CLINICAL AND EXPERIMENTAL STUDIES OF LOCOREGIONAL TUMOUR GROWTH IN
COLORECTAL CANCER.

by

Diana Helen Reinbach

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The experimental animal work explored factors such as mode of
cancer cell delivery, tissue injury and suture material used on
cancer cell adherence to an anastomosis and tumour growth. Results
show that viable circulating tumour cells implant at a colonic
anastomosis and cause tumour growth in a time dependent fashion. A
comparison of the potential for intraluminal (IL), intraperitoneal
(IP) and circulating tumour cells to adhere to a colonic anastomosis
showed that adherence is significantly higher for IL and IP
compared to circulating cells. Histological and ultrastructural
findings suggest that tumour cells from solid organ metastases
used to sites of tissue injury (colonic anastomosis) and lead to
tumour growth. Finally, results have shown that the suture material
used to repair a colotomy (tissue injury) is more important than the
mucosal injury itself in tumour cell adherence, with braided material
(milk) adhering significantly better than monofilament suture
material (polypropylene).

The role of growth factors was evaluated in a series of
experiments: the findings show that the vector used for growth
ABSTRACT

Local recurrence has been reported in 10-25% of patients following potentially curative resection of colorectal cancer and is a major factor limiting survival. The causes of locoregional recurrence have not been fully defined and this work investigates some factors that may be important.

The clinical part of this project studies the effect of a surgeon’s specialty interest on specimen resection length. Results show that surgeons with an interest in colorectal surgery perform a more extensive resection of left sided colon and rectal cancer than surgeons with gastrointestinal or other surgical interests.

The experimental animal work explores factors such as mode of tumour cell delivery, tissue injury and suture material used on tumour cell adherence to an anastomosis and tumour growth. Results show that viable circulating tumour cells implant at a colonic anastomosis and cause tumour growth in a time dependant fashion. A comparison of the potential for intraluminal (IL), intraperitoneal (IP) and circulating tumour cells to adhere to a colonic anastomosis demonstrates that adherence is significantly greater for IL and IP tumour cells compared to circulating cells. Further experimental findings suggest that tumour cells from solid organ micrometastases may seed to sites of tissue injury (colonic anastomoses) and lead to tumour growth. Finally, results have shown that the suture material used to repair a colotomy (tissue injury) is more important than the tissue injury itself in tumour cell adherence, with braided material (silk) adhering significantly more tumour cells than monofilament suture material (polypropylene).

The role of growth factors was evaluated in a series of experiments: the findings show that the vector used for growth
factor delivery (bovine collagen) promotes tumour growth in this animal model. There was no evidence of tumour promotion due to growth factors alone, however, the overwhelming effect of collagen was such that it may have masked any effect from growth factors.
ACKNOWLEDGEMENTS

I am indebted to a large number of people who have contributed towards the work described in this thesis.

First and foremost thanks must go to Mr Patrick J O'Dwyer, Senior Lecturer in the University Department of Surgery of the Western Infirmary, Glasgow for his untiring support, valuable advice and continual interest.

I must also express my gratitude to Professor Pierre Guillou my supervisor in the University of London and to Professor David George of the University Department of Surgery at the Western Infirmary in Glasgow for their assistance and support whilst carrying out my thesis work.

I would like to thank Mr John McGregor a colleague in the University Department of Surgery for his advice, particularly with cell culture techniques. Drs Stephen Dahill and Iain Brown of the University Department of Pathology, Western Infirmary, Glasgow for their expert analysis of histological and immunocytochemistry results respectively in the animal studies. Dr Robin Leake, Department of Biochemistry, University of Glasgow for advice and invaluable assistance given with radioimmunoassay of growth factors and Dr Gordon Murray, Senior Lecturer in Medical Statistics in the Department of Surgery for assistance in the statistical interpretation of findings.

I acknowledge and thank several members of the technical staff of the University Department of Surgery. Mr Colin Hughes was responsible for animal management, Mr Alan Fleming for assistance with intracarotid injection of tumour cells and Mr Alan McIntyre for advice in the technique of radioisotope use.
My thanks are also due to the technical staff of the Department of Pathology who contributed to the preparation of histological slides and to Ms D Aitken and S A Cameron for the scanning electron micrographs.

This work was funded by grants from the research support group of the Greater Glasgow Health Board and from the Royal College of Surgeons of Edinburgh.

Lastly but not least I thank Mrs Jean Kennedy for her expert secretarial skills.
DECLARATION

The compilation of this thesis has been entirely my own work. It has not been previously submitted. The clinical and experimental data were obtained from August 1990 to July 1992 whilst I was employed full time by the Western Infirmary, Glasgow, as Gastroenterology Research Registrar.

The majority of work in this thesis is original. Some experiments have repeated previous work using a different cell line and animals to confirm that the results are reproducible and to allow valid comparisons with experimental data in this thesis (Experiment 1:- Viable circulating tumour cells and anastomotic tumour growth). Also, some of the comparisons have not been made before (Experiment 2:- Adherence of intraluminal, intraperitoneal and intraarterial tumour cells to colonic anastomosis and normal colon). The remaining experimental designs were entirely original in concept although established experimental methodologies have been utilised (Experiment 3:- Can tumour cells from distant micrometastases cause anastomotic tumour growth; Experiments 4.1, 4.2:- Relative importance of tissue injury and suture material in tumour adherence; Experiments 5.1-5.3:- The role of growth factors).

I took advice from a number of sources when designing the project and benefitted from the past experience of other researchers both external and internal to the department. As a result I was able to introduce the use of growth factors in an animal model to the department and the assessment I made of colonic resection and surgical specialty interest has been expanded to an ongoing clinical trial.
The following procedures were performed exclusively by the author: Collection and analysis of all clinical data. Animal operating:- laparotomy and colonic anastomoses, sacrifice and post mortem analysis of rats, tissue sampling, coding of specimens, freezing and storage of colonic specimens in liquid nitrogen prior to radioimmunoassay and immunohistochemistry. All cell culture techniques including radiolabelling of cells, assessment of samples for radioactivity. Preparation and application of growth factors in a collagen gel, assessment of histopathology slides by linear planimetry, statistical analysis of growth factor work and evaluation of all results.

As discussed in the acknowledgements I had some assistance with intracarotid injection of tumour cells, remaining statistical analysis and radioimmunoassay work.

The histopathological assessment of animal work was performed by one histopathologist who was unaware of the code of the sample. However, all analysis of the results was performed by the author. Immunohistochemistry was performed by Dr Iain Brown and scanning electron micrographs by Ms D Aitken and S A Cameron.

I certify that I have personally seen all the reference articles quoted except for a few of the earlier historical articles.
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CHAPTER 1

LOCOREGIONAL TUMOUR RECURRENCE IN COLORECTAL CANCER
1.1 INTRODUCTION

Colorectal cancer remains the second most frequent cause of cancer related deaths in the Western world. Only carcinoma of the bronchus in males and breast in females are more prevalent. It accounts for 14% of all cancer related deaths from malignant disease (Silverberg & Lubera, 1988) and in England and Wales approximately 8,500 men and 9,000 women die of the disease each year (OPCS 1990 DH2 No 17). In common with other malignancies the stage of the disease and extent of spread of the large bowel cancer is a major factor in determining the outcome (Whittaker et al, 1976; Goligher, 1984a). At the time of presentation approximately 50-60% of patients with large bowel cancer are thought to have undergone 'curative' resection (Phillips et al, 1984a; Gill et al 1978; Slaney 1971; Umpleby et al, 1984a). Of these patients, the 5 year survival rate has been reported between 50-60% (Phillips et al, 1984a; Umpleby et al, 1984a; Jatzko et al, 1992; Galanduik et al, 1992; McArdle et al, 1990) and for those that survive this time most will have been cured, however a small percentage of 3-5% will go on to develop metachronous tumour in the remaining colon (Hughes et al, 1981). Over the past 30 years there has been little change in the outcome despite advances in surgical, medical, radiation oncology and anaesthetic technique. For all patients who undergo potentially 'curative' surgery attempts at improving long term survival should therefore focus on methods of reducing the risk of developing recurrent disease.

1.2 PATTERNS OF RECURRENT DISEASE

Recurrence of colorectal cancer is either distant/systemic recurrence or local recurrence or a combination of both.
1.2.1. DISTANT DISEASE

The development of distant recurrence is most frequent in the liver, 15-20% of all patients coming to surgery with colorectal cancer will have overt metastatic liver disease (Oxley et al, 1969; Stearns et al, 1979; Bengtsson et al, 1981; Neilson et al, 1973). In a clinical study of 71 patients who had undergone potentially ‘curative’ colorectal cancer surgery, 24% (17) had occult hepatic metastases detected by post operative computerised tomography (CT) and ultrasound scanning (US) and of these only one patient survived 5 years (Finlay and McArdle, 1986). Autopsy studies have shown that 50-80% of patients who die from colorectal cancer have hepatic metastases (Cedermark et al, 1977; Welch and Donaldson, 1979; Berge et al, 1973; Shindo, 1974). Once established the prognosis is poor, with median survival of between 5-9 months (Jaffe et al, 1968; Woods et al, 1976; Wanebo et al, 1978), although latterly encouraging results from resection of isolated liver metastases or metastases limited to one lobe have been reported (Sugihara et al, 1993; Savage and Malt, 1992).

Metastatic disease limited to the abdominal cavity such as diffuse intraperitoneal seedlings is always associated with concomitant local failure and/or distant metastases and rates of 7-26% following ‘curative’ resection have been reported (Chung et al, 1988; Gunderson et al, 1985). Sugarbaker (1989; 1991) has looked extensively at treatment of peritoneal carcinomatosis. His work has suggested that cytoreductive surgery followed by intraperitoneal chemotherapy can produce long term disease free survival in a disease that previously had a uniformly lethal outcome. This correlated with low tumour aggressiveness such as pseudomyxoma peritonei of colonic...
origin, adequate cytoreductive surgery and the use of intraperitoneal chemotherapy.

The lung is the most common extra-abdominal site of metastases from colorectal cancer. Autopsy studies have found pulmonary metastases in 30-50% of patients with colorectal cancer (Welch and Donaldson, 1979; Shindo, 1974). In most cases these are multiple and associated with disseminated deposits elsewhere. In a minority they are isolated either singly or as multiple deposits within a lobe and in this group surgical resection offers good long-term prospects (Hughes et al, 1981; Pollard et al, 1989; Wilkins et al, 1989), with survival up to 58 months following surgery reported (Mori et al, 1991). Less common sites of distant recurrence include bone, brain or spinal cord and organ metastases to adrenal, thyroid, pancreas and spleen (Welch et al, 1979; Shindo, 1974). Outlook is poor and the prospect of cure is unlikely as disease is usually widespread by this time. A major advance in the treatment has been the combination of 5-Fluouracil and Folinic Acid, with a highly significant improvement in the objective tumour response in six of seven trials. The results of two of the studies have also demonstrated significant improvements in patient survival (Mayer et al, 1989). However, other treatment options of radiotherapy or surgery offer palliation only rather than cure (Hughes et al, 1989; Pollard et al, 1989; Williams et al, 1985; Welsh and Donaldson, 1978).

1.2.2. LOCOREGIONAL DISEASE

The incidence of clinically reported local recurrence ranges from 2.6% (Heald and Ryall, 1986) to just less than 40% (Pahlman and Glimelius, 1984) for the rectum and between 10-25% for colonic cancer.
(Gill and Morris, 1978; Slaney, 1991; Umpleby et al, 1984a; Jatzko et al, 1992; Galanduik et al 1992; Rich et al, 1983; Willett et al, 1984; McDermott et al, 1985). These differences may arise because of varying definitions of local recurrence and methods of diagnosis. For the purpose of this thesis the definition used is that of the large bowel cancer project: local recurrence is said to occur when there is convincing evidence of recurrence of the cancer at an anastomosis, in the region of the anastomosis, in the abdominal/perineal wound, colostomy or drain site but NOT hepatic or peritoneal seedlings (Phillips et al 1984a).

Post mortem studies and second look laparotomy series suggest that clinical series underestimate the true incidence of local failure (Gunderson and Sosin, 1974; Wangensteen et al, 1954; Gilbert et al, 1984; Gunderson et al, 1984). In the Minnesota reoperative series, results were taken from patients who were reoperated on at 6-12 monthly intervals following 'curative' resection of Dukes B or C rectal cancer: 48% of recurrences were locoregional alone, 8% were due to distant metastases alone, and 44% both distant and local disease (Gunderson and Sosin, 1974) and suggests that the more thoroughly recurrence is sought by special investigation the more frequently it will be found.

The reported incidence of anastomotic recurrence is also variable from 1.6% (Hardy et al, 1971) to 25% (Judd and Ballagie, 1952), although it is more generally reported to develop in 5-15% of patients following potentially curative colorectal surgery (Jatzko et al, 1992; McDermott et al, 1985; Wangensteen et al, 1954. Cole, 1952; Beal and Cornell, 1952; Wheelock et al, 1959; Wright et al 1969; Hojo, 1986; Stulc et al, 1986). Aste et al (1982) undertook a clinical study utilising colonoscopy in regular post operative
review and found an 18% suture line recurrence rate. This variability seems to reflect differences in definition.

Tumour growth at an anastomosis may indicate recurrent disease in the pelvis and is not synonymous with isolated anastomotic tumour growth, a mechanism proposed by Labow et al (1975) and previously recognised by others (Hardy et al, 1971; Morson et al, 1963). Interestingly, anastomotic recurrence is reduced at anastomoses when one side is ileal. Wright et al (1969) report an anastomotic recurrence rate of 0.7% in 272 patients following ileocolic anastomosis compared to 12.2% in 586 patients after colocolic anastomosis.

Although the exact incidence remains unclear, it is apparent that locoregional tumour growth is a major factor limiting survival in colorectal cancer. It is said characteristically to develop early, the majority (70-80%) of local recurrences present within two years of operation (Umpleby and Williamsom, 1987; Tyndale et al, 1984; Welch and Donaldson, 1978; Polk and Spratt, 1971). Phillips et al (1984a) found the risk of local recurrence increased rapidly with time to reach a peak between 9-12 months of 2%, falling thereafter to a steady rate of 0.75-1% per three month period over the following four years. The high percentage seen in the first two years mainly being a reflection of the greater numbers at risk early in follow-up. At least 5% of local recurrences present after 5 years (Cass et al, 1976) and the diagnosis is generally regarded as carrying a poor prognosis.

The reported 'curative' re-resection rates in selected cases of less than 15% confirms the poor outlook for local recurrence (Welch and Donaldson, 1978; Polk and Spratt, 1971; Gunderson et al, 1974; Beart and O'Connell, 1983; Willett et al, 1984; Welch and Donaldson,
1978). Stulc et al (1986) reported median survival of 5 months in those who had no further surgery, 14 months for those who had a palliative resection and 23 months for those who underwent 'curative' resection. Vassilopoulous et al (1981) have shown that complete re-resection of recurrent tumour may be associated with an acceptable long-term prognosis in selected cases: in this series a 5 year survival of almost 50%, with a median survival time of 59 months.

Since the majority of regional recurrences occur within 24 months of a potentially curative resection, it has been argued that follow-up studies should be intensive during this period to maximise protection of recurrence at an early and potentially curable stage (Vignati and Roberts, 1993; Kelly and Daly, 1992; Ovaska et al, 1989); however, the value of such a regime is debatable. In a prospective follow-up study of patients who had undergone potentially curative resection of colorectal carcinoma, Schiessal et al (1986), demonstrated that 41% of all recurrences could be diagnosed by carefully planned follow-up, which included regular clinical examination, assessment of carcinoembryonic antigen (CEA) levels and colonoscopy: with a potentially curative re-resection rate of 40%. In contrast, Tornqvist et al (1982) and Camunas et al (1991) assessed the impact of intensive follow-up and concluded that even though it detected recurrence more frequently before symptoms developed, it did not affect the rate of curative re-operation and therefore was not useful.

The role of CEA as an early indicator of recurrent disease remains contentious. Northover (1986) concluded that CEA, although not uniformly sensitive or specific, is a reliable marker of recurrent disease. The rate of increase of slope of CEA concentration may be more important than the absolute value:
patients with a steeper slope are more likely to have disseminated disease than patients with a gentler slope (Staab et al, 1978; Wood et al, 1980; Boey et al, 1984). Several studies have suggested improved selection of patients for curative re-resection of locally recurrent cancer can be achieved by monitoring CEA levels after primary resection (Attiyeh and Sterns, 1981; Minton et al, 1984; Staab et al, 1985). Martin et al (1985) looked at 146 CEA directed second look laparotomies: 58% of patients found to have an elevated CEA underwent curative re-resections and the 5-year survival rate was 23.9%. However, the survival curve of the CEA determined re-operative group and the symptomatically determined group were the same. More recently, Martin and Carey (1991) have reported the use of radioimmunoguided surgery (RIGS) improves selection and survival of those operated on for cure, with 5-year survival rates of 60%. This approach is limited by the specificity of the available monoclonal antibodies and it remains to be conclusively proved that there is a long-term improvement in survival of locoregional recurrence by CEA monitoring.

There is considerable evidence to support the polyp cancer sequence (Heald and Bussey, 1975; Morson et al, 1974; Bussey et al, 1967; Muto et al, 1975): patients with multiple carcinomas are more likely to have associated polyps and there is an increased likelihood of a second carcinoma developing if polypi are associated with a carcinoma (Entline, 1975; Lasser, 1978; Hancock, 1975; Heald and Bussey, 1975; Cunliffe et al, 1984). The role of post-operative colonoscopy for detection of missed synchronous and metachronous tumours is well established; its use in the detection of local recurrence, appears limited and optimum time intervals for examination have not been fully defined. Finan et al (1987)
concluded that full examination of the colon in all patients presenting with a primary colorectal carcinoma is mandatory and should be by pre or per-operative colonoscopy. It is widely accepted that a colonoscopy 3-6 months post-resection should be performed to identify missed synchronous tumours: followed by periodic colonoscopic surveillance at variable time intervals for patients found at the time of curative resection to have several adenomatous polyps, especially if one was larger than 1 cm or associated with villous change (Vignati and Roberts, 1993; Kelly and Daly, 1992; Granqvist and Karlsson, 1992; Kagan and Steckal, 1991; Greg and Millar, 1989; Michael et al, 1988). Barlow and Thompson (1993) have suggested that post operative colonoscopy is unlikely to benefit the majority but may be extremely important for young fit patients with continuing polyp formation who are at high risk of developing metachronous tumours.
1.3. GEOGRAPHICAL INCIDENCE.

The incidence of locoregional recurrence does not seem to vary widely between differing countries in the Western world. The United Kingdom large bowel cancer project reviewed 2336 patients who underwent 'curative' resection of large bowel cancer (Phillips et al, 1984a). Follow up information was available on 95% and of these patients 14% developed a local recurrence. Galanduik et al (1992) assessed data on 818 patients from the Mayo clinic, Rochester and Creighton University, Omaha, USA. who had undergone 'curative' resection for Dukes B2 or C carcinoma of the colon and rectum. Locoregional recurrence alone was found in 41 (12%) patients. Jatzko et al (1992) in Austria assessed 575 patients, 479 of whom had undergone 'curative' resection of colorectal cancer. The recurrence rate varied within the study dependant on site of tumour, a recurrence rate of 4% was noted for colonic resection whereas it rose to 13.4% in rectal cancer treated by anterior resection and 14.3% in those treated by APR. McDermott et al (1985) from Melbourne, Australia reported on a series of 1008 patients, 107 (11%) developed local recurrence without systemic spread.

1.4. FACTORS PREDISPOSING TO LOCAL RECURRENCE.

1.4.1 PATIENT ATTRIBUTES

Dukes (1940) examined the incidence of lymph node metastases in 1000 rectal cancer specimens and found it to be 72% in those less than 40 years and 51% in those aged 40-59 years, suggesting a pathologically more advanced tumour in the younger age group.

A consistently higher local recurrence rate has been reported following curative resection in patients below the age of 60 years (Moosa et al 1975; Rees et al, 1975). Malcolm et al (1984) examined
recurrence patterns in 285 patients following curative resection of colorectal cancer. The recurrence rate was 38% in the younger patient (<60 years) compared to 24% in those over 60 years. Galloway et al (1984) examined retrospectively the management of colorectal cancer in 481 consecutive patients, 7.5% (36) of whom were less than 50 years of age. They found that a higher proportion of the younger group (<50 years) had metastatic disease at the time of treatment. Histological grading of tumours did not differ from the older group (>50 years), however only 17.5% of the younger group presented within three months of symptoms compared to 50% of the older group. Interestingly, the median survival was significantly better in the younger group (p<0.02) and may be a reflection of the smaller numbers.

A report from Umpleby and Williamson (1984a) concentrated on the features of large bowel cancer in 85 patients under 40 years: 14% (6) developed anastomotic recurrence out of a total of 42 patients who underwent a restorative procedure. This is relatively high when compared to other studies. However, outcome was similar to that of all ages, any unfavorable pathological features being balanced by improved survival following emergency procedures.

Unfortunately, only the study by Galloway et al (1984) took account of other prognostic variables such as Dukes staging and tumour histology. None of the other studies described above standardised their groups for the influence of such prognostic variables and it is therefore difficult to draw firm conclusions from this data.

The situation was clarified by the large bowel cancer project (Phillips et al, 1984a). Results indicated that local recurrence decreases with age (p<0.01) even after standardising each group for
sex and Dukes staging, thus providing good evidence that age exerted an independent influence on local recurrence. In contrast to this, Chapius et al (1985) in a multivariate analysis of survival of 709 patients with large bowel cancer found that the survival worsened progressively in patients over 61 years and that women had a marginally better 5 year survival rate than men. More recently, Wiggers et al (1992) concluded that neither age nor sex had prognostic significance for survival, particularly when the analysis is restricted to disease-related survival and that death from other causes significantly affected overall survival.

1.4.2. TUMOUR CHARACTERISTICS.

1.4.2.1. Tumour stage.

Numerous studies have shown that tumour stage is the most important influence on the development of local recurrence in both colonic and rectal tumours. Rich et al (1983) looked at 142 patients who underwent curative surgery for rectal cancer. Local recurrence was documented in 8% stage A tumours, 31% stage B tumours and 50% stage C tumours. Several prospective studies have since confirmed this linear relationship between stage and local recurrence (Phillips et al, 1984a,c; Feil et al, 1988; Rubbini et al, 1990). In a retrospective study looking at 1008 patients who underwent curative resection of rectal cancer, McDermott et al (1985) found similar findings for tumour stage and local recurrence and this was statistically significant (p<0.001). Willett et al (1984) have confirmed tumour stage has the same effect in cases of colonic cancer.

In addition, many authors (Moosa et al, 1975; Dukes and Bussey, 1950; Spratt & Spjut, 1967; Jass et al, 1986; Wolmark et al, 1986,
Grinnell, 1939) have demonstrated that the number of lymph nodes involved influences local recurrence and survival regardless of depth of invasion.

1.4.2.2. Grade.

Dukes (1940) showed that tumour grade is closely correlated with tumour stage and several investigators have shown a relationship between risk of local recurrence and histological grade (Olson et al, 1980; Chung et al, 1983; McDermott et al, 1984). However, a higher proportion of tumours with advanced stage will exhibit high grade features. From published studies it is difficult to be certain that tumour grade has an independent effect from tumour stage. The large bowel cancer project (Phillips et al, 1984a) corrected for Dukes staging and found as a result histological grade to be independently insignificant in its effect on local recurrence.

1.4.2.3. Tumour invasion and fixity.

Many authors have shown in retrospective and prospective studies that the groups at highest risk of local failure were those with extension through the bowel wall (Whittaker and Goligher, 1976; Lockhart-Mummery, 1976; Michelessi et al, 1990; Morson et al, 1963; Moosa et al, 1975; Rao et al, 1981; Gunderson & Sosin, 1974;) Chung et al (1983) studied 251 patients with colorectal cancer following curative primary resection to determine patterns of failure. The groups at highest risk of local failure were those with extension of tumour through the bowel wall whether nodes were involved or not. Rich et al (1983) and Cass et al (1971) similarly found that depth of tumour penetration into the bowel wall influenced local recurrence rates. Shepherd & Jones (1971) demonstrated that for patients with
Dukes' B tumour there was a steady worsening of prognosis with increasing local spread beyond the bowel wall.

The large bowel cancer project (Phillips et al, 1984a) demonstrated an association between tumour mobility and local recurrence: freely mobile tumours having a local recurrence rate of 11% compared with a local recurrence rate of 21% in others. Both Wood et al (1981) and Habib et al (1983) found that tumour fixity was an important prognostic factor for survival when due to direct tumour invasion, a finding confirmed by others, but not when due to local inflammatory reaction (Durdley & Williams, 1984)

1.4.2.4. Tumour perforation and obstruction

Slanetz (1984) studied the effect of inadvertent intraoperative perforation on survival and recurrence in colorectal cancer in 174 curative resections with spillage: overall 5 year survival was 29% and in 67 patients where the cancer itself was disrupted during dissection the 5 year survival fell to 14% in the colon and 9.3% in the rectum. Local recurrence occurred in 65% with spillage through the tumour and 87% of Dukes C cases perforated during surgery.

Patients with obstructing tumours have been reported to have an increased risk of local recurrence (Phillips et al, 1984a) and poor survival (Chapius et al, 1985). The large bowel cancer project (Phillips et al, 1984a) demonstrated tumour obstruction and perforation independently exert an effect on local recurrence rates after standardisation for age and Dukes classification. In contrast, in a prospective randomised investigation of obstructing tumours, Garcia-Valdecasas et al (1991) found that these tumours were associated with an advanced tumour stage. After correcting for this in a multivariate analysis, obstruction disappears as a factor
predictive of local recurrence. It seems the poor outcome in obstructing colorectal cancer may be due to its locally advanced nature.

1.4.2.5. Site of tumour.

Most investigators have shown that tumour site influences local recurrence rates, particularly in the rectum (Gilchrist and David, 1947; Stearns and Binkley, 1953; Morson et al, 1963; Labow et al, 1975; Theile et al, 1982). Tumours below the peritoneal reflection have been shown to have a higher local failure rate with incidence of local recurrence increasing with decreasing distance from the anal verge (Stearns & Brinkley, 1953; Moosa et al, 1975; McDermott et al, 1985; Theile et al, 1982). Rich et al (1983) reviewed results of surgical treatment alone for 142 cases of carcinoma of the rectum and rectosigmoid colon. They were able to show a high pelvic failure rate (27%, 25/92) is found in the low (extraperitoneal) rectal tumours. Interestingly in this study, tumours above the peritoneal reflection treated by any operation had a higher local failure rate (59%, 16/27), however this may be a reflection of the smaller numbers in this group. In contrast, others have shown no difference in incidence of local failure according to location within the colon, but did not look at different sites within the rectum (Chung et al, 1983; Cass et al, 1976; Phillips et al, 1984).

Most surgeons would still regard the risk of local failure as being greatest when operating deep in the pelvis. At least in part, this may be due to the easier detection of local recurrence at these lower levels compared to the peritoneal cavity. However, it may also reflect anatomical difficulties and surgeon related factors rather than effect of the tumour itself (Goligher, 1984b).
1.5. PROCEDURE RELATED VARIABLES.

1.5.1. Operative technique.

The earliest attempts at rectal excision were made via a perineal approach. Using this technique Miles (1908) reported a 3 year recurrence rate of 95% and from post mortem studies he concluded that an upward zone of spread exists which was impossible to clear. This lead to the development of radical procedures including Miles description of a combined abdomino-perineal approach to excision of the rectum (Miles, 1908; 1910). Once anaesthetic and medical advances made it a safer procedure in the 1930's, it became standard practice for rectal and rectosigmoid cancer.

The previous four decades have seen a shift away from the combined abdomino-perineal excision (APR) of lesions in the mid/low rectum in favour of sphincter preservation and restorative procedures with the attendant advantages of avoidance of a stoma. In St Marks hospital the frequency with which restorative procedures were performed (mostly anterior resection) increased gradually from 16.9% of all cancer operations for the 4 year period 1948-52 to 41.9% for the period 1968-72 (Lockhart-Mummery et al, 1976). This change in surgical treatment has not compromised outcome. It has been conclusively demonstrated that the operative mortality is comparable to or lower than that associated with APR (Whittaker et al, 1975; Hughes et al, 1980; Williams, 1984). In addition the quality of life following a restorative procedure is better than following an APR with its resultant stoma (Williams and Johnston, 1983).

With the exception of the large bowel cancer project (Phillips et al, 1984a) current evidence suggests that when recurrence and survival are correlated with tumour stage, differentiation and the
extent of local spread, sphincter saving resection (SSR) can be regarded as being as curative as abdominoperineal resection (APR) (McDermott et al, 1985; Rich et al, 1983; Nicholls et al, 1979; Jones and Thomson, 1982; Colombo, 1987 Phiels et al, 1983; Kirwan et al, 1988; Leff et al, 1985; Gillen and Peel, 1986; Williams, 1984). The large bowel cancer project (Phillips et al 1984a,b) demonstrated a small but significant increase in the risk of local recurrence associated with sphincter preservation and the statistical significance of this was increased if the patients were standardised for Dukes classification. In this study 848 patients survived 'curative' resection which was either an anterior resection or APR on whom follow up information was available: of these 124 (15%) developed a local recurrence, 18% of patients who had anterior resection developed local recurrence compared with 12% of those undergoing APR (p=0.02, $X^2$ 5.45).

The wide availability of the circular stapling devices has enabled many surgeons to perform restorative operations. Initial reports on local recurrence rates following stapled anterior resection suggested a higher local recurrence rate with this technique. Hurst et al (1982) reported 11 early anastomotic recurrences following 34 stapled low anterior resections. All of these occurred in patients with locally advanced lesions and lymph nodes although resection margins were clear of tumour. Anderberg et al (1984) described a local recurrence rate of 24% in a series of 38 rectal cancer patients similarly managed and Bisgaard et al (1986) highlighted the risk of potentially increased local recurrence following low anterior resection with a stapled anastomosis. Other studies have reported similar findings (Reid et al, 1984; Rosen et
al, 1986) however, they are difficult to evaluate as numbers are small and the studies were not controlled or randomised.

The majority of more recent articles have not confirmed the initial results of higher than expected local recurrence rates following stapled anterior resection (Leff et al, 1985; Rosen et al, 1985; Wolmark et al, 1985; Kennedy et al, 1985; Odou et al, 1986; Amato, 1991). Kennedy et al (1985) found that the use of the circular stapler was associated with a significant reduction in the number of patients with middle rectal cancers requiring APR, their local recurrence rate before and after introduction of this technique was similar. Similarly, Odou et al (1986) found no significant change in local recurrence rates following the introduction of circular stapling even though the frequency with which low anterior resection was performed increased from 42% to 62% of all procedures. Leff et al (1985) found no difference in local recurrence rates when comparing stapled and sutured anastomoses despite the stapler being used to treat lower rectal lesions. Wolmark et al (1985) used data from two multicentre prospective randomised trials of adjuvant chemotherapy in colorectal cancer. No difference was found in terms of local recurrence rate or survival, however randomisation was not sutures vs staples but of various radio and chemotherapeutic regimes.

In marked contrast, a randomised prospective study assessing sutured vs stapled colorectal anastomosis in 294 patients undergoing curative resection of colorectal cancer found a significant difference in local recurrence rates ($p<0.05$): 22% in the sutured group compared to 12% in the stapled group (Akyol et al, 1991). There was also a significantly higher cancer specific mortality in the sutured group ($p<0.01$) and it was suggested that in colorectal cancer surgery the use of the stapling instrument for anastomotic
construction could be associated with a reduction in the incidence of recurrence and mortality rate by as much as 50%. Randomisation occurred only after the surgeon was satisfied that either technique was applicable thus ensuring comparability of tumour clearance between both groups, however a criticism of the study is that no account is taken of surgeon related variables. An update from the most recent data in this study has confirmed that the increased incidence of local recurrence and cancer specific mortality persists (personal communication, unpublished data, J Docherty, 1993).

1.5.2. Surgeon related variable.

The large bowel cancer project assessed local recurrence rates for different grades of surgeon and found that there was no statistically significant difference in local recurrence as regards grade of surgeon (Phillips et al, 1984a). However, there was a wide range of local recurrence rates between individual consultant operators, with rates ranging from less than 5% to greater than 20%. After stratification by age and Dukes staging, the statistical significance remained: some surgeons consistently had an increased incidence of local recurrence for both sexes and for each level of the Dukes classification. The variance was such that it was suggested that the surgeon is an independent prognostic factor that needs to be considered with Dukes staging. Fielding et al (1980) have demonstrated surgeon related variables with regard to anastomotic technique. They reported dehiscence rates varying from below 5% to over 30%, a variation that could not be accounted for in clinical mix of patient population, confirming that the operator is an independent variable factor in colorectal surgery and by implication possibly in 'curative' resection and frequency of local
recurrence. In a clinical study McArdle and Hole (1991) looked retrospectively at 645 sequential patients operated on for colorectal cancer by 1 of 13 surgeons, none of whom had a specialist interest in colorectal surgery. Results indicated a significant variation in patient outcome amongst surgeons after surgery for colorectal cancer and that these differences compromised survival. It was suggested that considerable improvement in survival might be achieved if such surgery were undertaken by surgeons with a special interest in colorectal surgery or surgical oncology.

One of the comments in a recent comprehensive review of adequate distal margin of resection for adenocarcinoma of the rectum by Phillips (1992) was that the clearance margin is less important than the training and skill of the surgeon performing the operation.

1.6. MECHANISMS OF LOCAL RECURRENCE.

The causes of local recurrence have not been fully defined and possible mechanisms are listed below.

1. Inadequate resection of the primary tumour.
2. Implantation of viable exfoliated tumour cells.
3. Tumour disruption.
4. Promotion of carcinogenesis by foreign materials at the anastomosis

1.7. INADEQUATE RESECTION

The first and probably most obvious cause is inadequate resection of the primary tumour leaving behind foci of residual disease. It is generally accepted that an adequate and therefore 'curative' resection involves removal of all macroscopic tumour and a length of adjacent normal colon and the associated lymph drainage zone. Radical excision is not synonymous with 'curative' surgery as
a palliative procedure can be radical whilst a simple local excision might be thought of by the surgeon as curative. Extended or radical surgery can involve en bloc resection of involved adjacent viscera and long term survival has been obtained, with reported 5 year survival rates of 32-54% (Curley et al, 1992; Jeekel, 1987). Radical resection may include extended lymph node excision. Jatzko et al (1992) recently reported that extending resection margins to encompass neighbouring lymph drainage zones for tumours located close to border zones resulted in no mortality or recurrence in 28 patients undergoing 'curative' resection for colorectal cancer, with a mean follow up of 44 months.

The importance of removing the draining lymph nodes en bloc with the primary tumour was recognised as early as 1908 by Lord Moynihan. Despite this, there remains no general agreement among surgeons as to what represents an adequate resection for colorectal cancer and unlike breast cancer surgery there have been no clinical trials in this area. The available data is retrospective, often with small numbers and for that reason likely to be unreliable. In a retrospective study comparing local segmental resection and radical left hemi-colectomy for the treatment of carcinoma of the sigmoid colon and upper rectum in 107 patients, Busutill et al (1977) found a lower suture line recurrence: 4.3% versus 18.7% and a higher five year survival rates, 70% versus 56% for the local resection group. In contrast, results from a prospective study comparing left hemicolectomy with segmental colectomy for curative resection of left colonic carcinoma (Rouffet et al, 1994) show there was no significant difference in post-operative morbidity, mortality, median survival and actuarial survival between groups. However, carcinoma
of the rectum and recto-sigmoid were specifically excluded in this study, so results may not be truly comparable.

Most studies examine the type of resection performed for colorectal cancer and centre around high versus low ligation of the inferior mesenteric artery or aorto-pelvic and internal iliac lymphadenectomy for carcinoma of the sigmoid and rectum. Pezim and Nicholls (1984) found no survival advantage for high ligation in a series of over 1300 patients. An update on this study looking at patients most likely to benefit from high ligation confirmed the original findings (Surtees et al, 1990) Results from extended lymphadenectomy have been equally disappointing in improving the survival rates and have been associated with a high incidence of genitourinary complications (Glass et al, 1985; Hojo et al, 1989).

It is now generally accepted that a 5cm distal clearance margin is unnecessary for rectal cancer and that submucosal infiltration, lymphatic metastasis and islands of tumour tissue are rarely present more than 2cm away from the macroscopic edge of the tumour (Williams et al, 1983; Kirwan et al, 1988). Williams et al (1983) have shown that a wide distal resection margin is rarely necessary. Fifty APR specimens for carcinoma were examined histologically for distal intramural spread, in 36 (76%) no such spread was seen and spread of less than 1cm occurred in 7 (14%) specimens. In only 5 patients was there evidence of distal intramural spread greater than 1cm, all of whom had poorly differentiated Dukes C carcinoma and who were dead or dying from distal metastases within 3 years of surgery. In this study outcome of patients following potentially 'curative' surgery of rectal carcinoma was not adversely affected by a distal resection margin of less than 5cm for either locoregional recurrence or survival.
Kirwan et al (1988) histologically examined distal doughnut rings from a series of 20 anterior resections with stapled anastomosis. In 19 of the 20 patients no tumour tissue was encountered. In 1 patient tumour cells were present in 1 of a total of 16 blocks. The tumour was Dukes C with involvement of the highest node and distal submucosal spread in the pathology specimen for a distance of 1.5cm. Submucosal spread was not seen in the other 19 tumours. This study concluded the mean margin of resection (2.5cm) in this group of patients was justified by the pathology findings.

Distal intramural spread beyond 1cm is rare except in cases with advanced disease where the prognosis is poor. Pollett and Nicholls (1983) were unable to find any case in the literature even when treated by APR that had survived 5 years when distal intramural spread was greater than 1.5cm. These patients die of distant metastases before they develop local recurrence and this provides further support that the classical margin for resection is not necessary in a low anterior resection of rectal cancer.

Williams et al (1985) compared the results of sphincter saving resection (SSR) for low and mid rectal cancers with abdominoperineal resection (APR). Results indicated that SSR did not appear to carry an increased risk of recurrent disease compared with APR after an equivalent follow-up period, and it was suggested that a 2cm margin of distal clearance would suffice. Other studies have also shown no difference in locoregional recurrence rates between SSR and APR (see 1.51). This in contrast to Phillips et al (1984a,b) who found a significantly greater risk (p<0.02) of developing local recurrence following anterior resection compared to APR (see 1.51).

A suggested reason for the difference was the possibility of less perineal tissue being excised when attempting to preserve the
sphincters. Support for this comes from the work by Heald et al (1982), who have hypothesized that rectal cancer is confined initially to the mesorectum and that total excision of this would reduce local recurrence. This study described 5 cases of minute foci of adenocarcinoma in the mesorectum several centimetres distal to the lower edge of the rectal cancer and in 2 of the cases there was no evidence of lymphatic spread of tumour. A second study by Heald and Ryall (1986) reported local recurrence rates after total mesorectal excision: 115 patients underwent 'curative' anterior resection with an average follow up of 4.2 years and a local recurrence rate of 2.6%. A further update reported on 192 patients who underwent anterior resection and 21 who had APR: 79% had 'curative' sphincter saving surgery and 4(2.6%) developed local recurrence (Heald and Karanjia, 1992). More recently, independent assessment of Heald's work on total mesorectal excision (TME) by Macfarlane et al (1993) indicates results are superior to the best reported from conventional surgery plus radiotherapy or combined chemotherapy: 5% local recurrence at 5 years compared with 25% and 13.5% respectively; and 22% overall recurrence compared with 62.7% and 41.5% respectively (Moertal et al, 1990; Krooke et al, 1991). It was suggested that meticulous TME can improve cure rates and reduce the variability of outcome among surgeons. Support for this comes from Nilsson (Onkologisk Forum. Tranheim. November, 1992) who reported a reduction in local recurrence rates from 33% to 2 out of 41 (4.9%) cases by application of this technique.

Further support comes from Quirke et al (1986) who assessed the degree of lateral spread by histopathological study of the resected specimen in patients undergoing SSR of rectal adenocarcinoma without using the technique of TME and related this prospectively to the
short term local recurrence rate. There was spread to the lateral resection margin in 14 of 52 (27%) operative specimens examined and 12 of these proceeded to develop local recurrence. It was concluded that in rectal adenocarcinoma local recurrence is mainly due to lateral spread of the tumour and has previously been underestimated.

Marks (1993) commented that local recurrences may be determined by the "completeness" of removal of the meso-rectum and that the involvement of the lateral resection margin was a poor prognostic factor: patients from an earlier study with involved lateral margins all died of distant metastases (Cawthorne et al, 1986).

The significance of lateral node dissection for advanced rectal cancer at or below the peritoneal reflection has been investigated by Moriya et al (1989). On pathological examination the overall incidence of lateral node metastases was 18.1% (42/281) in all patients: for Duke’s C carcinoma patients, 36% had lateral node metastases and in 6% of this sub-group (42/114) metastatic spread was limited to the lateral nodes. The incidence of local failure in those who had extended dissection was lower than that after conventional dissection (12% vs 17%), however, the difference was not significant. Moriya et al (1989) recommended systemic lymphadenectomy with lateral node dissection be performed for advanced rectal cancer at or below the peritoneal reflection. Although local recurrence may be caused by lateral node metastases, this is not always clinically apparent. Extended pelvic lymphadenectomy is associated with frequent complications to urinary and sexual function, other studies have failed to show favourable results and therefore clinical benefit has not been established (Deddish, 1960; Bacon, 1960; Glass et al, 1985; Hojo et al, 1989).
1.8. IMPLANTATION OF TUMOUR CELL

1.8.1. History

The mechanism of tumour cell implantation as a cause of local recurrence was first proposed by Gerster (1885) and shortly afterwards by Lack (1896). Gerster (1885) suggested that local recurrence was the result of cancer cells being spread by contaminated instruments and the surgeons hands. Sir Charles Ryall (1907; 1908) thought cancer to be a spreading infective process and stressed the importance of minimising the spread of the infection. His theory was that tumour cell could implant on all freely cut tissue, and 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.

The hypothesis of tumour cell implantation is supported by reports of recurrent tumour developing in the abdominal wound (Ryall, 1907; Lawrie, 1906), colostomy site (Mayo, 1913) and on raw mucosal surfaces such as haemorrhoidectomy scars, anal fissure and fistulae (Le Quesne and Thomson, 1958; Killingbeck et al, 1965; Guiss, 1954; Rollinson and Dundas, 1984) after resection of a colonic carcinoma.

1.8.2. Clinical evidence

Gordon-Watson (1938) suggested that free tumour cells could be implanted into the bowel wall by the needle during suturing and may grow directly on the raw anastomosis. It became a widely accepted belief that exfoliated tumour cells were likely to be responsible for local recurrence (Gordon-Watson, 1938; Goligher et al, 1951) and as a result measures to kill the cells or limit their spread became widespread. Isolation of the tumour with tapes before surgery or irrigation of the bowel lumen with cytotoxic agents became standard
practice in colorectal surgery (Cole, 1952; Cole et al, 1954; Morgan, 1955; Southwick et al, 1962). Strong support for implantation comes from the observed reduction in the incidence of suture line recurrence following measures to kill or limit the dissemination of desquamated tumour cells (Morgan, 1955; Southwick et al, 1962; Long and Edwards, 1989; Keynes, 1961). Prior to the introduction of these measures the incidence of suture line recurrence was reported at 8.5%-36% (Cole, 1952; Beal and Cornell, 1956; Wheelock et al, 1959; Wright et al 1969; Keynes, 1961; Floyd et al, 1965). At St Marks Hospital the routine use of 0.2% mercuric perchloride for bowel lavage reduced the incidence of anastomotic recurrence from 13% to less than 3% per year (Keynes, 1961).

Further evidence supporting implantation of exfoliated tumour cells was provided by McGrew et al (1954), who performed Papanicoulou stains on smears taken from the lumen of 50 specimens of large bowel cancer. Malignant cells were found to be present in 42% of proximal and 65% of distal resection margins at an average distance of 21 and 10cm respectively from the macroscopic edge of the tumour, the percentage of positive smears being inversely proportional to the distance from the tumour. Rygick et al (1969) demonstrated apparently viable cells in the washings taken from surgical instruments and from surgeons hands. Pomeranz and Garlock (1955) studied a series of smears taken from the serosa of 20 colonic carcinomas and found malignant cells to be present in 2 cases. It was suggested that the dissemination of these cells from the serosal surface would explain peritoneal and abdominal wound recurrences.

The presence of intraluminal exfoliated tumour cells in patients with large bowel cancer became such a well recognised phenomenon that it was suggested that detection of such cells may be
of value in the diagnosis of this disease (Oakland, 1961; Rosenberg and Giles, 1977). Oakland (1961) used exfoliative cytology to confirm or exclude the diagnosis of carcinoma in 97 patients with equivocal endoscopic and/or radiological findings. Of 24 patients eventually found to have carcinoma 18 were first diagnosed by exfoliated cytology. The viability of these desquamated tumour cells and their ability to give rise to recurrent tumour was questioned (Rosenberg, 1978; Rosenberg et al, 1978). Although it was possible to demonstrate the presence of large numbers of exfoliated colorectal cancer cells by in vivo colonic lavage and ex vivo manipulation of the surgical resection specimen in Hartmans solution, it was found that the cells isolated were unable to exclude the supravital dye trypan blue. Viable cells could only be demonstrated in tumour homogenate cell suspensions. Therefore, it was concluded that although exfoliated tumour cells were present in the operative field during colorectal cancer surgery, these cells were unlikely to be viable and therefore incapable of giving rise to recurrent tumour growth.

More recent work by Umpleby et al (1984b) supports the viability of exfoliated colorectal tumour cells. Preoperative colonic lavage with Hartmans solution in 19 patients with colonic cancer found malignant cells in the washings of 14 cases, with a median viability of 92%. The resection margins of the surgical resection specimen were also irrigated and malignant cells with a median percentage viability of 70% were recovered from 57% (17 of 30) of the proximal and 84% (21 of 25) of the distal resection margins. The number of cells obtained was inversely proportional to the distance of the macroscopic tumour edge to the distal resection margin, tumour cells being found up to 35cm away from the tumour edge.
at the proximal resection margin and 20cm from the distal resection margin. Skipper et al (1987) have reported in vitro monolayer growth of tumour cells obtained from washing of the bowel lumen, mesorectum and serosal surface, luminal mucus specimens and post dissection lavage of the tumour bed in patients undergoing potentially 'curative' colorectal cancer surgery. All colonies stained positive for epithelial markers and carcinoembryonic antigen. The same group proceeded to demonstrate that exfoliated malignant cells isolated by the same method were capable of proliferating in immune deprived mice (Fermor et al, 1985).

Ambrose et al (1989) compared the assessment of free malignant cells in the peritoneal cavity by cytology and immunoperoxidase monoclonal antibody staining. The correlation between the assessment was disappointingly poor and it was thought this may be due to changed antibody expression by the tumour cells. A more recent paper by Leather et al (1993) looked at detection and enumeration of circulating tumour cell from the mesenteric vein by immunocytochemistry in patients operated on for colorectal cancer. Definite morphological evidence of malignancy was observed in three patients and suspicious features in a further seven. Immunocytochemistry confirmed these findings in all three of the malignant but only one of the suspicious cases. Results supported the use of immunological markers to detect and enumerate malignant cells. The difference in outcome between the two studies may be due to methodology and choice of antibody and suggests that further assessment of intraperitoneal and intraluminal cells by immunocytochemistry is indicated as this is a powerful tool for detecting and enumerating malignant cells.
1.8.3. Experimental evidence

Animal work has demonstrated that malignant cells are capable of implanting at a suture line and giving rise to tumour growth (Vink, 1954; Cohn and Attick, 1960; Waltzer and Altemeier, 1965; Broyn and Helsinger, 1972; Broyn, 1972; Skipper et al, 1989; O'Dwyer et al, 1985; McGregor et al, 1989). Haverbeck and Smith (1959) 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 synergystic mice. Gubareff and Suntzeff (1962) similarly demonstrated the transfer of highly malignant mouse rhabomyosarcoma by suture material and found that pretreatment of the suture material effectively decreased the tumour transportation. Certain suture materials can potentiate tumour growth when implanted into experimental animals with an inoculum of tumour cells. Pendergrast et al, 1976 implanted sutures subcutaneously in rats with an inoculum of B16 melanoma cells and found that all suture types potentiated tumour growth with large numbers of tumour cells. However, when a subclinical dose of tumour cells (approx 100 cells) were used, silk and to a lesser extent steel were found to increase tumour occurrence whereas nylon, polyglycolic acid and chromic catgut did not potentiate tumour growth and results were attributed to the physical characteristics of the suture. More recently, it has been demonstrated that tumour cells differentially adhere to suture materials and that in-vivo tumour growth correlated well with the in vitro tumour cell adherence characteristics of the different suture materials (O'Dwyer et al, 1985; McGregor et al, 1989 ref 9.7). Experimental studies assessing the use of iodinised catgut sutures have shown them to be effective in preventing implantation in experimental animal models (Cohn et al, 1963; Keller et al, 1966;
Herster and Sbuelz, 1966). Cohn (1967) and Yu and Cohn (1968) demonstrated that Pearce Brown tumour cells in a rabbit model did not grow on the mucosal surface of bowel but they would produce anastomotic tumours by seeding onto the serosal surface of the bowel or into the bowel wall via the suture material. The passage of shed intraluminal colorectal cancer cells across a sealed anastomosis has been shown by Leather et al (1991) using immunocytochemistry to identify malignant cells, confirming previous work by O'Dwyer and Martin (1989) who were able to demonstrate that intraluminal tumour cells can leak through a watertight anastomosis and cause extraluminal tumour growth in a Wistar/Firth rat colon cancer model. Tumour growth did not occur intraluminally in either the sham laparotomy or anastomosis group and suggests that anastomotic tumour growth associated with extraluminal tumour growth does not necessarily indicate invasion inwards from an extramural focus of cancer cell left behind from incomplete resection.

1.9. TUMOUR DISRUPTION.

Slanetz (1984), Phillips et al (1984a) and Zirngibl (1990) have demonstrated significantly increased local recurrence rates with tumour perforation. (see 1.4.2.4.) Disruption of the tumour may result in dissemination of tumour cells and previous discussion (see 1.8.1-1.8.3) has indicated that such tumour cells could 'seed' the operative site or be transferred to other areas by hands/instruments leading to tumour growth.

1.10. METACHRONOUS CARCINOGENESIS

Approximately 20-30% of all local recurrence arise 2 years or more after primary resection for large bowel cancer, Umpleby and
Williamsom (1987) have suggested that this may represent metachronous carcinogenesis at the anastomosis. Colonic neoplasm is recognised as a multifocal disease, adenomas are multiple in 28%-35% of patients at the time of diagnosis (Bussey, 1978; Gilliespie et al, 1979; Goligher, 1984c) and carcinomas are multiple in 3-5% of cases, either as synchronous or metachronous lesions (Heald and Bussey, 1975; Bussey, 1978; Enker and Dragaceviv, 1978; Goligher, 1984d; Finan et al, 1987). The presence of adenomas at the time of resection of a colonic carcinoma approximately doubles the risk of developing a metachronous large bowel cancer (Adson, 1967; Bussey et al, 1967; Morson, 1974) and the risk increases to 10% at 25 years (Muto et al, 1975). The strong association of long-standing ulcerative colitis and familial adenomatous polyposis coli with the development of carcinoma supports the theory of an unstable epithelium (Bussey, 1978; Riddell et al, 1978). Williamson (1981) and Williamson et al (1982) have proposed that the adaptive hyperplasia of the mucosa which occurs at the anastomosis following colonic resection is important in increasing the susceptibility to malignant change. An alternative theory from Rubio and Nylander(1982a) suggests that environmental carcinogens may promote cellular abberations in the colonic mucosa and when these cells are stimulated to divide by the trauma of surgical resection, malignant transformation takes place.

1.10.1. Experimental evidence.

Experimental studies have indicated that surgical trauma may act as a co-carcinogen. Rous and Kidd (1941) demonstrated repeated injury can promote the reappearance of tar induced ear tumours which had previously regressed. Gottfried et al (1961) reported an earlier
appearance and more rapid progression of dibenzpyrene induced tumours in mice following repeated skin wounding, or more notably repeated laparotomy. The same applies to carcinogen induced experimental models of colorectal cancer. Using the azoxymethane rat colonic carcinoma model, intestinal resection at different levels in the small and large bowel results in a consistent preference for tumour to develop at or immediately adjacent to the site of an anastomosis (Williamson, 1981; Williamson et al, 1979; Williamson et al, 1982; Rainey et al, 1985; Williamson and Rainey, 1984).

Roe et al (1987) have studied the relationship between the proliferation status of the mucosa and the susceptibility of an anastomosis to tumour development using azoxymethane in a Sprague-Dawley rat model. Colonic anastomosis displayed increased susceptibility to tumour cell development up to 3 months post operation. Cell proliferation was increased in the immediate area of the anastomosis throughout this time, as demonstrated by morphometry and thymidine labelled autoradiography. Pozharriski (1975) demonstrated that the general proliferation response of colonic mucosa to injury only lasts 40-50 days, however the presence of a solitary suture implanted in a rat caecum will act as a small focus for enhanced cell proliferation for a significantly greater period. Administration of dimethylhydrazine 2 months following injury led to a marked increase in tumours found in the caecum and it was suggested that the rise in incidence of tumours following injury may be due to a greater number of stem cells entering into the mitotic cycle at which stage they are susceptible to carcinogenic influences. Other workers have shown that large bowel tumours in experimental animals develop preferentially at sites of colonic anastomosis irrespective
of whether carcinogen administration precedes or follows surgery (Rubio et al, 1982a,b; Steele et al, 1981).

Phillips and Cook (1986) looked at the effect of various sutures on the incidence of chemically induced rodent tumours. Dimethylhydrazine was administered to Wag rats 2 months after construction of a colonic anastomosis with either steel or silk sutures. Their results showed that colonic primary tumours preferentially develop around a previously constructed anastomosis and that the choice of suture material can influence this, with a significantly greater number of anastomotic tumours in the steel suture group. An explanation for this may be that the steel suture persisted in greater numbers for a longer period of time than silk and could have resulted in a more pronounced or widespread increase in cellular proliferation.

McGregor et al (1991) looked at the effect of different suture materials on colorectal carcinogenesis in animals that had been treated with azoxymethane for 12 weeks prior to operation. Results again confirm that the type of suture material can significantly influence local tumour development in the post initiation phase of carcinogenesis. Interestingly, in this study stainless steel was associated with significantly less local tumour growth than either polyamide or polyglycolic acid sutures and it was concluded that stainless steel may be associated with a lesser risk of promoting a metachronous suture line tumour compared with conventional suture material. The explanation for this was unclear, as cell kinetic studies by the same author show no difference between the suture materials used, although the incidence of tumours was markedly different (McGregor, 1988). More recently, work by Uff et al (1993)
has clarified suture related factors in tumour cell adherence (ref 9.7).

1.10.2. Clinical evidence

Filipe and Branfoot (1974) described abnormalities of mucosal mucin in apparently normal colonic mucosa of patients with colorectal cancer. This field change or 'transitional' mucosa comprises of normal sulphomucin predominate pattern to abnormal sialomucin predominate pattern and similar changes have been identified in the colonic mucosa of experimental animals treated with carcinogens known to promote colorectal neoplasia (Filipe, 1975)

Sunter (1985) studied biopsies taken from the anastomotic site of 28 patients following curative resection of colorectal cancer. In 11 (39%) there was non specific inflammatory changes and in 7 there was transitional changes of the mucosa which he regarded as potentially malignant. Dawson et al (1987a) have also reported abnormally high sialomucin levels in at least one resection margin of large bowel resection specimens; however, there was no direct evidence to suggest that these alterations in mucin patterns are preneoplastic. They may simply represent a non specific response to chronic injury (Issacson & Attwood, 1979) or be a feature of immature colonic mucosa at a site of hyperplasia (Olubuyide et al, 1985).

There is now accumulating evidence that sialomucin may be of predictive value in identifying patients at high risk of developing local recurrence. Dawson et al (1987b) reported results from a prospective study of 358 patients in which the presence of sialomucin at either resection margin was determined from the surgical resection specimen in the immediate post operative period. A sialomucin predominate pattern was observed at resection margin in approximately
30% and this proved to be of significant prognostic value for survival and development of local recurrence. Similar findings were reported by the same group for a retrospective study (Habib et al, 1984).

While there is no direct supportive evidence, it seems plausible that a proportion of local recurrences may represent the development of a second primary tumour at or adjacent to the anastomosis site. Local recurrence would be expected to present early and metachronous carcinogenesis may be particularly applicable to the 20-30% that occur after the second post operative year.

1.11. SUMMARY.

It is clear from the preceding data that local recurrence is a significant factor limiting survival after potentially 'curative' colorectal cancer surgery. Although inadequate excision is undoubtedly the major cause, it seems likely that other mechanisms are involved and that much work needs to be done to understand fully the causes of local recurrence.

Results of several studies have supported surgeon variability which may contribute to local recurrence and outcome following curative colorectal surgery. Strong evidence of implantation of tumour cells at an anastomosis as a cause of local recurrence has been presented and the role of suture material in tumour cell adherence has been examined. Other factors such as metachronous carcinogenesis have been explored.

1.11.1. Outline of thesis.

The aim of this research work was to investigate further factors that may be important in the aetiology of locoregional
recurrence in colorectal cancer and is divided into two main parts: clinical work and experimental studies in an animal model.

The clinical work investigates the effect of the surgeons speciality interest on the type of resection performed for colorectal cancer.

Experimental studies in a Fisher rat model investigate

1. The potential for viable circulating tumour cells to implant at a colonic anastomosis.
2. A comparison of the potential of intra-arterial, intraluminal and intraperitoneal tumour cells to adhere to a colonic anastomosis and normal colon.
3. The potential for tumour cells from a distant metastasis to cause tumour growth at a colonic anastomosis.
4. The relative importance of tissue injury and suture material on tumour cell adherence.
5. The role of growth factors in anastomotic recurrence.

The following chapters look at the background of colonic anastomoses and the role of other factors that may be important in local recurrence.
Chapter 2

A DESCRIPTION OF INTESTINAL ANASTOMOSES
2.1. SUTURES

The term suture is used to describe the surgical insertion of a stitch and similarly applies to the material used for closing a surgical or accidental wound by stitching.

A brief history and description of materials relevant to intestinal suturing follows.

2.1.1. History.

Majno (1975) has extensively investigated the history of sutures and the following is a brief description of some of his findings. The use of sutures has been reliably described in the classical writings of India and Greece. Various materials were recommended in the early Greek and Indian texts such as flax, hemp, bark fibre or 'the hair of a woman'. Other implements used include insect mandible described in ancient Hindu texts and acacia thorns through the wound edges, thought to be still used in East Africa. The use of catgut was first described in Greece (approximately AD 150). More recently amongst Lister's many advances to medicine was the development of chromatising catgut (Lister, 1908).

Lord Moynihan (1920) defined the ideal characteristics of a suture. He stated that it should: achieve its purpose, i.e. be sufficient to hold parts together, be free of infection and non irritant. In the early days of intestinal suturing the choice of material was limited and lay between catgut and nonabsorbable materials such as silk or linen. Modern man-made fibre technology has made available a much wider range of sutures both absorbable or non absorbable and monofilament or multifilament (braided). Currently sutures fulfil these requirements to differing degrees, braided materials may be easier to handle than monofilament materials.
but they can be associated with an increased risk of submucosal damage, greater infection and tumour cell implantation due to the potential for bacteria/tumour cell to lodge in the structure of the material (see 3.1.2; 1.8.3). The choice of suture material used is based on absorption and handling qualities, and this has largely remained a personal one.

2.1.2. Absorbable sutures

An absorbable suture is degraded to soluble products in the body tissues by hydrolytic and/or enzymatic degradation and disappears from the operative site usually within 2-6 months (Devi and Vasudevan, 1985). It can be man-made or derived from natural materials.

Surgical catgut is derived from either the serosal connective tissue of bovine intestine or the submucosal fibrous tissue of sheeps' intestine and is essentially collagen (Salthouse, 1983). It has been widely used throughout surgical history because of its absorbability and good knotting qualities. Untreated catgut is rapidly digested, however it can be made more resistant to tissue degradation and absorption in vivo by immersing it in chromium salt solutions as reported by Lister in 1908 (Devi and Vasudevan, 1985). In vivo, there is variable but vigorous inflammatory response over the first week, which has subsided and is considered minimal by day 14 (Salthouse, 1980). The degradation of chromic catgut largely depending on the presence of proteolytic enzymes, usually supplied by macrophage activity and is absorbed in approximately 90 days (Salthouse et al, 1969).

Modern man-made fibre technology has made available a much wider range of sutures and their use has increased over the past two
decades. Their breaking strength, tissue reaction and absorption are more uniform and predictable than natural collagen-based sutures such as catgut (Pavan et al, 1979) although they do not handle or knot well, particularly in the monofilament form. They are absorbed in vivo by non enzymatic hydrolysis which provokes less tissue reaction than enzymatic digestion (Devi and Vasudevan, 1985). Examples include monofilament Polydioxanone (PDS) which produces minimal tissue response in vivo. It is absorbed in approximately 150-200 days (Durdey and Bucknal, 1984), with over 50% of its strength remaining after 4 weeks compared to 1-5% with other absorbable sutures (Ray et al, 1981). Braided absorbable sutures such as Polyglycolic acid (Dexon) and polyglactin 910 (Vicryl) have a greater degree of softness and flexibility than most other synthetic sutures (Devi and Vasidevan, 1985). Polyglactin 910 (Vicryl) in vivo loses 50% of its strength by 2 weeks and is absorbed completely by 84 days (Salthouse, 1983). It is a popular suture that is often used as an alternative to chromic catgut and along with Polyglycolic acid (Dexon) is in widespread use for the construction of intestinal anastomoses.

2.1.3. Non-absorbable sutures

Non-absorbable sutures become encapsulated in a fibrous sheath, are resistant to biodegradation, and remain in the tissue as a foreign body unless surgically removed from the tissue or naturally extruded (Devi & Vasudevan, 1985). They may be protein-based, or made from synthetic or inorganic material.

Silk is a braided material, it has excellent handling and knotting qualities which lead to its extensive use throughout surgical history (MacKenzie, 1971). Although silk is considered a
non absorbable suture it is very slowly degraded in vivo, and loses over 50% of its strength by 7 days.

Nylons (Polyamides) can be braided (Nuralon) or monofilament (Ethilon). In vivo, nylon becomes encapsulated by a thin well defined band of fibrous tissue within 2-3 months (Salthouse, 1980) and loss of tensile strength is slow, with only a 20-30% loss over 9-10 months (MacKenzie, 1971) Specific braiding technique allied to controlled wax impregnation have provided multifilament nylon with handling qualities close to those of silk. In contrast, monofilament nylon and prolene (Polypropylene) a monofilament polyolef based suture with similar properties to nylon remain difficult sutures to knot and handle and this has restricted their use.

Stainless steel is the only commonly used metallic suture. In vivo, the tissue response can vary from being almost inert to reactive, but this subsides within 2-3 months when the suture becomes encapsulated by a well defined band of collagen (Salthouse, 1983).

2.2. ANASTOMOTIC HEALING

2.2.1. Histological features

All wounds go through several well recognised stages of repair which are usually considered as three overlapping phases.

1. The lag phase is an acute inflammatory response of neutrophils and lymphocytes initially, and after 24 hours blood monocytes modulating to macrophages, which clears the wound of debris (0-4 Days).
2. A phase of granulation in which fibroblasts proliferate and lay down immature collagen (3-14 Days).
3. A phase of maturation where collagen remodels and the wound reaches maximum strength (10-80 Days).

In experimental animals one week after anastomosis the inverted cuff of bowel is covered with low cuboidal epithelium. By three weeks the mucosa is hyperplastic (Herrman et al, 1964) and it may be 12 weeks before the normal mucosal pattern is restored (Roe et al, 1987).

2.2.2. Anastomotic strength.

During the lag phase of healing following a bowel anastomosis the wound is weak and reliant on support from the repair (i.e.sutures/staples). Wound strength increases rapidly during the second phase of granulation and by the end of this time (10-14 days) the intestinal anastomosis does not require additional support.

Anastomotic healing is not only dependent on the normal healing processes of any wound but also the influence of suture material on this process. Currently an anastomosis is repaired and therefore supported by sutures or staples. These materials are variable in their properties and the vast majority remain for a significantly longer time than is necessary, even if they are absorbable.

2.3. Disadvantages of anastomotic repair

2.3.1. Sutures and staples.

The passage of a needle and suture material through the bowel wall is traumatic and may on occasions result in the interference of the local blood supply due to damage of structures in its path (Ordman & Gillman, 1966). Lord et al (1978) have shown that materials such as catgut, silk and Dexon may damage the submucosa
with their roughened surface and this carries a potential risk as the submucosa has been shown to be important to anastomotic integrity.

2.3.2. Adverse effect on healing.

All foreign bodies in a wound such as sutures or staples provoke an inflammatory response which is dependent on the material used. With non-absorbable sutures this is followed by a fibroplastic response and the formation of a collagen sheath around the suture. Chronic inflammation will persist around an absorbable suture until absorption is complete and and is dependent on the type of suture material used, those with delayed absorption will evolve a collagen sheath (Postlethwait et al, 1959; Postlethwait, 1970). Suture associated inflammation may be more prolonged than that related to the wound healing itself; in experimental skin wounds 40 days after suture removal fibrosing granulomatous tissue was still evident along the suture tract (Ordman and Gillman, 1956). Comparison of intestinal suturing techniques has shown that methods which cause less inflammation regain anastomotic strength at a faster rate (Getzen and Holloway, 1966; Letwin and Williams, 1967). In rat experimental anastomosis where accurate serosal apposition was achieved with minimal colonic inversion, healing was by primary intention; if a larger cuff was inverted, healing was by secondary intention (Hermann et al, 1964). Sunter et al (1985) took colonoscopic biopsies from a series of 28 patients who had undergone resection for carcinoma between 6-24 months preceding investigation. All anastomosis had been similarly constructed in 2 layers using silk and catgut. In 5 patients visible inflammation was noted and seen in another 6 patients on histological analysis, with distorted
cryptal architecture and occasional granulation related to suture presence.

2.3.3. Anastomotic sepsis.

The presence of sutures in a wound have been shown to increase the susceptibility of tissues to bacterial infection (Elek and Cohen, 1957). Further work has indicated that the degree of infection (see 3.2.2.) was dependent on the suture material used (Varma et al, 1974; Edlich et al, 1973). Katz et al (1981) demonstrated that the properties of the suture material influenced bacterial adherence and this experimentally correlated well with the degree of infection. Matheson and Irving (1975) developed the seromuscular suturing technique as a method of avoiding bacteria traversing suture tracts and giving rise to perianastomotic infection.

2.3.4. Suture implantation metastases

Implantation of exfoliated tumour cells by sutures is thought to be one of the causes of anastomotic recurrence.

McGrew et al (1954) suggested that contamination of the suture with intraluminal tumour cells and subsequent implantation of the cells during passage of the suture was the mechanism of local recurrence. Bacteriological studies (see 3.2.2.) have shown bacterial adherence to suture material and experimental studies (see 1.8.3) also have proven that sutures are able to transfer malignant cells from a solid tumour mass or from a cell suspension in large enough numbers to result in tumour growth in a recipient animal (Haverbech and Smith, 1959; Gubareff and Suntzeff, 1962). Pretreatment of the suture material effectively decreased the tumour
implantation and transportation and implantation (Cohn et al 1963; Gubareff and Suntzeff, 1962).

Anastomotic material may have a second potential role in implantation metastases in that they may act as a nidus for implanted tumour cells within the colonic wall, allowing cells to multiply and establish their growth advantage. Tumour cells adherence to suture material has been confirmed by O'Dwyer et al (1985) and McGregor et al (1989) and this correlated with vivo tumour growth studies (see 3.2.2).

2.4. ANASTOMOTIC TECHNIQUES

2.4.1. Sutured

The earliest description of successful anastomosis of divided bowel dates from 1743. In his account of 'Bubonocoele Incarcerata' Heister (Ravitch, 1984) describes Rhamorius as having resected a strangulated hernia and achieving union of the bowel ends by inserting one into the other in an intussucepting technique. The only suture used was a piece of string loosely holding the bowel ends together and anchoring the anastomosis to the mouth of the wound. Despite this success it was almost 150 years before intestinal suturing was attempted with any degree of regularity.

Many of the basic principles necessary for successful intestinal suturing were identified in the last century. Benjamin Travers (1812) recognised that it was possible to obtain primary intestinal wound healing by suturing and demonstrated this successfully by constructing an end to end everting anastomosis in dogs. In 1826 Lembert proposed the key to successful intestinal wound healing lay in achieving accurate serosa to serosa apposition with an inverting anastomosis. Czerny (1880) modified this by adding a second layer of sutures to approximate the mucosa and this was
followed by numerous other techniques for inverted anastomosis. These varied in terms of interrupted vs continuous sutures or the number of layers (Halstead, 1887; Connell, 1897; Gambee, 1951). Halstead (1887) demonstrated that the true holding layer of the anastomosis was the submucosa and not the serosa and associated adhesions as Lembert had assumed. A two layered surgical technique was usually recommended to achieve serosa to serosa apposition (Czerny, 1890) as it was assumed to offer greater security. However, complications such as anastomotic stenoses and obstruction remained a well recognised risk despite several surgeons (Halstead, 1887; Kerr, 1923) having advised against too much inversion. Such risks led to the development of new suture techniques which secured accurate serosa to serosa apposition of the bowel edge with at most only a minimal degree of inversion (Gambee, 1951; Gambee, 1956).

Experimental studies have shown more inflammation and avascular necrosis of the inverted cuff (Sako and Wangensteen, 1951) which rendered them weakened in experimental studies of intestinal bursting strength (Letwin & Williams, 1967). In recent years the interrupted subseromuscular closure (Matheson & Irving, 1975) has gained popularity. This technique has a low sepsis and anastomotic leak rate which may be as a consequence of the reduced interference with blood supply and resultant diminished mucosal necrosis.

In 1952 Hertzler and Tuttle demonstrated in experimental animals that everted anastomoses with mucosa to mucosa contact would heal satisfactorily. Immediate interest developed and further animal studies in dogs comparing everted and inverted anastomoses found no difference as judged by reticulum and collagen formation; however the everted anastomosis were associated with a wider stoma (Gertzen, 1966a) Initial clinical studies were favorable (Gertzen,
1966b) and Goligher et al (1970) undertook a prospective randomised controlled study of a single layer everting suture vs inverting two layered anastomosis. This study was terminated prematurely when it became 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.

2.4.2. Anastomotic staple guns

The increasing use of stapling instruments in Gastrointestinal surgery over the past two decades has confirmed the reliability of this mucosa to mucosa anastomotic healing in man. The long established belief of serosa to serosa contact as being essential to the intestinal wound healing can no longer be considered to be absolutely necessary.

2.4.3. History

The application of mechanical devices during the construction of Gastrointestinal anastomoses has not been restricted to comparatively recent times. From the earliest days of intestinal surgery to the present day many many instruments have been described. Many of the earliest implements were devised simply as aids to conventional anastomotic techniques, others were designed totally to replace manual suturing methods. This subject has been extensively reviewed by Steichen and Ravitch (1984a) and only a brief review of the more important developments is described.

Humer Hultl presented the first intestinal staple instruments (Fischer/Hultl) to the second annual meeting of the Hungarian Surgical Society in May 1908 (Steichen & Ravitch, 1984b; Robicsek, 1980). The instrument comprised of two large metal jaws, one of
which housed the steel wire staples, the other acting as the anvil against which the staples were formed. Once the jaws were closed 4 parallel rows of staples were produced allowing division of the viscus between the middle two rows without spillage of the intestinal contents. This instrument was time consuming, heavy and costly and an alternative lighter instrument was developed by von Petz in 1923. This model and its various modifications were used for over 30 years.

Neither the Hultl or von Petz instruments were intended for permanent construction of a Gastrointestinal anastomosis. They were 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 in the Scientific Institute for Experimental Surgical Apparatus in Moscow. Early versions of the three principle anastomotic staplers currently available were developed: a linear anastomotic stapler (Amosov, 1961), a device for side to side gastrointestinal anastomosis (Svinkin, 1964) and an instrument for inverting end to end circular anastomosis (Androsov, 1970). In 1958, Dr Mark M Ravitch, a visiting American surgeon, was impressed by the idea of mechanical closure of intestinal wounds. With Ravitch acting as an advisor, the first American stapling instruments were launched by the United States Surgical Corporation in 1967. The instruments had major advantages, they were both smaller and lighter, with staples supplied in a preloaded sterilised disposable cartridge, all factors which considerably facilitated their use. Since their introduction various modifications have occurred including adjustable staple height, choice of anastomotic diameter, removable head to facilitate pursestring placement and straight or angled shafts to improve access.
2.4.4. Complications

The introduction of the circular staplers coincided with a trend towards increasing restorative colorectal surgery (Goligher, 1979; Heald, 1980). Although some surgeons would disagree as to its advisability (McGinn et al, 1985) it has been estimated that the introduction of the circular staplers has resulted in an additional 15 of every 100 rectal cancer patients being spared a permanent colostomy (Goligher, 1982). As a result, at least 60-70% of all patients with a primary rectal cancer may be able to undergo an SSR (Heald, 1980; Goligher, 1982).

Initial reports were favorable. Goligher et al (1979) reported their experience with the Russian SPTU, using their own historical controls for comparison. There was no difference in clinical leak rates between the stapled and sutured groups. However, post operative contrast enema studies indicated a remarkably high radiological leak rate of 29% in patients with a sutured anastomosis compared to 6.5% in the stapled group. In an uncontrolled non-randomised study, Adloff et al (1980) compared 26 stapled colorectal anastomoses with 25 single layer handsewn anastomoses. Ten faecal fistulas developed in each group and there were two intraperitoneal anastomotic leaks in the sutured group compared with none in the stapled group. Other authors have confirmed the reliable construction of low colorectal anastomoses using the circular stapler (Detry & Kestons, 1981; Scher et al, 1982; Shahinian et al, 1980).

Randomised trials comparing stapled with single or two layered anastomoses have not confirmed any advantage of stapling over suturing in terms of anastomotic leak or septic complication, although the anastomotic construction was faster in the stapled group (Beart & Kelly, 1981; Brennan et al, 1982, Everett et al, 1986).
Both Brennen et al (1982) and Everett et al (1986) found this was partially offset by the incidence of technical problems with the stapler.

The majority of staplers in use are made of stainless steel. Stapled anastomoses heal by primary intention with minimal inflammatory response (Ballantyne, 1984) and are stronger in the lag phase than sutured anastomosis. With time the staples become encased in a thin fibrous capsule and all stapling devices utilise the same 'B' configuration of staples which has remained unchanged from the original Hultl/Fischer stapler to the present day. The theoretical principle behind this 'B' configuration is that the approximation of the tissues is sufficient to produce effective union of the bowel ends whilst the microvasculature is able to pass through the loops in the 'B' and ensure a good blood supply to the healing anastomosis.

2.5. Sutureless techniques

2.5.1. Clinical compression devices: History

The earliest device known to be developed primarily for the purpose of creating an anastomosis was presented by Felix-Nicholas Denans (1826) to a meeting of the Societe Royale de Medicine de Marseille. He reported successful construction of end to end small bowel anastomosis in dogs using this device but it is not known if he attempted his technique in clinical practice. Further mechanical devices were designed by Senn (1893), Bonnier (1885), Abbe (1889) and Murphy (1892).

The most successful implantable device was the Murphy's button. This consisted of two metal rings which trapped the cut ends of the transected bowel maintaining apposition by means of a lateral spring to allow healing. The compressed bowel was rendered ischaemic,
leading to necrosis and sloughing which released the ‘button’ into the faecal stream. The device was widely used but early problems of obstruction arose due to the narrow lumen of the button and later obstruction was due to anastomotic stenosis. In 1893 Senn stated ‘If any internal aids to circular suturing are used, they should be composed of entirely absorbable materials and employed in such a way as to not produce marginal gangrene and with a central opening large enough to allow free faecal circulation’.

2.5.2. New Designs.

Recently, there has been renewed interest in ‘sutureless’ technique resulting in new designs, all utilising the principle of compression to unite the bowel ends: Biofragmentable rings, AKA guns, Polypropylene rings and Magnetic rings. With time the device is passed into the faecal stream leaving behind a sutureless and healing colonic anastomosis. The subject has been extensively and comprehensively reviewed by McCue and Phillips, (1991) and the reader is referred to this article. It was concluded that compression devices have produced initial clinical results comparable with conventional suture and stapled anastomoses. McCue et al (1992) have shown experimentally that a sutureless anastomosis is associated with fewer induced anastomotic tumours.

2.6. Experimental:

2.6.1. Adhesive anastomoses and laser welding

Other methods of ‘sutureless’ anastomoses are experimental. With currently available materials glued anastomosis are not safe for clinical use. Use of synthetic agents such as plastic adhesives has been associated experimentally with a high anastomotic failure rate.
and mortality in both small and large bowel and these techniques have not been used in humans. Although initial experimental results using the fibrin glue in pig ileum and dog colon were promising (Hjortrup et al., 1986; Kjaegaard et al., 1987), further studies in rats have suggested the anastomosis is weak in the critical lag phase and that clinical attempts to use fibrin glue as a sole method of anastomosis would be unwise (Haukipuro et al., 1988).

This is in contrast to initial experimental results from laser welding which appear highly promising (Sauer et al., 1989a; Costello et al., 1990). At optimum power levels laser welded wound strength is equivalent or greater than sutured enterotomies at all time points (Sauer, 1989b; Mercer et al., 1987; Cespanyl, 1987; Vlasak et al., 1988) Near normal transmural histology is restored by three weeks in marked constrast to sutured anastomosis (Cespanyl et al., 1987). Earliest experimental results of laser welded ileal anastomoses (Sauer et al., 1989b; Costello et al., 1990) indicate that wound integrity, degree of tissue reaction, and bursting pressure were comparable to standard sutured anastomoses, although laser welded anastomoses appeared stronger between one to two weeks with less inflammation.

2.7. Summary

There is considerable experimental and clinical evidence to support the theory that local recurrence of colorectal cancer may be due to the surgical implantation of viable exfoliated malignant cells. Results from retrospective and prospective randomised trials have suggested that anastomotic technique exerts a marked effect on long term survival and local recurrence rates.
Experimental work has shown that anastomotic suture material may be a major factor in implantation and subsequent tumour growth at an anastomosis. Work within this thesis investigates the role of suture material in tumour cell adherence at colonic anastomoses.
CHAPTER 3
OTHER FACTORS IMPORTANT IN LOCAL RECURRENCE
3.1. INTRODUCTION

Tumour growth at injury sites in colorectal cancer has been observed at anastomosis, abdominal wounds, drain and colostomy sites and on raw mucosal surfaces such as haemorrhoidectomy scars (Ryall, 1907; Lawrie, 1906; Le Quesne and Thomsom, 1958; Killingbeck et al, 1965; Guiss, 1954; Rollinson and Dundas, 1984). The reasons are poorly understood and several factors are thought to be important.

3.2. TUMOUR CELLS

3.2.1. Mode of transport

Experimental work by Skipper et al (1988) has demonstrated that circulating tumour cells can cause anastomotic tumour growth. This is a time dependant phenomenon and only occurs if surgery precedes tumour cell injection. Following intracardiac injection of MC28 sarcoma cells in a Lister rat model tumour growth occurred on the serosal aspect of the anastomosis only if the surgery preceded tumour cell injection. Maximum growth occurred if cells were injected between days 2 and 8, with a peak between days 5-7 post anastomosis. If surgery followed tumour cell injection even by as little as 1 hour, then no growth occurred. Following injection of OES5 breast carcinoma cells on day 5 post anastomosis, perianastomotic tumour growth was seen in 2 out of 5 animals. The variability in tumour cell enhancement at different time points in the healing process remains unclear.

Fidler (1991; 1976,) has shown that tumours gain access to vascular spaces and circulate in the bloodstream and lymphatics. Using radiolabelled tumour cells Skipper et al (1988) also quantified the increased trapping of tumour cells at an anastomosis. This did not reach statistical significance and was only 1.5 - 1.6 times
greater than normal colon. Circulating tumour cells are thought to remain viable for 24-48 hours following resection of a primary carcinoma in man. Therefore, circulating tumour cells following resection would seem an unlikely cause of loco-regional recurrence. However, if such cells were being shed constantly from micrometastases elsewhere in the body then they could be a cause of such recurrences.

There is good evidence in the literature for the presence of micrometastases at the time of surgery. In a comprehensive clinical study, Finlay and McArdle (1986) assessed 71 patients who were considered by the surgeon on the basis of routine palpation at laparotomy to have had a 'curative' resection for colorectal cancer. In the immediate post-operative period routine ultrasound (US) and computerised tomography (CT) scans of the abdomen were performed and patients allocated to one of two groups on the basis of these results. Twenty four patients had a positive or an equivocal scan result and were grouped as having suspected occult hepatic metastases. Forty six patients had a negative scan and were presumed to have disease free livers. One patient died five days after surgery and necropsy revealed small hepatic metastases 0.5cm in diameter. Of the 24 patients in the suspected occult hepatic metastases group, 16 had progressively enlarging hepatic lesions clearly demonstrated on sequential scanning. One of these patients survived 5 years. In contrast, 5 of the 54 patients without evidence of occult hepatic metastases at the time of operation (46 of whom had a negative scan and 8 of whom had a false positive scan) died of disseminated disease. It was concluded that 30% of patients who undergo potentially curative resection of colorectal cancer had occult liver metastases at the time of surgery.
Areas of tissue injury such as those created by 'curative' surgery would appear to provide a suitable area for circulating tumour cells to adhere to and grow (see 3.3). Alexander et al (1985) postulated the production in host tissues of growth factors needed for the isolated cancer cells to grow and this is expanded in section 3.4. The role of circulating tumour cells from micrometastases is investigated in chapter 8.

Several studies have shown that viable tumour cells are present intraluminally and intraperitoneally following 'curative' colorectal cancer surgery (Umpleby et al, 1984b; Skipper et al, 1987; see 1.84). Work from this laboratory (McGregor, 1988) and by Skipper et al (1989) shows that intraluminal and intraperitoneal injection of tumour cells results in anastomotic tumour growth in 100% of animals. Clearly, tumour cells have the ability to implant and lead to tumour growth at a colonic anastomosis; however the relative importance of different modes of delivery remains in doubt and will be examined in chapter 7.

3.2.2. Tumour cell adherence to suture material.

Katz et al (1981) have shown that surgical sutures potentiate the development of wound infection. Using radiolabelled bacteria, adherence to braided sutures was 5-8 fold greater than to monofilament nylon. The wound infection rate correlated with the adherence characteristics and differential removal rate of glutaraldehyde fixed bacteria from the suture material. These results support the hypothesis that adherence of bacteria plays a significant role in the induction of surgical wound infection.

In recent years several studies have demonstrated that certain suture materials will enhance anastomotic tumour growth in an
experimental animal model (O’Dwyer et al, 1985; O’Dwyer and Martin, 1989; McGregor et al, 1991; McGregor, 1988; McGregor et al, 1989). O’Dwyer et al (1985) looked at the ability of different suture materials to localise radiolabelled tumour cells. Their results showed that this occurred maximally and significantly with braided material such as silk and least with monofilament materials such as prolene and stainless steel.

Work by McGregor (1988) and McGregor et al (1989). compared braided ((polyamide and polyglycolic acid) and monofilament (polypropylene and stainless steel) sutures with respect to their ability to entrap and transfer free Mtln3 cells from the colonic lumen of a rat. The braided materials transferred greater numbers of cells compared to the monofilament suture (p<0.001), but significant differences were also observed between polyglycolic acid and polyamide (p<0.001) and between polypropylene and stainless steel (p<0.05) In vitro assessment of tumour cell adherence confirmed Mtln3 cells adhere in significantly greater quantities to braided sutures (p<0.001) and that this was supported by in vivo growth studies. The reasons for the variability of tumour cell adherence to different suture materials have been clarified by Uff et al (1993).

Sutures have also been shown to potentiate colorectal carcinogenesis both at the suture line (Phillips and Cook, 1986; McGregor et al, 1989) and in normal colon (Porharriski, 1975; see 1.9)

3.3 TRAUMA

Experimental studies have demonstrated that tissue injury enhances tumour growth from locally implanted tumour cells (Jones &
Rous, 1914; Robinson & Hoppe, 1962; Skipper et al, 1989), or from cells that reach the site of injury via the circulation (Skipper et al, 1988; Alexander and Altemeier, 1964; Fisher et al, 1967, Murphy et al, 1988)

In 1914 Jones and Rous demonstrated that the resistance of the peritoneal lining to tumour cell implantation was largely or completely abolished by preliminary injection of a mechanical irritant (Kieselguhr lycopodium). Alexander and Altemeier (1964) examined the susceptibility of injured abdominal musculature and splenic/perisplenic tissue to haematogenous metastases in rabbits. A striking increase in the number of metastases was seen in the damaged tissue although the mode of injury and type of tissue seemed to have little effect on the development of tumour. In contrast, Fisher et al (1967) suggested such growth is related to the severity of trauma. Chromium$^{51}$ labelled tumour cells were injected intravenously or intra-arterially into normal rats or those subjected to either surgical, mechanical, or chemical trauma of a hind limb. The results demonstrated that tumour growth was related to the severity of the injury and that this appeared to correlate with the number of circulating tumour cells that localise to the site of tissue injury, with clean surgical incisions localising the least number of tumour cells and less likely to support tumour growth than either mechanical or chemical trauma. The mechanisms of enhanced tumour growth at injury sites is unclear.

Murphy et al (1988) investigated the mechanisms of organ selective tumour growth by blood born cancer cells. Sites of tissue injury were the preferred sites of tumour colonisation and could not be accounted for by the increased delivery of tumour cells to those regions. Results from studies in which the interval between trauma
and inoculation of tumour cells was varied, as well as promotion of intraperitoneal tumour growth by a macrophage infiltrate suggested that the macrophage component of the inflammatory infiltrate promoted tumour growth. Macrophages produce a number of growth factors including platelet derived growth factor and epidermal growth factor which are known to be mitogenic to tumour cells. The role of growth factors is examined below.

3.4. INTEGRINS AND GROWTH FACTORS

Integrins are a widely expressed family of cell surface adhesion receptors. Experimental work suggests they have an important role in cellular attachment to extracellular matrix, and may mediate cell to cell adhesion events as well as acting as signalling receptors (Hynes, 1992; Nigam and Pignatelli, 1993). In vitro studies have shown that attachment and spread of colon cancer and sarcoma cells to plastic dishes are mediated by laminin and fibronectin respectively, members of the integrin family (Viodavsky and Gospodarowicz, 1981). Stallmach et al (1992) reported a decreased expression of integrins in human colon carcinoma, suggesting this may contribute to the altered adhesion and migration properties of these tumour cells. Lindmark et al (1993) presented data that suggests that determination of the pattern of expression of the integrin subunits $\alpha_2$ and $\alpha_3$ in the preoperative biopsy and surgical specimen could be used as a prognostic indicator.

Growth factors are small polypeptides that bind to specific cell receptors. This binding produces an intracellular response that results in growth and proliferation but may also be inhibitory (Goustin et al, 1986; Arbet, 1990). Tumour cells produce growth factors and growth factor receptors and disorders of their control is
thought to confer on them a growth advantage compared to normal tissue (Steele, 1989; Goustin et al, 1986; Arbet, 1990; Wigley et al, 1986). Loss of TGF-β control is thought to occur when colon cells transform into malignant cells (Lahm & Odartchenko, 1993). Epidermal growth factor receptors have been identified in colorectal cancer cells (Steele et al, 1990). Epidermal growth factor (EGF), transforming growth factors alpha and beta (TGF-α, TGF-β), platelet derived growth factor (PDGF), basic fibroblast growth factor (β-FGF), Bombesin, Interleukin 2 (IL2), and colony stimulating growth factor have been identified in the conditioned media of various neoplastic cells (Anzano et al, 1989) and application of EGF and β-FGF has been shown to stimulate human colon cancer cells to proliferate in vitro (Whitehead et al, 1990). Growth factors have also been increasingly implicated in wound healing (ten Dijke and Iwata, 1989), and wound healing is associated with increased synthesis of growth factors in the cornea in rabbits (Barnes, 1988) and wound fluid extracts in rats (Grotendorst et al, 1989; Cromack et al, 1987). Numerous studies have shown that application of these growth factors (EGF, TGF-β, β-FGF) to incisional wounds increases tensile strength and angiogenesis, as well as cellularity and collagen content (Slavin et al, 1992a; Slavin et al, 1992b; Mustoe et al, 1987, 1989; Brown et al, 1988; Curtsinger et al, 1989; Kingsnorth and Slavin, 1991; Laato et al, 1986; Kingsnorth et al, 1990). These growth factors have been shown to be chemotactic and mitogenic for cells in vitro. Work by Kingsnorth et al (1990) has demonstrated an increase in tensile strength of intraperitoneal anastomotic wounds in pigs after intraperitoneal infusion of EFG.

Bracken (1991) and Bracken et al (1991) examined the healing of rat colonic anastomoses. Immunohistology showed a fibronectin
reaction from day 1, maximum on day 5; changes were also noted in collagen and laminen levels. Results also indicated an enhancement of the EGFR gene expression suggesting that anastomotic healing is associated with the presence of EGF or an EGF like substances and that its activity increases during the first post-operative week. It is possible that growth factor levels at different time points in healing may provide a suitable environment for circulating tumour cells to adhere to and grow and could thus explain Skipper et al’s findings. Anastomotic dehiscence may stimulate growth factor production and could explain the findings by Akyol et al (1991) and Fujita et al (1993) that an anastomotic leak increased local recurrence rates. The role of growth factors in anastomotic healing and tumour growth will be explored further in an experimental animal model (See chapter 10)

SUMMARY

In colorectal cancer there is potential for tumour cells to reach the anastomotic site either intraluminally, intra-arterially or intraperitoneally and lead to anastomotic tumour growth. Both suture material and trauma are important factors in tumour cell adherence. The role of growth factor is unclear. These factors will be investigated further in an experimental animal model in chapters 6-10.
SECTION 1

CLINICAL STUDIES.
CHAPTER 4

EFFECT OF THE SURGEON'S SPECIALITY INTEREST ON THE TYPE OF RESECTION PERFORMED FOR COLORECTAL CANCER.
4.1. INTRODUCTION

The large bowel cancer project (Phillips et al, 1984a) demonstrated a wide range of local recurrence rates between individual Consultant Surgeons, which varied between 5 and 20% and proved to be an independent prognostic factor following potentially 'curative' colorectal surgery. In a more recent study by McArdle and Hole (1991) similar findings were observed with local recurrence rates varying from 0-21% and survival at 10 years in patients following 'curative' resection varying from 20-62%. While some of the variations in outcome reflected differences in the patient population after adjustment for normal risk factors such as cardio-respiratory disease, elective versus emergency admissions, presence or absence of local spread etc., substantial differences remained.

A satisfactory explanation for differences in local recurrence and survival amongst surgeons performing colorectal cancer surgery has not been reached by either study (Phillips et al, 1984a; McArdle and Hole, 1991). Both show that there was a wide difference in technical complications such as wound or anastomotic dehiscence among surgeons but did not show correlation between such complications and recurrence. Neither study looks at the type of resection performed. Surgeons with an interest in colorectal cancer believe that a curative resection should include ligation of the major vascular pedicle or pedicles allowing for a wide excision of the lymph node bearing mesocolon and colon itself with wide tumour free margins.
4.2. AIM

To examine the type of resection performed by surgeons with a colorectal cancer interest and to compare this with surgeons of other specialty interests or gastroenterology interest.

4.3. Patients and methods

Over a one year period (1990) all patients undergoing surgery for colorectal cancer at the Western Infirmary and Gartnavel General Hospital in Glasgow were identified. In those undergoing curative resection the following details were recorded: Age, sex, site of tumour, length of resection specimen, resection margin, resection of clinically involved organs, histological appearance, number of lymph nodes retrieved by the Pathologist and in patient morbidity and mortality. There were three groups of surgeons, those with a colorectal cancer interest, group CCI; a gastroenterology interest, group GII and with other surgical interests such as vascular or transplant surgery, group OSI.

4.4. Results

Over a one year period 158 colectomies were performed, 125 were thought curative. Seven patients had subtotal colectomy with ileorectal anastomosis and were thus excluded from evaluation. In one patient the resection length was not measured, while in another with biopsy proven caecal cancer the specimen was lost between theatre and the Pathology Department. This left 116 evaluable cases of which 35 were right sided and 81 were left sided or rectal cancers (Table 2.1).
The resection lengths in right sided colon cancer are similar for all groups (Table 2.2). Distal resection margins were also similar, 100mm for group CCI versus 90mm for groups GII and OSI. A median of 13.5 lymph nodes per patient were retrieved by the pathologist for group CCI compared with 7.5 for the other groups (Table 2.3: P= 0.08 Mann Whitney Test).

Highly significant differences (P <0.001) in the median length of colon resected in left sided and rectal cancers between the groups (Table 2.4). Surgeons with a colorectal interest resecting over twice as much colon as those in group OSI. The median distal resection margins (30 mm) for rectal cancers were similar in all groups. However, for sigmoid carcinomas the median distal resection margin was 20mm for groups GII and OSI combined compared with 55mm for Group CCI (Table 2.5: P<0.001). There is no significant difference in the number of patients undergoing abdomino-perineal resection of the rectum in either group, 4(10%) in group CCI versus 6 (15%) in groups GII and OSI. A median of 6 nodes per patient were retrieved by the pathologist for groups CCI compared with 5.5 for other groups (Table 2.3: P= 0.20).

The average age of patients was similar, 72 years for group CCI versus 69 years for groups GII and OSI. A higher percentage of patients were female in groups GII and OSI: 58% compared with 48% in group CCI, however analysis showed that resection length was not influenced by gender.

Surgeons in group CCI were more likely to remove adjacent clinically involved organs, 15% of all curative resections versus 0% for groups GII and OSI. These included partial cystectomy (4), bilateral oophorectomy or hysterectomy (2), tail of pancreas and spleen (1) and en bloc resection of sigmoid and small bowel in
locally advanced caecal carcinoma. Nevertheless, the percentage of patients undergoing palliative resection in all groups was the same at 21%.

A higher post-operative mortality was noted in group CCI (Table 2.6) with 4 (7%) deaths within 30 days of operation. Three of the deaths were cardiovascular related while one was due to post operative pneumonia in an 89 year old lady. One death (1.5%) occurred in group GII and OSI. This resulted from sepsis in a patient who underwent splenectomy for splenic injury occurring during sigmoid colectomy. No patient in group CCI had clinical evidence of anastomotic dehiscence while 1 (1.5%) occurred in Groups GII and OSI combined. This required relaparotomy with a Hartmanns procedure. The patient was successfully reanastomosed three months later.
### TABLE 4.1
TUMOUR SITE AND LENGTHS FOR CURATIVE RESECTION GROUP

<table>
<thead>
<tr>
<th>Group</th>
<th>Right colon</th>
<th>Transverse colon</th>
<th>Left colon</th>
<th>Rectosigmoid</th>
<th>Median tumour length</th>
<th>No node positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group CCI</td>
<td>11</td>
<td>2</td>
<td>3</td>
<td>38</td>
<td>40mm</td>
<td>12 (22%)</td>
</tr>
<tr>
<td>Group GGI + OSI</td>
<td>20</td>
<td>2</td>
<td>5</td>
<td>35</td>
<td>37.5mm</td>
<td>24 (39%)</td>
</tr>
</tbody>
</table>

Group CCI = Colorectal cancer interest  
Group GII = Gastroenterology interest  
Group OSI = Other surgical interest.

### TABLE 4.2 MEDIAN RESECTION LENGTHS FOR CURATIVE RIGHT COLON CANCER

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Resection Lengths (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI</td>
<td>7</td>
<td>250.0 (150-400)</td>
</tr>
<tr>
<td>GII</td>
<td>15</td>
<td>280.0 (200-400)</td>
</tr>
<tr>
<td>CCI</td>
<td>13</td>
<td>270.1 (200-400)</td>
</tr>
</tbody>
</table>

Data in brackets interquartile ranges.

No difference between groups using the Kruskall-Wallis Test comparing the median of the three groups.
### TABLE 4.3 LYMPH NODE RETRIEVAL

<table>
<thead>
<tr>
<th>Group</th>
<th>Right colon*</th>
<th>Left colon**</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI + GII</td>
<td>7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>CCI</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

* *P* = < 0.08 Mann Whitney test.

** P = 0.20

Group CCI = Colorectal cancer interest
Group GII = Gastroenterology interest
Group OSI = Other surgical interest.

### TABLE 4.4 MEDIAN RESECTION LENGTHS FOR CURATIVE LEFT SIDED COLON AND RECTAL CANCERS

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Resection Lengths(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI</td>
<td>21</td>
<td>130 (87.5-180)</td>
</tr>
<tr>
<td>GII</td>
<td>19</td>
<td>200 (150-220)</td>
</tr>
<tr>
<td>CCI</td>
<td>41</td>
<td>280 (220-320)</td>
</tr>
</tbody>
</table>

Data in brackets interquartile ranges.

p = <0.001 using Kruskall Wallis Test for comparisons of medians of the three groups.
### TABLE 4.5 DISTAL RESECTION MARGIN FOR CURATIVE SIGMOID COLON CANCER

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI + GII</td>
<td>11</td>
<td>20 (15-30)</td>
</tr>
<tr>
<td>CCI</td>
<td>13</td>
<td>55 (35-77)</td>
</tr>
</tbody>
</table>

Data in brackets are interquartile range

Differences significant between groups using Mann-Whitney Test at P = 0.0017

- Group CCI = Colorectal cancer interest
- Group GII = Gastroenterology interest
- Group OSI = Other surgical interest.

### TABLE 4.6 MAJOR POST-OPERATIVE COMPLICATIONS FOR CURATIVE RESECTION GROUP.

<table>
<thead>
<tr>
<th></th>
<th>Group CCI</th>
<th>Groups GII + OSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>4 (7%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>Anastomotic leak</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
</tr>
</tbody>
</table>
4.5. DISCUSSION

This study shows that the type of resection performed for curative colorectal cancer surgery correlated with the surgeons specialty interest. Surgeons with a special interest such as vascular or transplant surgery removed less than half as much of the left colon and rectum as surgeons with a colorectal interest. Surgeons with a gastroenterology or general surgery interest performed a resection that was intermediate between the colorectal and other specialty group. Operative mortality was higher in the colorectal cancer interest group. This was reflected by a high incidence of cardiovascular related complications which may have been prevented by better intra or perioperative care.

The number of lymph nodes retrieved from the mesenteric resection was less for the groups without a colorectal interest although this did not reach statistical significance. The method used for lymph node analysis was that performed as part of routine pathology within the hospital laboratory. This involved dissecting and palpating the omentum with removal and assessment of any nodes found and was obviously highly dependent on the individual pathologist performing the analysis. Techniques such as xylene clearance have been shown significantly to increase the number of lymph nodes recovered (Cawthorn et al, 1986; Pickren, 1975), but without affecting the final stage of the disease (Pickren, 1975). More recently, Haboubi et al (1992) reported that xylene clearance combined with immunohistochemistry changed the Dukes staging in 12 out of 41 cases of colorectal carcinoma, resulting in 55% of Duke's B becoming Dukes C by the detection of occult invasion by immunohistochemistry. Whether this is useful and how this
corresponds to patient survival is unclear as this study did not address these issues.

It has been shown that small lymph nodes (<5mm) in colorectal carcinoma commonly contain metastases yet they may easily be missed (Herrera-Ornelas, 1987). Occult metastasis has proved to be of significant prognostic value in axillary lymph nodes of breast carcinoma (Neville et al, 1990). Although Dukes staging has remained useful regarding prognosis (Dukes & Bussey, 1958), it is imprecise, approximately 25% of patients staged Dukes B die within 5 years, while 30% staged Duke’s C survive 5 years (Dukes, 1957). An important part of the staging is assessment of involvement of lymph nodes, particularly as the number of involved lymph nodes has been suggested as an independent prognostic indicator of colorectal cancer (Dukes and Bussey, 1958; Wolmark et al, 1986). Xylene clearance enables much more accurate assessment of lymph nodes in a specimen but is relatively time consuming and a technically difficult procedure which has restricted its use. Its place in the routine work of a laboratory is debatable and its application has been mainly as a research tool.

There is good evidence for a surgeon related variable in patient outcome following curative colorectal cancer surgery (McArdle and Hole, 1991; Phillips et al, 1984). This study has investigated surgeons with a specialty interest as a group and has not looked at individual surgeons. It is possible that the differences seen are a reflection of surgical skill independent of specialisation but results would still seem to suggest that individuals performing a wide excision of colorectal cancer are those with specialist training. The effect of this on outcome has not been assessed and is certainly a criticism of the study.
Other surgeon dependent factors need to be considered. According to some retrospective analysis, preoperative and peroperative blood transfusion may be associated with increased risk of tumour recurrence (Burrows and Tartter, 1982; Blumberg et al, 1985; Beynon et al, 1989; Liewald et al, 1990). In contrast, results from a multivariate analysis of a prospective study by Bentzen et al (1990) showed that patients receiving whole blood or packed blood cells fared no worse than non-transfused patients. These findings were supported by a more recent prospective study of perioperative blood transfusion by Sene et al (1993) which indicated the incidence of recurrence of colorectal carcinoma was no greater in the transfused group compared to non-transfused. The literature is conflicting and therefore the risk of blood transfusion remains unclear. This surgeon related variable was not assessed in this study and is unlikely to have affected the resection length obtained and for this reason its use in prospective studies is debatable.

Implantation of exfoliated tumour cells has been suggested as a cause for some local recurrences (see 1.82-1.83). The use of cytotoxic agents to prevent implantation of tumour cells by surgeons has been claimed to reduce the incidence of local recurrence (Keynes, 1961; Southwick et al, 1962). Based on experimental results indicating the presence of intraperitoneal, intraluminal tumour cells and cytotoxicity of specific solutions to these cells, their routine use in colorectal cancer surgery was recommended (Umpleby et al 1984b). When surveyed, 67% of surgeons in the south west of England used tumourcidal agents (Umpleby et al 1984b). More recently, unpublished data from the West of Scotland (Personal communication, J Docherty, 1993) has confirmed surgeon variability, with 70% of
surgeons using some form of tumourcidal agent. In this study, it proved impossible to ascertain if tumourcidal agents were used (and anyway the information is unlikely to have affected specimen resection length. However, it is an important surgeon dependent variable that could influence local recurrence rates and would be important to note in the ongoing study looking at the outcome of colorectal surgery in different specialty groups.

Whether length of resection affects outcome in colorectal surgery is unclear. There is no general agreement among surgeons as to what represents an adequate resection for colorectal cancer. Unlike breast cancer surgery there have been no clinical trials in this area. There is good evidence that a surgeon related variable can alter outcome of patients following colorectal cancer surgery. Results from study have shown a difference in resection lengths between surgeons of different specialty interests and to assess the effect on outcome an ongoing clinical trial is under way.

4.6. CONCLUSION

Surgeons with an interest in colorectal cancer surgery perform a more extensive excision for left sided colon and rectal cancer.
SECTION 2
EXPERIMENTAL STUDIES.
CHAPTER 5

MATERIALS AND METHODOLOGY.
5.1. INTRODUCTION.

The animal model and cells used were consistent throughout all experimental work. Similarly, the techniques of cell culture and radiolabelling were unchanged for each experiment and have been described together for convenience.

5.2. EXPERIMENTAL MATERIALS

5.2.1. Animals

Eight to ten week old Fischer F344 rats (Olac Laboratories Ltd. Bicester) were used. Animals were aged 8-12 weeks at the start of each experiment and were housed in fours in polypropylene cages with stainless steel mesh lids prior to laparotomy and individually post-laparotomy. Food and water were freely available and their diet consisted of high quality breeding formulation ("CRM", Biosure Ltd., Lavender Hill, Manea, Cambridgeshire). No measures were taken to avoid coprophagy and animals were not fasted prior to laparotomy. For all colonic anastomosis work the distal colon was used. The rats were housed in an animal facility in the Department of Surgery at the Western Infirmary under proper lighting and temperature control and were observed daily for fur loss, texture and weight loss. All animal work was licensed by the Home Office under the Animals (Scientific Procedure) Act 1986.

5.2.2. Tumour cell line.

The tumour cell line used in all experimental work was the Mtln3 clone of rat adenocarcinoma 13762NF. The parent cell line for the Mtln3 tumour was developed and characterised by Segaloff (1966). The Mtln3 clone was isolated from spontaneous lung metastases of the tumour by Neri et al (1982) and donated by Drs Neri and Nicholson MD,
Anderson Hospital, Houston, Texas, USA. Extensive experimental work within the Department of Surgery at the Western Infirmary has shown these cells to be synergistic with the strain of experimental animals used (McGregor, 1988; Akyol, 1990). At the time of commencing the study no known colonic adenocarcinoma cell lines were available and M1ln3 cells were used as they have proved suitable for colonic experimental work in the past (McGregor, 1988; Akyol, 1990).

5.2.3. Cell Culture Material

Cells were cultured in either 75cm² or 150cm² 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 Dulbecco's modified eagles medium (F10/DMEM) with 10% foetal calf serum (FCS) (all obtained from Flow Laboratories Ltd., Rickmansworth, Hertfordshire). No antibiotics were added to the medium and cultures were incubated at 37°C in equilibrium with 5% carbon dioxide in humidified CO₂ gas incubator.

5.2.4. Radioisotope

The isotope used for all experiments was Iodine¹²⁵, obtained in the form of I¹²⁵-I-iodo-2-deoxyuridine (IUDR) (Amersham International plc). This is a gamma emitter with a half life of 60 days. Full precautions for the handling of radioactive materials were taken as laid down by the Radiation Protection Officer of the University of Glasgow and Greater Glasgow Health Board. All radioisotope experiments were carried out in one designated laboratory.
5.3. EXPERIMENTAL METHODS

5.3.1. Preparation of fresh cell culture

All cell cultures were carried out under a laminar flow hood using sterile materials. Fresh stock of the Mtln3 clone was stored in liquid nitrogen in individual vials each containing approximately 1 million cells suspended in 1ml of culture medium with 10% dimethylsulphoxide (DMSO). Preparation of a fresh culture involved first thawing the frozen cells by placing a sealed vial in water at 37°C. The cell-suspension was then withdrawn using a sterile micropipette tip and placed in a 75cm\(^2\) tissue culture flask; a total of 24mls of the F10/DMEM/FCS culture medium was then added slowly over a 5 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 confluence growth was obtained, normally after a period of 4-5 days.

5.3.2. CELL PASSAGE

Once near confluence was observed the culture was ready for passage. All culture medium was withdrawn from the tissue culture flask by pipette and replaced with 10mls of Hanks’s balanced salt solution (Hank’s BSS, Gibco, Paisley, Scotland). Following the removal of this 5mls of 0.2% trypsin EDTA solution (Gibco) was added and incubated for 5-10 minutes until the cells had detached and 10 mlis (an excess) of F10/DMEM/FCS was then added to neutralise the trypsin activity, after which the cells were washed twice with F10/DMEM/FCS by centrifugation at 1200 rpm for 10 minutes. The cell pellet was resuspended in 1ml of F10/DMEM and the cell count viability assessed using Trypan blue exclusion test. Only those
with 95% or greater viability were used. Final dilutions were adjusted to give $1 \times 10^6$ cells in 0.2ml of medium. Each batch of cells was passaged a maximum of 6 times before being discarded to minimise problems of phenotypic drift (Neri and Nicolson, 1981).

5.3.3. Radiolabelling of tumour cells

Cells were radiolabelled with IUDR in order to quantify the number of tumour cells adhering to the anastomosis and normal colon. The process for cell labelling was as follows. Once a fresh batch of cells had been cultured successfully, $5 \times 10^5$ cells were introduced into a 150cm$^2$ flask in 40mls of F10/DMEM/FCS and cultured until 20-30% confluence growth was obtained. The medium was then changed and 2 Microcuries ($\mu$ci) of IUDR were added. Previous work in our laboratory has shown that optimum radiolabelling of the Mtln3 cell line occurs using a concentration of 0.05-0.07$\mu$ci IUDR/ml of culture medium (Purushotham et al, 1991). After 24 hours the cells were removed as previously described. Only those cells with >95% viability were used and final dilutions were adjusted to give $1 \times 10^6$ cells in 0.2mls of F10/DMEM medium. The radioactivity was assessed in a gamma counter as described.

5.3.4. Measurement of radioactivity

Radioactivity of all samples was measured in a ("Compugamma" LBK Pharmacia, Milton Keynes) gamma counter. Radioactive counts were acquired over a two minute period and a mean number of counts per minute calculated. On each occasion a labelled cell suspension containing $1 \times 10^6$ cells was measured and used to calculate the number of cells in each sample assessed.
5.3.5. Histopathology

All tissue samples for histopathology were initially fixed in 10% formal saline at the time of autopsy. Further preparation took place within the Department of Pathology at the Western Infirmary using standard techniques. Tissue dehydration and blocking in paraffin wax were carried out in a 22 hour cycle in a histokine automatic tissue processing machine as follows. The dehydration sequence comprised 1 hour in 50% alcohol followed by a further hour in 80% alcohol after which the sample was passed through the 3 beakers each containing 8% phenol in methylated spirit over an 8 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 2 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 chart. Sections of 5 micrometres were then cut and submitted for staining.

The procedure for tissue staining comprised a regressive haemotoxylin and eosin technique. Each tissue section was firstly dewaxed in xylol and alcohol and then stained in haemotoxylin 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 to further dehydration using alcohol followed by xylol prior to permanent mounting in D.P.X.
CHAPTER 6

Experiment 1.

VIABLE CIRCULATING TUMOUR CELLS AND
ANASTOMOTIC TUMOUR GROWTH.
6.1. INTRODUCTION

It is generally accepted that carcinomas spread via the lymphatic route and sarcomas via the bloodstream resulting in different metastatic patterns. Skipper et al's (1988) work has clearly demonstrated a time dependant enhancement of perianastomotic tumour growth following intracardiac injection of tumour cells. However, the vast majority of results were obtained from work using a sarcoma cell line, only limited work was performed with an adenocarcinoma cell line.

6.2. AIM

To determine if intra-arterial adenocarcinoma cells result in tumour growth at a colonic anastomosis in a pattern similar to that observed with a sarcoma cell line by Skipper et al (1988).

6.3 ANIMAL MODEL

Previous work at this laboratory has shown that Mtln\textsuperscript{3} adenocarcinoma cells will seed to a colonic anastomosis in Fischer rats if injected via the intracardiac route (Akyol, 1990). Post mortem studies of the animals showed widespread and uniformed sized nodules in the lungs, diaphragm and throughout the abdominal cavity (particularly the small bowel mesentery and adrenals) with the notable exception of the bowel, unless this was traumatised prior to tumour cell injection.

Under intraperitoneal midazolam (Hypnovel, Roche Products Ltd, Welwyn Garden City, England) and Fentanyl (Hypnorm, Janssen Pharmaceutical Ltd., Grove, Oxford, England) (\(\frac{1}{4}\) Midazolam, \(\frac{1}{4}\) Fentanyl, \(\frac{1}{4}\) sterile water) the right carotid artery was isolated between ligatures and opened. An 0.6mm outside diameter polythene
cannula (Boros Labs) was passed proximally into the left ventricle. The correct position was confirmed by noting the resistance of the aortic valve and the double wave form transmitted to the cannula and $1 \times 10^6$ MTLn3 cells suspended in 0.2ml of F10/DMEM were injected (Figure 4.1). The cannula was removed, the artery tied off, the skin closed with a single layer of 3.0 vicryl and the animal was allowed to recover.

6.4. EXPERIMENTAL DESIGN

<table>
<thead>
<tr>
<th>Groups</th>
<th>Timing of ICI in relation to anastomosis (Anast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minus 1/2hr</td>
<td>30 min prior to anast.</td>
</tr>
<tr>
<td>Day 0</td>
<td>30 min post anast.</td>
</tr>
<tr>
<td>Day 1</td>
<td>24hr post anast.</td>
</tr>
<tr>
<td>Day 3</td>
<td>3 days post anast.</td>
</tr>
<tr>
<td>Day 5</td>
<td>5 days post anast.</td>
</tr>
<tr>
<td>Day 7</td>
<td>7 days post anast.</td>
</tr>
<tr>
<td>Day 10</td>
<td>10 days post anast.</td>
</tr>
<tr>
<td>Day 14</td>
<td>14 days post anast.</td>
</tr>
<tr>
<td>ICI - Intracarotid injection</td>
<td></td>
</tr>
</tbody>
</table>
Eighty one animals divided into 8 groups were used. All animals had laparotomy with distal colonic anastomosis on day 0 and intracarotid injection (ICI) of $10^6$ Mtln3 tumour cells in 0.2ml of F10/DMEM at variable time points.

Halothane induction of anaesthesia was used for all animals. It proved to be technically difficult with an unacceptably high mortality to perform intracarotid injection of tumour cells using halothane inhalation anaesthesia and for this reason all animals had the procedure performed under intraperitoneal midazolam and fentanyl anaesthesia. This unfortunately has a narrow therapeutic ratio leading to excessive mortality if used repeatedly in the same animal and it was only used once for intracarotid injection of tumour cells. All animals were subjected to both anaesthetics although there obviously were differences in the timing of the administration.

In groups -1/2hour and day 0 procedures were performed under midazolam/fentanyl anaesthesia as the animals were given intracardiac injection of $1 \times 10^6$ Mtln3 cells under the same anaesthetic. In the other groups halothane anaesthesia was used on day 0 as the only procedure performed at this time was a colonic anastomosis. The distal colon was divided and a primary colonic anastomosis fashioned with 8-10 interrupted 5.0 silk sutures. The laparotomy incision was closed with 2 layers of 3.0 continuous vicryl and the animals allowed to recover. The ten animals in the day 0 subgroup underwent intracardiac injection of tumour cell after closure of the abdomen and 5 animals in group -1/2 hour had intracardiac injection of tumour cells prior to fashioning of the distal colonic anastomosis, both procedures being performed under the same anaesthetic. Under midazolam and fentanyl anaesthesia subgroups of 10 or more animals were given an intracardiac injection of $1 \times 10^6$ Mtln3 cells via the
right carotid artery as previously described on days 1, 3, 5, 7, 10, 14. Animals were allowed to recover and sacrificed by cervical dislocation under halothane anaesthesia on day 21 post intracarotid injection, or earlier if they become unwell or lost more than 10% of their initial body weight. The anastomosis was excised and sent for independent assessment of perianastomotic tumour growth by a pathologist in the department of histopathology at the Western infirmary. The technique for tissue sampling and preparation have been described in section 5.3.5.

6.5. RESULTS

All animals at post mortem had evidence of intraabdominal disease with obvious tumour in the small bowel mesentery and adrenals but NOT on untraumatised small or large bowel (Figure 6.1). Tumour was also seen in the lungs, pleura and diaphragm. Those in group -1/2 hour were noted to have less marked disease, although still present.

Two samples (Day 1) were not evaluable due to technical difficulties leaving 79 anastomoses for histopathological assessment. Standardized sections were taken transversely through the level of the anastomosis. Figure 6.1 illustrates the post-mortem appearance of tumour occurrence following ICI of Mtln3 tumour cells and shows widespread nodules throughout the abdominal cavity, particularly the small bowel mesentery, adrenals and spleen. No tumour growth was seen in the bowel unless traumatised prior to tumour cell injection. Figure 6.2 demonstrates tumour growth at the anastomotic site. Results show that tumour growth occurred at the anastomosis at all assessed time points following intracardiac injection of tumour cells (Figure 6.3). All animals developed perianastomotic tumour growth
if cells were injected on days 5-10 post anastomosis. The incidence of perianastomotic tumour growth was significantly less on days 0 and 14 compared with days 5, 7, 10. (P <0.05 Chi squared test with Yates correction).
Figure 6.1.
Post-mortem appearance of tumour occurrence following ICI Mtln3 tumour cells
Figure 6.2

Anastomotic occurrence after ICI of Mtln3 tumour cells.
Figure 6.3 Effect of timing of surgery on perianastomotic tumour growth following intracarotid injection of Mtln3 tumour cells.
6.6. DISCUSSION

These results are similar to Skipper et al's findings for a sarcoma cell line that tissue injury will enhance tumour growth at an anastomosis only if tumour cell injection follows surgery. However, the results of this study differ from Skippers in that there appears to be a higher incidence of tumour growth at all time points and this may be a reflection of the greater tumour burden used in this study (1 x 10^6).

Why no tumour growth occurs if surgery is performed after tumour cell injection in this model is unclear. The findings in this study agree with those of Skipper et al (1988). Work by Murphy et al (1988) suggests that tumour cells do not recirculate significantly after injection and are rapidly cleared from the bloodstream by implantation in organs. Although bioassay (see below) proved viable tumour cells in many organs for up to 24 hours after tumour cell injection, none were found in the rats bloodstream 5 minutes after intraarterial injection and this certainly would explain why no tumour growth occurs if a colonic anastomosis is created after tumour cell injection. It is possible that by the time the anastomosis was fashioned no viable circulating tumour cells remained in the bloodstream to implant at the anastomosis site. The bioassay used in this experiment involved intraperitoneal transplantation of "minced" organs into recipient rats, viable cells leading to obvious peritoneal tumour growth. Both Murphy et al (1988) and Skipper et al (1988) show increased trapping of tumour cells at a colonic anastomosis compared to normal bowel and therefore ischaemia of the anastomosis created by suture placement would seem to be an unlikely explanation.
Although experimental work has shown increased trapping of tumour cells at a colonic anastomosis compared to normal bowel, it was thought that the increased count seen with surgical trauma was insufficient to account for the observed marked susceptibility of the anastomosis to tumour growth (Murphy et al, 1988, Skipper et al, 1988). The work by Murphy et al (1988) would explain why no tumour growth is seen at an anastomosis if tumour cells are injected before surgery but it does not account for the time dependant enhancement of tumour growth at an anastomosis when cells are injected after surgery, so other factors in the healing process may be important. Growth factors have been increasingly implicated in wound healing (ten Dijke and Iwata, 1989), and wound healing has been associated with increased synthesis of growth factors in a rat model (Cromack et al, 1987; Grotendorst et al, 1989). Epidermal growth factor receptors have been identified in colorectal cancer cells (Steele et al, 1990) and growth factors have been identified in the conditioned media of various neoplastic cells (Anzano et al, 1989). It is possible that growth factor levels at different time points in healing may provide a suitable environment for circulating tumour cells to adhere and grow, and could explain results from this experiment and Skipper et al's (1988) findings.

6.7. CONCLUSION

Intra-arterial adenocarcinoma cells can implant at a colonic anastomosis and result in tumour growth in an experimental animal model and occurs only if colonic surgery precedes tumour cell injection.
CHAPTER 7

Experiment 2

ADHERENCE OF INTRALUMINAL, INTRAPERITONEAL AND INTRA-ARTERIAL TUMOUR CELLS TO A COLONIC ANASTOMOSIS AND NORMAL COLON.
7.1. INTRODUCTION

Increased trapping of tumour cells at an anastomosis has been demonstrated by Skipper et al (1988) although this did not reach statistical significance and was only 1.5 - 1.6 times greater than normal colon. Work from this laboratory (McGregor, 1988) and by Skipper et al (1989) shows that intraluminal and intraperitoneal injection of tumour cells results in anastomotic tumour growth in 100% of animals. However, the number of cells implanting was not quantified and therefore it is difficult to ascertain the relative importance of the different methods of tumour cell delivery. In colorectal cancer it is possible for tumour cells to reach the anastomotic site either intraluminally, intra-arterially or intraperitoneally and cause anastomotic tumour growth.

7.2. AIM

To compare the potential for intraperitoneal, intraluminal and intra-arterial cells to adhere to a colonic anastomosis and normal colon using radiolabelled tumour cells.

7.3. EXPERIMENTAL MODEL

Thirty three F344 Fischer rats underwent a laparotomy through a midline abdominal incision under midazolam and fentanyl anaesthesia (1/4 Midazolam, ¼ Fentanyl, 1/2 sterile water). A 1cm longitudinal colotomy was fashioned in the descending colon and this was immediately repaired with 4 interrupted 4.0 silk sutures. Following this $1 \times 10^6 \ 1^{125}$ IUDR labelled Mtln3 cells were introduced either intra-arterially (IA), intraluminally (IL) or intraperitoneally (IP) in separate groups of rats.
In group IA while still under Midazolam and Fentanyl anaesthesia all animals underwent cannulation of the right carotid artery via a transverse cervical neck incision as described in chapter 6. The cells were introduced into the right atrium following fashioning of the colotomy and closure of the abdomen in two layers with continuous 2.0 vicryl. The cells were injected in 0.2mls of F10/DMEM medium followed by introduction of a further 0.1ml of medium to flush the catheter. The neck incision was then closed in two layers of continuous 2.0 vicryl.

In group IL following closure of the colotomy a cannula was introduced rectally and passed 3cm proximal to the anastomosis. The bowel lumen was occluded 1cm above the anastomosis but below the tip of the cannula by digital pressure and the cells injected under low pressure and the cannula flushed with 0.1ml of unlabelled medium. The abdomen was closed in two layers of continuous 2.0 vicryl. (Figure 7.1).

In group IP following closure of the colotomy the colon was lifted medially and the cells placed in the left paracolic gutter away from the anastomosis and the abdomen was then closed in two layers of continuous 2.0 vicryl (Figure 7.2). One hour later all animals were killed by cervical dislocation while still under anaesthesia. The anastomosis and normal colon immediately adjacent to the anastomosis proximally were excised and assessed in the gamma counter for radioactivity. In group IL the 1cm of colon immediately distal to the anastomosis was excised as well as a further 1cm of proximal normal colon. The radioactivity was assessed as previously described.
7.3.1. Controls for Free $I^{125}$ IUDR

Experiments were repeated using two animals in each group with free $I^{125}$ IUDR in 0.2 ml of cell free F10/DMEM. The concentration of $I^{125}$ IUDR was calculated on the basis of radioactivity from previous washings of radiolabelled tumour cells. The purpose of this was to ensure that differences in radioactivity observed between groups represented differences in cell count and not adherence of non-cell bound $I^{125}$ IUDR.
Figure 7.1.

Intraluminal injection of Mtln3 tumour cells
Figure 7.2.

Intraperitoneal injection of Mtln3 tumour cells.
7.4. RESULTS

No significant difference could be demonstrated for the number of circulating cells adhering at a colonic anastomosis (colotomy) and normal and normal colon (table 7.1). In group IP significantly adhere to the colotomy site than normal colon (Table 7.2). A similar pattern is observed in group IL when normal proximal and distal colon are compared with the colotomy site (Table 7.3). This difference is statistically significant for distal normal colon but not proximal normal colon.

Both intraperitoneal and intraluminal tumour cells have a greater potential to adhere to a colonic anastomosis. Intraperitoneal cells were $35 \times (21889:625)$ and intraluminal cells $20 \times (12366:625)$ more likely to adhere to an anastomosis than intra-arterial cells.

7.4.1. Effect of free $^{125}$IUDR

Radioactivity count at the colotomy site was no different to that from normal colon when radiolabelled medium was injected either IP, IL or IA and would not account for the differences seen.

7.5. DISCUSSION

Results from this study demonstrates that both intraluminal and intraperitoneal tumour cells have a much greater potential to implant at a colonic anastomosis than circulating tumour cells. Work presented in this thesis and by Skipper et al (1988) has shown that circulating tumour cells will grow at a colonic anastomosis. Although circulating tumour cells can lead to anastomotic tumour growth it has been demonstrated that they are much less likely to adhere to an anastomosis than intraluminal and intraperitoneal cells and seem to have less potential to cause local recurrence.
Work by Umpleby et al (1984b) and Skipper et al (1987) has provided strong support for the presence of viable intraluminal and intraperitoneal tumour cells remaining after curative colorectal cancer surgery. This study shows that such cells will preferentially adhere to the anastomosis and may be an important cause of local recurrence. Up to one third of surgeons do not use any measures to kill or limit their spread (Umpleby and Williamson, 1984b) and results from this experiment would suggest that such measures should be undertaken in any colorectal cancer operation.

7.6. CONCLUSION

Viable intraluminal and intraperitoneal tumour cells have a significantly greater potential to adhere to a colonic anastomosis than intra-arterial tumour cells and this may be important when considering measures to prevent tumour cell implantation in colorectal cancer.
### Table 7.1

**TUMOUR CELL ADHERENCE TO NORMAL AND INJURED COLON**

**GROUP IA (N=12)**

<table>
<thead>
<tr>
<th></th>
<th>Median cell No</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomosis</td>
<td>625</td>
<td>(0-2997)</td>
</tr>
<tr>
<td>Normal Colon (NC)</td>
<td>0</td>
<td>(0-2320)</td>
</tr>
</tbody>
</table>

\[ p = 0.230 \text{ Wilcoxon matched pair test} \]

### Table 7.2

**TUMOUR CELL ADHERENCE TO NORMAL PROXIMAL AND DISTAL COLON AND COLOTOMY SITE IN GROUP IL (INTRALUMINAL INJECTION) (N=11)**

<table>
<thead>
<tr>
<th></th>
<th>Median Cell No</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomosis</td>
<td>12366</td>
<td>-</td>
</tr>
<tr>
<td>Proximal NC</td>
<td>9800</td>
<td>0.087</td>
</tr>
<tr>
<td>Distal NC</td>
<td>1800</td>
<td>0.024</td>
</tr>
</tbody>
</table>

\[ \text{Wilcoxon Test with Bonferroni correction} \]
Table 7.3

TUMOUR CELL ADHERENCE IN GROUP IP (INTRA-PERITONEAL INJECTION) (N=10)

<table>
<thead>
<tr>
<th>Median Cell No</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomosis</td>
<td>21889</td>
</tr>
<tr>
<td></td>
<td>(11270-56146)</td>
</tr>
<tr>
<td>NC</td>
<td>10705</td>
</tr>
<tr>
<td></td>
<td>(0-27025)</td>
</tr>
</tbody>
</table>

p = 0.019

Wilcoxon matched pair test
CHAPTER 8
Experiment 3

CAN TUMOUR CELLS FROM DISTANT MICROMETASTASES CAUSE ANASTOMOTIC TUMOUR GROWTH
8.1. INTRODUCTION

There is good evidence in the literature for the presence of micrometastases at the time of surgery (Finlay and McArdle, 1986).

Experimental studies in this thesis and work by Skipper et al (1988) have demonstrated that circulating tumour cells can cause anastomotic tumour growth. As circulating tumour cells are thought to remain viable for 24-48 hours following resection of a primary carcinoma in man it seem unlikely that they would account totally for the pattern seen. A possible source of tumour cells could be micrometastases as it has been shown that tumours can gain access to vascular spaces and circulate in the bloodstream and lymphatics (Fidler, 1991; 1976,). Areas of tissue injury such as those created by ‘curative’ surgery would appear to provide a suitable area for circulating tumour cells to adhere and grow.

8.2. AIM

To determine if tumour cells from pulmonary micrometastases can seed to a colonic anastomosis and cause anastomotic tumour growth.

8.3. ANIMAL MODEL

A pulmonary metastatic model has previously been developed in our laboratory using Mtln3 mammary adenocarcinoma cells in a Fischer F344 rat model (McCulloch and George, 1987). Briefly, intravenous injection of $10^4$ Mtln3 cells produces pulmonary metastases in 100% of rats after 12 days. Metastases to regional and mediastinal lymph nodes occur commonly but no metastases to other viscera have been detected. This is a virulent model and animals start to lose weight at 17 days to 3 weeks from tumour burden. Previous work from our laboratory has also shown that this cell line will seed to a colonic
anastomosis when injected intraluminally/intra-arterially and give rise to perianastomotic tumour growth (McGregor, 1988; Akyol, 1990).

8.4. EXPERIMENTAL DESIGN

Forty eight rats were given tail vein injection of $1 \times 10^6$ MTLn3 cells under Halothane anaesthesia on day 0. The animals were divided into four groups and the following procedures performed under intraperitoneal Midazolam and Fentanyl anaesthesia ($\frac{1}{4}$ Midazolam, $\frac{1}{4}$ Fentanyl, $\frac{1}{4}$ water).

**Group 1**

Sham laparotomy on day 10 post tail vein injection (TVI). The abdomen was opened and the distal colon handled but not traumatised for 5 minutes followed by closure of the abdomen in two layers of continuous 3.0 vicryl.

**Group 2**

Laparotomy followed by division of the descending colon and immediate repair with interrupted 5.0 silk sutures on day 7 post TVI with closure of the abdomen as Group 1.

**Group 3**

The same procedure as for Group 2 except on day 10 post TVI.

**Group 4**

The same procedure as for Group 2 except on day 14 post TVI.

The timing of tail vein injection was based on the results of experiment 1 in chapter 6, to provide the most fertile period in
anastomtic healing for viable tumour cells to implant and grow. The aim was to see if cells from micrometastases could implant and lead to tumour growth and therefore differences between the groups were not assessed and the number of animals used was correspondingly greatly reduced.

All animals post laparotomy were housed individually in polypropylene cages and allowed to recover following operation. All animals were sacrificed by cervical dislocation under halothane anaesthesia on day 21 post TVI, or sooner if they became unwell or lost weight.

8.5. RESULTS

All macroscopically abnormal lesions recorded at autopsy were submitted for detailed histological analysis by an independent observer. In addition a consistent area of the descending colon corresponding to the site of the anastomosis was examined microscopically irrespective of the macroscopic appearance in Group 1. The methods used for histological analysis were as described in chapter 5.

There were 5 anaesthetic deaths. One post tail vein injection and 4 post laparotomy. These animals have been excluded from the following data. Table 8.1 shows the histological results of tissues excised.

All 42 animals had pulmonary metastatic disease (Figure 8.1). Two animals had histological proof of abdominal wound tumour growth, and one had tumour growth at the anastomosis site (figure 8.2). Fourteen animals had tumour deposits found in subdiaphragmatic para-aortic lymph nodes examined.
TABLE 8.1.

HISTOLOGICAL RESULTS OF TISSUES EXCISED

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO</th>
<th>LUNG</th>
<th>ABDOMINAL</th>
<th>ANAST/COLON</th>
<th>LYMPH NODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (SHAM)</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>2 (day 7)</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3 (day 10)</td>
<td>13</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4 (day 14)</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>42</td>
<td>42</td>
<td>2</td>
<td>1</td>
<td>14</td>
</tr>
</tbody>
</table>
Figure 8.1.
Histological section of rat lung metastases
Figure 8.2

Histological section of anastomotic occurrence (High power)
8.6. DISCUSSION

The results demonstrate that tumour cells may seed from pulmonary micrometastases to sites of tissue injury (wound and anastomosis) in an experimental animal model. An interesting and unexpected finding was that 25% (14) of animals had tumour deposits in subdiaphragmatic lymph nodes examined. Autopsy studies from previous work in this laboratory has shown that in addition to pulmonary metastases in 100% of rats, metastases are commonly found in mediastinal lymph nodes but at no other sites (McCulloch and George, 1987). This may suggest a change in the behaviour of the tumour cell line or that the effects of anaesthesia or laparotomy could be influencing the pattern of lymph node spread (Radosevic-Stacic, 1989).

The mechanisms of metastatic tumour spread remain to be fully explained. The lymphatic and vascular systems have numerous connections and experimental evidence has shown that disseminating tumour cells may pass from one system to the other (Fisher & Fisher, 1966; del Regato, 1977; Zeidman & Buss, 1954; Fidler, 1976; 1991). Penetration of lymphatic vessels and subsequent passive transport in lymph leads to lymph node metastases. Histological proof of subdiaphragmatic lymph node metastases was seen in 50% of animals and in the animal with anastomotic occurrence. The node in this animal was not overtly diseased and there was no evidence of intraperitoneal disease. Retrograde lymphatic spread could be a cause of the anastomotic occurrence but it would seem an unlikely explanation for the two wound occurrences seen.

A further mechanism of metastasis is transcoelomic spread, as demonstrated by intraperitoneal metastases from ovarian tumours. Although there were pleural metastases, there was no evidence of
direct invasion of the diaphragm or of peritoneal metastases. Tumour cells from the affected intraperitoneal lymph node may have seeded to the anastomosis and cannot be discounted as a cause of tumour occurrence in the wound and anastomosis in this model.

The seed/soil hypothesis of Paget (1889) emphasised that the environment in which circulating tumour cells were trapped was important in the development of metastasis. In contrast to this Ewing (1928) stressed the importance of the mechanisms of circulation. The eventual site of metastases from circulating cells being governed by both haemodynamic and seed/soil factors (Viadana et al, 1978; Murphy et al, 1988). Experimental work by Fidler and Nicolson (1976) supports the concept of metastasis from metastases. Two weeks after normal tumour free mice were joined parabiotically to tumour bearing animals there was no evidence of tumour growth in the guest animals. However, when the parabiont mice were allowed to survive for four weeks after separation from the tumour bearing animals, 40% developed lung metastases and it was concluded that the metastases in the 'guest' mice were due to metastases from metastases.

Both Skipper et al (1988) and work presented in this thesis show that circulating tumour cell can implant at a colonic anastomosis and result in anastomotic tumour growth. A possible cause of the occurrences observed in the wound and anastomosis in this experiment is implantation of circulating tumour cell from pulmonary micrometastases although other methods cannot be excluded. Clinically it has been shown curative re-resection of locally recurrent tumour is possible and can be associated with acceptable operative morbidity mortality and longterm survival (Vassilipoulous et al 1981; Martin et al 1985; Martin and Carey 1991). However, if
such a patient had micrometastases as the source of tumour recurrence, such surgery would be unsuitable as this is now considered incurable and therefore careful patient selection prior to curative re-resection of local recurrence is required.

8.7. CONCLUSIONS

Tumour cells may seed from solid organ micrometases to sites of tissue injury; this however is unlikely to be a common cause of local recurrence. Nevertheless, patients who present with local recurrence should be fully investigated to exclude metastastic disease prior to definitive treatment.
CHAPTER 9
Experiment 4

THE RELATIVE IMPORTANCE OF TISSUE INJURY AND SUTURE MATERIAL IN TUMOUR CELL ADHERENCE
9.1. INTRODUCTION

Experimental studies have demonstrated that tissue injury enhances tumour growth from locally implanted tumour cells or from cells that reach the site of injury via the circulation (Jones & Rous, 1914; Skipper et al, 1988; Alexander and Altemeier, 1964; Fisher et al, 1967; Skipper et al, 1989.)

The mechanisms of enhanced tumour growth at injury sites remains to be fully defined. Studies by Fisher et al (1967) suggest that such growth is related to the severity of the injury (see 3.3.) In recent years several studies have demonstrated that certain suture materials will enhance anastomotic tumour growth in an experimental animal model (O’Dwyer et al, 1985; O’Dwyer and Martin, 1989; McGregor et al, 1991; McGregor, 1988; McGregor et al, 1989) (see 3.2.2.) The relative importance of suture material to tumour cell adherence in the presence of tissue injury has not been investigated.

9.2. AIMS

(1) To compare adherence of viable intraperitoneal tumour cells to normal and injured colon repaired with different suture materials.

(2) Examine in vitro adherence of tumour cells to suture materials.
9.3. EXPERIMENT 4.1: in vivo comparison of suture material and tissue injury

9.3.1. Animal model

Under intraperitoneal anaesthesia ($\frac{1}{3}$ water, $\frac{1}{3}$ midazolam, $\frac{1}{3}$ fentanyl) 23 animals underwent a laparotomy through a midline abdominal incision. A 1 cm colotomy was created in the descending colon. This was repaired with four interrupted 5.0 silk sutures (Ethicon Ltd. Edinburgh, UK) (Group IS). Four interrupted 5.0 prolene sutures (Ethicon Ltd. Edinburgh, UK) (Group IP) or four interrupted silk or prolene sutures followed by suture removal 10 minutes later when the anastomosis had sealed (Group IA). This was to create a sutureless anastomosis and to simulate tissue injury alone and was based on a method developed by McCue (1993). $1 \times 10^6$ $\text{I}^{125}$ IUDR labelled MTLn3 cells suspended in 0.2ml of F10/DMEM medium were then placed in the left paracolic gutter away from the anastomosis and the left colon. Following this the laparotomy incision was closed in two layers of continuous 2.0 vicryl. One hour later all animals were killed while still under anaesthetic by cervical dislocation, the anastomosis was excised, the radioactivity measured and cell counts calculated. A 1 cm length of normal left colon was also taken from all animals in the IA group for control cell count.

9.3.2. Controls for free $\text{I}^{125}$ IUDR

Experiments were repeated using 5 animals in each group with free $\text{I}^{125}$ IUDR in 0.2mls of cell free F10/DMEM. The concentration of $\text{I}^{125}$ IUDR was calculated on the basis of radioactivity from previous washings of radiolabelled tumour cells. The purpose of this was to ensure that differences in radioactivity observed between
groups represented differences in cell count and not adherence of non-cell bound $^{125}$IUDR.

9.4. EXPERIMENT 4.2: In vitro assessment of tumour cell adherence to suture material.

9.4.1. Experimental Model

A 5 cm length of 5.0 prolene and 5.0 silk were immersed in a tumour cell suspension of $5 \times 10^6$ Mtln3 cells/ml of F10/DMEM medium and incubated for one hour at room temperature. Following this the sutures were removed and scanning electron micrographs taken.

9.4.2. Electron microscopy

Scanning electron micrographs (SEM) of the prolene and silk suture material were obtained by fixing the sutures in 2% glutaraldehyde for 24 hours following removal from the tumour cell suspension, then rinsed in cacodylate buffer x 4 for one hour; post fixed in osmium tetroxide (Oxkem, Oxford, UK) for one hour before again rinsing in cacodylate buffer x 4 for a further hour. Sutures were then dehydrated for 15 minutes in 20%, 25% and 75% ethanol and 4 times in 1 hour in absolute alcohol, this was then followed by drying in a critical point drier mounted on aluminium stubs and sputter coated with gold/palladium. Micrographs were taken in a Joel/JSM 6,400 scanning electron microscope (Milton Keynes, UK).

9.5. RESULTS: EXPERIMENT 4.1

9.5.1. EFFECT OF COLONIC INJURY

Tumour cell adherence at the site of injury in group IA was not significantly different from that observed at normal or non-injured
left colon taken from the same animal (Table 9.1). Adherence was similar whether silk or prolene had been used to oppose the bowel wall in group IA with medium cell counts of 8,993 and 7,685 respectively ($p = \text{NS}$).

9.5.2. EFFECT OF SUTURE MATERIAL

Significantly increased tumour cell adherence occurred in group IS compared with all other groups (Mann Whitney U Test with Bonferroni correction) (Table 9.1). In contrast tumour cell adherence in group IP was similar to that observed in normal colon (Table 9.1).

9.5.3. EFFECT OF FREE $^{125}\text{I}UDR$

Radioactivity count at the colotomy site sutured with silk was low and was not significantly different to that from normal colon (Table 9.2) and this could not account for any differences observed between group IS and all other groups.
### Table 9.1

**TUMOUR CELL NUMBERS ADHERING TO NORMAL COLON (NC) AND ALL INJURY GROUPS**

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Cell No</th>
<th><strong>p Value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>16</td>
<td>7002 (453-19263)</td>
<td>-</td>
</tr>
<tr>
<td>IA</td>
<td>16</td>
<td>8602 (115-289146)</td>
<td>-</td>
</tr>
<tr>
<td>IP</td>
<td>7</td>
<td>7449 (5028-17630) NS</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>10</td>
<td>21888 (11270-51187) &lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

*Median cell No (range)*

**Compared with NC and IA groups (Mann-Whitney U Test with Bonferroni correction)**

---

### Table 9.2

**RADIOACTIVITY COUNTS FOR NORMAL COLON AND GROUP IS USING FREE 125IUDR**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO</th>
<th>Counts</th>
<th>p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>5</td>
<td>7.5 (0-11)</td>
<td>-</td>
</tr>
<tr>
<td>IS</td>
<td>5</td>
<td>0 (0-5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Median radioactive count (range)*
9.6. RESULTS: EXPERIMENT 2

Figure 9.1 Scanning electron appearance of 5.0 prolene (right) and silk (left) with tumour cells arrowed.

The scanning electronmicrographs clearly demonstrate silk as a braided material, the architecture of which provides a suitable environment for spherical tumour cells (arrowed) to adhere to. In contrast prolene is smooth and tumour cells (arrowed) flatten out in an attempt to obtain a hold, and the number of tumour cells is markedly less. These appearances further substantiate our findings with radiolabelled tumour cells.
9.7. **DISCUSSION**

Results from this study demonstrate that colonic injury repaired with silk sutures adhere significantly more intraperitoneal tumour cells than colonic injury alone or colonic injury repaired with prolene, indicating suture material used is more important than tissue injury in tumour cell adherence. There is strong evidence to support viable exfoliated intraluminal and intraperitoneal cancer cells remaining in significant numbers following potentially curative colorectal cancer surgery (Oakland, 1961; Rosenberg and Giles, 1977; Skipper et al, 1987; Umpleby et al, 1984b). However, it is not certain of the role that such cells play in colorectal cancer recurrence and this is reflected in the diverse attitudes of surgeons, up to one-third not using any method of preventing tumour cells implanting during colorectal cancer surgery (Umpleby and Williamson, 1984b; unpublished data, J. Docherty, 1993).

Previous studies examining adherence of tumour cells by various suture materials in vitro and in vivo have shown a significant difference between braided and monofilament materials and failed to take account of what effect tissue injury may have had on such localisation (O’Dwyer et al, 1985; McGregor et al, 1989; O’Dwyer, 1987). Secretion of adhesive glycoprotein such as laminin and fibronectin by regenerating endothelial cells and exposure of basement membrane collagen following surgical injury may provide an area that will adhere and support the growth of an increasing number of tumour cells (O’Dwyer et al 1985). The work by Fisher et al (1967) has indicated that the degree of injury created may be important in this context and suggested such factors play a significant role only when the injury is severe and caused by crushing or use of chemicals. In this study chronic injury was
created only by sharp instruments and loosely applied sutures which
were subsequently removed and did not adhere more tumour cells than
on the injured colon.

During this study the period in which viable cancer cells were
exposed to tissue injury is short. It is reasonable to assume that
viable exfoliated cancer cells may survive longer than one hour in
the bowel lumen or peritoneal cavity following colorectal cancer
surgery. Murphy et al (1980) demonstrated viable cancer cells by
bioassay in many organs up to 24 hours after intra-arterial
injection. The work by Fisher et al (1967) also demonstrated
significantly increased counts in areas of tissue injury 24 hours
after intravenous or intra-aortic injection of viable cancer cells.
However, this increase is only marginally higher than that obtained
after one hour in surgically traumatised animals, suggesting in this
situation the greatest localisation of tumour cells takes place
within the first hour of exposure.

Increased trapping of tumour cells is probably only one factor
explaining enhanced tumour growth at sites of tissue injury. Although
area of tissue injury does not seem to be important in tumour cell
adherence, increase in growth factor levels in response to healing
of injured tissues could be relevant. Viable cancer cells localised
to suture material may achieve a proliferative advantage by being
favourably positioned to cells involved in the healing process acting
as a paracrine source of growth factors (Steele, 1989). Work from
Loizidou et al (1991) supports this hypothesis; it was demonstrated
that fibroblasts from regenerating liver promoted significantly more
tumour in a colorectal cancer line when nude mice were inoculated
subcutaneously, and that tumours were tenfold larger than in
controlled animals. Similar results have also been demonstrated for
breast cancer cell lines using breast and skin fibroblasts (Morgan et al, 1987).

Uff et al (1993) have recently reported that both the physical configuration and chemical composition of the suture material influences the adherence of tumour cells. Results indicated radiolabelled rat colonic tumour cells (RCC5) preferentially adhere to protein based and multifilament sutures and this was confirmed by scanning electron micrographs. Protein based sutures have more polar groups available for hydrogen bonding, multifilament sutures display surface topography suitable for tumour cell adherence as well as a larger surface area. O'Dwyer et al (1985) have shown that adherence of tumour cells to sutures is dependent on fibronectin and other as yet unidentified factors. This would predict increased adherence to collagen based sutures such as catgut and silk and this has been reflected in results from Uff et al (1993). These findings would explain results from this experiment, silk is not only protein based but also multifilament and would be expected to adhere significantly more than monofilament non polar prolene.

9.8. CONCLUSION

This study demonstrates in an experimental animal model that suture type used to repair the injury is more important than injury itself in causing intraperitoneal tumour cells to adhere at a colonic anastomosis. It has also been shown that viable intraperitoneal tumour cells preferential adhere to injured colon. In a cancer with a high incidence of local recurrence following resection such factors may be important and from these findings the use of a suture with a low capacity to adhere tumour cells for an anastomosis in colorectal cancer surgery should be recommended.
CHAPTER 10.
Experiments 5.1- 5.3

THE ROLE OF GROWTH FACTORS.
10.1. INTRODUCTION

An anastomotic leak in colorectal surgery can be a catastrophic event associated with significant morbidity and mortality (Fielding et al, 1980) A study by Schrock et al (1973) investigating factors contributing to an anastomotic leak reported a mortality of 33% in the leak group compared to 2.6% in the no leak group. The large bowel project (Fielding et al, 1980) reported an overall leak rate of 13%: the presence of a leak was associated with a mortality of 22% compared to 7% in the intact group. Schrock et al (1973) found that a leak increased morbidity, with days in hospital rising from 25 days for the intact group to 45 days in patients with a leak. In addition, recent work from Akyol et al (1991) and Fujita et al (1993) have shown increased local recurrence rates and cancer specific mortality following curative resection of left sided colon and rectal cancers in those patients with an anastomotic leak.

Growth factor receptors have been identified on colonic cancer cells and growth factor have been identified in the conditioned media of various neoplastic cells (see 3.4). Since viable cells are present in the bowel lumen and circulation after potentially curative surgery, it is unclear what effect growth factor application would have on local tumour growth.

Trauma has been shown to enhance anastomotic tumour growth in many studies (see 3.3). More recently, it has been suggested that growth factors have a role in this phenomenon (Murphy et al, 1988; Alexander et al, 1988). Growth factors have been increasingly implicated in healing (see 3.4) and therefore may have a role in reducing leaks from high risk colonic anastomoses. The role of growth factors in anastomotic healing and tumour growth are investigated by experimental work in this chapter.
10.2. AIMS

Experiment 5.1.

To determine if there is a relationship between growth factor levels at different time points in anastomotic healing and the ability of circulating tumour cells to implant and grow at an anastomosis, leading to anastomotic tumour growth.

Experiment 5.2.

To determine if an anastomotic leak affects growth factor production.

Experiment 5.3.

To determine if growth factors applied at the anastomosis alter the pattern of tumour growth.

10.3. EXPERIMENTAL DESIGN

10.3.1. Experiment 5.1. The relationship of growth factors to anastomotic tumour growth.

Seventy animals underwent a laparotomy under halothane anaesthesia (Halothane MB, May and Baker Ltd, Dagenham, England) on day 0. The distal colon was divided and a primary colonic anastomosis was fashioned with 8-10 interrupted 5.0 silk sutures. The laparotomy incision was closed in two layers with continuous 3.0 vicryl and the animals allowed to recover.

In subgroups of 10, rats were killed on day 0, 1, 3, 5, 7, 10, 14 post laparotomy by cervical dislocation under halothane anaesthesia. The anastomosis was excised, snap frozen in liquid nitrogen and sent to the Pathology Laboratory for radioimmunoassay of
EGF and TGF-α, and immunohistocytochemical staining for EGF, TGF-β, 
β-FGF, laminin, collagen IV, fibronectin and EGFR.

The data from the animals used to investigate the effect of 
intra-arterial injection of adenocarcinoma cells were used to compare 
with the above group. This experiment was not repeated as the 
results were conclusive and the animals and methodology used was 
unchanged.

10.3.2. Experiment 5.2. The effect of an anastomotic leak on 
growth factor production

Thirty seven rats underwent a laparotomy under halothane 
anesthesia (May and Baker Ltd., Dagenham, England) on day 0. The 
distal colon was divided and immediately repaired with 4 interrupted 
5.0 silk sutures. Previous work in this laboratory has shown this 
to be a reliable model of a leaking colonic anastomosis (Akyol, 
1990). The abdomen was closed in 2 layers of 3.0 continuous vicryl 
and the animals allowed to recover. In subgroups of 5, the animals 
were sacrificed by cervical dislocation under halothane anaesthesia 
on day 0, 1, 3, 5, 7, 10, 14. The anastomosis was excised, snap 
frozen in liquid nitrogen and sent to the Pathology Laboratory for 
independent assessment of growth factor levels, laminin and 
fibronectin by immunocytochemical assay.

10.3.3. Experiment 5.3. The effect of application of growth 
facors on tumour growth at a colonic anastomosis.

Forty two rats underwent a laparotomy on day 0 using midazolam 
and fentanyl (¼ midazolam, ¼ fentanyl, ½ water) intraperitoneal 
anesthesia. The distal colon was divided and immediately repaired 
with 8-10 interrupted 5.0 silk sutures. The rats were then divided
into four groups. Group 1 acted as controls. Group 2 had 200μl of dilute collagen suspension (Zyderm II; Collagen Corporation, USA) applied around the anastomosis. Group 3 had 200μl of Zyderm II containing 5μg of human recombinant epidermal growth factor (EGF) (Sigma Chemical Co. Ltd. England) blended into a smooth suspension applied around the anastomosis. Group 4 had 200μl of Zyderm II containing 5μg of human recombinant basic FGF (β-FGF) (Sigma Chemical Co. Ltd. England) blended into a smooth suspension applied around the anastomosis. Previous experimental studies have shown that Zyderm II, a collagen implant composed of highly purified bovine dermal collagen dispersed in phosphate buffered physiological saline will delay release of growth factors, with a half life of 20 hours (Mustoe et al, 1987; Kingsnorth and Slavin, 1991). The amount of EGF and β-FGF used was derived from reported experimental work (Steele, 1990; Mustoe et al, 1987; Brown et al, 1988).

Following fashioning of the anastomosis with/without application of Zyderm II ± growth factor, the abdomen was closed in 2 layers of continuous 3.0 Vicryl. 1 x 10^6 Mtln3 tumour cells suspended in 0.2ml F10/DMEM medium were injected intra-arterially via the right carotid artery (see 6.3) and the neck wound was closed in a single layer of continuous 3.0 vicryl. All animals were then allowed to recover and were killed by cervical dislocation under halothane anaesthesia on day 21 post laparotomy, or sooner if they became unwell or lost more than 10% of initial body weight. A full post mortem was performed and the anastomosis excised and sent for independent histological assessment of perianastomotic tumour growth in the Department of Pathology.
10.4. Histological Analysis.
The method used is as described in chapter 5. From a standardised transverse section taken at the level of the anastomosis the area of tumour growth in each slide was assessed using a MOP AM02 linear planimeter (Kontron, Munich, Germany).

10.5 GROWTH FACTOR ANALYSIS

10.5.1. Radioimmunoassay.
Extractable EGF and TGF-α were initially assayed using polyclonal antibodies developed by Dr Harry Gregory (ICI Pharmaceuticals). The cross reactivity for EGF and TGF-α for this assay was 0.0004% and extraction efficiency for solid tumours is 80% (total extract). In this and other experiments the sensitivity of this assay is such that 20 picograms/ml extract wet weight was detected (Owens et al, 1991).

10.5.2. Extraction of EGF and TGF-α.
Frozen tissue specimens were removed from storage and allowed to thaw on ice. Once thawed colonic specimens were mopped with tissue paper to remove excess water. Specimens stored in sucrose/glycerol buffer were thoroughly rehydrated in homogenised buffer. Fresh homogenised buffer was prepared (20 mM HEPES, 12 mM EDTA and 0.5 mM PMSF adjusted to pH 7.4 with sodium hydroxide) and stored on ice. The colon was then cut into small 1 mm blocks, weighed and placed in a centrifuge tube on ice. Homogenising buffer (5 ml gm⁻¹ wet weight) was added. The tumour was homogenised on ice with an ultra turrax (Janke & Kunkel) with 2 x 15 second bursts at maximum speed but allowing the homogenate to cool between bursts. The homogenate was centrifuged at 1000g for 10 minutes and the resulting
supernatent was subjected to a higher spin speed (12,000g for 1 hour). The nuclear pellet from the first spin was resuspended in 3ml of homogenising buffer and stored at -20°C until required for DNA analysis (Modified Burton). The supernatant from the high spin speed was added to 2 volumes of ice cold alcohol and this was centrifuged at 1,000g for 30 minutes. The supernatant solution from the alcohol extraction was added to four volumes of ice cold alcohol ethyl acetate and placed in a fridge overnight (4°C). After 16 hours a crude extract precipitated to the bottom of the vessel. The supernatant was discarded, the crude extract was suspended in 2ml of 1 N acetic acid. The extract was stored at -70°C until required for lyophilisation.

10.5.3. Lyophilisation

Extracts were removed from -70°C and caps were loosened or the nescofilm pierced. Sodium hydroxide pellets were placed in the bottom of a dessicator and the samples placed above on a metal shelf. The dessicator was attached to a pump (Javac Double Stage high Vacuum Pump ID 60) through an ice cooled trap. The pump was switched on and the lyophilisation usually took over 16 hours. The lyophilised product was resuspended in 1ml of RIA buffer (0.2 M Na₂HPO₄, 0.2 M NaH₂PO₄, 0.1% sodium azide, 0.15 M sodium chloride, 0.01 M EDTA, 0.5% BSA, and pH to 7.4) and placed on ice or stored dry at -20°C.

10.5.4 Radioimmunoassay for EGF and TGF-α.

Lyophilised tumour extracts were removed from -20°C and thawed on ice. Each extract was resuspended in 1ml of RIA buffer and placed on ice. Standards were earlier prepared in RIA buffer using human recombinant EGF or TGF-α (in RIA buffer) and the actual values on the
standard curve were as follows: 0, 20pg, 50pg, 100pg, 250pg, 500pg, 750pg, 1ng, 5ng, 10ng.

Antibody dilutions were made up fresh with RIA buffer in the range of 1: 10,000 - 1: 20,00 for TGF-α and usually 1: 100,000 for EGF but these dilutions were varied slightly from one iodination to the next and the antibodies were placed on ice.

Iodination of peptides (EGF & TGF-α were human recombinant) was performed as a modification of Gregory et al (1988) by an 'in house' technique using iodogen and a column containing biogel P6. Free iodine (I\textsuperscript{125}) was purchased from NEN. The iodinated peptide came off the peak of the columns and the fraction with the maximum on competition assay was used for the radioimmunoassay. Finally the labelled peptides were made up in the RIA buffer to give 30,000 c.p.m./250μl. For every 1ml of labelled peptide 4μl of sheep's serum was added to reduce nonspecific binding.

Colonic extract (250μl) was added in duplicate to eppendorfs. The primary antibody (either anti-EGF or TGF-α) was added to each eppendorf in a volume of 250μl. Finally 250μl of I\textsuperscript{125} EGF was added to the eppendorf in which the primary antibody was anti-EGF and I\textsuperscript{125} TGF-α when the antibody was anti TGF-α. The eppendorfs were capped, gently vortexed and incubated at 4°C for 48 hours, the standards were treated in the same way.

Secondary antibody (donkey/antisheep - Scottish antibody production unit) at a dilution of 1: 15 (made up in RIA buffer) in a volume of 250μl was added to unknowns and standards. Incubation continued for a further 24 hours at 4°C.

All specimens were centrifuged at 40,000g in a refrigerated centrifuge (Sarstedt) for 20 minutes. The supernatant was removed with a pasteur attached to a water pump and the pellet remaining was
counted in a Thorn EMI 620 Turbo Multichannel gamma counter (60% efficiency). The peptide content was read off the standard curve.

The assays for EGF and TGF-α were sensitive over a range of 20 pg to 10 ng/ml and there was no cross reactivity between the two peptides. The efficiency of extraction was calculated to be 60% when various samples of placental tissue were spiked with a known amount of EGF and TGF-α and subsequently extracted which is similar to other assays reported. Corrections for extraction efficiency were not made and results of each extract were expressed as ng/mg DNA. The coefficient of variation was of the order of 5% (Owens and Leake, 1992).

10.5.5. Immunocytochemistry.
Antibodies screened:
β-FGF
Fibronectin
Laminen
FGF(Mouse) FGF(Rabbit)
EGF
TGF-β
EGFR

These antibodies were initially screened using an immunoperoxidase technique on frozen sections with diaminobenzidene as the chromagen. Results were very difficult to interpret as there was a non specific background "wash" of brown staining.

The system was changed to an alkaline phosphatase anti alkaline phosphatase (APAAP) technique, but this gave positive
staining of the intestinal mucosa native alkaline phosphatase which again prevented interpretation.

The final method employed used β-galactosidase as the chromagen; this gave much clearer results with little background "noise".

The antibodies were tested at various dilutions and incubation times to determine the optimum criteria for staining.

The various types of controls applicable to immunocytochemistry have been comprehensively detailed by Heydermen (1979) and are briefly summarised below.

The only true immunological negative control is the absorption control. This involves utilising excess specific antigen to absorb the antibody and abolish the immunoreactivity of that antibody. Absorption with similar quantities of related substances should not abolish the reaction. An absorption control is mainly performed by a pure immunology laboratory and relies on obtaining excess antigen which is difficult for most routine laboratories. In this particular experiment purified antigen was not available so it was not possible to use this technique.

Standard negative controls used by this and most laboratories are DAB alone and omission of one or more reagents or substitution with buffer. Other negative controls utilise irrelevant antibodies, non immune serum and antiseraum raised with another species. There are advantages and disadvantages to each method but no one has proved superior to the others. The antibodies used are commercially raised and therefore assumed to have been extensively tested and purified to exclude inappropriate antibodies, one of the criticisms of the above 'other' controls and means these techniques are comparable with the absorption control and were considered adequate for this experiment.
10.6. RESULTS

10.6.1. Radioimmunoassay.

Results of assays of ovarian and breast malignant tissue are shown (Figures 10.1, 10.2) to demonstrate reliability of the assay with results of Experiments 5.1. and 5.2.

EGF and TGF-α levels were found to be too low for reproducible assay. Initially it was thought that the sample size was too small and samples from the same group were combined in an effort to overcome this. Similar results were obtained and therefore further analysis was performed by immunocytochemistry to determine whether there were localised deposits of relatively high concentration of TGF-α and other growth factors. Colleagues (Lloyd et al, 1992) have shown changes in TGF-α distribution in prostatic sections in relation to the extent of the intra-epithelial neoplasia.

10.6.2. Immunocytochemistry

There was no identifiable positive staining with any of the antibodies under any of the above conditions as demonstrated with figures 10.3 - 10.12.
Figure 10.1

TGF-alpha assay results.
Figure 10.2

EGF assay results.
Figure 10.3. Immunocytochemistry for EGF. No specific EGF localisation is seen in the preparation. Brown staining is background and non specific in nature.

Figure 10.4. Immunocytochemistry for β-FGF. No specific FGF-β localisation is seen in the preparation. Brown staining is background and non-specific in nature. Immunocytochemistry: β-FGF 1/100. DAB. X75.
Figure 10.5. Immunocytochemistry for laminin. No specific laminin localisation is seen in the preparation. Brown staining is background and non specific in nature.

Immunocytochemistry: laminin 1/60. DAB. X75.
Figure 10.6. Immunocytochemistry - Mouse negative control (no primary antibody).
N.B. Background staining similar to that seen in Figure 10.3.
Immunocytochemistry: Mouse negative control DAB. X75.
Figure 10.7. Immunocytochemistry - Rabbit negative control (no primary antibody).

N.B. Background staining is less than that of the mouse negative control in Figure 10.6.

Immunocytochemistry: Rabbit negative control. DAB. X75.
Figure 10.8. Immunocytochemistry for Laminin. No specific laminin localisation is seen in the preparation. Blue staining is background and non specific in nature.

Immunocytochemistry: Laminin 1/60. β-galactosidase X75.
Figure 10.9. Immunocytochemistry β-FGF. No specific β-FGF localisation is seen in the preparation. Blue staining is background and non specific in nature.

Immunocytochemistry: β-FGF 1/100. β-galactosidase X75.
Figure 10.10. Immunocytochemistry - Negative control (no primary antibody).

N.B. Background staining is similar to that seen in figure 10.9.

Immunocytochemistry: Negative control. β-galactosidase X75.
Figure 10.11. Immunocytochemistry for fibronectin. No specific fibronectin localisation is seen in the preparation. Red staining is background and non specific in nature. Immunocytochemistry: Alkaline Phosphatase X75.
Figure 10.12. Immunocytochemistry: Negative control (no primary antibody).

N.B. Background staining similar to that seen in Figure 10.11.

Immunocytochemistry: Negative control. Alkaline Phosphatase X75.
10.6.3. Histopathology.

Experiment 3

Table 10.1 documents the area of tumour growth in each slide assessed with the linear planimeter and is shown graphically in figure 10.13.

Application of collagen with/without growth factors led to a significant increase in the number of animals developing tumour compared with the control group (Table 10.2) \( (P<0.05, \text{ chi squared test with Yates correction}) \), this did not reach significance for 

\[ \text{Table 10.1} \]

LINEAR PLANIMETRY OF HISTOLOGICAL SECTIONS

mm\(^2\) tumour in each group.

<table>
<thead>
<tr>
<th>Control</th>
<th>Collagen</th>
<th>Collagen +EGF</th>
<th>Collagen +FGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.37</td>
<td>1.26</td>
<td>0.37</td>
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<tr>
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<td>8.97</td>
<td>3.14</td>
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<tr>
<td>3.72</td>
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</tr>
</tbody>
</table>

Mann Whitney U test.
P < 0.001 comparing collagen alone and collagen + EGF to controls.
p = NS comparing collagen to collagen + growth factors.
Figure 10.13. Area of Tumour growth at the site of anastomosis assessed by linear planimetry.
Table 10.2.

HISTOLOGY RESULTS OF ANASTOMOSIS FOLLOWING APPLICATION OF GROWTH FACTORS.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>+ve Histol</th>
<th>*P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>5 (50%)</td>
<td>-</td>
</tr>
<tr>
<td>Collagen</td>
<td>9</td>
<td>9 (100%)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>+β-FGF</td>
<td>7</td>
<td>7 (100%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>+EGF</td>
<td>10</td>
<td>10 (100%)</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

* *X^2* test with Yates correction.
10.7. DISCUSSION

The final analysis of immunocytochemical growth factor assessment provided reliable data and fault within the method used to detect the growth factor seems unlikely. Similar analysis of other tissues has yielded positive results within the same laboratory again validating the method (Figure 10.1, 10.2). It may be that these results are accurate and that growth factors are not produced by the anastomotic tissue. This seems improbable as it has been shown that wound healing is associated with increased synthesis of growth factors in wound chambers in rats (Cromack et al, 1987; Grotendorst, 1989) and work by Bracken et al (1991) has shown an increased expression of EGF receptor gene in response to anastomotic healing. Other studies have shown application of growth factors to improve wound healing (Mustoe et al, 1987, 1989; Brown et al, 1988; Kingsnorth and Slavin, 1991: Kingsnorth et al,1990; Slavin et al, 1992a+b). It is possible that a rat colonic anastomosis (approximately 25milligrams) does not produce enough growth factor(s) to be detected by this method or that they can only be detected in the wound fluid; impossible to demonstrate using this model. An alternative explanation is that growth factor is present but tightly bound by its receptor in such a way that it is neither extracted by organic solvents nor is the appropriate epitope available for detection by the antibody. The process of microwaving or pressure cooking samples to expose epitopes is a relatively new innovation. It disrupts the chemical structure of the sample, particularly the cell membrane, exposing the antigenic epitope for antibody binding. The process was not in use in this laboratory during the time that this work was performed. If the binding remains negative it is still not possible to be sure that the epitope is not there as it may also
have been disrupted/altered by the process of microwaving or pressure cooking. The validity of using such antigen exposing techniques when the exact mechanism of their action is unclear is in doubt (Boon and Kok, 1989).

Application of collagen with/without growth factors led to a significant increase in the number of animals developing tumour and the area of tumour seen in histological section when compared with the control group. However, there was no significant difference seen between either of the above parameters between collagen alone and collagen plus growth factor. This is in contrast to results found by other authors (Morgan et al, 1987; Loizidou et al, 1991). It is possible that small differences caused by growth factors could have been masked by the overwhelming effect of collagen.

Collagens are one of the ligands that bind to integrins. These are a widely expressed heterogeneous group of cell surface adhesion molecules that allow anchorage, position, differentiation and growth and are thought to be the major receptors by which cells attach to extra-cellular matrix (Hynes, 1992). Collagen around an anastomosis may provide a suitable environment for tumour cells to adhere and lead to a lowering of the threshold at which a given number of tumour cells will cause tumour growth.

Work from Akyol et al (1991) and Fujita et al (1993) demonstrate an increase in local recurrence rates and cancer specific mortality following an anastomotic leak. Slavin et al (1992a and b) have investigated the effect of growth factors on healing of incisional and intestinal wounds. Zyderm II was used as a vector for growth factor delivery and results indicated that growth factors will improve wound healing in the model examined and that the observed effect was not due to collagen. EGF has been shown to be
chemotactic and mitogenic for tumour cells in vitro, various studies have shown EGF receptors on human colon cancer cells (Steele et al, 1990; Bradley et al, 1986;). and 6-FGF is a potent angiogenic factor which has also been shown to have chemotactic and mitogenic properties (Editorial, Lancet, 1990). Although application of growth factors has been shown to improve wound healing and could have a place in prevention of an anastomotic leak, the addition of growth factor(s) may further optimise conditions necessary to produce tumour growth at an anastomosis and requires further investigation prior to clinical use in colorectal cancer surgery. The use of collagen as the vehicle for delivery is likely to promote tumour growth and should therefore be avoided in colorectal cancer. Other agents potentially suitable for this purpose are currently under investigation in this laboratory.

10.8. CONCLUSION.

From this experimental data it would seem that growth factor levels do not vary during anastomotic healing or in the response to an anastomotic leak. This is at variance with the data for wound healing and may reflect inadequate samples.

Bovine Collagen (Zyderm II) promotes tumour growth in this animal model and is therefore not a suitable vector for application of growth factors to an anastomosis in colorectal cancer. There were no adverse effects from growth factor application and further assessment using other delivery vehicles are in progress.
CHAPTER 11
SUMMARY
There is no doubt that colonic cancer remains a serious health problem. Continuing research into various adjuvant therapies has not altered the place of surgery as the only potentially curative treatment option. There has been little change in the five year survival rate over the past four decades despite improved operative mortality and local recurrence remains a significant factor limiting survival following curative surgery for colorectal cancer. Although it has been shown that intensive follow up and further surgery can lead to early discovery and 'cure' of local recurrence, a better understanding of the mechanisms of local recurrence would aid prevention. The most common cause of local recurrence is inadequate resection and should be avoidable by a wide resection which is histologically free of residual disease. Measures to prevent the rarer cause of anastomotic seeding of free malignant cells are variable as shown by Umpleby and Williams (1984b). Clinical and experimental work in this thesis have looked at some of the factors that may be important in the aetiology of local recurrence.

The clinical work presented has examined the effect of a surgeon's specialty interest on specimen length following curative resection of colorectal cancer. Recently, there has been a trend toward increasing surgical specialisation and several studies have shown a surgeon dependant variable on outcome following colorectal cancer surgery (Phillips et al, 1984a; McArdle and Hole, 1991), although it is not clear why. Similarly, it has been shown that the outcome of aortic aneurysm repair, both emergency and elective, is dependent on the vascular experience/interest of the individual surgeon (CEPOD, 1987; Jenkins et al, 1986; Meddings et al, 1991). Our results may appear superficially to concur, results indicating those with a specialist interest in colorectal surgery resect
significantly more left colon than those with other specialty interests, surgeons with a gastroenterology interest performing a resection that was intermediate between these groups. However, the effect this has on local recurrence rates and outcome was not examined in this study and whether this can be accounted for by specialty interest is not clear, as it may well be surgeon dependant. Other surgeon dependant variables such as the use of cytotoxic agents are not reliant exclusively on specialty interest and could have an important influence on local recurrence rates although recent work casts doubt on this. Unpublished data by J. Docherty (personal communication, 1993) has concluded that tumouricidal agents are effective cytotoxic agents in-vitro but are only weakly cytotoxic in-vivo. Inactivation by organic matter is probably responsible for the discrepancy between in-vitro and in-vivo efficacy. In view of the proven potential damaging effects on the patient (Pusey et al, 1979; Phillips and Dudley, 1985) doubt about their clinical benefit in the peritoneal cavity are raised and further study is warranted to clarify this issue.

In the experimental work, Experiment I has confirmed Skipper’s work that tumour cells adhere to a colonic anastomosis in a time dependent fashion. Skipper’s work was performed mainly with a sarcoma cell line and results from this study demonstrated the same effect for adenocarcinoma cells. There was a difference in the degree to which this effect was seen which has been discussed previously (see 6.7).

The role of tumour cell adherence in relation to different modes of delivery has been investigated in Experiment 2. Tumour cells have potential to implant at a colonic anastomosis from either intraluminal, intraperitoneal or intra-arterial tumour cells. From
this experimental work it seems that the potential is greatest for IL and IP cells. Their presence following curative colorectal surgery has been clearly demonstrated. As discussed earlier, recent experimental work has indicated that the use of cytocidal solutions in vivo may not be as effective as in-vitro work would suggest (Personal communication J Docherty 1993). As the use of these solutions has been recommended as a preventative measure against IL and IP tumour cells further work is clearly indicated to assess their in vivo effect on colonic cancer cells. In addition, alternative cytocidal solutions could be investigated for both effect on tumour cells and toxicity to the patient.

Experiment 3 was designed to investigate whether circulating tumour cells from colonic micrometastases could account for some cases of tumour growth at an anastomosis. Although tumour growth was seen in the anastomosis of one animal and two abdominal wounds, it has proved impossible to say without doubt that implantation of circulating tumour cells from pulmonary micrometastases were responsible (see 8.6.). However, it is clear from experimental data that metastases from metastases can occur and therefore any patient presenting with anastomotic recurrence should undergo thorough investigation for evidence of distant disease prior to any major intervention.

Experiment 4 has confirmed that the type of suture material used to repair an injury is more important than the tissue injury itself in terms of tumour cells adherence. Electron micrographs have shown a difference in the surface topography of suture material and number of tumour cells adhering to material. Work published after this thesis was completed by Uff et al (1993) has clarified suture related factors important in tumour cell adherence. Their results
indicate that a suture material which is polar and/or braided has greater potential for tumour cell adherence. These results explain the findings of this experiment that silk, which is braided and polar, adheres significantly more tumour cells than nonpolar monofilament prolene. Obviously, it is impossible to perform surgery without trauma to tissues, but minimising this as well as utilising a suture with a low capacity to adhere tumour cells is recommended.

In experiments 5.1-5.3, the role of growth factors has been studied. Disappointingly, it proved impossible to find evidence of a variation in growth factor levels at different time points in anastomotic healing that may have explained the finding from Skipper et al (1988) and experiment 1 that tumour growth at an anastomosis varies at different time point in healing. Similarly, results from the effect of an anastomotic leak on growth factor production were unproductive. The external application of growth factors to a colonic anastomosis did not have any effect on tumour growth. However, the vehicle of delivery, bovine dermal collagen (Zyderm II), seemed to promote tumour growth and therefore its use in colorectal cancer should be avoided. It may be that any effect of growth factors was masked by that of the collagen.

11.2. FURTHER WORK

Although it has been shown that the surgeon's specialty interest does affect the type of resection performed, the effect of this on local recurrence and survival is not known. Prospective studies addressing this are in progress.

Growth factors have been shown to exert a beneficial effect on colonic healing. Collagen as a vehicle for growth factor delivery is unsuitable as it promotes tumour growth and other potentially suitable agents are under investigation.
PRESENTATIONS

Colonic injury and tumour cell adherence.

Tumour cell localisation in colonic injury and repair.

Can solid organ micrometastases metastasise to sites of tissue injury? British Association of Surgical Oncology Meeting, University of South Manchester, 10th July, 1992.

Adherence of viable circulating, intraluminal and intraperitoneal tumour cells to normal and injured colon. Combined Meeting of the Association of Surgeons of Great Britain and Ireland and Trinity 400, Trinity College, Dublin, 23rd September, 1992.

Effect of surgeon’s specialty interest on resection specimen lengths for colorectal cancer surgery. Surgical Research Society, Ninewells Hospital and Medical School, Dundee, 8th July, 1993.


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