) 2mm nodule proximal colon c) 3mm polyp proximal colon d) 10mm polypoidal tumour in mid-colon	a) adenomatous change b) moderately dysplastic tubulovillous adenoma c) moderately differentiated adenocarcinoma d) moderately differentiated adenocarcinoma

	e) 3mm nodule anastomotic area	e) severely dysplastic tubular adenoma
8	a) 3mm nodule mid-colon	a) moderately differentiated adenocarcinoma
	b) 3mm nodule in peri-anastomotic area	b) moderately differentiated adenocarcinoma
9	a) 3mm polyp mid-colon	a) moderately dysplastic tubular adenoma
	b) 2mm nodule anastomotic area	b) moderately dysplastic tubular adenoma
10	a) mucosal heaping mid-colon	a) normal
	b) anastomotic area normal	b) normal histology
11	a) 2mm nodule anastomotic area	a) severely dyasplastic tubulovillous adenoma
12	a) normal	a) foreign body granuloma related to suture. Reactive lymph nodes
13	a) 3mm polyp mid-colon	a) poorly differentiated adenocarcinoma
	b) anastomotic area normal	b) severe inflammation. Adenomatous change with dysplasia in mucosa
14	a) 2mm polyp in peri-anastomotic area	a) moderately differentiated adenocarcinoma
15	a) mucosal heaping of mid-colon	a) lymphoid aggregates
	b) anastomotic area normal	b) foreign body granuloma
16	a) no abnormality	a) serosal foreign body granulomata
17	a) 2mm nodule proximal colon	a) normal histology
	b) 4mm polyp mid-colon	b) moderately differentiated adenocarcinoma arising in sessile villous adenoma
	c) 3mm nodular lesion in anastomotic area	c) severely dysplastic tubulovillous adenoma
	d) 4mm polyp at anastomosis	d) severely dysplastic tubulovillous adenoma
18	a) right ear tumour	a) squamous carcinoma
	b) 3mm polyp proximal colon	b) severely dysplastic tubulovillous adenoma
	c) 4mm plaque mid-colon	c) severely dysplastic tubulovillous adenoma
	d) 2mm nodule anastomotic area	d) moderately dysplastic villous adenoma

	e) 4mm polypoidal lesion at anastomosis	e) moderately dysplastic tubulovillous adenoma foreign body granulomatous reaction to sutures
19	a) ulcerated left ear tumour b) no colonic lesion seen	a) squamous carcinoma b) foreign body granuloma around sutures

Appendix 3: Gross and Microscopic Pathology

Suture Material: Polyglycolic Acid

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) 3mm polyp suture area	a) tubular adenoma
	b) 4mm polypoidal lesion suture area	b) moderately differentiated adenocarcinoma arising in a tubulovillous adenoma
	c) 4mm polyp suture area	c) moderately dysplastic tubulovillous adenoma
2	a) ulcerated left ear tumour	a) squamous carcinoma
	b) large gastro-duodenal tumour mass	b) necrotic adenocarcinoma
	c) 2mm nodule proximal colon	c) poorly differentiated adenocarcinoma
	d) 3mm polyp mid-colon	d) moderately dysplastic tubular adenoma
	e) 2mm nodule suture area	e) moderately dysplastic tubular adenoma
	f) 3mm nodule suture area	f) moderately dysplastic tubular adenoma
3	a) ulcerated right ear tumour	a) squamous carcinoma
	b) 3mm polyp suture area	b) severely dysplastic tubular adenoma
	c) 2mm raised nodule suture area	c) lymphoid aggregates
4	a) 6mm polyp proximal colon	a) moderately dysplastic tubular adenoma
	b) 2mm nodule mid-colon	b) tubular adenoma
	c) separates 2mm nodule mid-colon	c) tubular adenoma
	d) 7mm polypoidal lesion in suture area	d) moderately dysplastic tubulovillous adenoma
	e) 8mm ulcerated tumour suture area	e) moderately differentiated adenocarcinoma
		f) marked foreign body giant cell reaction suture area
5	a) mucosal heaping proximal colon	a) normal histology
	b) 4mm nodule suture area	b) well differentiated adenocarcinoma
6	a) area of thickening in peri-anastomotic area	a) lymphoid aggregates

7	a) 2mm plaque proximal colon	a) poorly differentiated adenocarcinoma
	b) 10mm obstructing lesion suture area	b) moderately differentiated adenocarcinoma
	c) 10mm polypoidal lesion suture area	c) moderately differentiated adenocarcinoma
8	a) 12mm intussuscepting peri-anastomotic tumour mass	a) moderately differentiated adenocarcinoma
9	a) ulcerated ear tumour	a) squamous carcinoma
	b) 3mm nodule suture area	b) well differentiated adenocarcinoma
	c) 10mm polyp suture area	c) moderately differentiated adenocarcinoma arising in a tubulovillous adenoma
	d) 8mm peri-anastomotic polyp	d) well differentiated adenocarcinoma arising in a tubulovillous adenoma
10	a) 3mm nodule proximal colon	a) well differentiated adenocarcinoma arising in tubulovillous adenoma
	b) 3mm plaque mid-colon	b) poorly differentiated adenocarcinoma
	c) 4mm polypoidal lesion anastomotic area	c) moderately differentiated adenocarcinoma
		d) separate small focus of adenomatous change in peri-anastomotic area
11	a) left ear tumour	a) squamous carcinoma
	b) 2mm nodule anastomotic area	b) moderately differentiated tubulovillous adenoma
	c) 3mm ulcerated lesion in anastomotic area	c) well differentiated adenocarcinoma
12	a) normal	a) large foreign body granuloma surrounding suture material
13	a) 2mm polyp mid-colon	a) severely dysplastic tubulovillous adenoma
	b) suture area normal	b) normal histology
14	a) ulcerated left ear tumour	a) squamous carcinoma
	b) "vascular" cystic lesion on surface of liver	b) biliary hamartoma
	c) anastomotic area normal	c) foreign body granuloma
15	a) 3mm nodule proximal colon	a) moderately dysplastic tubular adenoma
	b) 7mm bleeding polypoidal lesion at anastomosis	b) severely dysplastic tubular adenoma
16	a) solid mass intra-peritoneal tumour	a) poorly differentiated adenocarcinoma

	b) anastomosis encased in tumour? primary or secondary	b) poorly differentiated adenocarcinoma in serosal fat. No mucosal abnormality
	c) 3mm nodule mid colon	c) poorly differentiated adenocarcinoma arising in tubular adenoma
17	a) 12mm obstructing tumour at anastomosis	a) poorly differentiated infiltrating adenocarcinoma
	b) 4mm polyp at anastomosis	b) severely dysplastic tubulovillous adenoma
18	a) 4mm polyp proximal colon	a) poorly differentiated adenocarcinoma with signet ring cells
	b) 3mm polyp anastomotic area	b) mildly dysplastic tubular adenoma
19	a) 5mm plaque mid-colon	a) moderately dysplastic tubulovillous adenoma
	b) 9mm polypoidal anastomotic tumour	b) moderately differentiated adenocarcinoma arising in tubulovillous adenoma
20	a) normal	a) serosal inflammation around sutures

Appendix 3: Gross and Microscopic Pathology

Suture Material: Stainless Steel

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 weeks

Animal	Macroscopic Pathology	Microscopic Pathology
1	a) normal	a) minimal granulomatous reaction around sutures
2	a) 2mm nodule anastomotic area	a) focus of glandular dysplasia
3	a) normal	a) normal
4	a) 2mm nodule at anastomosis	a) granulomatous inflammation no neoplastic tissue
5	a) 4mm ulcerated tumour proximal colon	a) poorly differentiated adenocarcinoma with prominent signet cells
	b) nodule proximal colon	b) moderately dysplastic tubulovillous adenoma
	c) localised mucosal heaping around sutures	c) poorly differentiated adenocarcinoma arising from tubulovillous adenoma
	d) multiple peritoneal seedlings	d) poorly differentiated adenocarcinoma
6	a) 4mm polyp mid-colon	a) poorly differentiated adenocarcinoma arising in dysplastic epithelium
	b) suture area normal	b) lymphoid aggregates at anastomosis
7	a) 3mm nodule at anastomosis	a) normal histology
8	a) 2mm nodule mid-colon	a) moderately dysplastic tubular adenoma
	b) blood in small bowel, no mucosal lesion found	
	c) suture area normal	c) superficial area of adenomatous change
9	a) 6mm ulcerated tumour in proximal colon	a) poorly differentiated adenocarcinoma with some signet cells
	b) multiple peritoneal tumour deposits	b) poorly differentiated adenocarcinoma
	c) multiple liver nodules	c) subcapsular tumour deposits
	d) multiple pulmonary nodules	d) metastatic adenocarcinoma
	e) suture area normal	e) serosal deposit of poorly differentiated adeno-carcinoma. Mucosa normal

10	a) small right ear tumour	a) squamous carcinoma
	b) blood in proximal small bowel, no source found	
	c) anastomotic area normal	c) normal histology
11	a) 2mm nodule mid-colon	a) severely dysplastic tubulovillous adenoma
	b) 6mm plaque in anastomotic area - bleeding	b) poorly differentiated adenocarcinoma with prominent signet cells
12	a) 3mm bleeding ulcerated lesion mid-colon	a) severely dysplastic tubulovillous adenoma
	b) suture area normal	b) normal histology
13	a) 3mm polyp mid-colon	a) moderately dysplastic tubulovillous adenoma
	b) anastomotic area normal	b) severely dysplastic tubulovillous adenoma
14	a) 3mm polyp mid-colon	a) moderately differentiated adenocarcinoma
	b) anastomotic area normal	b) normal histology
15	a) normal	a) minor metaplastic change of mucosa at anastomosis
16	a) ulcerated right ear tumour	a) squamous carcinoma
	b) 4mm plaque mid-colon	b) adenocarcinoma invading bowel wall from pancreas
	c) 4mm polyp mid-colon	c) papillary adenocarcinoma
	d) separate 4mm nodule mid-colon	d) moderately differentiated adenocarcinoma
	e) 4mm polypoidal lesion at anastomosis	e) severely dysplastic tubulovillous adenoma
	f) 2mm anastomotic polyp	f) severely dysplastic tubulovillous adenoma
17	a) 2mm polyp proximal colon	a) moderately dysplastic tubular adenoma
	b) anastomotic area normal	b) serosal foreign body granuloma around sutures
18	a) small left ear tumour	a) "sebaceous" cyst
	b) 3mm polyp mid-colon	b) severely dysplastic villous adenoma
	c) anastomotic area normal	c) giant cell reaction at anastomosis

APPENDIX 4

Experimental Colorectal Carcinogenesis:

Whole Crypt Metaphase Counts

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	8	9	10	7	11	6	10	9	10	12	8.90	0.54
40	19	15	22	17	22	21	26	21	24	19	20.60	1.20
60	32	31	33	38	28	36	35	32	37	38	34.00	1.05
80	34	39	42	47	44	39	41	46	37	39	40.80	1.28
100	44	51	48	46	43	45	47	38	49	39	45.00	1.32
120	59	43	51	46	50	57	60	44	51	50	51.10	1.89
140	64	56	67	54	68	73	57	63	67	70	63.90	2.01
160	80	68	84	71	77	75	62	73	61	64	71.50	2.46

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	3	6	4	6	5	4	6	6	3	5	4.70	0.45
40	8	10	10	11	15	11	10	10	9	16	11.00	0.80
60	16	22	20	21	22	14	16	15	20	20	18.60	0.96
80	31	23	24	27	25	27	32	29	29	28	27.50	0.92
100	31	35	27	29	30	36	29	33	28	33	31.10	0.96
120	38	35	33	33	34	33	36	37	34	37	35.00	0.60
140	35	41	37	38	35	37	41	38	37	37	37.60	0.65
160	50	48	39	48	47	43	42	44	41	46	44.80	1.12

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	17	16	9	13	11	12	13	11	11	10	12.30	0.80
40	14	16	21	20	20	17	15	19	19	20	18.10	0.77
60	26	31	26	34	27	28	33	31	34	34	30.40	1.07
80	34	32	38	33	39	37	35	37	38	35	35.80	0.74
100	43	42	38	42	40	44	45	39	46	41	42.00	0.82
120	53	42	46	40	38	40	42	48	42	45	43.60	1.42
140												
160	66	53	65	68	58	48	60	57	60	57	59.20	1.93

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	4	6	4	4	7	6	8	7	7	6	5.90	0.46
40	17	14	14	13	15	13	14	12	14	13	13.90	0.43
60	27	22	26	24	22	27	24	28	25	22	24.70	0.72
80	33	33	36	34	30	34	28	31	36	39	33.40	1.01
100	37	40	31	30	43	40	38	35	41	38	37.22	1.49
120	43	39	41	44	36	37	41	43	33	34	39.10	1.24
140												
160	39	43	40	42	39	45	43	46	44	46	42.70	0.84

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	9	9	10	13	9	15	11	13	11	10	11.00	0.65
40	17	16	16	18	17	21	19	18	20	19	18.10	0.53
60	23	25	23	24	28	30	26	26	25	26	25.60	0.69
80	29	27	29	28	30	33	35	34	33	32	31.00	0.87
120	43	40	38	42	36	37	39	40	39	42	39.60	0.72
140	51	52	50	49	44	52	42	50	40	43	47.30	1.44
160	51	65	57	49	63	49	48	56	47	46	53.10	2.15

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	8	9	11	12	7	12	9	10	14	10	10.20	0.66
40	15	13	16	18	15	16	17	18	17	15	16.00	0.49
60	22	23	17	21	25	20	21	21	16	22	20.80	0.84
80	24	26	23	26	28	27	26	24	20	21	24.50	0.82
120	31	22	28	28	30	31	32	27	34	33	29.00	1.11
140	32	33	36	35	33	41	33	38	43	36	36.00	1.16
160	56	44	52	41	45	38	48	34	40	43	44.10	2.07

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: None

Injections: Azoxymethane

Operative Procedure: Sham Laparotomy

Sacrifice Time: 4 Weeks

A. "Suture" Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	12	19	12	17	20	16	21	20	23	21	18.10	1.20
40	36	41	35	23	32	32	34	35	31	33	33.60	1.52
60	43	37	42	39	35	44	32	41	46	37	39.60	1.39
80	41	49	38	37	47	49	43	39	45	41	42.90	1.40
100	49	44	52	37	51	47	49	46	46	37	45.80	1.65
120	51	57	56	57	56	51	48	49	56	51	53.20	1.11
140	57	58	57	60	51	51	55	59	46	54	54.80	1.38
160												

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	21	16	9	12	15	19	23	25	18	21	17.90	1.57
40	29	35	28	33	34	31	32	30	25	33	31.00	0.97
60	40	36	35	37	45	40	37	29	41	38	37.80	1.34
80	44	42	49	34	41	43	36	43	34	39	40.50	1.52
100	38	47	49	35	42	38	41	46	34	39	40.90	1.61
120	47	43	46	48	45	44	41	47	52	49	46.20	1.00
140	56	44	61	50	56	49	52	46	51	60	52.50	1.79
160												

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	6	9	8	7	9	8	6	6	7	6	7.20	0.39
60	12	13	12	10	15	14	14	13	15	16	13.40	0.56
90	20	20	17	19	23	17	16	14	15	19	18.00	0.86
120	25	28	28	24	26	24	23	27	22	24	25.10	0.66
150	30	29	28	29	33	32	27	26	29	27	29.00	0.70
180	38	32	38	32	36	32	34	31	32	34	33.90	0.82

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	6	8	8	9	9	10	9	9	12	12	9.20	0.57
60	14	12	16	17	12	15	14	17	15	17	14.90	0.61
90	16	19	17	21	19	15	20	18	20	14	17.90	0.74
120	23	20	19	22	25	26	23	25	25	24	23.20	0.73
150	28	25	28	29	29	23	28	27	22	26	26.50	0.78
180	27	31	31	29	27	28	35	31	30	31	30.00	0.76

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	6	9	7	10	7	7	5	7	8	8	7.40	0.45
60	15	21	25	19	27	26	24	23	26	27	23.30	1.24
90	36	34	36	27	31	33	35	36	26	28	32.20	1.25
120	38	29	36	28	29	29	29	35	34	28	31.50	1.20

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	7	4	10	6	7	8	7	6	8	6	6.90	0.50
60	12	13	15	10	15	12	16	13	20	11	13.70	0.92
90	21	21	27	24	25	22	27	23	26	23	23.90	0.72
120	25	30	24	25	24	21	21	25	19	23	23.70	0.96

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	10	13	11	12	12	10	11	15	14	14	12.20	0.55
60	17	25	22	23	22	19	23	21	24	22	21.80	0.74
90	31	27	28	32	25	25	28	28	23	26	27.30	0.87
120	32	35	32	36	39	35	37	39	36	37	35.80	0.77

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	13	14	12	16	15	17	12	14	10	16	13.90	0.69
60	23	26	24	25	26	23	24	26	26	23	24.60	0.43
90	31	27	31	29	28	29	28	27	26	23	27.90	0.75
120	37	38	36	37	34	36	39	36	35	39	36.70	0.52

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: None

Injections: Saline

Operative Procedure: Sham Laparotomy

Sacrifice Time: 4 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	11	13	12	13	10	15	13	16	14	18	13.50	0.75
60	22	19	24	15	18	20	16	20	22	22	19.80	0.90
105	37	31	35	30	34	28	30	25	30	30	31.00	1.11
150	37	41	46	47	41	45	47	44	40	42	43.00	1.05

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	9	11	10	11	12	13	9	10	13	9	10.70	0.50
60	18	22	24	23	29	25	26	23	29	29	24.80	1.13
105	41	34	45	37	44	38	48	35	37	35	39.40	1.53
150	33	49	36	36	41	49	33	42	45	34	39.80	2.00

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	5	3	6	5	5	4	4	6	4	3	4.50	0.34
30												
45	15	14	16	16	21	18	13	15	10	14	15.20	0.93
60	28	29	29	21	27	21	29	25	28	27	26.40	0.98
75	33	26	34	32	36	24	31	33	33	28	31.00	1.20
90	30	35	33	35	32	36	36	38	32	40	34.70	0.96
105	41	50	43	41	51	33	42	41	39	49	43.00	1.76
120	52	44	47	39	50	45	51	44	57	43	47.20	1.67
150	46	54	55	47	49	59	60	43	52	48	51.30	1.79
180	55	62	63	46	69	57	60	69	81	54	61.60	3.09

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	2	3	2	6	5	4	4	5	5	6	4.20	0.47
30	7	8	7	9	9	10	8	7	5	8	7.80	0.44
45	14	12	15	16	13	9	14	14	12	15	13.40	0.64
60	21	16	15	15	17	16	19	18	17	18	17.20	0.59
75	20	29	21	25	27	22	19	22	27	24	23.60	1.06
90	24	22	25	22	25	24	23	24	21	30	24.30	0.80
105	26	27	33	38	24	28	30	28	30	34	29.80	1.32
120	26	24	29	26	24	29	29	26	27	28	26.80	0.61
150	31	35	38	27	39	36	35	42	37	38	35.80	1.34
180	43	47	37	46	52	55	44	40	43	46	45.30	1.67

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	3	5	7	4	7	4	6	9	7	9	6.10	0.66
30	17	14	13	18	14	21	14	19	20	18	16.80	0.90
45	24	22	27	29	25	33	23	29	24	26	26.20	1.06
60	23	30	33	28	35	33	30	32	31	34	30.90	1.10
75	38	42	41	36	37	40	35	42	37	40	36.80	1.86
90	46	44	46	35	45	41	41	36	52	45	43.10	1.60
105	54	45	47	46	51	41	43	50	41	42	46.00	1.42
120	45	48	54	57	44	47	42	56	42	51	48.60	1.78
140	42	43	49	44	42	43	61	42	45	44	45.50	1.85
160	60	47	53	41	63	52	56	54	62	44	53.20	2.36
180	56	61	57	78	86	61	54	67	80	65	66.50	3.52

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	5	6	8	9	9	12	10	8	7	7	8.10	0.64
30	9	14	14	12	12	13	10	15	10	12	12.10	0.62
45	19	15	19	15	17	18	17	17	16	18	17.10	0.46
60	35	41	26	27	30	15	25	31	23	25	27.80	2.23
75	22	27	22	24	29	27	24	25	23	24	24.70	0.73
90	31	23	31	34	28	30	26	29	32	28	29.20	1.00
105	34	30	28	29	29	31	35	31	30	26	30.30	0.84
120	32	33	35	35	30	29	35	40	33	30	33.20	1.03
140	31	25	32	32	39	36	28	27	36	38	32.40	1.51
160	23	22	44	21	27	24	22	25	24	27	25.90	2.11
180	46	43	40	34	48	44	35	43	39	36	40.80	1.51

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	17	13	16	12	20	18	15	17	15	13	15.60	0.79
40	27	29	31	31	34	28	26	27	31	26	29.00	0.84
60	37	35	35	34	38	37	33	38	38	31	35.60	0.76
100	42	46	40	45	47	39	48	40	47	42	43.60	1.07
120	54	54	53	59	52	52	63	57	57	58	55.90	1.12
160	54	57	52	67	69	58	61	55	65	57	59.50	1.83
180	75	78	77	66	68	66	74	63	62	65	69.40	1.90

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	8	6	7	9	8	7	6	7	7	10	7.50	0.40
40	14	16	16	17	18	13	16	18	17	15	16.00	0.52
60	23	26	24	28	20	22	27	25	23	28	24.00	0.85
100	27	29	32	33	28	31	30	27	29	32	29.80	0.68
120	33	42	43	40	35	39	36	38	41	39	38.60	1.00
160	44	47	39	51	38	43	46	41	52	45	44.60	1.47
180	59	61	58	54	59	54	60	61	58	57	56.10	2.06

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: None

Injections: Azoxymethane

Operative Procedure: Sham Laparotomy

Sacrifice Time: 12 Weeks

A. "Suture" Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	7	8	9	9	11	9	10	7	14	9	9.30	0.65
30	10	16	14	18	21	16	14	17	14	14	15.40	0.93
45	18	22	21	18	19	20	19	16	25	29	20.70	1.21
60												
75	28	37	27	37	29	25	26	22	33	31	25.50	1.58
90	24	32	23	26	25	18	22	20	22	21	23.30	1.22
105	28	23	35	27	32	30	31	32	24	27	28.90	1.20
120	27	35	39	37	35	38	29	34	33	32	33.90	1.21
135	46	41	45	36	39	38	45	37	48	39	41.40	1.34
150	40	47	34	49	57	43	48	36	45	44	44.30	2.11
165	39	40	38	39	43	49	36	39	61	49	43.30	2.42
180	52	78	52	62	58	54	46	71	60	56	58.90	3.01

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	9	6	10	8	9	8	10	8	11	10	8.90	0.46
30	13	8	15	19	14	17	18	10	19	15	14.80	1.17
45	10	13	20	18	17	19	16	12	17	21	16.30	1.14
60	24	17	19	16	24	21	25	19	20	14	19.90	1.16
75	30	27	25	21	23	17	29	22	26	20	24.00	1.31
90												
105	42	32	28	30	24	27	25	31	32	32	30.30	1.60
120	33	26	32	28	32	27	24	29	33	31	29.50	1.00
135	26	48	39	30	34	28	40	32	29	40	34.60	2.20
150	30	25	36	25	32	24	32	23	33	28	28.80	1.41
165	43	50	39	38	41	45	48	36	37	44	42.10	1.49
180	43	45	42	39	48	55	39	46	41	43	44.10	1.52

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Times: 12 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	5	6	5	9	5	9	8	7	9	10	7.30	0.62
40	11	12	15	12	14	13	19	15	18	14	14.30	0.82
60	11	12	20	16	14	16	17	19	19	14	15.80	0.96
80	13	17	25	19	25	23	21	24	24	20	21.10	1.24
100	23	18	17	36	20	24	18	22	24	26	22.80	1.75
120	23	24	20	19	19	20	19	23	20	15	20.20	0.83
140	21	30	24	22	24	27	26	27	25	26	25.20	0.83
160	33	36	37	34	38	34	35	35	37	37	35.60	0.52

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	4	4	2	4	3	4	5	6	5	4	4.10	0.35
40	9	7	8	9	9	8	7	7	8	10	8.20	0.33
60	3	3	4	3	6	5	6	4	6	4	4.40	0.40
80	18	16	18	16	17	15	14	19	16	14	16.30	0.54
100	11	13	12	11	14	13	11	17	11	13	12.60	0.60
120	18	19	16	21	19	20	21	18	18	17	18.70	0.52
140												
160	31	29	23	28	29	30	24	30	32	31	28.70	0.94

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	7	6	6	4	6	4	7	6	5	7	5.80	0.36
40	11	9	10	9	8	10	10	9	8	9	9.30	0.30
60	17	16	14	14	13	15	13	15	14	15	14.60	0.40
80	23	15	18	16	16	14	15	14	19	15	16.50	0.89
100	23	26	22	28	25	26	30	21	30	28	25.90	1.01
120	34	25	30	32	22	24	28	33	31	30	28.90	1.28
140												
160	34	38	35	34	33	33	37	33	35	34	34.60	0.54

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	5	8	5	4	7	6	6	5	5	6	5.70	0.37
40	6	4	6	4	9	5	6	4	3	5	5.20	0.53
60	8	10	10	7	8	7	10	9	5	7	8.10	0.53
80	11	12	10	15	9	16	14	14	16	13	13.00	0.78
100	20	18	21	19	20	25	20	15	22	21	20.10	0.82
120												
140												
160	32	38	40	36	27	29	33	35	39	38	34.70	1.38

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	8	6	13	12	7	8	10	9	11	7	9.10	0.74
40	14	19	17	15	16	21	15	18	16	15	16.60	0.69
60	28	21	31	27	33	24	31	26	28	29	27.80	1.12
80	30	36	37	26	30	36	26	32	31	39	32.30	1.44
100	36	33	38	38	33	37	25	29	33	32	33.40	1.31
120	45	34	43	36	31	41	35	35	42	41	38.30	1.47
160	46	45	48	45	41	49	44	40	43	47	44.80	0.92
180	44	46	46	48	44	56	39	42	49	44	45.80	1.45

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	7	5	4	8	5	5	6	3	5	4	5.20	0.47
40	6	10	12	11	13	14	11	14	12	14	10.70	1.24
60	13	16	18	20	14	20	23	21	17	22	18.40	1.07
80	27	32	28	22	26	30	28	26	31	23	27.30	1.02
100												
120	34	39	30	42	29	41	37	35	34	35	35.60	1.35
160	40	46	39	40	49	33	39	42	42	39	40.90	1.37
180	39	47	45	40	46	42	38	48	47	40	43.20	1.20

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: None

Injections: Saline

Operative Procedure: Sham Laparotomy

Sacrifice Time: 12 Weeks

A. "Suture" Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	6	4	5	1	2	3	2	2	1	4	3.00	0.54
45	17	14	20	13	21	17	22	12	19	20	17.50	1.11
75	26	27	21	24	22	21	31	22	24	21	23.90	1.04
90	32	27	32	29	24	29	31	25	29	28	28.60	0.86
120	42	35	36	30	38	41	38	31	40	38	36.90	1.26
135												
165	49	46	50	58	39	53	46	48	50	49	48.80	1.56
180	47	54	61	55	59	48	52	54	49	51	54.00	1.48

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	5	6	5	3	7	4	5	8	3	4	5.00	0.52
45	12	18	20	18	21	19	14	19	18	20	17.90	0.89
75	28	24	26	27	26	31	25	29	31	26	27.30	0.76
90												
120	38	36	42	38	39	33	44	41	39	45	39.50	1.15
135	39	39	41	35	40	45	39	42	43	36	39.90	0.96
165	55	49	50	50	45	45	46	48	62	46	49.60	1.68
180	52	50	47	51	56	49	55	57	48	50	51.50	1.09

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	14	11	11	14	13	12	11	10	17	15	12.80	0.70
40	23	26	31	25	28	22	18	22	23	25	24.30	1.14
60	37	29	33	28	33	28	35	34	32	35	32.40	0.99
80	41	46	45	41	39	47	40	33	43	39	41.40	1.30
100	44	48	55	54	52	50	56	55	46	47	50.70	1.36
120	65	68	63	52	58	54	54	67	59	52	59.20	1.96
160	68	82	66	73	77	64	76	60	62	70	69.80	2.26

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	5	6	7	3	7	8	9	4	6	3	5.80	0.65
40	14	18	16	11	15	15	14	19	13	14	14.90	0.74
60	19	27	24	24	26	19	25	23	27	21	23.50	0.95
80	26	34	37	35	29	38	31	35	29	30	32.40	1.25
100	41	37	42	38	36	42	43	37	44	38	39.80	0.92
120	56	52	58	50	48	57	52	50	50	47	52.00	1.20
160	58	57	71	62	70	60	62	66	69	62	63.70	1.59

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	11	14	12	11	13	15	12	15	14	9	12.60	0.62
40	18	24	22	28	23	19	20	25	18	24	22.10	1.05
60	32	34	33	29	30	35	34	32	33	30	32.20	0.63
80	42	45	37	44	47	38	40	41	43	35	41.20	1.19
100	44	48	48	57	45	48	54	46	49	51	49.00	1.27
120	61	57	69	67	57	55	63	51	52	57	58.90	1.90
160	72	62	76	61	72	79	62	69	81	67	70.10	2.27

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	8	9	6	10	6	11	14	9	11	8	9.20	0.77
40	13	18	15	14	17	23	18	19	20	17	17.40	0.93
60												
80	27	33	35	33	34	29	38	27	39	33	32.80	1.31
100	45	36	41	44	38	36	38	43	36	40	39.70	1.09
120	46	51	45	54	51	48	55	48	52	41	49.10	1.37
160	66	68	51	63	59	51	57	59	61	52	58.70	1.92

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	16	21	20	17	20	21	22	16	23	23	19.90	0.85
40	36	30	30	36	32	26	28	35	34	32	31.90	1.08
60	41	39	40	43	39	35	39	39	43	38	39.60	0.75
80	55	61	49	57	48	52	42	49	51	50	51.40	1.67
100	61	52	64	48	66	61	59	52	46	54	56.30	2.17
120	68	62	58	63	49	63	56	54	58	61	59.20	1.71
160	82	70	80	85	77	69	77	72	70	80	76.20	1.79

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	12	16	12	10	11	12	11	14	11	9	11.80	0.63
40	11	16	15	15	14	16	18	16	18	17	15.60	0.65
60	15	25	22	20	20	26	20	23	19	27	21.70	1.16
80	29	34	37	26	37	36	28	30	31	36	32.40	1.29
100	34	40	36	41	41	40	39	41	47	35	39.40	1.19
120	51	39	50	46	48	47	55	42	41	43	46.20	1.58
160	80	52	49	46	43	42	39	43	59	42	49.50	3.87

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	11	11	11	13	11	16	12	14	10	14	12.30	0.60
60	16	14	13	17	14	17	14	13	16	14	14.80	0.49
90	20	23	29	21	24	27	24	27	27	19	24.10	1.07
120	23	29	31	38	32	26	30	25	26	29	28.90	1.35

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	4	2	6	5	7	6	5	5	4	5	4.90	0.43
60	9	17	15	17	17	10	20	14	16	15	15.00	1.05
90	21	19	16	17	19	21	18	13	18	19	18.10	0.75
120	16	20	24	21	27	18	21	20	19	22	20.80	0.98

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	9	9	12	4	7	10	12	12	9	8	9.20	0.80
60	21	31	34	32	28	28	35	32	33	29	30.30	1.28
90	40	33	31	33	27	28	33	31	29	32	31.70	1.15
120	39	42	37	39	36	34	40	34	37	31	36.90	1.04

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	4	7	3	3	4	7	6	7	6	7	5.40	0.54
60	21	23	24	19	21	23	28	26	23	20	22.80	0.88
90	26	24	24	31	22	27	26	23	24	24	25.10	0.81
120	31	33	27	34	28	31	28	29	28	31	30.00	0.75

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	10	6	9	11	13	8	11	7	9	10	9.40	0.65
60	13	9	16	12	11	12	15	14	10	17	12.90	0.82
90	21	19	26	28	22	25	25	20	27	23	23.60	0.97
120	26	30	33	29	30	34	27	29	31	28	29.70	0.79

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
30	7	6	4	8	3	9	6	4	4	6	5.70	0.62
60	13	17	10	15	15	12	18	11	13	15	13.90	0.81
90	20	19	22	20	18	23	15	19	19	23	19.80	0.77
120	27	25	31	24	29	30	22	26	25	28	26.70	0.90

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	11	10	9	14	20	11	8	14	9	15	12.10	1.16
30	21	16	20	19	22	17	20	20	25	15	19.50	0.93
45	29	33	29	32	32	35	26	27	32	26	30.10	0.99
60	30	41	37	29	40	38	42	32	45	41	37.50	1.72
75	36	38	34	46	34	47	36	41	42	42	39.60	1.49
90	46	53	47	50	55	55	50	56	63	47	52.50	1.67
105	57	46	63	61	46	45	57	62	66	51	55.40	2.48
120	50	76	63	50	63	48	47	57	72	58	58.60	3.14
150	66	72	58	55	70	57	81	60	75	62	65.50	2.74
180	73	68	61	86	57	68	62	75	70	64	68.40	2.63

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	5	6	3	7	6	4	8	2	8	5	5.40	0.64
30	10	12	7	14	16	15	12	10	11	15	12.20	0.89
45	21	21	17	24	26	19	28	16	19	23	21.40	1.22
60	22	26	31	36	30	28	31	30	27	29	29.00	1.16
75	30	35	38	32	23	27	26	23	36	27	31.70	1.80
90	30	38	34	39	40	30	28	37	42	36	35.40	1.50
105	35	37	38	51	40	49	38	42	35	38	40.30	1.75
120	43	42	44	45	46	51	45	43	40	43	44.20	0.93
150	34	38	31	35	30	36	45	29	35	36	34.90	1.45
180	49	28	43	29	34	44	33	30	46	26	36.20	2.67

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	4	9	7	6	10	10	3	6	8	7	7.00	0.75
30	8	14	12	14	11	13	13	12	11	16	12.40	0.69
45	15	22	21	23	29	18	27	19	25	29	22.80	1.50
60	30	38	37	28	36	39	37	38	30	27	34.00	1.48
75	37	39	34	39	41	45	26	39	33	31	36.40	1.73
90	35	38	46	52	41	46	41	42	39	44	42.40	1.53
105	45	46	55	44	43	51	49	55	51	53	49.20	1.42
120	57	60	62	65	56	62	67	69	63	69	63.00	1.45
135	64	69	81	72	76	58	66	82	62	72	70.20	2.52
150	78	72	62	69	66	72	68	80	78	87	73.20	2.38
180	94	102	84	71	76	89	74	82	68	79	81.90	3.37

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	6	6	7	9	5	6	10	11	9	7	7.60	0.64
30	13	11	14	10	16	15	16	15	13	11	13.40	0.69
45	25	23	19	25	21	24	28	21	24	25	23.50	0.82
60	27	31	23	30	23	32	27	28	29	29	24.50	1.14
75	31	22	24	27	30	29	23	25	25	26	26.20	0.95
90	36	33	37	34	29	31	34	37	39	35	34.50	0.95
105	39	36	35	38	32	33	36	38	41	36	36.40	0.86
120	31	29	31	29	32	39	38	34	32	37	33.20	1.15
135	41	46	36	42	45	45	37	41	46	36	41.50	1.28
150	49	43	46	49	39	48	41	42	38	48	44.30	1.33
180	51	46	50	48	46	50	60	47	52	45	49.50	1.38

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15												
30	8	10	6	5	11	12	5	6	10	7	8.00	0.82
45	14	12	9	15	13	14	15	14	16	14	13.60	0.62
60	19	26	27	22	27	24	23	26	31	25	25.00	1.03
75	29	27	31	27	29	29	26	28	28	32	28.60	0.58
90	33	29	27	36	39	32	36	31	37	35	33.50	1.20
105	30	38	37	40	49	36	46	39	47	45	40.70	1.87
120	49	47	54	60	51	45	52	57	48	54	51.70	1.48
160	51	49	47	44	41	39	50	51	47	53	47.20	1.45

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	2	6	6	5	7	8	6	9	10	7	6.60	0.70
30	12	11	13	9	15	14	10	11	14	13	12.20	0.61
45	19	16	17	15	18	22	19	25	14	22	18.70	1.10
60	23	22	28	28	30	25	23	28	27	28	26.20	0.87
75	39	28	30	39	38	40	38	29	33	35	34.90	1.45
90	28	36	39	37	39	37	38	33	33	30	35.00	1.21
105	41	37	42	31	42	37	30	30	34	36	36.00	1.49
120	49	46	52	41	49	55	46	49	48	47	48.20	1.18
160	41	40	42	38	42	42	45	54	58	54	45.60	2.22

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyamide

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	7	6	7	7	10	8	7	8	9	8	7.70	0.37
40	11	10	14	13	15	11	17	18	14	17	14.00	0.88
60	23	20	19	24	19	17	21	16	22	24	20.50	0.89
80	28	35	22	25	34	32	25	27	27	30	28.50	1.33
100	40	35	39	43	38	41	43	38	45	46	40.80	1.09
120	47	48	46	40	35	42	44	48	42	45	43.70	1.29
140	56	48	54	43	42	45	45	40	46	39	45.80	1.76
160	51	48	55	51	54	49	50	42	55	44	49.90	1.39

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	5	7	9	6	10	6	8	9	6	7	7.30	0.52
40	15	16	9	18	16	11	19	16	14	14	14.80	0.95
60	22	20	21	25	25	18	24	23	22	25	22.50	0.75
80	32	33	34	30	28	36	27	30	29	28	30.70	0.93
100	35	36	31	36	27	34	28	25	39	37	32.80	1.50
120												
140	33	42	37	39	38	29	33	38	29	32	35.00	1.40
160	43	41	40	47	42	39	40	42	37	34	40.50	1.11

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Polyglycolic Acid

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 Weeks

A. Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	11	7	13	10	9	12	13	14	9	11	10.90	0.69
40	11	20	19	13	21	23	16	18	20	20	18.10	1.18
60	33	27	34	29	25	24	27	33	25	34	29.10	1.28
80	32	20	31	20	26	41	28	28	33	31	29.00	1.97
100	31	33	38	44	38	37	32	35	32	29	34.90	1.40
120	36	34	37	40	33	36	42	39	37	35	36.90	0.88
140	42	45	50	53	44	49	41	52	57	55	48.80	1.76
160	49	47	65	58	42	43	51	51	54	58	52.80	2.74

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	3	3	7	5	10	3	7	7	7	5	5.70	0.73
40	6	8	12	7	9	8	10	8	11	9	8.80	0.57
60	17	11	12	11	15	10	14	17	12	13	13.20	0.79
80	18	13	16	12	14	13	17	16	16	15	15.00	0.62
100	19	17	18	16	18	16	19	19	22	18	18.20	0.55
120	23	20	17	19	23	20	26	22	23	26	21.90	0.92
140												
160	34	35	35	30	33	27	29	26	27	28	30.40	1.12

Appendix 4 - Whole Crypt Metaphase Counts

Suture Material: Stainless Steel

Injections: Saline

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 12 Weeks

A. Perianastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	8	8	7	6	9	6	4	7	8	9	7.20	0.49
40	16	11	14	16	15	14	13	12	14	16	14.10	0.55
60	21	21	19	21	27	21	26	27	29	17	15.60	0.98
80	33	34	42	38	34	30	39	32	33	27	34.20	1.40
100												
120	35	36	35	35	36	37	25	38	31	30	33.80	1.25
160	37	39	42	45	52	36	44	35	35	38	40.30	1.73

B. Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	5	7	6	10	9	10	7	7	9	9	7.90	0.55
40	14	12	11	12	9	12	11	12	13	14	12.00	0.47
60	16	12	16	15	16	19	14	19	17	14	15.80	0.70
80	21	26	19	20	18	25	15	24	16	26	21.00	1.29
100	25	29	33	32	28	26	34	35	32	32	30.60	1.08
120	28	34	35	33	29	36	29	35	43	40	34.20	1.53
160	31	30	35	28	45	35	36	30	29	28	32.70	1.66

Dynamic Cell Population Kinetics - Reproducibility Study

Suture Material: Polyamide

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 4 Weeks

A. Recounted Values for Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	13	10	7	9	12	10	6	9	11	8	9.50	0.69
40	21	17	23	19	26	15	18	22	24	20	20.50	1.07
60	38	29	34	30	26	31	34	33	27	35	31.70	1.19
80	29	48	38	44	36	41	46	39	41	43	40.50	1.72
100	42	48	40	39	52	46	41	47	39	50	44.40	1.51
120	50	48	56	42	55	56	48	51	54	49	50.90	1.41
140	58	67	69	72	52	61	57	66	54	59	61.50	2.12
160	67	79	69	87	59	75	81	71	70	76	73.40	2.51

B. Variation from Original Values

Time (mins)	Original mean	Original sem	Recount mean	Recount sem	% variation from original mean
20	8.90	0.54	9.50	0.69	+ 6.74%
40	20.60	1.02	20.50	1.07	- 0.49%
60	34.00	1.05	31.70	1.19	+ 6.76%
80	40.80	1.28	40.50	1.72	- 0.74%
100	45.00	1.32	44.40	1.51	- 1.33%
120	51.10	1.89	50.90	1.41	- 0.20%
140	63.90	2.01	61.50	2.12	- 3.76%
160	71.50	2.46	73.40	2.51	+ 2.66%

Dynamic Cell Population Kinetics - Reproducibility Study

Suture Material: Polyamide

Injections: Saline

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Recounted Values for Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	9	5	5	0	7	8	5	3	6	7	5.50	0.82
40	6	11	9	9	6	10	7	12	6	8	8.40	0.69
60	5	12	8	6	3	6	4	8	10	7	6.90	0.86
80	20	21	13	15	19	16	16	15	13	17	16.50	0.87
100	15	10	19	15	17	12	19	16	12	15	15.00	0.94
120	23	15	21	19	19	22	16	21	19	22	19.70	0.83
160	24	29	35	25	30	29	32	30	27	31	29.90	1.03

B. Variation from Original Values

Time (mins)	Original mean	Original sem	Recount mean	Recount sem	% variation from original mean
20	4.10	0.35	5.50	0.82	+ 34.15%
40	8.20	0.33	8.40	0.69	+ 2.44%
60	4.40	0.40	6.90	0.86	+ 56.82%
80	16.30	0.54	16.50	0.87	+ 1.23%
100	12.60	0.60	15.00	0.94	+ 19.05%
120	18.70	0.52	19.70	0.83	+ 5.35%
160	28.70	0.94	29.90	1.03	+ 4.18%

Dynamic Cell Population Kinetics - Reproducibility Study

Suture Material: Polyglycolic Acid

Injections: Azoxymethane

Operative Procedure: Implantation of Sutures

Sacrifice Time: 12 Weeks

A. Recounted Values for Suture Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	9	2	13	7	10	6	9	9	7	6	7.80	0.93
30	22	13	18	20	14	21	17	12	15	20	17.20	1.12
45	19	32	29	25	28	28	32	24	35	26	27.80	1.46
60	37	22	31	32	35	23	39	33	36	30	31.80	1.78
75	41	36	34	39	44	41	29	40	39	41	38.40	1.37
90	35	51	40	48	39	39	49	42	44	40	42.70	1.63
105	58	49	62	40	54	58	41	45	45	46	49.80	2.44
120	42	52	56	45	61	53	44	58	56	54	52.10	2.02
140	50	44	43	49	41	56	48	45	45	47	46.80	1.35
160	51	65	53	59	55	68	51	45	56	50	55.30	2.23
180	60	71	49	59	69	55	78	72	66	59	63.80	2.81

B. Variation from Original Values

Time (mins)	Original mean	Original sem	Recount mean	Recount sem	% variation from original mean
15	6.10	0.66	7.80	0.93	+ 27.87%
30	16.80	0.90	17.20	1.12	+ 2.38%
45	26.20	1.06	27.80	1.78	+ 6.11%
60	30.90	1.10	31.80	1.78	+ 2.91%
75	36.80	1.86	38.40	1.37	+ 4.89%
90	43.10	1.60	42.70	1.63	- 0.93%
105	46.00	1.42	49.80	2.44	+ 8.26%
120	48.60	1.78	52.10	2.02	+ 7.20%
140	45.50	1.85	46.80	1.35	+ 2.86%
160	53.20	2.36	55.30	2.23	+ 3.95%
180	66.50	3.52	63.80	2.81	- 4.06%

Dynamic Cell Population Kinetics - Reproducibility Study

Suture Material: Polyglycolic Acid

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Recounted Values for Rectum

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	15	12	7	9	10	13	8	8	7	10	9.90	0.85
40	19	14	15	21	23	17	19	15	20	18	18.10	0.91
80	31	35	29	30	39	32	29	37	35	35	33.20	1.10
100	36	47	38	46	49	35	41	41	37	46	41.60	1.61
120	56	48	51	55	46	50	57	52	54	53	52.20	1.11
160	49	61	57	59	56	61	53	59	60	55	57.00	1.22

B. Variation from Original Values

Time (mins)	Original mean	Original sem	Recount mean	Recount sem	% variation from original mean
20	9.20	0.77	9.90	0.85	+ 7.61%
40	17.40	0.93	18.10	0.91	+ 4.02%
80	32.80	1.31	33.20	1.10	+ 1.22%
100	39.70	1.09	41.60	1.61	+ 4.82%
120	49.10	1.37	52.20	1.11	+ 2.24%
160	58.70	1.92	57.00	1.22	- 2.90%

Dynamic Cell Population Kinetics - Reproducibility Study

Suture Material: Stainless Steel

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time: 4 Weeks

A. Recounted Values for Peri-anastomotic Area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
20	13	25	14	18	19	24	21	22	23	14	19.30	1.40
40	39	29	33	25	32	26	29	31	31	36	31.10	1.35
60	30	47	39	44	36	40	42	35	42	38	39.30	1.54
80	44	48	50	42	58	51	42	53	56	49	49.30	1.75
100	51	65	59	59	64	53	61	58	54	56	58.00	1.45
120	69	60	58	65	55	63	54	54	59	63	60.00	1.59
160	75	86	64	72	77	88	69	75	68	70	74.40	2.43

B. Variation from Original Values

Time (mins)	Original mean	Original sem	Recount mean	Recount sem	% variation from original mean
20	19.90	0.85	19.30	1.40	- 3.02%
40	31.90	1.08	31.10	1.35	- 2.51%
60	39.60	0.75	39.30	1.54	- 0.76%
80	51.40	1.67	49.30	1.75	- 4.09%
100	56.30	2.17	58.00	1.45	+ 3.02%
120	59.20	1.79	60.00	1.59	+ 1.35%
160	76.20	1.79	74.40	2.43	- 2.36%

Dynamic Cell Population Kinetics - Reproducibility Study

Suture Material: Polyamide

Injections: Azoxymethane

Operative Procedure: Colotomy and Re-suture

Sacrifice Time:- 12 Weeks

A. Recounted Values for Peri-anastomotic area

Time (mins)	Counts in Individual Crypts 1	2	3	4	5	6	7	8	9	10	Mean	SEM
15	9	7	12	8	9	10	9	14	18	10	10.60	1.04
30	11	18	15	17	15	16	19	13	13	15	15.20	0.77
45	28	23	20	29	28	25	26	22	21	19	24.10	1.14
60	29	30	41	36	38	33	36	43	35	38	35.90	1.40
75	39	38	35	45	36	49	44	38	39	41	40.40	1.38
90	54	43	48	47	53	51	56	48	50	51	50.10	1.20
105	52	46	59	54	48	45	57	55	50	59	52.50	1.63
120	68	52	59	47	61	54	58	59	51	58	56.70	1.87
150	65	50	72	49	55	59	62	52	54	57	57.50	2.28
180	86	55	69	67	67	75	58	61	67	59	66.40	2.89

B. Variation from Original Values

Time (mins)	Original mean	Original sem	Recount mean	Recount sem	% variation from original mean
15	12.10	1.16	10.60	1.04	- 12.40%
30	19.50	0.93	15.20	0.77	- 22.05%
45	30.10	0.99	24.10	1.14	- 19.93%
60	37.50	1.72	35.90	1.40	- 4.27%
75	39.60	1.49	40.40	1.38	+ 2.02%
90	52.50	1.67	50.10	1.20	- 4.57%
105	55.40	2.48	52.50	1.63	- 5.23%
120	58.60	3.14	56.70	1.87	- 3.24%
150	65.50	2.74	57.50	2.28	- 12.21%
180	68.40	2.63	66.40	2.89	- 2.92%

APPENDIX 5

^{51}Cr Labelled Cell Experiments

Radioactivity of Sutures and Derived Cell Numbers

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

"In Vivo" Transfer of Tumour Cells from Rat Caecum

Assay 1

Background C.P.M.	82	1 million cells:	729489
	80		648005
	85		706267
Mean:	82	mean:	694587

1 million cells corrected for background: 694505

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	1140	1058	1523
	1312	1230	1771
	2477	2395	3448
	4465	4383	6311
polyglycolic acid	3718	3636	5236
	5447	5365	7725
	4360	4278	6160
	3326	3244	4671
stainless steel	237	155	223
	366	284	409
	180	98	141
	554	472	680
polypropylene	256	174	251
	144	62	89
	235	153	220
	123	41	59

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

"In Vivo" Transfer of Tumour Cells from Rat Caecum

Assay 2

Background C.P.M.	84	1 million cells:	229922
	86		226657
	85		224489
Mean:	85	mean:	227023

1 million cells corrected for background: 226938

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	891	806	3552
	391	306	1348
	1086	1001	4411
	715	630	2776
polyglycolic acid	3069	2984	13149
	3437	3352	14771
	2149	2064	9095
	2080	1995	8791
stainless steel	850	765	3371
	244	159	701
	169	84	370
	404	319	1406
polypropylene	167	82	361
	391	306	1348
	188	103	454
	172	87	383

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

"In Vivo" Transfer of Tumour Cells from Rat Caecum

Assay 3

Background C.P.M.	87	1 million cells:	156491
	87		155373
	87		149428
Mean:	87	mean:	153764

1 million cells corrected for background: 153677

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	574	487	3169
	708	621	4041
	917	830	5401
	1672	1585	10314
	615	528	3436
	1107	1020	6637
	1497	1410	9175
	2419	2332	15175
polyglycolic acid	2210	2123	13815
	3042	2955	19229
	2804	2717	17680
	3719	3632	23634
	1901	1814	11804
	4792	4705	30616
	4318	4231	27532
	2867	2780	18090
stainless steel	194	107	696
	135	48	312
	189	102	664
	163	76	495
	153	66	429
	173	86	560
	153	66	429
	341	254	1653
polypropylene	241	154	1002
	237	150	976
	269	182	1184
	407	320	2082
	214	127	826
	492	405	2635
	273	186	1210
	332	245	1594

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

"In Vivo" Transfer of Tumour Cells from Rat Caecum

Assay 4

Background C.P.M.	82	1 million cells:	260136
	90		262079
	84		266897
Mean:	85	mean:	263037

1 million cells corrected for background: 262952

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	806	721	2742
	1422	1337	5085
	1532	1447	5503
	2417	2332	8869
	828	743	2826
	1347	1262	4799
	835	750	2852
	1174	1089	4414
polyglycolic acid	5004	4919	18706
	3808	3723	14158
	5191	5106	19418
	5784	5699	21673
	1631	1546	5879
	6706	6621	25180
	2973	2888	10983
	2802	2717	10333
stainless steel	164	79	300
	274	189	719
	168	83	316
	224	139	529
	446	361	1373
	388	253	962
	366	281	1069
	203	118	449
polypropylene	648	563	2141
	535	450	1711
	988	903	3434
	395	310	1179
	717	632	2403
	490	405	1540
	665	580	2206
	565	480	1825

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

In Vitro Tumour Cell Adhesion Assay

Assay 1

Background C.P.M.	104	1 million cells:	273420
	99		265928
	100		262700
Mean:	101	mean:	267349

1 million cells corrected for background: 267248

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	2782	2681	10032
	2734	2633	9852
	1021	920	3442
polyglycolic acid	4502	4401	16468
	2421	2320	8681
	7315	7214	27095
stainless steel	180	79	296
	174	73	273
	226	125	468
polypropylene	193	92	344
	122	21	79
	116	15	56

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

In Vitro Tumour Cell Adhesion Assay

Assay 2

Background C.P.M.	102	1 million cells:	348521
	104		376752
	100		358266
Mean:	102	mean:	361180

1 million cells corrected for background: 361078

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	2672	2570	7116
	3759	3657	10125
	2768	2666	7381
polyglycolic acid	8835	8733	24179
	4697	4595	12722
	5382	5280	14619
stainless steel	413	311	861
	202	100	277
	202	100	277
polypropylene	341	239	662
	241	139	385
	174	72	199

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

In Vitro Tumour Cell Adhesion Assay

Assay 3

Background C.P.M.	81	1 million cells:	401403
	84		404470
	79		414900
Mean:	81	mean:	406924

1 million cells corrected for background: 406843

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	650	569	1399
	489	408	1003
	785	704	1730
polyglycolic acid	1382	1301	3198
	2312	2231	5484
	1157	1076	2645
stainless steel	234	153	376
	166	85	209
	151	70	172
polypropylene	139	58	143
	119	38	93
	122	41	101

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

In Vitro Tumour Cell Adhesion Assay

Assay 4

Background C.P.M.	89	1 million cells:	746288
	81		776934
	79		752372
Mean:	83	mean:	758531

1 million cells corrected for background: 758448

Suture Material	C.P.M.	Corrected for Background	No of Cells
polyamide	1380	1297	1710
	1546	1463	1929
	1888	1805	2380
polyglycolic acid	1859	1776	2342
	3237	3154	4159
	3621	3538	4661
stainless steel	325	242	319
	274	191	252
	396	313	413
polypropylene	472	389	513
	209	126	166
	186	103	136

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

Controls: In Vivo Tumour Cell Transfer Study

Assay 1

Suture Material	Counts per minute
polyamide	236
	209
	178
	193
polyglycolic acid	341
	490
	179
	256
stainless steel	208
	452
	232
	321
polypropylene	590
	289
	155
	183

Assay 2

Suture Material	Counts per minute
polyamide	365
	171
	261
	264
polyglycolic acid	459
	395
	411
	415
stainless steel	130
	407
	198
	142
polypropylene	698
	374
	694
	424

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

Controls: In Vitro Tumour Cell Adhesion Study

Assay 1

Suture Material	Counts per minute
polyamide	206
	184
	188
polyglycolic acid	411
	272
	201
stainless steel	135
	117
	114
polypropylene	139
	149
	99

Assay 2

Suture Material	Counts per minute
polyamide	499
	504
	197
polyglycolic acid	589
	739
	348
stainless steel	187
	219
	230
polypropylene	159
	162
	243

Appendix 5 - ^{51}Cr Labelled Mtln3 Cell Experiments

Controls: In Vitro Tumour Cell Adhesion Study

Assay 3

Suture Material	Counts per minute
polyamide	187
	163
	136
polyglycolic acid	138
	131
	137
stainless steel	159
	152
	149
polypropylene	106
	103
	97

Assay 4

Suture Material	Counts per minute
polyamide	379
	428
	-
polyglycolic acid	327
	294
	272
stainless steel	235
	237
	337
polypropylene	221
	202
	130

APPENDIX 6

Anastomotic Materials and Implantation Metastasis:

"In Vivo" Tumour Growth Experiments

Appendix 6 - Mtln3 Cell Animal Studies: Pathology

Experiment 1: Suture Materials and the Growth of Implanted Tumour at a Colonic Anastomosis

a) Polyamide Suture

Animal	Macroscopic Appearances at Autopsy
1	8mm tumour upper caecal pole. No metastases
2	Extensive tumour mass invading abdominal wall. Widespread peritoneal deposits. Blood stained ascites.
3	5mm tumour mass at anastomosis
4	Large tumour mass surrounding caecum. Peritoneal deposits. Blood stained ascites.
5	8mm bleeding tumour at caecal anastomosis. Scattered peritoneal deposits with blood stained ascites.
6	6mm tumour mass upper caecal pole
7	3mm tumour nodule at suture line. No metastases
8	5mm caecal tumour. Scattered tumour deposits over serosal surface of caecum.

b) Polyglycolic acid Suture

Animal	Macroscopic Appearances at Autopsy
1	Extensive tumour mass invading adjacent viscera. Multiple peritoneal deposits with blood stained ascitic fluid.
2	Died immediately post-operatively.
3	7mm nodular tumour arsing from anastomosis
4	Bleeding tumour mass arising from upper caecal pole. Scattered peritoneal deposits and multiple liver nodules. Blood stained ascites.
5	Two distinct nodules, 3mm and 5mm in diameter, arising from anastomotic suture line.
6	Extensive invasive tumour mass originating from upper caecal pole and invading adjacent viscera and abdominal wall. Multiple peritoneal deposits and blood stained ascitic fluid
7	Large (10mm) primary tumour mass along suture line. Scattered tumour nodules over serosal surface of caecum.
8	4mm nodular tumour mass at caecal anastomosis.

c) Stainless Steel Suture

Animal	Macroscopic Appearances at Autopsy
1	6mm nodular tumour mass upper caecal pole arising from anastomotic suture line.
2	Found dead 3rd post-operative day. Marked intra-peritoneal bleeding.
3	Extensive tumour mass encasing upper pole of caecum and invading adjacent viscera. Multiple peritoneal tumour deposits and liver metastases. Blood stained ascites.
4	8mm tumour mass arising from upper pole of caecum. Scattered tumour nodules on serosal surface of caecum
5	Very pale and listless on day 3 post-operatively. Intra-peritoneal bleeding from caecal anastomosis.

6	Multiple small nodules of tumour along entire length of caecal wound.
7	7mm tumour nodule arising from caecal anastomosis.
8	Extensive primary tumour mass involving upper caecal pole with invasion of adjacent viscera, multiple peritoneal and diaphragmatic tumour deposits and blood stained ascites.

d) Polypropylene Suture

Animal	Macroscopic Appearances at Autopsy
1	Multiple small nodules over upper pole of caecum. No gross evidence of tumour spread.
2	7mm nodular tumour arising from region of caecal anastomosis. No obvious metastases.
3	Multiple small tumour nodules along anastomotic suture line and also arising from serosal surface of remainder of upper caecal pole.
4	Extensive tumour mass encasing upper caecal pole and invading abdominal wall and small bowel. Extensive peritoneal spread with blood stained ascitic fluid.
5	Friable 7mm nodular lesion arising from upper caecal pole in region of suture line. Multiple small tumour nodules over serosal surface of caecum but no further dissemination.
6	Two separate areas of tumour growth from upper caecal pole. Four deposits of tumour in left lobe of liver.
7	8mm nodular mass of friable tumour arising form upper caecal pole. Multiple peritoneal tumour deposits with blood stained ascites.
8	Multiple small tumour nodules upper pole of caecum distributed along anastomotic suture line and over adjacent serosal surface

Appendix 6 - Mtln3 Cell Animal Studies: Pathology

Experiment 2:

Suture Materials and the Implantation of Viable Tumour Cells

1. Inoculum of 10^4 Mtln3 Cells

a) Polyamide Suture

Animal	Pathological Findings
1	Two distinct 5mm diameter nodular tumour masses surrounding sutures
2	8mm tumour nodule adjacent to sutures
3	15mm tumour mass arising from upper caecal pole at site of suture implantation
4	Two separate 7mm tumour masses. Multiple small peritoneal and diaphragmatic tumour deposits with some blood stained ascites
5	Extensive intra-peritoneal tumour with blood stained ascites
6	Invasive 10mm diameter tumour of upper caecal pole. Multiple peritoneal tumour deposits with some blood stained ascitic fluid
7	Two 6mm nodular deposits of tumour adjacent to sutures. Scanty peritoneal tumour deposits.
8	Extensive tumour mass encasing upper pole of caecum invading small bowel. Blood stained ascitic fluid.

b) Polyglycolic Acid Suture

Animal	Pathological Findings
1	3mm nodular tumour deposit adjacent to sutures
2	3mm nodular tumour arising from site of suture implantation
3	Total of 6 nodular tumour deposits, all 2mm in diameter, on upper pole of caecum adjacent to sutures
4	Extensive tumour mass involving upper caecal pole (25mm diameter). Multiple peritoneal and diaphragmatic metastases with blood stained ascitic fluid.
5	Multiple small tumour deposits scattered over serosal surface of upper caecal pole
6	20mm diameter tumour nodule arising from caecum adjacent to sutures. Scattered peritoneal deposits. Minimal ascites
7	15mm nodular tumour of upper caecal pole. Multiple tumour deposits scattered throughout peritoneal cavity. Blood stained ascites
8	Invasive tumour mass arising from caecum. Multiple tumour deposits in liver and throughout peritoneal cavity. Blood stained ascitic fluid.

c) Stainless Steel Suture

Animal	Pathological Findings
1	Six nodular deposits of tumour, each 2mm diameter and directly related to the sutures
2	10mm tumour mass upper pole of caecum. Multiple small tumour nodules over serosal surface of caecum and scattered peritoneal deposits. Minimal blood stained ascitic fluid
3	Separate 10mm and 5mm tumour masses related to site of suture implantation. Small nodular deposits on serosal surface of adjacent caecum
4	15 mm tumour mass upper caecal pole. No spread
5	Extensive invasive tumour mass encasing upper caecal pole and adjacent viscera. Widespread dissemination throughout the peritoneal cavity with blood stained ascites
6	6mm nodular tumour deposit localised to upper caecal pole. No evidence tumour dissemination
7	Multiple small tumour nodule along line of sutures and spreading to serosal suface of adjacent caecum
8	9mm tumour mass involving upper caecal pole. Scattered peritoneal tumour deposits and subcapsular deposits on liver. Minimal ascites

d) Polypropylene Suture

Animal	Pathological Findings
1	3mm nodular tumour arising from suture area
2	5mm nodular tumour adjacent to sutures
3	Separate 10mm and 15mm tumour masses upper caecal pole. Multiple peritoneal and diaphragmatic tumour deposits. Blood stained ascites
4	Extensive tumour mass (25mm) involving upper pole of caecum. Multiple intra-peritoneal deposits with blood stained ascites
5	20 mm diameter tumour mass arising from line of sutures. Multiple peritoneal tumour deposits with blood stained ascites
6	15mm tumour related to sutures. Scattered peritoneal tumour deposits and some blood stained ascitic fluid
7	Multiple tumour nodules on serosal surface of upper caecal pole adjacent to sutures
8	10mm nodular tumour of upper caecal pole. Occasional scattered peritoneal tumour deposits with minimal ascites

2. Inoculum of 10^3 Mtln3 Cells

a) Polyamide Suture

Animal	Pathological Findings
1	5mm nodular tumour arising adjacent to sutures
2	10mm nodular tumour of upper caecal pole
3	intra-operative death
4	Four separate 2mm tumour nodules directly related to site of suture implantation
5	8mm diameter nodular tumour upper caecal pole. Scattered peritoneal tumour deposits. Minimal ascitic fluid
6	Two separate 5mm tumour nodules of upper caecal pole

b) Polyglycolic Acid Suture

Animal	Pathological Findings
1	5mm nodular tumour adjacent to sutures
2	Extensive invasive tumour mass encasing upper pole of caecum. Multiple deposits throughout peritoneal cavity with blood stained ascites
3	Two separate 3mm nodular tumour deposits adjacent to sutures on upper caecal pole
4	10mm tumour nodule upper caecal pole with adjacent small nodules on caecal serosa
5	Multiple small tumour deposits over entire upper caecal pole. No evidence of further dissemination
6	Extensive intra-peritoneal tumour originating from area of caecum. Copious blood stained ascitic fluid

c) Stainless Steel Suture

Animal	Pathological Findings
1	No macroscopic or microscopic tumour
2	Single 2mm tumour nodule directly related to site of suture implantation
3	2mm tumour nodule adjacent to sutures
4	No macroscopic tumour but on microscopy small focus of tumour on caecal serosa
5	Multiple small tumour deposits on serosa of caecum surrounding site of implanted sutures
6	5mm nodular tumour mass upper caecal pole

d) Polypropylene Suture

Animal	Pathological Findings
1	Multiple small tumour deposits on caecal serosa
2	No macroscopic abnormality. Small serosal tumour deposit on microscopy
3	Normal to both macroscopic and microscopic examination
4	10mm nodular tumour upper caecal pole in relation to implanted sutures
5	Two distinct 3mm nodular tumours upper caecal pole

6 No macroscopic abnormality. Evidence of serosal tumour on microscopy

Appendix 6 - Mtln3 Cell Animal Studies: Pathology

Experiment 3

The Implantation of Mtln3 Tumour Cells at Intact Colonic Anastomoses

a) Polyamide Suture

Animal	Pathological Findings
1	Large tumour mass arising from descending colon widespread dissemination throughout peritoneal cavity with blood stained ascitic fluid
2	10mm tumour mass arising from region of colonic anastomosis. No evidence of spread
3	Widespread intraperitoneal tumour with blood stained ascitic fluid. Descending colon encased in tumour mass
4	5mm nodular tumour at anastomosis
5	8mm tumour mass arising from anastomosis
6	Large invasive tumour mass originating from descending colon. Scattered peritoneal tumour

b) Polyglycolic Acid Suture

Animal	Pathological Findings
1	20mm tumour mass arising from region of colonic anastomosis. Scattered peritoneal deposits.
2	7mm nodular tumour localised to anastomosis
3	Extensive intra-peritoneal tumour with solid mass encasing distal colon. Blood stained ascites
4	15mm nodular tumour of distal descending colon
5	Widepread intra-peritoneal tumour originating from large tumour mass of distal descending colon. Marked ascites
6	15mm nodular tumour at anastomosis with scattered peritoneal deposits.

c) Stainless Steel Suture

Animal	Pathological Findings
1	Large tumour mass arising from region of colonic anastomosis. Multiple peritoneal deposits with blood stained ascitic fluid
2	Widespread intra-peritoneal tumour. Blood stained ascites
3	20mm tumour mass arising from anastomotic suture line. Scattered peritoneal deposits
4	Widespread intra-peritoneal tumour with marked blood stained ascitic fluid
5	15mm nodular tumour localised to anastomosis
6	Solid mass of tumour encasing distal colon. Scattered peritoneal tumour deposits

d) Polypropylene Suture

Animal	Pathological Findings
1	Widespread intra-peritoneal tumour with profuse blood stained ascitic fluid
2	8mm nodular tumour localised to anastomosis
3	Large invasive tumour mass arising from distal colon, involving small bowel and associated with multiple peritoneal deposits and blood stained ascites
4	20mm primary tumour mass arising from anastomosis. Multiple small tumour deposits on surface of liver and on peritoneum
5	Extensive intra-peritoneal tumour with copious blood stained ascitic fluid
6	7mm nodular tumour localised to anastomosis

e) Sham Operated (Control) Animals

Animal	Pathological Findings
1	Normal macroscopically and microscopically
2	Normal macroscopically and microscopically
3	Normal macroscopically and microscopically
4	Normal macroscopically and microscopically
5	Normal macroscopically and microscopically
6	Normal macroscopically and microscopically

Appendix 6 - Mtln3 Cell Animal Studies: Pathology

Experiment 4

The Transplantation of Tumour Cells by Suture Materials

1. Animals Sacrificed on Day 21 Post-operatively

a) Polyamide Suture

Animal	Pathological Findings
1	Multiple small tumour nodules (1-2mm) around surrounding sutures
2	3mm nodular tumour in relation to one suture
3	20mm tumour mass arising from upper caecal pole. Scattered peritoneal tumour deposits
4	Multiple small tumour nodules surrounding site of penetration of bowel wall by sutures
5	No macroscopic or microscopic evidence of tumour
6	Widespread intra-peritoneal tumour with blood stained ascites
7	Disseminated intra-peritoneal tumour
8	Disseminated intra-peritoneal tumour

b) Polyglycolic Acid Suture

Animal	Pathological Findings
1	15mm nodular tumour of upper caecal pole
2	Multiple small tumour nodules surrounding sutures
3	Large primary invasive tumour mass. Multiple peritoneal deposits and blood stained ascites
4	Multiple small tumour nodules around sutures
5	Multiple small tumour nodules around sutures
6	Disseminated intra-peritoneal tumour. Blood stained ascites
7	Large primary tumour of caecum with peritoneal dissemination
8	Disseminated intra-peritoneal tumour with blood stained ascitic fluid

c) Stainless Steel Suture

Animal	Pathological Findings
1	No evidence of tumour
2	No evidence of tumour
3	No evidence of tumour
4	Multiple small tumour nodules around sutures
5	Multiple small tumour nodules around sutures
6	No evidence of tumour
7	No evidence of tumour
8	8mm nodular tumour of upper caecal pole

d) Polypropylene Suture

Animal	Pathological Findings
1	No evidence of tumour
2	No evidence of tumour
3	No evidence of tumour
4	No evidence of tumour
5	No evidence of tumour
6	Three small tumour nodules around sutures
7	No evidence of tumour
8	No evidence of tumour

2. Animals Only Sacrificed When Unwell

a) Polyamide Suture (Sacrifice Day 26)

Animal	Pathological Findings
1	Disseminated intra-peritoneal tumour with blood stained ascitic fluid
2	Large primary tumour mass of upper caecal pole. Multiple peritoneal tumour deposits and ascites
3	Disseminated intra-peritoneal tumour with blood stained ascites
4	Disseminated intra-peritoneal tumour with blood stained ascites
5	20mm tumour mass upper caecal pole with scattered peritoneal tumour nodules
6	Disseminated intra-peritoneal tumour with blood stained ascites

b) Polyglycolic Acid Suture (Sacrifice day 26)

Animal	Pathological Findings
1	Found dead day 26. Disseminated intra-peritoneal tumour. Copious blood stained ascitic fluid
2	Large invasive tumour mass encasing upper caecal pole. Multiple peritoneal and diaphragmatic tumour deposits
3	Extensive intra-abdominal tumour with ascites
4	Extensive intra-abdominal tumour with ascites
5	Found dead day 26. disseminated intra-abdominal tumour with ascites
6	Extensive intra-abdominal tumour with ascites

c) Stainless Steel Suture (Sacrifice Day 33)

Animal	Pathological Findings
1	Disseminated intra-abdominal tumour with blood stained ascitic fluid
2	Large invasive tumour mass arising from upper caecal pole. Multiple tumour deposits in liver and throughout peritoneal cavity
3	No evidence of intra-abdominal tumour
4	8mm nodular tumour arising from area of sutures

5	Disseminated intra-abdominal tumour
6	10mm tumour localised to upper caecal pole

d) Polypropylene Suture (Sacrifice Day 36)

Animal	Pathological Findings
1	10mm nodular tumour upper caecal pole with scattered peritoneal tumour deposits
2	Disseminated intra-abdominal tumour and ascites
3	Multiple small tumour nodules surrounding area of suture implantation on upper caecal pole
4	Disseminated intra-abdominal tumour with marked blood stained ascites
5	No evidence intra-abdominal tumour
6	15mm nodular tumour arising from area of suture implantation

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SUMMARY OF RELEVANT PRESENTATIONS AND PUBLICATIONS

The following list summarises the current presentations and publications relevant to the work described in this thesis.

Presentations

1. Sutures or Staples in Colonic Surgery?: A Prospective Randomised Comparison. **Surgical Research Society.** January, 1987.

2. Sutures and Staples in Upper GI Surgery. **1st British Symposium on Surgical Stapling.** October, 1987.

3. The Role of Stapling in Emergency Gastro-Intestinal Surgery. **1st British Symposium on Surgical Stapling.** October, 1987.

4. The Role of Suture Materials in Implantation Metastasis: An Experimental Study. **British Association of Surgical Oncology.** December, 1987.

5. Anastomotic Materials and Colorectal Carcinogenesis. **Surgical Research Society.** January, 1988.

6. The Role of Suture Materials in Implantation Metastasis: An Experimental Study. **Surgical Research Society.** January, 1988.

7. Sutures versus Staples in Gastro-Intestinal Anastomoses - A Prospective Randomised Controlled Trial. **Academic Departments of Surgery of Europe Meeting.** April, 1988.

8. Sutures versus Staples in Gastro-Intestinal Anastomoses. **Sutures en Chirurgie Viscéral.** Luxembourg, June, 1988.

9. Surgical Stapling in Emergency Gastro-Intestinal Surgery. **Sutures en Chirurgie Viscéral.** Luxembourg, June, 1988.

10. A Critical Comparison of Sutured and Stapled Large Bowel Anastomoses. **The International Society of University Colon and Rectum Surgeons.** July, 1988.

11. Do Anastomotic Sutures Promote Colorectal Carcinogenesis? **The International Society of University Colon and Rectum Surgeons.** July, 1988.

12. The Adherence of Adenocarcinoma Cells to Anastomotic Sutures. **The International Society of University Colon and Rectum Surgeons.** July, 1988.

Publications

1. McGregor JR, Galloway DJ, Bell G, Sugden BA, Munro A, George WD. Sutures or Staples in Colonic Surgery?: A Prospective Randomised Comparison (abstract). Br J Surg 1987; **74**: 540.

2. McGregor JR, Galloway DJ, McCulloch P, George WD. The Role of Suture Materials in Implantation Metastasis (abstract). Eur J Surg Oncol 1988; **14**: 350-351.

3. McGregor JR, Galloway DJ, Jarrett F, George WD. Anastomotic Materials and Colorectal Carcinogenesis (abstract). Br J Surg 1988; **75**: 603.

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6. Galloway DJ, McGregor JR, McCulloch P, George WD. The Role of Suture Materials in Implantation Metastasis (abstract). Gastroenterology 1988; **94** (2): A 141.

7. Galloway DJ, McGregor JR, George WD. Sutures or Staples in Colorectal Surgery?; A Prospective Randomized Comparison (abstract). Gastroenterology 1988; **94** (2): A 140.

8. McGregor JR, Galloway DJ, McCulloch P. George WD. Anastomotic Suture Materials and Implantation Metastasis: An Experimental Study. Br J Surg 1988-89 (in press).