A THESIS ON

HISTOCHEMICAL CHANGES AT THE RESECTION MARGIN AND THEIR RELATIONSHIP TO RECURRENT COLORECTAL CANCER

By

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"We have scotch'd the snake not killed it;

She'll close and be herself,

whilst our poor malice

Remains in danger of her former tooth"

William Shakespeare
Macbeth
Act III Scene II

ABSTRACT

Local recurrence of colorectal cancer occurs in up to 40% of patients following 'curative' resection. There is a need for a test which will identify before operation, patients who are at greatest risk of local recurrence so that meticulous radical surgery and adjuvant radiotherapy may be included in the treatment. This thesis assesses the potential of one possible test.

Colorectal carcinomas are surrounded by a zone of altered mucin (sialomucin) secreted by goblet cells. In the first study the depth of invasion of 72 colorectal carcinomas was compared with the size of sialomucin band. There was a significant difference ($p<0.05$) in the length of sialomucin adjacent to carcinomas that invaded other structures compared to those which had not. If the band was >3 cm then there was a 70% probability of tumour extension to adjacent structures.

A multicentre study prospectively assessed whether a sialomucin band of >3 cm could predict patients who would develop local recurrence. Multivariate analysis of 358 patients followed for 2 years (median) suggested that a band of >3 cm was a significant predictor ($X^2=4.59$, $p<0.006$) of local recurrence coming after Dukes' stage and hospital of operation in predictive power. Furthermore, the presence of sialomucin in the resection margin was the strongest of all

variables as a predictor of local recurrence (X^2=14.19, $p<0.001$).

The prevalence of sialomucin was studied in neoplastic and non-neoplastic conditions in both man and in a rat model of neoplasia and inflammation. While sialomucin was produced in response to inflammation (5% prevalence), it was much more prevalent adjacent to neoplasia (polyps 80%, carcinoma 86%) or where there was a risk of neoplasia (cancer-prone colon 26%, irradiated colon 34%, ulcerative colitis with dysplasia 83%). Results of the animal study were similar. Sialomucin is a cancer associated but not cancer specific abnormality.

Sialomucin is as accurate as serum CEA monitoring as a predictor of local recurrence (44% sensitive, 75% specific, 70% accurate) but has the advantage of being a pre-operative test (eg, biopsy at colonoscopy) which can be used in the majority of colorectal carcinomas to identify patients at risk of local recurrence.

STATEMENT OF ORIGINALITY

The work in this thesis was performed whilst I was a Research Fellow in Surgery at the Royal Postgraduate Medical School, London, between August 1984 and August 1985 and latterly as Surgical Registrar in the Department of Surgery at Charing Cross and Westminster Medical School. Whilst, of course, I had considerable technical help with the preparing and cutting of slides, I have undertaken much of the histological work load myself, with the exception of actually cutting the blocks. I personally collected all of the data from the London end of the multicentre trial, whilst Mr N A Habib FRCS, Lecturer in Surgery, Bristol Royal Infirmary, collected the patient data from the Bristol side. I recorded the results and following advice compared them statistically.

I personally performed the animal experiments and the art work.

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CHAPTER 1

BACKGROUND

1.1 LOCAL RECURRENCE OF COLORECTAL CANCER - The Clinical Problem

1.1.1 Introduction

Resection of the colon for carcinoma was first performed by Reybard in 1843. The patient, a 26-year-old male, died from recurrent disease one year later (Reybard 1843). Eighty years later, with the advent of anaesthesia and antiseptics, 25 per cent of all cases of colorectal cancer were considered operable (Goligher, 1984) but half of these patients undergoing surgery died in hospital. Seventy-five per cent of those who survived tumour resection eventually succumbed to recurrent disease.

Today, in England and Wales, after another interval of 80 years, 50% of patients who develop a carcinoma of the colon or rectum can be expected to undergo resection with curative intent and to survive the operation. Subsequently, however, at least 14% of patients develop local recurrence (Phillips et al, 1984a), and 18% distant recurrence (McDermott et al, 1985).

Whilst there has obviously been a marked improvement in outlook over this 160 year period since the first colonic resection for cancer, the overall prognosis following curative resection has not improved over the past thirty years (Lockhart-Mummery et al, 1976; Umpleby et al, 1984).

It is particularly disappointing that up to 40% of patients who undergo apparently curative resection develop local or regional tumour recurrence, suggesting a failure by the surgeon to achieve complete local control of the disease.

1.1.2 The Effect Of Local Recurrence On Survival Following 'Curative' Resection Of Colorectal Cancer.

Local recurrence is usually associated with relentless progression of symptoms resulting in death. The mean survival varies from 7 to 11 months from time of diagnosis of local recurrence (Pestana, 1964, Polk and Spratt, 1971, Welch and Donaldson, 1978). In reported series local recurrence is responsible for death in approximately 50% of cases of colorectal cancer.

Floyd (1965) reported on 50 patients who underwent re-exploration or had a post mortem following recurrent carcinoma. Eighteen patients (36%) had locally recurrent disease. Only 9 of the 41 patients who died (22%) succumbed to distant metastases. Moertel (1973) confirmed that 50 per cent of patients who have undergone 'curative' resection of primary colorectal cancer, die from the local effects of recurrent disease (eg intestinal or ureteric obstruction or pelvic sepsis). Cass et al (1976) studied 105 patients with recurrent cancer. Sixty-three patients (60%) developed local recurrence alone, while 15 patients (14%) developed both local and distant metastases, and 27 patients (26%) had

distant metastases alone. Local recurrence without clinical evidence of distant metastasis was the most common cause of death during the first 5 years after primary resection.

1.1.3 What Causes Local Recurrence?

Local recurrence must be a consequence of malignant cells remaining after operation at the site of tumour excison. There are three possible factors which might influence this: tumour biology, patient response and surgical technique.

(i) Tumour Biology

Gilbertsen (1960) reported a 24 per cent local recurrence rate after total rectal excision and noted that the rates of local recurrence were less for Dukes' A tumours (9.1%) compared to Dukes' B (16.7%) and Dukes' C (40.8%) tumours. A recent study of 1008 patients undergoing total rectal excision or anterior resection has confirmed these findings (McDermott et al, 1985).

Broder (1925) was the first to attempt to grade carcinoma on the proportion of differentiated cells. Dukes' (1932) pointed out that tumour differentiation was not uniform and proposed a classification based on cell pattern. Grinnell (1939) studied 223 cases of carcinoma of the colon and rectum and graded cancers according to glandular arrangement, invasiveness, nuclear polarity and number of mitoses into 3 grades. Each grade was then related to survival. It was

found that the higher the grade, the worse the subsequent survival. It was concluded that this system was not as good as Dukes' classification in prognostic terms, but it was a useful adjunct to it. The most poorly differentiated tumours, however, have been shown to be associated with distal intramural spread and a subsequent high rate of local recurrence (Williams et al, 1984). While tumour differentiation contributes prognostic information about both survival and recurrence, if Dukes' staging is included in the analysis it ceases to be a significantly prognostic predictor. Moreover, grading is a subjective interpretation (Blenkinsopp et al, 1981; Thomas et al, 1983) further decreasing its ability as a prognostic indicator.

Tumour cell DNA measured by flow cytometry has been shown to influence subsequent survival (Armitage et al, 1985). In this study the DNA content was measured from paraffin block material in 134 cases. Seventy-two specimens (55%) had an aneuploid configuration, not related to pathological stage or grade. Only 14 patients (19%) survived 5 years compared to 27 patients (43%) with the diploid (normal) configuration. These findings were not confirmed however by Rognum et al (1987). The corrected 5 year survivals in this series were 64% for diploid configuration and 49% for aneuploid, not significantly different.

(ii) The Patient

Site of tumour: Gilchrist and David (1947) reported a recurrence rate of 16.1 per cent after abdomino-perineal excision for rectal carcinoma situated completely or partially below the peritoneal reflection. This compared with a local recurrence rate of 3.6 per cent for lesions above the peritoneal reflection. Gilbertsen (1960) confirmed these findings. Stearns and Binkley (1953) reported a 21.9 per cent incidence of local recurrence of rectal cancer and again reported an inverse relationship between recurrence and the level of the tumour above the anus. When considering colonic carcinoma, Cass et al (1976) found that local recurrence was greater for caecal and rectosigmoid carcinomas compared to those in the transverse or descending colon. Russell et al (1984) reported a local recurrence rate of 34% following excision of colonic cancer.

Extent of local invasion of tumour: Russell et al (1984) demonstrated that the risk of local recurrence is related to degree of differentiation of the tumour and depth of penetration of the bowel wall (16% recurrence rate with tumour confined to muscularis propria compared to 48.5% recurrence rate of patients with tumours adherent or invading surrounding structures). In this series, 34% (64/180) of patients underwent re-operation. Two modes of local

recurrence which were identified were: peritoneal seedling and retroperitoneal recurrence.

Rich (1983), in a series of 142 patients with rectal and rectosigmoid carcinomas recorded that the degree of extra rectal extension after curative resection is an independant factor influencing the risk of local recurrence. This applies even in node positive patients. The risk of local recurrence in node positive patients in whom tumour is confined to the bowel wall (36%) was less than that in node negative patients where there was extension through the wall (54%).

(iii) The Surgeon

Technical difficulty: Failure to resect growth that has penetrated the bowel wall into surrounding structures (eg, perirectal fat, other organs) could be expected to be important in the development of local recurrence. This may be particularly difficult in excision of rectal carcinoma where adequate pararectal tissue margins may be difficult to achieve because of the encroaching pelvic side wall (Morson, 1963). Quirke et al (1986) examined whole mount transverse sections of specimens taken from 52 rectal cancer patients. They found spread to the lateral resection margin in 14 of 52 patients (27%), 12 of whom subsequently developed local recurrence. The specificity, sensitivity and positive predictive values of lateral spread for local recurrence were

92, 95 and 85% respectively. In comparison with a similar retrospective group of rectal cancer specimens, no specimen was shown by routine tissue sampling to have lateral spread. It was concluded that lateral spread is under estimated and is an important factor in local recurrence.

Inadequate excision of the primary tumour may also occur because microscopic spread of tumour in the bowel wall is beyond the macroscopic extent of the tumour seen by the surgeon (Grinnell, 1954). The extent of microscopic spread is particularly important in sphincter saving resection procedures where adequate distal clearance may be compromised (Morson et al, 1963; Grinnell, 1954; Quer et al, 1953; Goligher, 1984). A 'safe' distal margin of clearance has been determined at 5 cm since Goligher (1951) first reported a distal margin of 0.6 cm in two cases of recurrent cancer. More recently, Williams et al (1983) questioned this 5 cm rule. In a study of 50 rectal carcinoma specimens obtained from potentially curative abdomino-perineal resection, 76% of patients were found to have no distal intramural spread, 14% had spread of 1 cm or less, and the remainder 2 cm or less. Survival and recurrence rates were comparable in patients with either a small or wide distal margin of clearance. Pollett and Nicholls (1983) confirmed these findings. This suggested that the 5 cm rule could be revised and that more patients could undergo sphincter-saving surgery without an increased risk of recurrence, or reduction in survival.

The incidence of local recurrence varies with operation for rectal cancer. Early reports demonstrated a 35.7% local recurrence rate after anterior resection (Garlock and Ginzberg, 1950). Similar figures have been reported by Hurst (1982) after using the automatic stapling gun. These are not, however, universal. Goligher (1951) and Cole (1952) had local recurrence rates of 10% whilst Phillips et al (1984b) identified a local recurrence rate of 18% following anterior resection compared to 12% for abdomino perineal excision. Although there appears to be a higher incidence following the former operation, Williams (1984) was not able to substantiate this in a substantial review of the literature. Heald et al (1982) described microfoci of carcinoma several centimetres distal to the lower edge of the tumour in the mesorectum, and subsequently recommended removal of the entire structure. Heald and Ryall reported their experience with this approach in 1986. Of 115 patients undergoing 'curative' surgery, 3 pelvic recurrences (2.6%) occurred after a mean follow-up of 4.2 years with no examples of anastomotic recurrence. Heald concluded that it was safe to limit distal clearance and preserve the sphincters provided that the meso-rectum was excised intact with the cancer.

More radical operations have been suggested by Hojo and Koyama (1982) who described extended pelvic lymph node dissections for rectal cancer. Decreased local recurence

rates for Dukes' B cancers were observed, but have not been confirmed by Enker et al (1986) although both centres do report increased survival rates.

Implantation metastasis: Because colorectal cancer can recur in the wound, local implantation of viable malignant cells has been suggested as a mechanism of local recurrence. As early as 1908, Sir Charles Ryall advocated treating carcinoma as an infection suggested that avoidance of local cell spillage would prevent recurrence (Ryall, 1907, 1908). Cole (1952) reported a 16% incidence of suture line recurrence in 55 cases undergoing colonic resection thought to be due to implantation. Implantation was advanced as the explanation for suture line recurrences after partial colectomy (Morgan, 1955). McGrew et al (1954) demonstrated viable carcinoma cells in 42% of the proximal and 65% of the distal ends of specimens at a mean distance of 21 and 10 cm from the tumour respectively. Similarly, Vink (1954) demonstrated viable exfoliated cells in an experimental animal model, and suggested they played a part in local recurrence.

Rosenburg, however, (1978, 1979), failed to demonstrate viable exfoliated cells (as demonstrated by trypan blue exclusion) and therefore questioned whether implantation was important in local recurrence. Recently, Umpleby et al (1984), has revived the implantation controversy by demonstrating large numbers of viable cells. Exclusion of

vital dyes however does not mean that cells are capable of tumour growth in vivo. Current practice in the UK suggests that a third of all General Surgeons have abandoned any form of prevention of cell spillage, presumably in the belief that this is not a significant cause of local recurrence despite the literature (Umpleby and Williamson, 1984).

The incidence of anastomotic recurrence varies from 4-7.9% (Deddish and Stearns 1961, Morson et al, 1963). Most anastomotic recurrences are in fact examples of extraluminal recurrence that has invaded the anastomosis which is the most vulnerable part of the wall (Umpleby, 1987). This does not however account for all anastomotic recurrence. Some 'early' anastomotic recurrence may be the result of implantation metastasis whilst some 'late' recurrence could result from metachronous tumour (Umpleby, 1987).

1.1.4 Treatment of Local Recurrence.

Most recurrent cancer is incurable by the time diagnosis is established (Goligher, 1984). In a review of 177 patients with recurrent disease, Welch and Donaldson (1979) found that 154 patients (87%) could only be offered palliative treatment. Twenty-eight percent (23/83) of patients who were re-explored underwent resection with curative intent and of these 7 patients (4% of the total with local recurrence) survived for five years. Radiotherapy may help to palliate unresectable local recurrence (Black and Kelly, 1955).

However, Welch and Donaldson (1978), found that radiotherapy produced symptom relief in only half of the patients who were treated. It appears that surgery offers the best chance of cure. Attempts to improve the results of surgical resection therefore have been based on early diagnosis and re-operation.

Re-resection for cure: Surgical resection is possible if recurrence is diagnosed early. Bacon and Berkeley (1959) resected recurrent tumour in 38 (out of 43) patients, and reported a 40 per cent 5 year survival. Ellis (1975) reported 12 favourable outcomes of 42 patients re-explored for suspected recurrent cancer. Fourteen patients had recurrent tumour and a further 5 patients had metachronous carcinoma. Gilbertsen reported on a series of patients in whom a 'second look' laparotomy was routinely performed before symptoms and signs occurred. The thirteen years experience (Gilbertsen and Wangensteen, 1962) of this approach resulted in an 11.8 per cent salvage rate. Berge et al (1973), reported a similar experience. The two major disadvantages with this approach are:

1) The 10% operative mortality associated with the major surgical resection which is frequently required.

2) Large numbers of patients have to undergo surgery for a small number who will ultimately benefit.

Because of this, routine 'second look' surgery has not gained general acceptance. The world experience in the treatment of recurrent colorectal cancer was reviewed by August (1984) who concluded that 20% of local recurrences were curable by further surgery. This suggests that only a 3% improvement in overall survival for colorectal cancer could be achieved by an aggressive surgical approach to local recurrence.

1.1.5 Would Treatment Be More Effective If Local Recurrence Was Diagnosed Earlier?

Three approaches to earlier detection of local recurrence have been tried. Firstly patients can be "followed-up" very closely by regular and frequent clinical examination, secondly patients can be investigated regularly (for example by routine CT scan), and thirdly the serum CEA can be monitored.

Frequent clinical surveillance: The mainstay for detection of recurrent colorectal cancer in most hospitals is routine clinical examination at the out-patient clinic. In an attempt to see if closer out-patient attendances could detect recurrence earlier, Cochrane et al (1980) reviewed 406 patients with large bowel cancer attending one colorectal out-patient follow-up clinic. Fifty-eight per cent of patients who developed recurrence were diagnosed outside routine out-patient follow-up appointment times. It was suggested that education of the patient in the symptoms and

signs of recurrence would be more effective in the early detection of recurrence than routine out-patient examination. More frequent follow-up increased diagnosis of recurrence, although subsequent treatment did not improve survival. Similar conclusions were reached by Tornqvist et al (1983).

Frequent radiological investigation: Intravenous urography, ultrasound and computerised tomography (Zelas, 1980; Reznek et al, 1983) are able to give the most useful information about recurrence. Barium enema if relied upon for detecting recurrence is dependant on a post-operative control study (Fleischner and Berenberg, 1956). Colonoscopy is replacing barium enema as the investigation of choice. In a comparison of radiological diagnostic methods available for detecting local recurrence after anterior resection, Grabbe and Winkler (1985) found CT scanning to be most useful at diagnosing local recurrence. When comparing the different modalities they found a sensitivity for CT of 78%, CEA 76%, Barium Enema 66%, Endoscopy 61% and Clinical Examination 30%, in 51 patients studied.

Serum CEA monitoring: Gold and Freedman first described carcinoembryonic antigen (CEA) in 1965. Since then a rise in serum CEA has been shown to be associated with a variety of malignant and benign conditions. Serum CEA is raised in up to 70% of cases of colorectal cancer (Martin et al, 1976; Mach et al, 1974) It has been suggested that serum CEA level

reflects tumour bulk (Wood, 1980). Thus, after primary tumour excision, growth of a small focus of remaining tumour might be detected by a rise in serum CEA.

Many reports have suggested that post-operative elevation of serum CEA can identify early recurrence (Wood et al, 1980 and Tate, 1982). This would then enable surgeons to re-operate earlier in suitable cases with a corresponding better chance of salvage (MacKay et al, 1974). Most workers have identified that CEA rises about 4 months prior to recurrence (Northover, 1986). Wood (1980) has identified 2 modes of post-operative elevation of CEA - 'slow rise' and 'fast rise'. 'Slow' rise is when serum CEA concentrations remain below 75 ug/l for at least 12 months whilst 'fast rise' is when serum concentrations reach 100 ug/1 within 6 months. In this series the majority (65%) of patients with a 'fast rise' developed distant metastases, whilst the majority of patients with a 'slow rise' (64%) developed local recurrence alone.

Moertel (1978) questioned the value of CEA in early diagnosis of recurrence, suggesting that the anxiety and expenditure produced by a CEA follow-up policy was not justified. In his series only 9 of 36 (25%) patients with recurrent disease developed a rise in CEA. Sugarbaker et al (1976) assessed the value of CEA monitoring as a predictor of recurrent disease, after primary colorectal cancer excision. Thirty four patients had monthly CEA estimations, 3-monthly physical

examinations, routine biochemical tests and plain X-ray examinations. Serial CEA was shown to be the earliest indicator of recurrence in just over half (58%) of the patients whilst physical examination detected a further 25% of cases. Attiyeh and Stearns (1981) operated on 32 patients with a rising CEA titre and no evidence of distant spread. They concluded that one third of patients could be expected to be cured by re-operation with early recurrence detected by CEA post-operatively.

Encouraging results have been reported from CEA directed second-look surgery (Minton et al, 1985; Martin et al, 1985). At present, however, there is no conclusive evidence that an approach of aggressive CEA based follow-up and second look surgery reduces mortality or prolongs survival (Northover, 1986).

It can be seen that while strategies such as close surveillance, intensive investigation and aggressive surgery may result in small improvements in survival after local recurrence, they cannot cure the majority of affected patients. A more effective approach would be to identify before primary surgery those patients at risk of local recurrence, and then to be prepared for a more radical primary resection and to consider subsequent adjuvant treatment.

1.1.6 Can We Predict Who Will Develop Local Recurrence?

Because depth of tumour penetration is an important factor for local recurrence, preoperative prediction of local invasion could be expected to predict those cancers that will recur.

Pre-operative digital assessment of local invasion of colorectal cancer is limited to the rectum. Nicholls et al (1982) found that assessment of local invasion by digital examination was highly subjective and was only 75% accurate, in the hands of experienced clinicians. Pre-operative detection and assessment of local spread of rectal cancer by computerized tomography has been shown to be 94% sensitive, 90% specific and 93% accurate (Williams et al, 1985; Dixon et al, 1981). CT scanning is not freely available and is expensive. Endoluminal ultrasound may be useful in assessing local spread from the rectum (Benyon et al, 1986). Thirty-eight patients were studied by intra-rectal ultrasound and the results compared with the pathology of resected specimens. There was a good correlation (0.92 Rank Spearman) between both ultrasound prediction of spread and invasion, whilst invasion of the muscularis propria could be predicted with high sensitivity (96%) specificity (92%) and predictive value (96%). A further benefit was the ability to detect enlarged mesorectal lymph nodes (which CT is not able to do). Similar results were reported by Romano et al (1985).

Colonic tumours however cannot be visualised by this method at present, as they are beyond the length of the probe.

Post-operative prediction of local recurrence has been recently enhanced by the ability to recognise lateral spread of tumour on the resected specimen (Quirke et al, 1986). Similarly imprint and 'scrape' cytology of the lateral pelvic walls and tumour bed have been shown to be reliable indicators of inadequate tumour clearance (Hall et al, 1987).

Some patients at risk of developing local recurrence after excision of rectal cancer can now be identified before operation with ultrasound and CT scan. However, there is still a need for a reliable pre-operative indicator of patients at high risk of local recurrence after primary tumour excision of colon and some rectal carcinomas.

1.1.7 If Local Recurrence Is Predicted, Can It Be Prevented?

Prevention of implantation of viable malignant cells: Pre-operative cytotoxic irrigation of the intestinal lumen - for example, with mercuric bichloride - has been advocated by Keynes (1961) who noted a fall from 13 to 2.6% in local recurrence rates following this measure. The control patients were historical and, therefore, these conclusions are open to criticism. Isolation of the tumour with tapes

(Cole, 1952) and ligation of the inferior mesenteric vein prior to handling the tumour were also recommended as ways of reducing local recurrence (Morgan, 1955; Southwick, 1962). Turnbull (1967) advocated the 'no touch' technique and suggested that survival in Dukes' stage C cancers 5 years' was improved from 28% to 57% at 5 years.

Wider radical surgery: Heald et al, (1986) have advocated wide local clearance of rectal tumours, particularly of the mesorectum and has demonstrated a low (2.6%) local recurrence rate. Enker (1979, 1986) has demonstrated that extended pelvic lymphadenectomy increases survival and reduces local recurrence in mid rectal cancers. This was confirmed by Hojo (1986). These procedures, however, do involve increased mortality and morbidity and are unnecessary for patients not at risk of local recurrence after conventional excision.

Adjuvant chemotherapy: Higgins et al (1978) in a prospectively randomised trial, compared adjuvant 5-Fluoro-Uracil (5FU) with primary tumour excision alone. There was no evidence that local recurrence rates were reduced by adjuvant chemotherapy.

Adjuvant radiotherapy: Adjuvant radiotherapy has been more extensively studied for rectal than for colonic carcinoma. A variety of regimens have been tried: pre-operative, post-

operative and combined pre and post-operative regimens ("sandwich therapy") in varying doses.

The Veterans Administration Surgical Adjuvant Group (VASAG) conducted a randomised trial of pre-operative radiotherapy of 2000 cGy in two weeks compared with surgical resection only (Higgins et al, 1978). Although there was no improvement in overall survival between the 2 groups, those patients receiving radiotherapy and abdomino-perineal excision did have a significantly improved survival. Analysis of the lymph nodes of resected specimens demonstrated a reduction of positive nodes for tumour in the irradiated group compared with control. It was suggested that pre-operative radiation may destroy foci of carcinoma, perhaps converting the tumour to a more favourable stage.

Further autopsy studies on 180 patients suggested a smaller number of patients with local recurrence than in the control non-irradiated group, although this was not significant. Similar findings were reported from Toronto (Rider et al, 1977). Higher dose (5000 cGy) pre-operative radiotherapy may reduce local recurrence still further, but controlled trial data is not available (Mendenhall et al, 1985; Stevens et al, 1976). The MRC trials in this country have failed to show any survival benefit (Duncan, 1985a) in two low-dose pre-operative regimes (500 cGy and 2000 cGy).

Post-operative adjuvant radiotherapy has not been extensively studied. Gunderson (1976) in a small series of 31 patients who had undergone surgery with curative intent, reported that there was only one local failure (3%).

Combined pre- and post-operative radiotherapy ("sandwich" technique) has been assessed at the Thomas Jefferson University Hospital. Mohiuddin (1985) reported a comparison of all 3 adjuvant radiotherapy approaches. Patients receiving pre-operative radiation alone showed a reduction of distant metastases (24%), but no effect on local recurrence. Patients receiving post-operative radiation alone showed a reduction in local recurrence (11%), but no reduction in distant metastases. Those who received the sandwich regimen showed reductions in both local recurrence and distant metastases.

It appears that adjuvant radiotherapy can influence the prevalence of node positivity and disease free interval, and that it may also reduce local recurrence. However, if used as an adjuvant 60-80% of patients would be irradiated unnessarily since they will never develop local recurrence.

1.1.8 Conclusions

The treatment of established local recurrence by either radiotherapy or surgery is unlikely to provide a solution in most cases, unless it can be diagnosed much earlier. The

elimination of local recurrence appears to lie in meticulous radical surgery, prevention of spillage of cells, and adjuvant radiotherapy. This would be facilitated by pre-operative identification of patients at increased risk of local recurrence so that the operation can be carried out by a suitably experienced surgeon, and radiotherapy given where indicated. Endorectal ultrasound may be of value in the pre-operative assessment of rectal carcinoma, but cannot be used in all rectal carcinomas, or in colonic carcinoma. CT scan will have to become cheaper and more widely available to be of general use.

There is a need for a simple test that could identify early - preferably before operation - the patient at risk of local recurrence following excision of primary colorectal carcinoma. Ideally the test would be accurate, simple and cheap.

Greaves et al (1980) reported that the length of the sialomucin band surrounding colonic carcinoma 'Transitional mucosa' (TM) was related to subsequent survival following excision of Dukes' B carcinomas. An inverse correlation was seen between survival and length of TM adjacent to Dukes' B carcinoma ($p=0.005$). It was suggested that this was related to increased amounts of sialic acid in TM which may depress tumour immunogenicity (Currie and Bagshawe, 1968).

This raises the possibility that increased sialic acid adjacent to primary colorectal carcinoma may reflect a different biological behaviour of the tumour compared to those tumours where less sialomucin is present. This difference in biological behaviour might be a significant factor in local tumour invasiveness. Hence, extent of sialomucin might be associated with risk of local recurrence.

1.2 SIALOMUCINS AND MALIGNANCY

1.2.1 Function of Mucus

The functions of mucus are:

1. To lubricate colonic mucosa to assist in the passage of faeces.
2. To protect colonic mucosa, from both damaging intestinal luminal content and the shear forces generated by peristalsis. Mucosal integrity prevents bacterial infection and chemical injury.
3. To facilitate passage of small molecules into luminal absorptive cells (Allen, 1981).

Other functions of mucus, for example providing a defensive barrier and regulating absorption, have been proposed but not fully evaluated (Allen, 1980). The integrity, quality and quantity of the mucus gel may become deranged in a number of diseases, including bacterial and parasitic infection, cystic fibrosis and chronic inflammatory bowel disease (Forstner et al, 1981).

1.2.2 Composition of Mucus

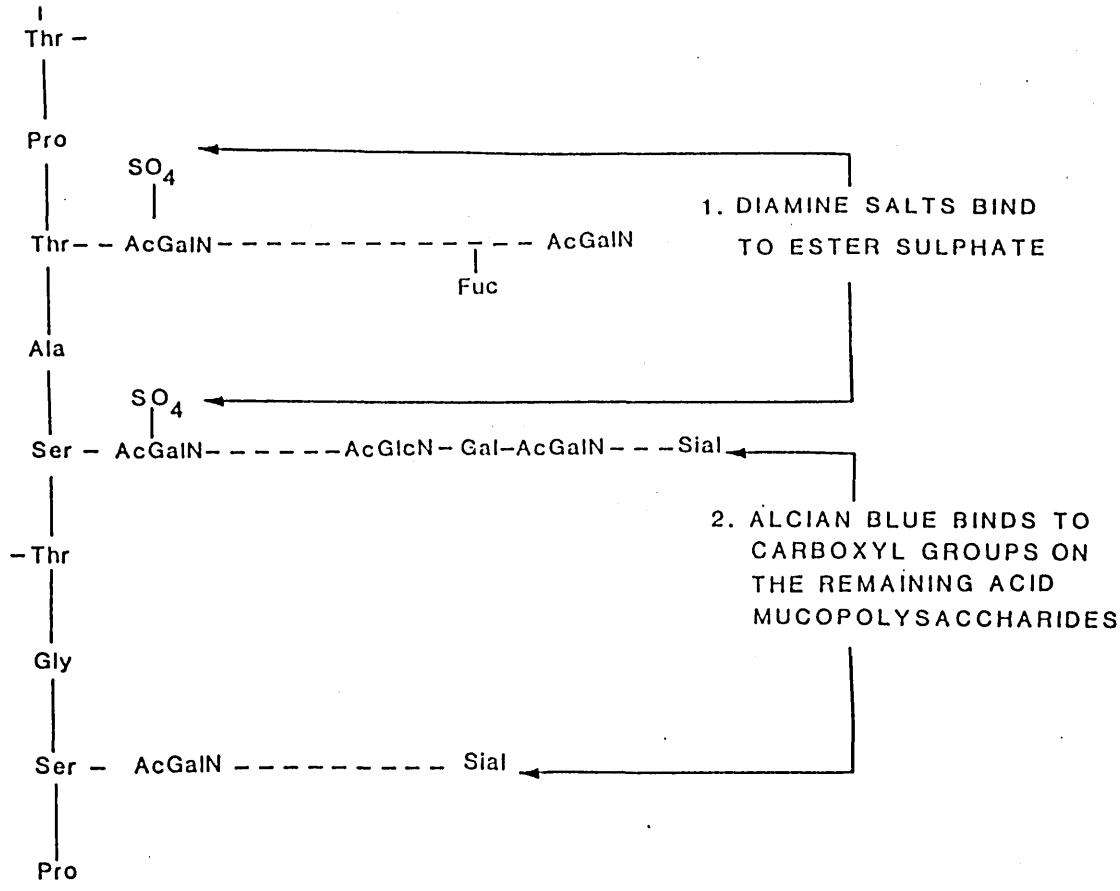
Colonic mucus is made up of varying molecular weight (0.5->6 x 10^6 daltons) glycoproteins consisting of an 800 amino acid peptide core and numerous side chains (Lamont and Ventola, 1980; Silverberg, 1982; Forstner, 1978; Feizi et al, 1984).

The peptide core contains threonine and proline, and small amounts of alanine and glycine. The carbohydrate side chains are always attached to either serine or threonine by o-glycosidic linkages (Goodman, 1975). As well as n-acetyl galactosamine (the first carbohydrate in the side chain), the sugars galactose, n-acetyl glucosamine, fucose and sialic acids are attached closest to the peptide core, along with variable amounts of ester sulphate (Forstner, 1978; Goodman, 1975), (Figure 1). Little is known about the secondary or tertiary structure of colonic mucin because their extensive glycosylation has frustrated attempts to isolate intact peptides.

Individual molecules of mucus glycoprotein are linked to each other by disulphide bonds which result in the typical characteristics of mucus, its ability to form loosely structured gels and its slimy quality (Goodman 1975).

1.2.3 Synthesis

Mucus is synthesised in colonic mucosal goblet cells which are distributed throughout the colon. These are less numerous than absorbtive cells (1:10 goblet:absorptive on the luminal surface and 1:5 in the cell crypts; Goodman, 1975). Both goblet and absorptive cells arise from pluripotential stem cells in the base of the colonic crypts (Shamshuddin et al, 1982). As these cells migrate towards the lumen they differentiate into either goblet or absorptive cells, and



Thr = Threonine,Pro =.Proline,SO4 = Ester sulphate,AcGlcN = N-acetyl-glucosamine,--= o-glycosidic linkage,Fuc = Fucose,Ala = Alanine,Ser = Serine,AcGalN = N-acetylgalactosamine,Gal = Galactose,Gly = Glycine, Sial = Sialic acid(terminal sugar).

Figure 1 Oligosaccharide linkages in intestinal mucus and mode of action of the high iron diamine alcian-blue stain.
Diamine salts bind to the ester sulphate groups.
Alcian-blue binds to the carboxyl groups on the remaining non-sulphated acid mucopolysaccharides.

lose the capacity to proliferate. Goblet cells are highly specialised cells (Figure 2) whose ultrastructure reveals their specialisation for the manufacture, storage and synthesis of mucin (Neutra, 1982). On electron microscopy, in addition to the rough endoplasmic reticulum, the region above the nucleus is occupied by an elaborate Golgi apparatus which is essential for the glycosylation process required to produce mucin glycoprotein. It is here that sulphation of mucus is thought to occur. At the apex of the goblet cell is a cup-like structure (the theca) which contains closely opposed vacuolar structures and whose shape is maintained by a vertical array of microtubular structures surrounding the mucin droplets (Neutra and Forstner, 1986). At the luminal end of the cell the mucin containing vesicles are closely opposed to the apical membrane and may be discharged selectively or en mass by membrane fusion and exocytosis.

The first stage of mucus production occurs on the ribosomes of the rough endoplasmic reticulum where a peptide chain is synthesized (Phelps, 1978). This is transported through the smooth endoplasmic reticulum to the Golgi apparatus, where sugars are added in sequence under the influence of specific glycosyl transferases (Phelps, 1978). Finally the terminal sugars are attached which are responsible for the antigenic and histochemical characteristics of mucus (Goodman, 1975).

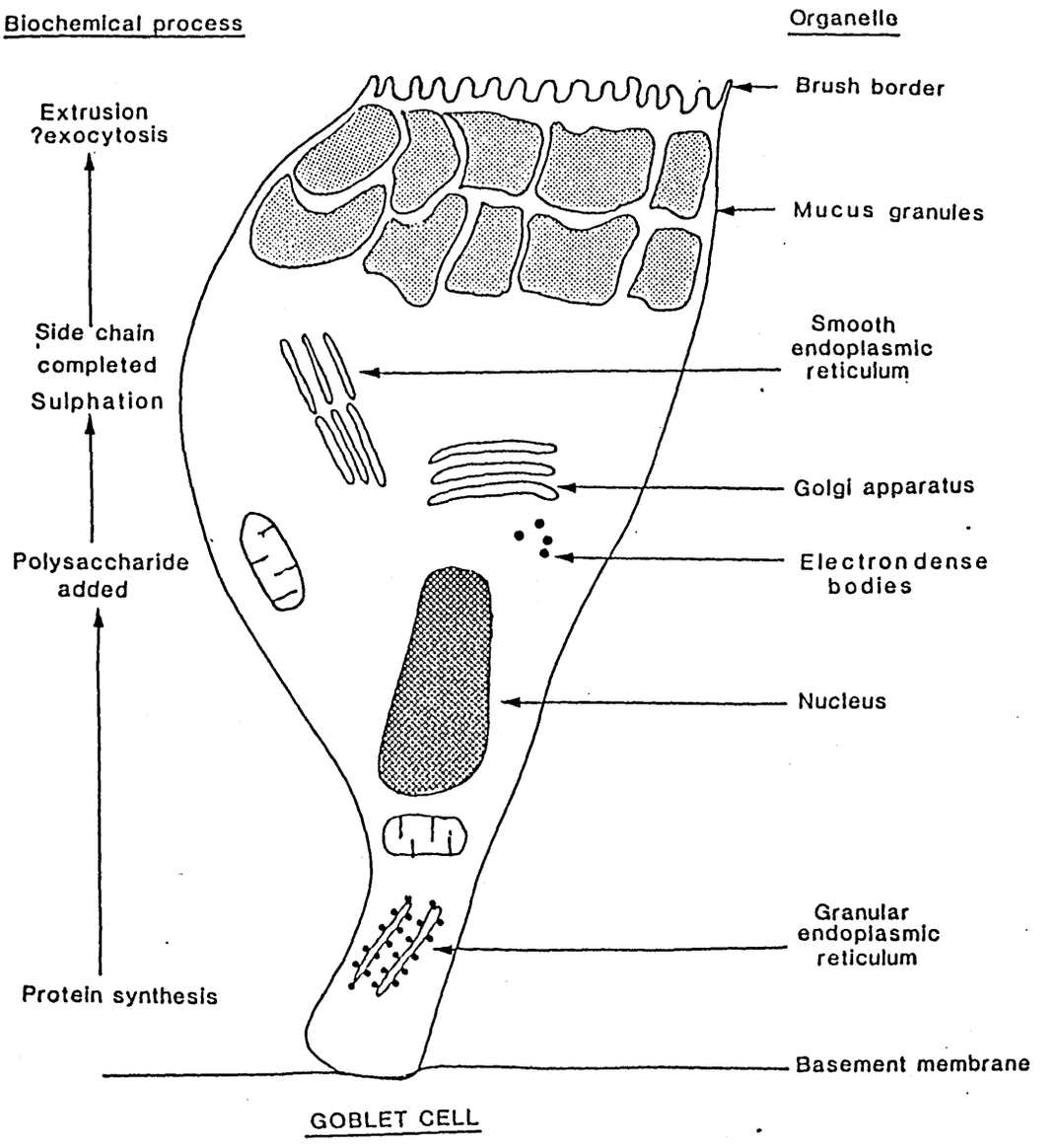


Figure 2 A colonic goblet cell.
Organelles responsible for mucus secretion are shown on the right hand side and the corresponding metabolic functions of these organelles are on the left hand side.

It is not clear whether mucus is released from goblet cells by exocytosis or controlled apocrine release. Like many exocrine cells, goblet cells maintain a slow base line secretory rate that involves intermittant release of single secretory granules (Specian and Neutra, 1980), counteracted by new production in the Golgi region of the cell (Neutra and Leblonde, 1966). Many factors can accelerate this process, for example cholera toxin (Forstner, 1981), mediators of the immune response and muscarinic cholinergic agents (Neutra et al, 1982) whereas peptide hormones (eg, secretin, substance P, VIP) have no effect (Neutra et al, 1982). Whilst dietary factors, for example fibre quality and content, may affect mucin synthesis in the small intestine the mechanism and their relevance in the colon are not known (Sherman, 1985). Once mucus is released from the crypt orifices it forms a continuous layer on the colonic surface. Mucus is then lost from the colonic wall, either by attachment to faeces or by bacterial degradation. This occurs because bacteria may produce extracellular glycosidases, which after cleavage of the sialic acid in mucus progressively split off the sugar moeities (Variyam & Hoskins, 1981; Prizant & Konigsberg, 1981).

1.2.4 Mucin Varieties

"Mucin" was first defined by Eichwald in 1865, who suggested that it was a "moeity with all the properties of a genuine protein and a moeity released under certain conditions as a sugar". Over the years many names have been given to these

substances (mucopolysaccharides, glycosaminoglycannes, mucosubstances and glycocongigates). Cook (1984) suggested that while the terminology is unimportant in itself, whichever terms are used should be consistent. In this thesis the term mucin is reserved for those mucosubstances which are present in epithelia, whilst mucopolysaccharides are reserved for those mucosubstances present in connective tissue (Jeanloz, 1960). In simple terms these compounds are hexosamine containing polysaccharides which are covalently bound to varying amounts of protein. Free hexose groups are often available, together with certain acidic moeities, the presence of which influences markedly the histochemical reactions.

The presence of faeces, bacteria, secretory proteins and other contaminants complicate the chemical analysis of colonic mucins. Preliminary purification by gradient ultra-centrifugation (Starkey et al, 1974) or gel exclusion chromatography (Lamont & Ventola, 1980) is required prior to mucin chemical analysis. An alternative means of identifying the type and distribution of colonic mucus is by histochemical methods. Histochemical techniques provide less quantitative information about mucus composition, and they focus on only two components of mucin - sialic acid and sulphate. However, histochemistry is particularly suitable for the study of mucins in malignancy because sialic acid and sulphate in particular are altered in malignancy (Filipe, 1969). The various types of mucins that may be identified by histochemical methods are shown in Table 1 (Cook, 1984).

Table 1 Histochemical characterisation of mucins (Cook, 1984)

Type	Source of Origin	Popular Name
ACIDIC		
Strongly Sulphated		
(a) Connective tissue	Fibroblasts Chondrocytes Osteocytes	Keratin sulphate Heparin Chondroitin Sulphate A Chondroitin Sulphate B Chondroitin Sulphate C Chondroitin
(b) Epithelial	Bronchial Serous glands	-
Weakly Sulphated	Colonic goblet cells	Sulphomucin
Sulphated Atypical	Tracheobronchial mucus glands	-
Carboxylated mucins		
(a) Enzyme labile	Bronchus Submandibular gland Intestine	Sialomucins
(b) Enzyme resistant	Large bowel Stomach Bronchus	
Sulphated mucins	Prostatic cancer	-
Non-sulphated carboxylated	Fibroblasts Synovial fluid	Hyaluronic acid
NEUTRAL	Brunners glands Stomach Prostate	Fucose and mannose containing mucins Immunoglobulins

The histochemical methods used for detecting mucins depend on the groups present in the carbohydrate moeity (Figure 1). Available staining methods are listed in table 2 (Filipe, 1969). The methods available for the identification of mucosubstances can be divided into oxidation methods, for example, Periodic Acid Schiff reaction, metachromasia, basic dyes; for example alcian blue, and the diamine methods. More recently mucopolysaccharides have been investigated using lectin probes (Boland et al, 1984). The basic dye and diamine methods used in this thesis are considered below.

Alcian blue

The alcian dyes are cationic dyes (positively charged) and form electro-static bonds with certain tissue polyanions bearing either carboxyl or sulphate groups. Alcian blue was first introduced by Haddock in 1948 which was then adapted as a histochemical technique by Stevens (Cook, 1984). The basic reaction between the dye and acid group in acid mucosubstances is a reaction between the cationic dye and the ionised acid radical which is pH dependant. By varying the pH of the basic dye it is possible to differentiate between the carboxyl groups from phosphates and sulphates (the main acid radicals in acid mucosubstances). At pH 2.5 sialomucin and weakly acidic sulphated mucins are ionised and show basophilia with alcian blue, whilst the strongly sulphated

Table 2 Histochemical methods used for visualising mucosubstances (Filipe, 1969)

Method	Substances Visualized
Periodic-acid-Schiff (PAS)	Glycogen; neutral mucosubstances
Diastase-PAS	Loss of PAS reactivity in sites containing glycogen
Alcian blue (pH 2.5)-PAS	Neutral mucosubstances, magenta Acid mucosubstances, blue
Mild methylation-alcian blue-PAS Saponification-alcian blue-PAS	Carboxyl groups of acid muco-substances lose basophilia after methylation and regain it after saponification
Colloidal iron	Acid mucosubstances
Hyaluronidase-colloidal iron	Removes hyaluronic acid from acid mucosubstances
Aldehyde fuchsin-alcian blue	Sulphated mucosubstances: purple; Non-sulphated acid mucosubstances: blue
Alcian blue, pH 0.5	Sulphated acid mucosubstances
High iron diamine-alcian blue	Sulphated mucosubstances: black-brown Sialomucins: blue
Strong methylation-high iron diamine-alcian blue	Stain of all acid mucosubstances occluded
Neuraminidase-colloidal iron Neuraminidase- alcian blue-PAS	Removes sialic acid from mucosubstances

acid mucins remain un-ionised and do not react. The linkages between dye and ionised acid are probably salt.

Diamine methods

The essential reaction occurs between the diamines and the per-iodate engendered aldyhyde groups. The para and meta forms react with the acid mucosubstances to form an intense brown/black stain which leaves the non-sulphated acid mucosubstances unstained, which are then counter-stained by alcian blue at pH 2.5. The oxidization which is needed for this process is provided by ferric chloride, which also decreases the pH of the solution and prevents the carboxyl groups from reacting, thereby a clear distinction between the acidic sulphomucins and sialomucins can be made.

1.2.5 Mucins in Malignancy

Much of the available information about histochemical alterations of colonic mucus in malignancy comes from Dr M I Filipe at Guys Hospital Medical School, London, and Dr C F Culling, University of British Columbia, Vancouver. In 1968, the first reports of alteration in colonic mucus in malignancy came from Goldman and Ming who demonstrated a reduction of sulphated mucins in most colonic tumours. Gad (1969) confirmed these findings and demonstrated an increase in sialomucins in colonic carcinomas.

Filipe (1969) used the high-iron diamine alcian-blue (HID-AB) stain (Spicer, 1965), periodic acid-Schiff (PAS), Hale's

colloidal iron and several alcian blue methods to study mucosubstances produced by normal, benign, and malignant colorectal mucosa. Neutral mucosubstances were found to be periodate reactive, giving a positive PAS reaction and not being modified after diastase digestion. Acid mucosubstances stained blue with the alcian blue (pH 2.5) - PAS method whilst the high-iron diamine alcian-blue method stained the sulphated acid mucosubstances brown and the nonsulphated blue. Normal colonic mucosa showed a predominance of sulphomucins (staining brown), occupying the lower third to in some cases the entire crypt, although small amounts of sialomucin (staining blue) were found in the upper part of the crypts.

Both types of acid mucins are present in the surface epithelium with either predominating. Goblet cells in the surface epithelium or in the upper part of the crypt contain either sulphated or non-sulphated mucins or both. In the latter case non sulphated mucins are found in the zone between the nucleus and the goblet region of the cell. Sulphated mucins are found from the centre of the goblet cell up to the luminal border.

In 'transitional' mucosa (TM), ie, mucosa which is macroscopically and microscopically normal but lies adjacent to an epithelial neoplasm there is both a qualitative and quantitative change in mucin (Filipe, 1969; Filipe, 1979). Sulphomucin is reduced and sialomucin increased in proportion

to proximity to the tumour. There is a progressive increase in crypt sialomucin content until the crypt contains only sialomucin adjacent to an epithelial neoplasm. The tumours themselves have little or no mucin secretory activity and this is also found in the epithelium adjacent to the tumour.

The Vancouver group used the periodic acid-thionine Schiff/potassium hydroxide/periodic acid Schiff stain to study colonic mucin. This stain indicates the degree of O-acylation of sialic acid which appears to be reduced in malignancy (Culling et al, 1977; Reid et al, 1984).

1.2.6 Mucins in Transitional Mucosa (TM)

Transitional mucosa has two typical features on routine histological examination:

i) elongation and branching of mucosal crypts

ii) dilatation of goblet cells (Filipe and Branfoot, 1974).

Filipe and Cooke (1974) confirmed by chemical analysis that hexosamines and sialic acid were present in scrapings taken from normal and 'transitional' mucosa. Transitional mucosa showed an absolute increase in both total hexosamines and sialic acid compared to normal mucosa. This was accompanied by an increase in neuraminidase sensitive sialic acids. Paper chromatography demonstrated a change in mobility of TM sialic acid compared to normal mucosa which suggested a blocking of sialic acid by O-acetylation in normal mucosa. They proposed (Filipe and Cooke, 1974) that the epithelial

cells in TM may be immature, resulting in an increase in hexosamine and a higher percentage of neuraminidase sensitive sialic acids.

Filipe and Branfoot (1974) demonstrated that the length of TM from the tumour was related to the Dukes' stage of the tumour. However, there was no correlation between length of TM and differentiation or histological pattern of the tumour. They also noted that sialomucin could be seen both in the resection margin of specimens folowing colon resection and in isolated patches of sialomucin elsewhere in otherwise normal mucosa at a distance from colon carcinoma. This spread of sialomucin was more marked in association with tumours of the left colon than the right, where sialomucin in the lower 1/3 of the crypts was seen. They suggested that sialomucin production may represent a cellular response to an as yet unknown stimulus or a transformation to fetal type of epithelium associated with the carcinogenic stimulus rather than a secondary effect arising from the close presence of adjacent tumour.

Greaves et al (1980) demonstrated that the average length of transitional mucosa surrounding the tumour was 3.4 cm (range 0-19.5 cm). The mean length in 17 right-sided colon carcinoma specimens was 1.2 cm compared to 3.7 cm on the left side. There was no association between length of TM and Dukes'stage of the tumour. However, there was a significant ($p<0.005$) relationship between extent of TM and survival in

patients with Dukes' B carcinomas which was independant of tumour site. Isolated patches of sialomucin were also found at distances separate from the tumour.

Electron microscopy of TM: Studies of TM with electron microscopy (Dawson and Filipe, 1976) demonstrate a persistance of immature and intermediate cells at higher levels than normal in the crypts. These immature and intermediate cells appear to be shed into the crypts prematurely. Goblet cells are increased in size and number. Membrane limited electron dense bodies and an enlarged Golgi zone showing increased secretory activity are also found in TM. These findings suggested that TM could be either a regenerative process or a premalignant event.

Riddell and Levin (1977) correllated TM with scanning electron, transmission electron and light microscopy in 13 patients. They found loss of normal architecture and crypt distortion in nine cases. They confirmed a tendancy for cells to be shed into the crypt lumen at an early stage in development. Similar changes were seen in adjacent adenomas and in one patient in 'normal tissue'. The consistent finding of immature cells in the crypt lumen suggests that cell maturation may be deranged in TM, as well as in neoplastic epithelium.

1.2.7 Does Sialomucin Indicate Carcinogenesis?

Filipe (1969) suggested that TM might indicate a neoplastic change before polypoid or dysplastic change was evident. The evidence for an association between sialomucin and neoplasia comes from the experimental model of large bowel cancer induced in rats by administration of 1,2 dimethylhydrazine. Filipe (1975) demonstrated that predominant sialomucin secretion occurred in areas of colonic mucosal dysplasia before the appearance of a polyp or carcinoma. Traynor et al (1983) suggested that sialomucin secretion is part of a field change throughout the colon - appearing some 16 weeks before the appearance of adenomas. Further experimental work (Filipe, 1977; Filipe et al, 1982) confirmed that invasive cancers were preceded by mucosal changes including excess sialomucin production, as well as conventional dysplasia. Other workers have confirmed these findings (Shamsuddin et al, 1981). Sialomucin change has also been found in situations associated with increased colorectal cancer risk, eg, ureterosigmoidostomy (Strachan et al, 1985).

These histochemical findings are supported by biochemical studies where a generalised decrease in 35 sulphate uptake (which reflects sulphomucin depletion) is seen throughout the colon in association with neoplastic field change (Springer et al, 1970).

Sialomucin was also found in dysplastic mucosa in patients with chronic ulcerative colitis (Ehsanullah et al, 1982a). This was in contrast to 65 cases of non-specific proctitis

where sulphomucins were found in all but one case. Sialomucin secretion was related to the activity, duration and extent of ulcerative colitis. All biopsies with dysplasia, except one, showed predominant sialomucin staining. During remission, all patients with sialomucins on colonic biopsy also showed dysplasia, whilst during active disease a number of biopsies showed increased sialomucin without dysplasia. Sialomucins were also demonstrated in 10/20 patients with Crohn's disease.

Further studies (Fozard et al, 1987) have assessed the role of the HID-AB stain in cancer surveillance of patients with chronic ulcerative colitis. In this study, 115 pre-operative colonoscopic biopsies from 14 patients were assessed using the HID-AB stain and compared to the subsequent colectomy specimens (6 contained carcinoma, 8 did not). Sialomucin correctly identified the colectomy containing carcinoma in 5/6 cases. Thus, HID-AB stain may help to identify low grade dysplasia which can easily be misdiagnosed (Riddell et al, 1983).

Jass et al (1986) was not able to reproduce these findings. Biopsy specimens were obtained from 11 colitis patients shortly before colectomy for cancer and these were matched for age, sex and duration of disease, with biopsies from patients without cancer. No difference in HID-AB staining was found between the two groups. This has stimulated some debate. Jass suggests that Fozard has over-diagnosed low grade dysplasia. Fozard suggests that Jass's control group

is unreliable, since they have not undergone colectomy and dysplastic change has not been identified - a finding which is at variance with his and other workers (Ransohoff et al, 1985) experience of ulcerative colitis. The uncertainty about the significance of low-grade dysplasia in chronic ulcerative colitis has not been resolved, nor is the significance of a positive HID-AB test clear.

1.2.8 Are Sialomucins a Non-Specific Epiphenomenon?

Not all the studies of transitional mucosa have supported the view that sialomucin change associated with TM is neoplastic. Isaacson and Attwood (1979) failed to demonstrate specificity of the histological and histochemical changes in mucosa adjacent to colonic carcinoma. They described sialomucin rich mucosa adjacent to anal squamous cell carcinoma, malignant melanoma and malignant lymphoma. This suggested that TM is a secondary non-specific change. TM was also demonstrated in solitary ulcer syndrome and colostomies prone to mucosal prolapse. It was suggested that damage produced by mucosal prolapse resulted in TM production during regeneration.

Litinsky and Riddell (1981) demonstrated TM overlying benign mesenchymal tumours, metastatic tumours from non-colonic sites, and endometriosis. Lapertosa et al, (1984), were able to find TM in 2/15 patients with non-carcinomatous colonic neoplasms. Franzin et al (1981) also described TM in solitary ulcer syndrome (confirmed by Ehsanullah et al, 1982b) as well as in a variety of other situations including

ischaemic colitis, adjacent to metaplastic polyps and polypectomy scars. TM has also been found in diverticular disease (Rhatigan and Saffos, 1979) and Crohn's disease (Ehsanullah et al, 1982a).

Lev et al (1985) compared transitional mucosa in the colonic resection margins of specimens from three patients with diverticular disease, three with metastases to the colon, one epidermoid carcinoma of the anus, one sigmoid volvulus and two normal controls (one patient, a diabetic, had a total colectomy for constipation, the other a sub-total colectomy with large areas of normal sigmoid mucosa). They found no differences between these specimens to suggest that transitonal mucosa was associated with neoplasm. The findings suggested that TM was a non-specific response to injury or inflammation. They also concluded that sole use of the HID-AB method could provide misleading results.

1.2.9 How Reproducible is Mucin Histochemistry?

The divergence of results discussed above may be because different laboratories are unable to obtain similar results with apparently similar methods. Filipe (1984) blamed a variation in staining techniques with HID-AB when comparing her results to Franzin (1983). In this case, the water rinse, pH of the solutions, and the commercial source of the reagents were thought to be crucial. Greaves et al (1980) reported 'slight' variations between batches and controls. When 16 different hospitals were asked to assess the technique, the variation in results was marked (Barr, 1978).

The discrepancies appear to be due in part to different published methods. According to Sheahan et al (1970) the method described by Spicer (1965) is misleading because it describes 10 per cent ferric chloride instead of 10 per cent ferric ion. Textbooks vary in the recommended concentration of this compound. According to Sorvari (1972) differences in results can occur by using 10 per cent instead of 60 per cent ferric chloride. The histochemical features of TM could be diagnosed in normal tissues merely by altering the concentration of ferric ion in the staining. Similarly the diamine salts used are also known to be unstable. Caution should, therefore, be exercised when comparing different centres' results (Cook, 1988).

1.2.10 Conclusions

Ehsanullah et al (1982a) suggested that the association between dysplasia and abnormal mucins could be explained by impaired cell maturation in dysplastic epithelium, with the gradual replacement of mature by immature and intermediate cell types. This would result in deficient glycoproteins being produced which were similar to those found in neoplasia and in fetal gut mucus (Lev, 1965). Fozard et al (1987) have suggested that the finding of sialomucin in the presence of active inflammation and in the absence of dysplasia could represent early changes of carcinogenesis preceeding morphological atypia. However, it is clear that transitional mucosa is not exclusively cancer associated since there is

widespread agreement concerning its presence in association with a number of benign conditions where mucosa is injured.

The association between TM and colorectal cancer can be attributed to mucosal injury where the TM is adjacent to the tumour. However, the patches of TM scattered discontinuously throughout the neoplasm-prone colon could not be attributed to mucosal injury. It seems that sialomucin change is a result of an alteration in cell proliferation which can be induced either by injury or by neoplasia.

CHAPTER 2

EXPERIMENTAL HYPOTHESIS AND OUTLINE OF STUDY

2.1 HYPOTHESIS

There is a need for a simple pre-operative test which identifies patients at increased risk of local recurrence following excision of primary colorectal carcinoma. Colonic mucosal sialomucin appears to indicate a variation in tumour biology which is associated with poor survival after primary tumour resection. Thus, sialomucin change might be a suitable pre-operative indicator of patients at risk of local recurrence.

The hypothesis which is tested in this thesis is that sialomucin change adjacent to primary colorectal carcinoma reflects local tumour invasion and hence subsequent risk of local recurrence after primary tumour excision.

2.2 OUTLINE OF STUDIES

The first study (Chapter 3) has assessed whether size of sialomucin band adjacent to colorectal carcinoma correlates with depth of tumour invasion through the colon wall.

As a result of the findings of the first study, a more ambitious multi-centre study (Chapter 4) was established to provide the necessary power for the relative importance of the many factors contributing to local recurrence to be assessed. The objective of this larger study was to

determine whether size of sialomucin band correlated with probability of local recurrence; and to assess the relative importance of sialomucin band and sialomucin extending to resection margin as risk factors in comparison with other known local recurrence risk factors.

These findings raised the question of the biological significance of colonic mucosal sialomucin change: neoplasm-related or non-specific injury response? The final part of the thesis (Chapter 5) has assessed the prevalence of sialomucin change in various neoplastic and inflammatory conditions both in man and in animal models. The aim has been to determine whether increased prevalence is associated with neoplasia and pre-neoplasia or whether it is more dependant on extent of mucosal injury.

CHAPTER 3

IS THERE AN ASSOCIATION BETWEEN DEPTH OF INVASION OF PRIMARY COLORECTAL CANCER AND ADJACENT MUCIN CHANGE?

3.1 AIM

The aim was to identify the length and distribution of sialomucin secreting mucosa surrounding human colorectal carcinoma and to relate the findings to known prognostic determinants of local recurrence and survival.

3.2 METHODS

Patients who had undergone excision of primary colo-rectal carcinoma were studied. Tumours were categorized into right-sided lesions (caecum up to but not including splenic flexure) and left sided lesions (splenic flexure to rectum).

The excised colon was opened and fixed in 10% formal saline solution immediately after excision. An entire strip of colonic mucosa was excised incorporating the centre of the tumour and both resection margins (Figure 3). Longitudinal blocks were taken along the length of the specimen and processed in paraffin. Five micron thick sections were cut and stained with Haematoxylin and Eosin and the high-iron diamine alcian-blue stain.

The high-iron diamine alcian-blue stain

In view of the controversy (see Chapter 1) surrounding the high-iron diamine alcian-blue technique, I initially assessed a group of test slides using different concentrations of

Ferric Chloride. These were 1.4 ml of 10% Ferric Chloride (Spicer, 1965); 5.6 ml of 10% Ferric Chloride (Cook, 1972) and 1.4 ml of 30% Ferric Chloride (Cook, 1984). Using 1.4 ml of 10% Ferric Chloride, I was unable to get colonic goblet cells to stain for sulphomucin. Using the remaining 2 concentrations, however, there was adequate staining of both sulphomucin and sialomucin secreting colonic goblet cells. Providing the method produces reliable results with consistent discrimination between sulpho- and sialomucin colonic cells, then the methodology can be considered valid (Cook, 1988). Caution, however, should be used when interpreting different laboratories' results because of discrepancies in the methodology. In this thesis, I have used the method described by Cook in 1972. The procedure is as follows:

(a) 120 mg N N dimethylmetaphenylenediamine hydrochloride is added to 20 mg N N dimethylparaphenylenediamine hydrochloride in 50 ml of water

(b) This is then added to 5.6 ml 10 per cent Ferric Chloride solution.

(c) The sections are stained for 18 hours with this mixture and then rinsed in water and counterstained for 10-20 minutes with alcian-blue (1 in 3% acetic acid). If the diamine stain is not taken up by the goblet cells the staining procedure stages (a) and (b) were repeated.

Control slides were placed with each batch to ensure uniformity of the staining reaction. Gloves were used when handling N N dimethylmeta and paraphenylenediamine hydrochlorides in view of possible carcinogenic effects that can occur as a result of skin absorption.

(d) All sections were then cleared in Xylene and mounted for examination under light microscopy.

The tumours were staged according to Dukes' classification and Broder's differentiation by a Consultant Pathologist (HCR) while the mucin stains were independantly examined by 2 surgeons (PMD and NAH). Three mucin patterns were identified on the HID stain. These were a purely sulphomucin secretion (Plate 1), a mixed sulphomucin and sialomucin pattern (Plate 2) and a purely sialomucin pattern (Plate 3). The first two patterns were considered to be normal.

Depth of invasion was classified according to the UICC TNM classification (Spiessel et al, 1982). The length of transitional mucosa was assessed using a graticule eyepiece. Measurements were made in centimetres. Agreement between the assessors about mucin staining patterns was reached in all cases using a definition of abnormal which required an entire microscope field to contain exclusively sialomucin. Transitional mucosa surrounding the tumour was recognised by exclusive sialomucin secretion on HID-AB stains, and on H + E

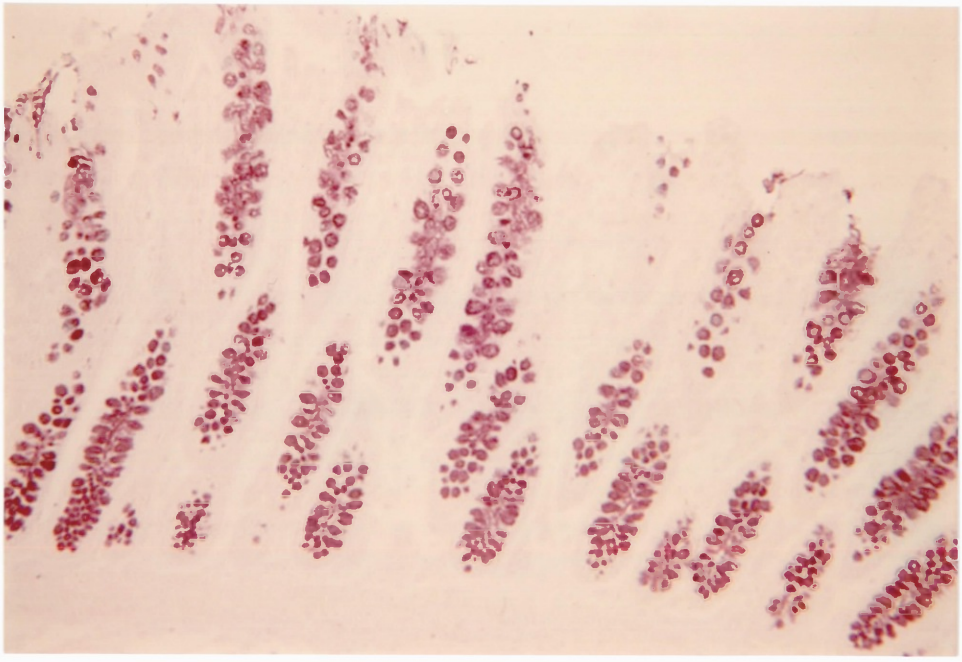


Plate 1 Human colonic mucosa-normal sulphomucin pattern. This is normal colonic mucosa stained by the HID-AB stain (x60). The glands are lined by cells containing brown/black staining sulphomucin.

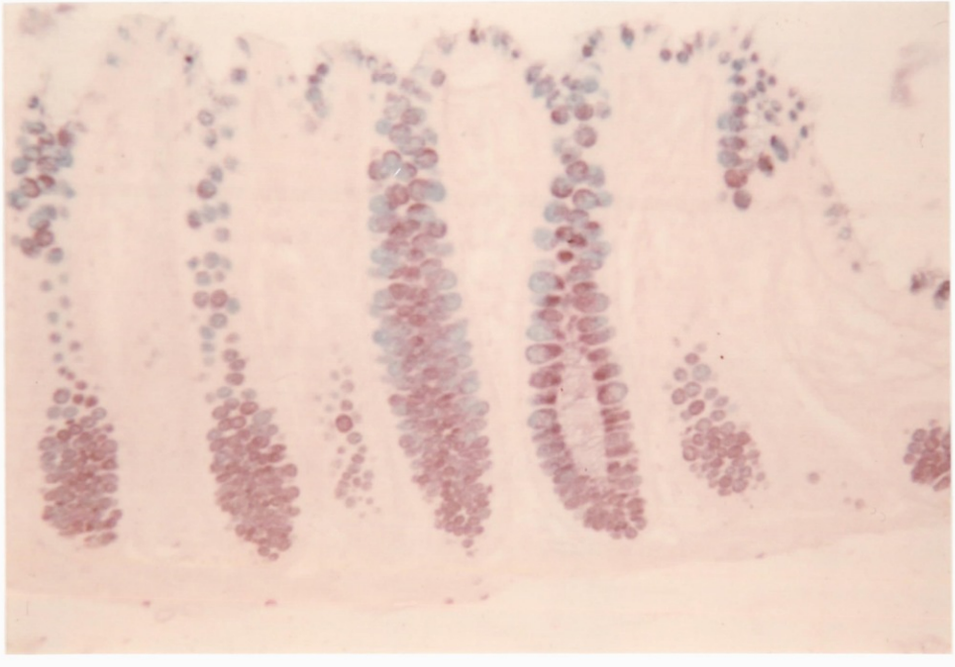


Plate 2 Normal human colonic mucosa stained by the HID-AB stain (x60). In this case a mixed pattern is seen. The glands are lined by cells secreting a mixture of sulphomucin in the base of the crypts and sialomucin towards the luminal surface. This is considered a normal variant.

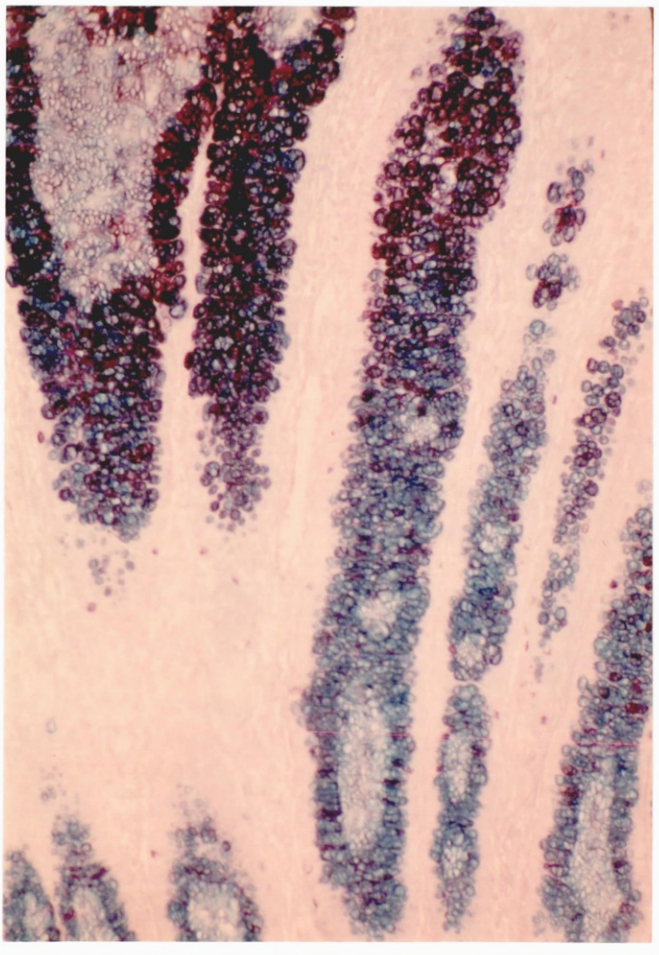


Plate 3 Human colonic mucosa-transitional mucosa. This section demonstrates transitional mucosa adjacent to a carcinoma. The glands are lined by cells which stain blue with the high-iron diamine alcian-blue stain (x60). There is no brown sulphated mucin.

sections by an increase in depth of colonic mucus crypts and extensive branching of the colonic glands. Isolated non-contiguous patches of sialomucin secretion were identified by exclusive sialomucin secretion since these areas appeared completely normal on H and E sections. Comparisons between length of transitional mucosa and various histological parameters were analysed by the Mann-Whitney U test or the students 't' test for unpaired data.

3.3 RESULTS

Seventy two specimens were studied. Details of the sites of tumours and operations performed are given in Table 3. There were 35 females (mean age 73.6 years) and 37 males (mean age 56.4 years). Transitional mucosa was not present in ten specimens which have therefore excluded from subsequent analysis. The Dukes' staging and histological differentiation of the remaining 62 cancers is shown in Table 4. Transitional mucosa could not be measured proximal to the tumour in 8 cases due to the proximity of the tumour to the ileum and in 3 cases due to the proximity of the tumour to the anal margin.

Table 3 Sites of tumour and operations performed in 72 colorectal carcinomas

Site of Primary Tumour	n	(%)	Operation Performed	n	(%)
Caecum	8	10.9	Right hemicolectomy	17	23.6
Ascending colon	3	4.1	Left hemicolectomy	5	6.8
Hepatic flexure	1	1.3	Sigmoid colectomy	25	34.7
Transverse colon	2	2.7	Anterior resection	12	16.4
Splenic flexure	4	5.4	Abdomino-perineal excision	7	9.7
Descending colon	5	6.8	Hartmann's	3	4.1
Sigmoid colon	27	37.0	Total colectomy	3	4.1
Rectum	23	31.5			
TOTAL	73			72	

(Includes 1 double primary)

Table 4 Length of TM (cm) with change in Dukes' stage, differentiation and invasion of the primary tumour.

Parameter		n	Length of TM (cm) mean	sd	p
Dukes' stage	A	6	2.44	2.05	
	B	32	3.20	3.22	ns
	C	24	3.14	2.73	
Differentiation	Well	11	3.98	2.53	
	Moderate	40	3.04	3.04	ns
	Poor	8	3.20	3.21	
Depth of invasion (UICC 'T' stage)	T1	1	1.0	-	
	T2	5	2.73	2.15	
	T3	46	2.87	2.82	<0.05*
	T4	10	4.82	3.40	

* Mann-Whitney U Test

The sites of sialomucin production are seen in Table 5. Three patterns of sialomucin distribution surrounding colorectal cancer were recognised (Figure 3):
Type 1 : Transitional mucosa extends to the resection margin without interruption. Type 2 : TM extends to the distal resection margin but not to the proximal, isolated patches are seen in the proximal segment. Type 3 : TM extends for a short distance proximally and distally, isolated patches intervene before the resection margin.

The mean length of transitional mucosa extending away from primary tumour (either in proximal or distal segment) was 3.13 ± sd 2.91 cm, range 0-17.1 cm). The mean proximal length of TM was 2.92 ± sd 2.49 cm and the mean distal length 2.49 ± 2.45 cm. There was no significant difference between these groups. There was no signifiant difference between right (mean 4.01 ± 4.65 cm) and left sided cancers (2.96 ± 2.25 cm).

Table 5 Sites of sialomucin production (n=62)

Site	No/Total	%
Resection margin		
Proximal	9/54	16.6
Distal	15/59	25.4
Sialomucin patches		
Proximal	15/54	27.7
Distal	6/59	10.1
Direct extension (TM)		
Proximal (RM)	5/9	55.5
Distal (RM)	12/15	80.0

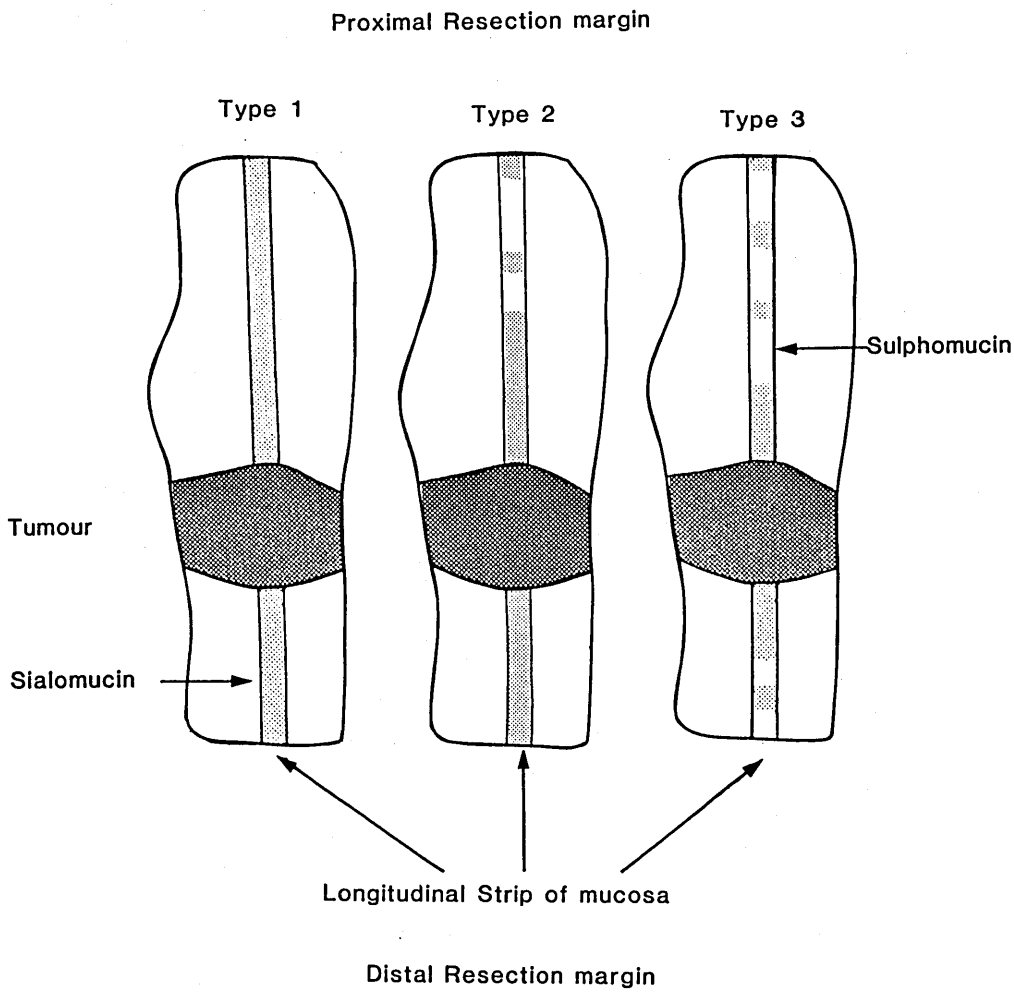


Figure 3 Patterns of sialomucin distribution adjacent to colorectal carcinoma.

Type 1 distribution.
Transitional mucosa extends without interruption to both resection margins.

Type 2 distribution.
Sialomucin extends to the distal resection margin. The proximal resection margin contains a similar length of transitional mucosa and further patches of sialomucin secretion which may or may not be present at the line of resection.

Type 3 distribution.
Transitional mucosa extends for a variable distance surrounding the tumour. Variable numbers of patches of sialomucin occur either proximal or distal to this.

There was a significant difference ($t=2.0$, $p<0.05$) in the length (Figure 4) of transitional mucosa between tumours which had penetrated other structures (T_4) (parietal peritomeum, bladder, uterus, pelvic sidewall) and those that had not (T_{123}). If the band of sialomucin was >3cm then there was a 70% chance that the tumour had invaded other structures. If the band was <3cm then there was a 66% chance that the tumour had not invaded other structures. There was no correlation between length of transitional mucosa and Dukes' stage (Figure 5) or histological differentiation (Figure 6). When the depth of invasion was stratified by Dukes' stage the association between length of transitional mucosa and depth of invasion was most marked in Dukes' C tumours but not Dukes' B carcinomas (Figure 7). There was no significant correlation between maximum diameter of the tumour and length of transitional mucosa ($r=3.77$ - Rank Spearman).

Isolated patches of sialomucin were found in 20 specimens (Figure 3). These occured at various distances from the tumour in both proximal and distal segments. Patches were found in 14 proximal segments, 5 distal segments and in one case in both proximal and distal segments. The isolated patches occurred without pattern or regularity, the maximum number being 11 in a 16 cm fixed specimen of proximal bowel (Table 5).

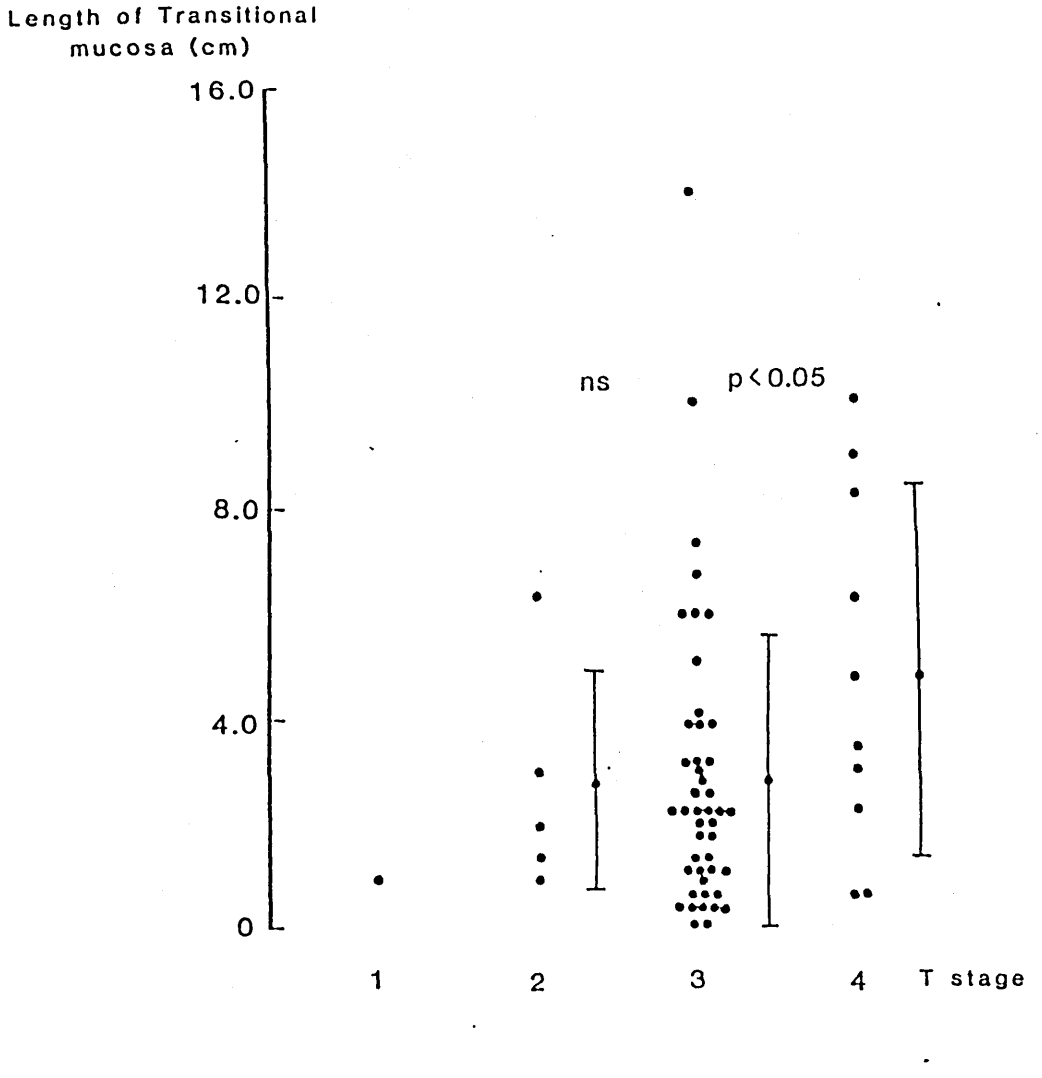


Figure 4 Length of transitional mucosa - depth of invasion. Tumours that had extended to adjacent structures (Stage T4) had a significantly increased length of transitional mucosa.

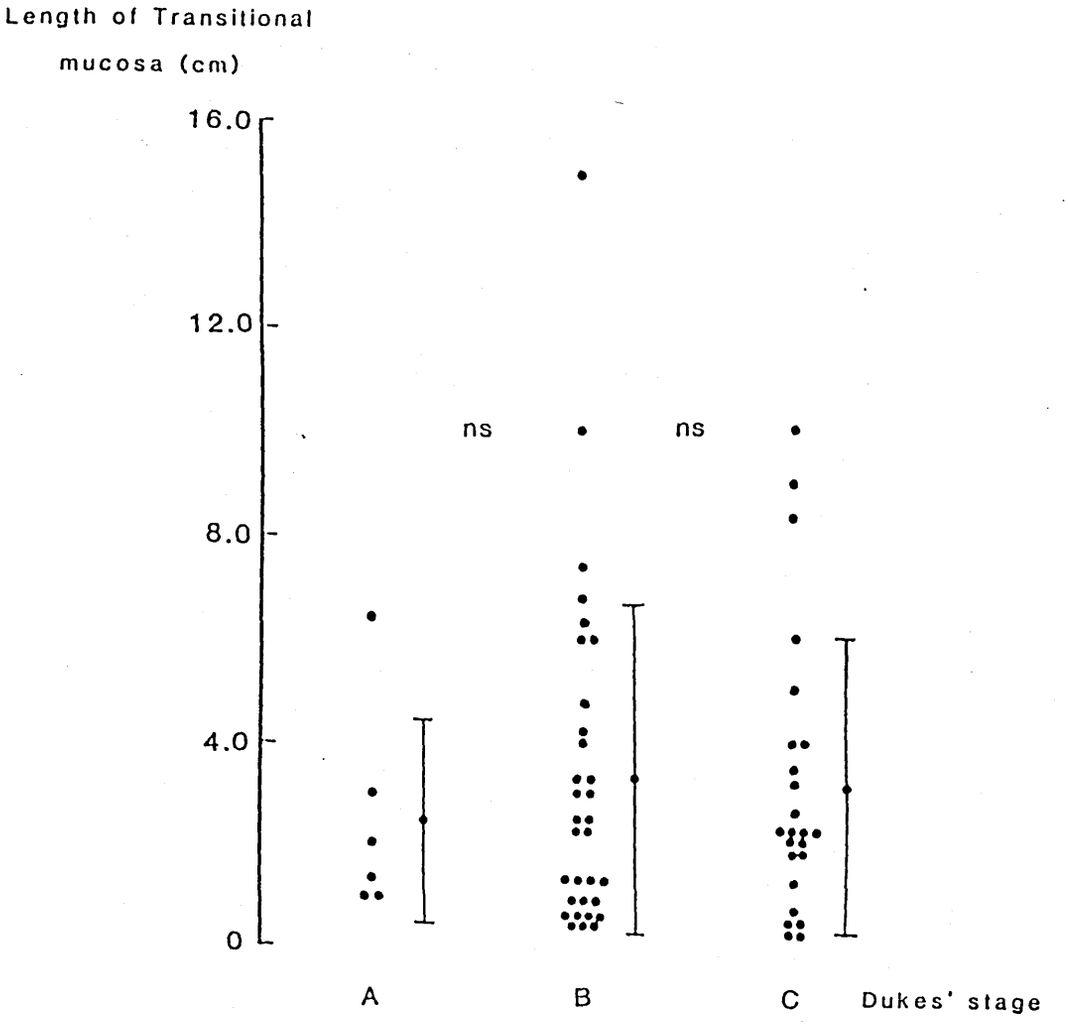


Figure 5 Length of transitional mucosa - Dukes' stage. There was no significant difference in length of transitional mucosa in any Dukes' stage.

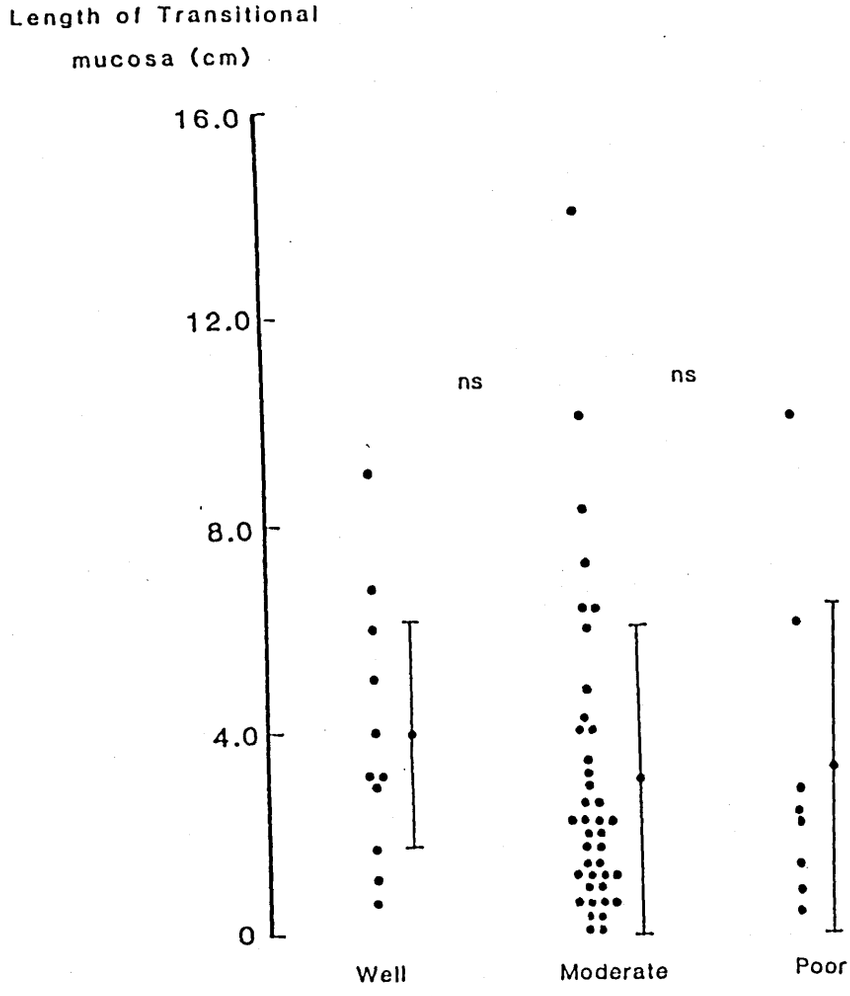


Figure 6 Length of transitional mucosa - differentiation of primary tumour.
There was no significant difference in the length of transitional mucosa with change in differentiation of the primary.

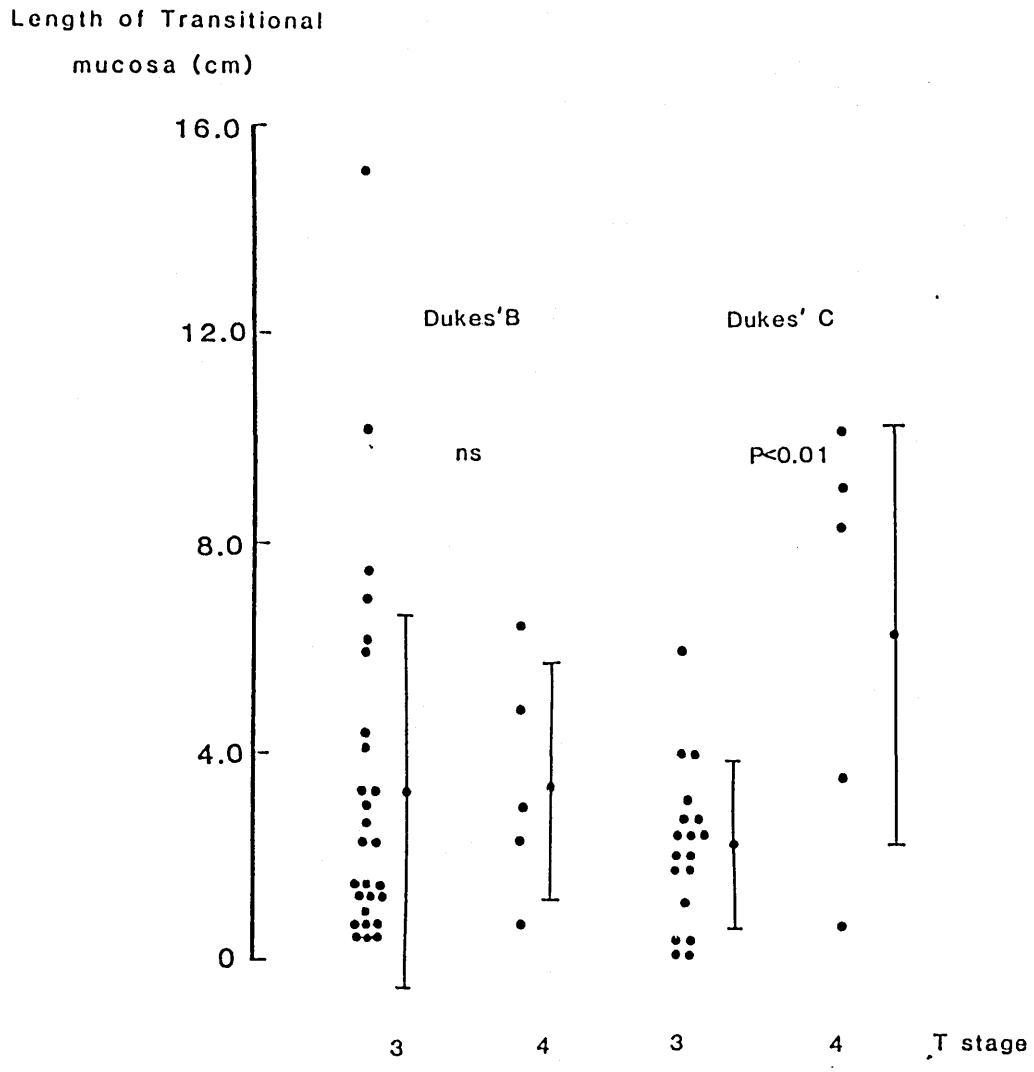


Figure 7 Length of transitional mucsoa - Dukes' B and C cancers .
There was no significant difference in length of transitional mucosa with depth of invasion for Dukes' B carcinomas but a significant difference for Dukes' C.

Sialomucin zone extended without interruption to the proximal resection margin in 5/9 (55.5%) cases with sialomucin at the proximal margin. It extended into the distal resection margin in 12/15 (80%) cases with sialomucin at distal margin. Sialomucin in the proximal resection margin was due to an isolated patch in 4 cases (44.5%) (Table 5). The appearance of sialomucin at the resection margin occurred significantly more often with the more invasive tumours (6/10, for T4 vs 10/45 for T3, $X^2=5.892$, $p<0.02$).

3.4 SUMMARY OF THE FINDINGS

* A transitional zone of sialomucin producing mucosa was present in 86% (62/72) of colonic cancers (mean length 3.13 ± sd 2.91 cm)
* There was a significant increase in length of sialomucin surrounding tumours which had penetrated other structures. If the band was greater than 3 cm then there was a 70% chance of the primary tumour extending to other structures, whereas if less than 3 cm there was a 66% chance that it had not.
* There was no relation between length of sialomucin zone and site of tumour, Dukes' stage, differentiation or tumour size.

CHAPTER 4

DOES THE SIZE OF SIALOMUCIN BAND PREDICT PATIENTS WHO ARE AT RISK OF LOCAL RECURRENCE AFTER EXCISION OF PRIMARY COLORECTAL CANCER?

4.1 AIM

In the previous chapter it has been shown that length of sialomucin band adjacent to colonic adenocarcinoma is significantly greater where the tumour has invaded through the bowel wall. The purpose of this study is to assess whether the extent of sialomucin found in association with colonic carcinoma correlates with local recurrence after primary tumour excision.

In the pathological study described in the previous chapter it was evident that a sialomucin zone of >3cm was associated with a 70% probability that the tumour had invaded adjacent structures whereas a zone of <3cm was associated with 66% probability that adjacent structures were not involved. In this clinical study the correlation between a sialomucin band of >3cm or more in the resected specimen and subsequent risk of local recurrence has been examined.

Factors which affect local recurrence were reviewed in Chapter I. These vary with both the patient and the surgeon, as well as the tumour. The association between tumour penetration and mucin change which has been demonstrated, suggests that while sialomucin change may provide information about local tumour behaviour it does not provide insight into host-related or surgeon-related factors affecting local recurrence. Sialomucin might, however, indirectly provide

some insight into surgeon-related variables. A long sialomucin band could indicate a local recurrence-prone-tumour. Where this band has been included in the mucosal resection margin, surgical resection could be inadequate - both laterally and above and below the tumour.

The three objectives of this clinical study were to assess whether:

1) The incidence of local recurrence is greater where the sialomucin band is >3 cm compared to <3 cm.

2) The incidence of local recurrence is greater where sialomucin is found in a colonic or rectal mucosal resection margin.

3) Length of sialomucin band is an independant variable influencing local recurrence when compared with other known variables such as Dukes' stage, surgeon and site of tumour.

4.2 METHODS

4.2.1 Patients Studied

Between January 1983 and December 1984, 391 patients with a histological diagnosis of adenocarcinoma of the colon or rectum underwent 'curative' resection in 8 participating

hospitals (see Appendix A). The abstracted protocol (Appendix B) was sent to all participating surgeons and pathologists. Surgeons performed the primary tumour resection according to their usual practice. Thirty-three patients did not survive the peri-operative period (30 days) and were excluded from analysis. The remaining three hundred and fifty-eight patients were followed-up for a median of 2 years (range 6-54 months) or until death. Patient information was stored on computer and analysed by SPSS (Nie et al, 1975). Survival was measured from the time of primary tumour resection until death. Cause of death was obtained from the death certificate, or where possible from post-mortem.

Evidence for tumour recurrence was derived from either histopathology, radiography (CT, barium enema, ultrasound etc) or obvious clinical signs.

4.2.2 Definitions

Curative Resection: Removal of the primary tumour with absence of tumour from resection margins on histological examination, in patients with no evidence at laparotomy of metastasis to liver, peritoneum or other abdominal organs (Phillips et al, 1984a).

Local Recurrence: Radiological or histological evidence of recurrence of cancer at or in the region of the anastomosis, in the abdominal wound, drain site or perineum, but not hepatic or peritoneal metastases (Phillips et al, 1984a).

Histological Preparation and Examination

Specimens were examined by a Consultant Pathologist in the hospital of operation, and were classified according to Dukes' staging and Broder's differentiation.

Sections were obtained from the tumour, proximal and distal resection margins of the specimen and from the mucosa surrounding the tumour. They were processed routinely, embedded in paraffin wax and 4-5 micron thick sections stained with either haematoxylin and eosin or the high iron diamine alcian-blue (Chapter 3).

Three patterns of staining were recognised with HID-AB (Plates 1-3):

(a) purely sulphomucin staining;

(b) a mixed pattern, and;

(c) purely sialomucin staining.

As before (Chapter 3), only the sialomucin predominant pattern (c) was considered abnormal. The HID-AB stained sections were examined independently by two assessors (PMD, NAH). A Consultant Pathologist (HCR) examined a random

sample of 100 specimens for mucin status, differentiation and Dukes' stage.

4.2.3 Statistical Methods

Each prognostic variable was analysed as a survival-predictor by life table analysis (Peto, Pike et al, 1978). Differences in observed survival between groups were tested for statistical significance using the Lee Desu statistic (1972 - appendix D). Survival and local recurrence were also analysed by multivariate analysis using the Cox regression model (Cox, 1972). This provides an estimate of the relative strength as a predictor of survival or recurrence of each of a number of variables. A summary of the Cox proportional hazards regression model is given in Appendix D. Deaths from causes other than cancer were treated as censored observations at the time of death. Prognostic variables for the events, 'distant recurrence', 'local recurrence', and 'survival' were chosen by the formal stepwise regression method using the programme BMDP2L (WJ Dixon, BMDP Statistical package UCLA 1983). Prognostic variables were thereby selected in steps until no further significant effect upon the events could be demonstrated.

4.3 RESULTS

There were 189 females (mean age 66.6 years) and 169 males (mean age 64.6 years). Details of primary tumour site and operation performed are shown in tables 6 and 7. The presence or absence of a zone of sialomucin >3cm from the tumour was determined by an analysis of those patients with a

distal resection margin greater than 3cm. It was assumed that if sialomucin was present at the distal resection margin then the zone of sialomucin had extended >3cm. The following data refers to presence or absence of sialomucin at the resection margin only.

One hundred and six patients (29.6%) had evidence of sialomucin secretion (sialomucin positive) in one or other resection margins. It was found in the proximal resection margin in 51 (18.6%) cases and in the distal resection margin in 72 (22.4%) cases. The ileum constituted a proximal resection margin in 84 cases and the anal margin a distal margin in 37 cases.

Table 6 Site of primary carcinomas and incidence of sialomucin change in 358 patients undergoing curative resection.

Site	Total		Sialomucin Positive	
	n	(%) of all tumours	n	(%) of tumours at that site
Caecum	51	(14.2)	9	(17.7)
Ascending colon	24	(6.7)	3	(12.5)
Hepatic flexure	7	(2.0)	1	(14.2)
Transverse colon	11	(3.1)	2	(18.1)
Splenic flexure	9	(2.5)	3	(33.3)
Descending colon	15	(4.2)	2	(13.3)
Sigmoid colon	111	(31.0)	45	(40.5)
Rectum	125	(34.9)	41	(32.8)
Multiple	5	(1.4)	0	(0)
Total	358		106	

Table 7 Operations performed and incidence of sialomucin change in each operation category

Operation Performed	Total		Sialomucin Positive	
	n	(%) of all operations	n	(%) of that operation
Right hemicolectomy	95	(26.5)	14	(14.7)
Transverse colectomy	3	(0.8)	0	(0)
Left hemicolectomy	21	(5.9)	6	(28.5)
Sigmoid colectomy	102	(28.5)	40	(39.2)
Anterior resection	81	(22.6)	29	(35.8)
AP resection	42	(11.7)	10	(23.8)
Hartmann's	6	(1.7)	4	(66.6)
Total colectomy	8	(2.2)	3	(37.5)
Total	358		106	

There was no significant difference in Dukes' stage of the primary tumour or Broder's (1925) classification of degree of differentiation in tumours where sialomucin was present at the resection margin compared with those where it was absent (Table 8). Similarly, no difference was observed between the age, sex or distance of the resection margins (proximal and distal) from the primary in either group. Twenty patients underwent emergency resection, no difference in outcome being observed between those with sialomucin present and sialomucin absent. There was no relationship between tumour size and the appearance of sialomucin at either resection margin.

One hundred and ten patients (30.7%) developed recurrence (Table 9) within the 24 month median period of follow-up (range 12-54 months).

Table 8 Incidence of sialomucin change by Dukes' stage and Broder's classification

	Total		Sialomucin Positive	
	n	(%)of total	n	(%) of that stage/grade
Dukes' Stage				
A	42	(11.7)	7	(16.6)
B	206	(57.5)	68	(33.0)
C	110	(30.7)	31	(28.1)
Broder's Differentiation				
Well	55	(15.4)	16	(29.0)
Moderate	259	(72.3)	75	(28.9)
Poor	33	(9.2)	10	(30.0)
Variable	11	(3.1)	5	(45.5)
TOTAL	358		106	

Table 9 The sialomucin status of the resection margin and tumour recurrence

Type of Recurrence	Total Recurrence		Sialomucin Positive	
	n	(%) of total	n	(%) type of recurrence
Local alone	41	(11.4)	13	(31.7)
Local and distant metastases	40	(11.1)	21	(52.5)
Metastases alone	39	(10.8)	9	(23.0)
Total	110		43	

Excludes metachronous tumours (n = 6)

Local and distant recurrence was confirmed by biopsy in 40 cases, ultrasound in 23 cases and CT scan of the pelvis in 12 cases. The remaining 35 patients were diagnosed from clinical signs. Diagnosis was confirmed at necropsy in 8 patients. The mean time to recurrence was 11.3 months in the sialomucin positive group and 12.6 months in the sulphomucin group. Twenty-three patients have undergone further laparotomy, 7 of whom have had further tumour excised. Six patients had metachronous tumours in the sialomucin negative group.

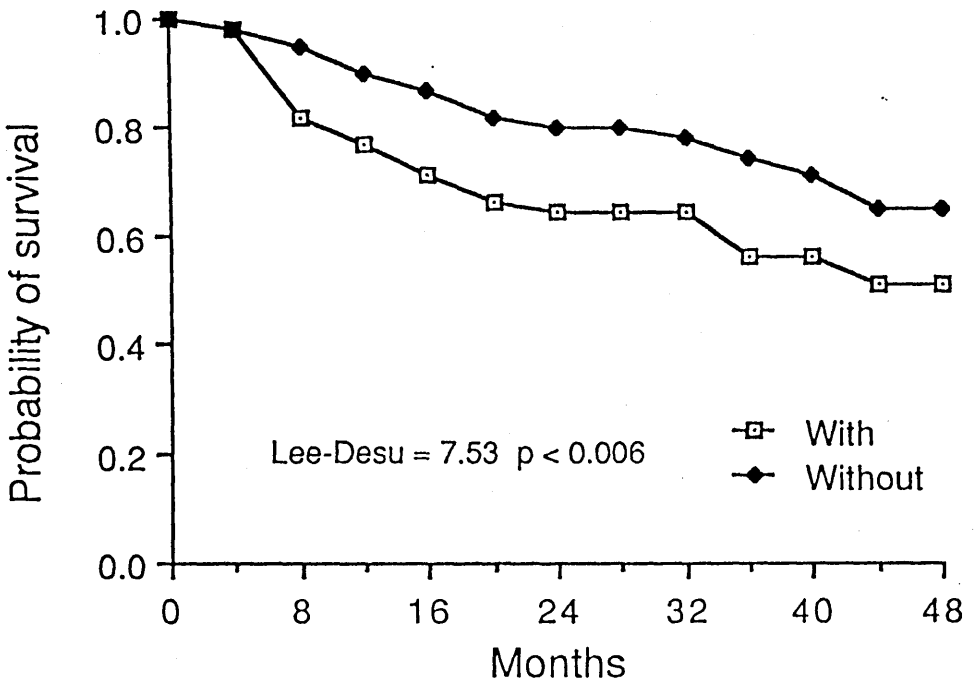
4.3.1 Sialomucin As A Predictor Of Survival

Eighty-six patients have died as a result of the carcinoma, 41/106 (38.6%) in the sialomucin resection margin positive group and 45/252 (17.8%) in the sulphomucin group. The prognostic variables entered into the Cox Model are shown in Table 10. The model was constructed using either a sialomucin band of >3cm or the presence of sialomucin at the resection margin as a variable. Patients with a sialomucin band of greater than 3 cm had a significantly shorter ($p<0.006$) survival, compared with patients in whom the sialomucin band was less than 3 cm (Figure 8). Similarly, patients in whom sialomucin was present at the resection margin had a significantly ($p<0.0001$) shorter survival than those in whom sialomucin was not present at the resection margin (Figure 9).

Table 10 Summary of variables entered into the Cox Regression Model

Variable	Category and Codes		Number of observations	Number of deaths	4-year survival
Sex	Male	1	169	53	55%
	Female	2	189	51	58%
Age	<70	1	217	39	80%
	>70	2	141	19	85%
Site	Colon	1	230	57	58%
	Rectum	2	128	46	52%
Stage	A	1	41	4	87%
	B	2	206	49	62%
	C	3	111	51	36%
Differentiation	Well	1	56	12	65%
	Moderate	2	257	67	60%
	Poor	3	34	18	39%
Depth of Invasion (T)	T_1	1	8)		
	T_2	2	30)	4	79%
	T_3	3	203)		
	T_4	4	20)	53	65%
Size	<5 cm	1	140	37	61%
	>5 cm	2	85	20	61%
Zone of Sialomucin	<3 cm	1	146	28	66%
	>3 cm	2	58	26	50%
Sialomucin at Resection Margin	Present	1	106	47	39%
	Absent	2	252	57	67%
Clearance of Distal Margin	<5 cm	1	182	43	60%
	>5 cm	2	124	13	85%

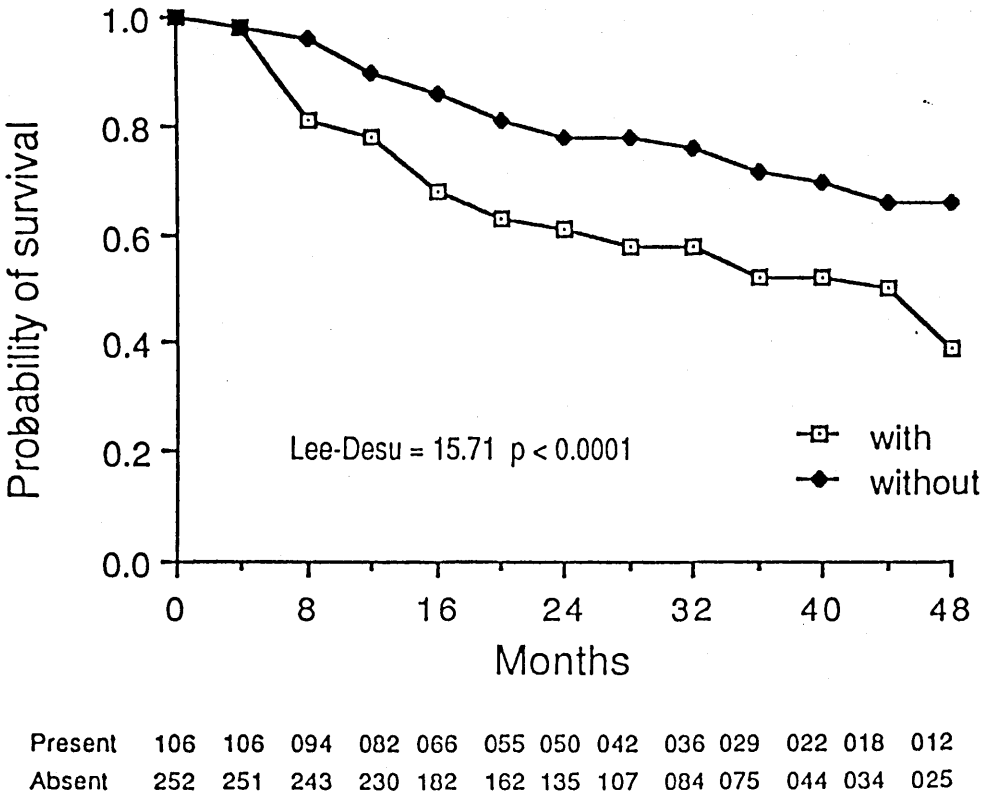
As would be expected, actuarial life table analyses demonstrate a significantly shorter survival for patients with Dukes C cancer (compared to A and B) poor differentiation, fixity and invasion of the tumour to deep structures or blood vessels. The life table analyses of



With	058	058	051	044	034	030	028	024	021	015	013	011	008
Without	146	146	141	133	108	098	086	067	056	051	029	021	016

No of patients at risk

Figure 8 Life-table of survival for colorectal carcinoma in which the sialomucin extended for >3cm compared to carcinomas where the sialomucin did not. It may be seen that survival was significantly shorter in patients where the band of sialomucin was longer than 3cm.



Present	106	106	094	082	066	055	050	042	036	029	022	018	012
Absent	252	251	243	230	182	162	135	107	084	075	044	034	025

No of patients at risk

Figure 9 Life-table of survival for colorectal carcinomas in which sialomucin extended to the resection margin compared to carcinomas in which there was no sialomucin at the resection margin. Survival was significantly shorter in patients when sialomucin extended to the resection margin.

these predictors are in Appendix C. The Cox Model identified Dukes' stage as the most important variable predicting survival, followed by a sialomucin band of >3 cm (Table 11). In the resection margin model, Dukes' stage, sialomucin, differentiation of the tumour and the distal margin (cm) of excision were selected (Table 12).

Table 11 Selection of regression model for 'survival' related parameters (n=146).

	X^2 to enter into model		
Parameters	Step 0	Step 1	Step 2
Operation	0.95	0.11	0.02
Site	0.45	0.07	0.03
Dukes	13.04	13.04	14.57
Differentiation	0.32	0.43	0.20
Size	0.04	0.01	0.01
Hospital	0.42	0.89	2.97
Sialomucin>3cm	7.23	8.77	8.77
Sex	0.25	0.41	0.44
Depth	1.87	0.00	0.49

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.

Table 12 Selection of regression model for 'survival' related parameters (n=305)

Parameters	X^2 to enter into model				
	Step 0	Step 1	Step 2	Step 3	Step 4
Operation	1.29	1.13	0.10	0.01	0.51
Site	0.44	0.56	0.00	0.07	0.18
Dukes	18.89	18.89	19.79	19.36	20.32
Differentiation	6.88	6.75	5.72	5.72	4.65
Distal Margin	4.68	7.02	5.65	4.58	4.58
Hospital	0.57	0.15	0.07	0.14	0.92
Sialomucin*	12.64	13.55	13.55	12.51	11.37
Sex	0.45	0.58	0.35	0.24	0.18
Overall likelihood ratio X^2		p<0.00001 18.46 (df=1)	p<0.00001 32.67 (df=2)	p<0.00001 39.24 (df=3)	p<0.00001 45.16 (df=4)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.
* at resection margin

4.3.2 Sialomucin As A Predictor Of Local Recurrence

Local recurrence was demonstrated in 71/358 (19.8%) patients of whom 22 had a sialomucin band of >3 cm and 37 showed sialomucin at a resection margin. The site of primary tumour, Dukes' staging and differentiation of the original primaries in patients who developed local recurrence are shown in Table 13. Regression analysis of local recurrence related parameters demonstrated that a band of sialomucin >3cm from the tumour was a significant and independant predictor of local recurrence following Dukes' Staging and Hospital of operation (Table 14). The presence of sialomucin at the resection margin was the most important predictor of local recurrence, being a stronger predictor than Dukes' stage and distal margin of excision (Table 15).

Table 13 Site of tumours, Dukes' stage and degree of differentiation of primary carcinoma in the 71 patients with evidence of local recurrence

Site of Tumour	n	(% of all tumours at that site)
Caecum	9	(17.6)
Ascending colon	2	(8.3)
Hepatic flexure	2	(28.5)
Transverse colon	1	(9.1)
Descending colon	3	(33.3)
Sigmoid colon	21	(18.9)
Rectum	32	(25.6)
Multiple	1	(16.6)
Total	71	

Dukes' Stage	n	(% of all tumours of that stage)
A	3	(7.1)
B	37	(17.9)
C	31	(28.2)
Total	71	

Differentiation	n	(% of all tumours of that degree of differentiation)
Well	9	(16.3)
Moderate	47	(18.1)
Poor	11	(33.3)
Variable	4	(66.6)
Total	71	

Table 14 Selection of regression model for 'local recurrence' related parameters (Sialomucin >3 cm)

	X^2 to enter into model			
Parameters	Step 0	Step 1	Step 2	Step 3
Operation	0.18	0.00	0.14	0.87
Site	0.67	0.30	0.04	0.25
Dukes	4.53	4.53	4.93	6.58
Differentiation	0.01	0.02	0.05	0.18
Size	0.00	0.02	0.01	0.00
Hospital	3.72	4.11	4.11	5.71
Sialomucin >3 cm	2.46	3.00	4.59	4.59
Sex	0.58	0.74	1.09	1.77
Depth	1.96	0.36	0.57	0.15
Overall likelihood ratio X^2		$p<0.03$ 4.47 (df=1)	$p<0.01$ 8.42 (df=2)	$p<0.006$ 12.19 (df=3)

Underline denotes parameter has been entered into the model;
X^2 denoted by underline denotes value to remove from model.

Table 15 Selection of regression model for 'local recurrence' related parameters (n=305) (Sialomucin at resection margin)

	X^2 to enter into model			
Parameters	Step 0	Step 1	Step 2	Step 3
Operation	0.35	0.06	0.07	1.15
Site	0.84	0.03	0.07	0.17
Dukes	8.79	9.53	9.53	9.87
Differentiation	3.66	3.09	2.81	2.19
Distal Margin	3.67	3.58	3.92	3.92
Hospital	0.18	0.72	1.52	0.54
Sialomucin*	12.50	12.50	13.24	12.28
Sex	0.36	1.01	0.56	0.79
		p<0.0002	p<0.00001	p<0.0001
Overall likelihood ratio X^2		14.09 (df=1)	23.11 (df=2)	27.98 (df=3)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.
* at resection margin

The mean distance of the proximal resection margin from the tumour was 11.9 cm (sd ± 8.5). The mean distance of the distal resection margin was 6 cm (sd ± 5.4). There was no significant differences between these distances in patients who did not develop recurrence and those who did. There is no significant association between tumour recurrence and tumour site. No significant difference in recurrence rates between hospitals was observed. The survival curve of patients with evidence of local recurrence is shown in Appendix C.

4.3.3 Sialomucin As A Predictor Of Distant Metastases

Thirty-nine patients (10.8%) developed distant metastases alone without evidence of local recurrence. The majority of these occurred in the resection margin sialomucin negative group, 30/252 (11.9%) compared to 9/106 (8.4%) in the resection margin positive group.

Regression analysis demonstrated that the most important predictor of distant metastasis was Dukes' stage in the resection margin and >3 cm model (Tables 16&17). Neither sialomucin band >3 cm nor at the presence of sialomucin at the resection margin were selected by either model as predictors of metastasis.

Table 16 Selection of regression model for 'distant recurrence' related parameters (n=305)

Parameters	X^2 to enter into model Step 0	Step 1
Operation	0.64	0.45
Site	0.16	0.09
Dukes	28.62	28.62
Differentiation	0.11	0.11
Distal Margin	0.07	0.81
Hospital	2.61	1.29
Sialomucin*	0.33	0.13
Sex	0.18	0.25
Overall likelihood ratio X^2		p<0.00001 28.80 (df=1)

* at resection margin

Table 17 Selection of regression model for distant metastases related parameters (n=146)

Parameters	X^2 to enter into model Step 0	Step 1
Operation	0.68	0.01
Site	0.03	0.46
Dukes	26.58	26.58
Differentiation	1.21	0.52
Depth	4.00	0.46
Distal Margin	0.05	0.09
Hospital	0.45	0.05
Sialomucin >3 cm	0.09	0.00
Sex	0.02	0.04
Overall likelihood ratio X^2		p<0.00001 22.89 (df=1)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.

4.4 SEPARATE ANALYSIS OF PATIENTS UNDERGOING COLO-RECTAL ANASTOMOSES AND RECTAL CARCINOMA EXCISION

In view of the uncertain influence of the ileal or anal mucosa at the resection margin, the absence of the distal margin after abdomino-perineal resection and the fact that the anal mucosa may normally secrete sialomucin (Fenger, 1978) an analysis was conducted of colorectal restorative procedures only (ie excluding right hemicolectomy and abdomino-perineal excision).

4.4.1 Results

There were 101 male and 106 females (mean age for both groups 66.2 years, median 68 years). The sites of the primary tumour and the operations performed are shown in Tables 18 and 19.

Table 18 Site of primary carcinoma in 207 patients undergoing curative restorative resection with colocolic or colorectal anastomosis.

Site	Total		Sialomucin positive	
	n	%	n	(% of tumours at that site)
Transverse colon	2	(1.0)	2	(100.0)
Splenic flexure	6	(2.9)	3	(50.0)
Descending colon	15	(7.2)	2	(13.3)
Sigmoid colon	105	(50.7)	42	(40.0)
Rectum	79	(38.2)	28	(47.2)
Total	207		75	

Table 19 Restorative operations with colocolic or colorectal anastomosis performed by presence of sialomucin at resection margin

Operation	Total		Sialomucin positive	
	n	(% of total)	n	(% of operations at that site)
Transverse colectomy	3	(1.4)	0	(0)
Left hemicolectomy	21	(10.1)	6	(28.5)
Sigmoid colectomy	102	(49.3)	40	(39.2)
Anterior resection	81	(39.1)	29	(35.8)
Total	207		75	

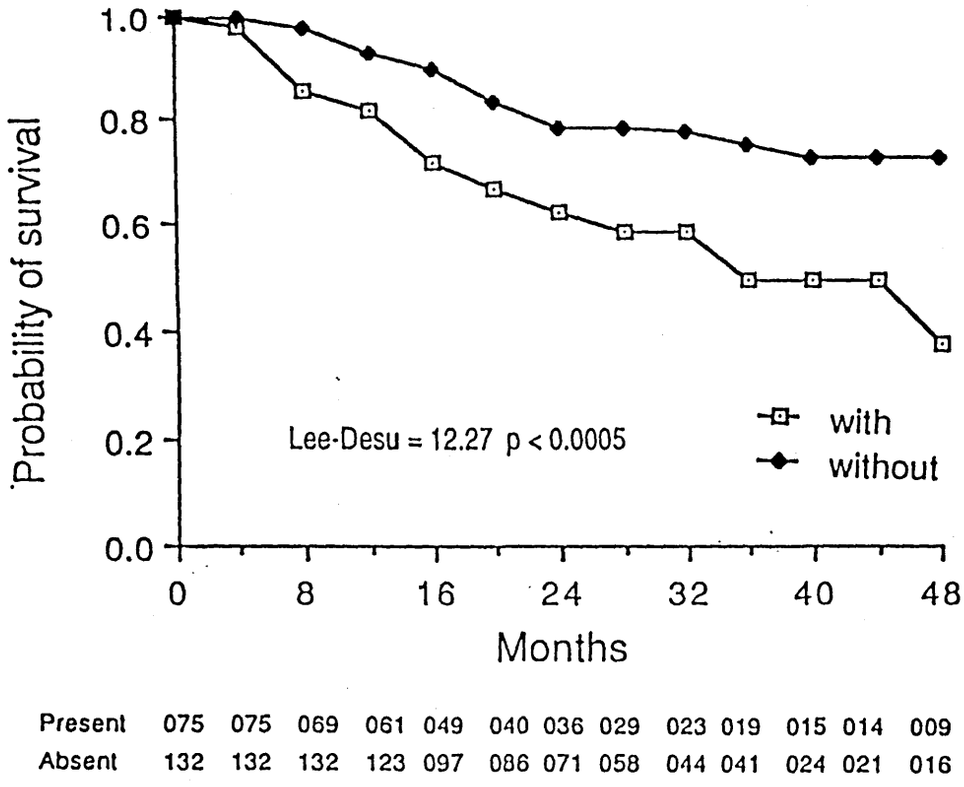
Sialomucin was exclusively secreted at the proximal resection margin in 36 (17.4%) cases and at the distal resection margin in 56 (27.1%) cases. The Dukes' Stage and Broders differentiation are shown in Table 20.

Table 20 Dukes' stage and Broders' differentiation of the primary carcinomas in the sialomucin positive and negative groups.

Dukes' Stage	Total		Sialomucin positive	
	n	(%)	n	(%)
A	30	(14.5)	6	(20.0)
B	116	(56.0)	48	(41.3)
C	61	(29.5)	21	(34.4)
Total	207		75	
Broders differentiation				
Well	35	(37.1)	13	(37.1)
Moderate	152	(34.8)	53	(34.8)
Poor	16	(37.5)	6	(37.5)
Variable	4	(75.0)	3	(75.0)
Total	207		75	

4.4.1.1 Sialomucin As A Predictor of Survival

Fifty-nine patients (28.3%) have died, 34 (57.6%) in the sialomucin resection margin positive group and 25 (42.4%) in the negative group. Twenty-eight patients died in the >3 cm group (positive) and 26 in the negative group. Life table analysis of the presence or absence of sialomucin in the resection margin is shown in Figure 10. Regression analysis demonstrated that sialomucin in the resection margin and at >3 cm were the most important survival predictors of survival. This was followed by Dukes' stage and distal margin of excision of the tumour (Table 21 and 22). Life table analysis for the prognostic variables, site of tumour, Dukes' classification, histological differentiation, depth of invasion and fixity are shown in Appendix C.



Present	075	075	069	061	049	040	036	029	023	019	015	014	009
Absent	132	132	132	123	097	086	071	058	044	041	024	021	016

No of patients at risk

Figure 10 Life-table of survival in patients undergoing colorectal or colo-colic anastomosis. There was a significantly worse survival in patients where sialomucin was present at the resection margin compared to those in whom sialomucin was absent from the resection margin.

Table 21 Selection of regression model for 'survival' related parameters (n=182)

	X^2 to enter into model			
Parameters	Step 0	Step 1	Step 2	Step 3
Operation	1.28	0.68	1.77	0.36
Site	0.65	0.25	0.99	0.12
Dukes'	9.90	10.43	10.43	9.87
Differentiation	7.09	5.29	3.27	3.73
Distal Margin	3.84	5.11	4.55	4.55
Hospital	0.16	0.59	1.02	0.37
Sialomucin*	13.76	13.76	14.29	14.35
Sex	0.10	1.00	0.32	0.84
		p<0.0001	p<0.00001	p<0.00001
Overall likelihood ratio X^2		14.76	24.34	30.60

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.
* at resection margin

Table 22 Selection of regression model for 'survival' related parameters (n=88)

Parameters	X^2 to enter into model Step 0	Step 1	Step 2
Operation	0.19	0.11	0.19
Site	0.10	0.82	0.14
Dukes'	7.05	9.32	9.32
Differentiation	0.00	0.17	0.67
Distal Margin	0.10	0.02	0.01
Hospital	0.48	0.44	2.46
Sialomucin >3 cm	7.37	7.37	8.61
Sex	1.41	2.85	1.65
Overall likelihood ratio X^2		p<0.004 7.96 (df=1)	p<0.0002 16.72 (df=2)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.

* at resection margin

4.4.1.2 Local recurrence

Forty-five patients showed evidence of local recurrence (Table 23). Regression analysis selected the presence of sialomucin in a resection margin as the most important predictor for local recurrence. This was followed by the Dukes' stage of the lesion and sex of the patient (Table 24). The regression model for >3cm did not select any parameter for predicting recurrence.

There was no significant difference between patients with or without sialomucin at the resection margin in proximal (median 12.0 cm) or distal (median 4.0 cm) excision margins, site of primary tumour, depth of invasion or tumour fixity. The mean time to recurrence for both groups was 11.0 months.

Table 23 Sialomucin at the resection margin and tumour recurrence in patients undergoing colocolic or colorectal anastomosis

Type of Recurrence	Total n	(% of group)	Sialomucin Positive n (% of total)
Local alone	19	(30.6)	11 (57.8)
Local and metastasis	26	(41.9)	14 (53.8)
Metastasis	17	(27.4)	5 (29.4)
Total	62		30

Table 24 Selection of regression model for 'local recurrence' related parameters (n=182)

	X^2 to enter into model			
Parameters	Step 0	Step 1	Step 2	Step 3
Operation	2.41	1.59	2.76	4.43
Site	2.82	1.98	3.27	5.08
Dukes	6.46	7.13	7.13	5.27
Differentiation	3.92	2.74	1.49	2.70
Distal Margin	1.30	1.97	1.61	2.64
Hospital	1.33	2.09	2.53	2.54
Sialomucin*	9.77	9.77	10.45	12.46
Sex	3.90	6.92	5.07	5.07
Overall likelihood ratio X^2		p<0.0002 10.52 (df=1)	p<0.0002 17.12 (df=2)	p<0.00001 22.85 (df=3)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.

*at resection margin

Table 25 Selection of regression model for 'distant recurrence' related parameters (n=182)

Parameters	X^2 to enter into model Step 0	Step 1
Operation	0.18	0.01
Site	0.25	0.04
Dukes	14.30	14.30
Differentiation	0.80	0.20
Distal Margin	1.43	1.77
Hospital	0.00	0.00
Sialomucin*	0.01	0.01
Sex	0.07	0.00
Overall likelihood ratio X^2		$p<0.0003$ 13.17 (df=1)

* at resection margin
Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.

4.4.1.3 Distant Metastasis

Seventeen patients developed distant metastases alone (27.4%). Twelve of these occurred in the sialomucin negative group.

Regression analysis (sialomucin at resection margin) selected Dukes' staging as the most important prognostic predictor of distant recurrence (Table 25). The >3 cm sialomucin regression model did not select any parameter for distant metastasis.

4.4.1.4 Rectal Carcinoma

A separate analysis of survival related parameters was performed for rectal carcinoma (n=65). They were categorised into high (greater than 10 cm), middle (6-10 cm) and low (0-6

cm) from the anal verge. Regression model analysis demonstrated that the presence of sialomucin at the resection margin was the most important predictive factor of survival. This was followed by the height of the tumour, the distal margin of excision, the depth of invasion of the tumour and the histological differentiation (Table 26). In the local recurrence >3 cm model, however, regression analysis selected hospital of operation alone (Table 27).

Table 27 Cox Regression for Rectal Cancer (n=44) Selection of regression model for survival related parameters

	X^2 to enter into model	
Parameters	Step 0	Step 1
Operation	0.65	1.08
Size	0.64	0.84
Dukes	1.31	0.42
Differentiation	1.88	0.65
Depth	0.42	0.95
Hospital	4.83	4.83
Sialomucin >3 cm	1.18	1.54
Sex	0.24	1.06
Overall likelihood ratio X^2		$p<0.04$ $X^2=4.14$ (df=1)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.

Table 26 Cox Regression for Rectal Carcinoma (n=65)
Selection of regression model for survival related parameters

	X^2 to enter into model					
Parameters	Step 0	Step 1	Step 2	Step 3	Step 4	Step 5
Operation	2.91	1.67	0.09	0.83	0.39	0.23
Dukes	1.73	5.22	5.98	5.07	1.26	1.14
Differentiation	5.12	5.60	4.22	5.18	4.91	4.91
Distal Margin	1.88	5.64	6.31	6.31	7.76	9.09
Size	0.24	0.08	0.07	0.00	0.40	0.04
Depth (T)	2.07	2.25	4.38	5.83	5.83	5.57
Hospital	0.61	1.29	0.51	0.14	0.02	0.32
Height	6.89	6.00	6.00	6.66	8.84	7.85
Sialomucin*	12.40	12.40	11.51	14.98	16.83	16.91
Sex	0.01	0.31	0.21	0.69	0.02	0.48
Overall likelihood ratio X^2		p<0.0003 13.22 (df=1)	p<0.0001 19.34 (df=2)	p<0.00001 24.20 (df=3)	p<0.000001 29.28 (df=4)	p<0.000001 38.82 (df=5)

Underline denotes parameter has been entered into the model; X^2 denoted by underline denotes value to remove from model.
* at the resection margin

4.5 Summary of the Results

* The presence of a band of sialomucin >3 cm from the primary tumour was a significant and independent predictor of local recurrence but was a less strong predictor than Dukes' Stage.

* The presence of sialomucin at the resection margin was the strongest independent predictor of local recurrence - even above Dukes' stage - which was identified.

* Overall, sialomucin did not predict which patients would develop distant metastases.

* Several predictors - Dukes' stage, differentiation, depth of invasion and distal margin of excision -of survival and recurrence have been confirmed.

* Sialomucin at the resection margin predicted for rectal cancer survival but not for local recurrence.

* The ability of sialomucin at the resection margin to predict for local recurrence remains after the influence of the ileal and anal margins have been removed.

CHAPTER 5

IS SIALOMUCIN CHANGE A PROLIFERATIVE RESPONSE TO NEOPLASIA OR TO INJURY?

5.0 INTRODUCTION

Studies, reviewed in Chapter 1, suggest that colonic goblet cell secretion changes from normal sulphomucin to sialomucin in cells which are adjacent to carcinoma. There is also evidence for sialomucin change in non-neoplastic colonic disease, but the difficulty is that some of these non-neoplastic disorders - ulcerative colitis, Crohn's disease and perhaps radiation colitis - may predispose to malignant change. This raises the question of whether sialomucin change is purely a consequence of inflammation, producing an increase in cell proliferation and inducing a change in cell morphology. Alternatively mucin change could be associated with the cellular changes which result in malignancy, and would be expected to predict malignant potential and behaviour. The studies in the previous chapters suggest that sialomucin change is a significant predictor of local tumour behaviour but not distant metastasis. Either of the above aetiologies of sialomucin could be consistent with this. Sialomucin would be more prevalent if there was more inflammation around the tumour, as is the case with more locally invasive growths. Alternatively locally invasive growths may be more biologically agressive and more likely to be associated with a field of sialomucin change.

An attempt has been made to classify the aetiology of sialomucin change by examining a variety of inflammatory bowel disorders, some with considerable malignant potential -

ulcerative colitis and others with little malignant potential - Crohn's disease, radiation colitis and finally diverticular disease with no malignant potential. The first objective was to establish whether the prevalence of sialomucin change is increased in association with conditions which have a malignant potential - particularly in association with severe dysplasia which is an indicator of increased malignant potential (Morson and Pang, 1974).

The second objective was to compare the prevalence of sialomucin in the above non-malignant conditions with that
(a) adjacent to neoplastic polyps
(b) in the carcinoma-prone colon after removal of the carcinoma.

These questions were also addressed in an animal model of colonic inflammation and repair where the prevalence of sialomucin change was compared with that in an animal model of colonic cancer in order to establish whether sialomucin was produced equally readily by the two different proliferative stimuli.

5.1 HUMAN INFLAMMATORY BOWEL DISEASE

5.1.1 Patients and Methods

Patients studied

Eighty-eight patients were studied. Thirty-two patients had histologically proven ulcerative colitis. Specimens from

ulcerative colitis patients were obtained by colonic resection (12 patients) and sigmoidoscopic biopsy (20 patients). Tissue blocks from a further 12 patients with ulcerative colitis associated with dysplasia were kindly provided by Dr B C Morson, Consultant Pathologist, St Mark's Hospital. Two of the patients in this dysplasia group had concomitant carcinoma.

Ten patients had histologically proven Crohn's colitis, of whom 2 had undergone colectomy with ileo-rectal anastomosis and 8 sigmoidoscopic biopsy. Ten patients with diverticular disease of whom 2 had undergone sigmoid colectomy and 8 endoscopic biopsy of the affected lower sigmoid colon.

Patients with Crohn's disease, diverticular disease and non-dysplastic ulcerative colitis were categorised into a group of patients with active disease at the time of the biopsy samplings and a group whose disease was in remission.

Twenty-six patients who had been irradiated for pelvic malignancy (cervix, 17 patients; endometrium, 3 patients; ovary, 2 patients; prostate, 3 patients; and teratoma, 1 patient) were also studied. Biopsies were taken from colectomy specimens in 20 of these patients who had undergone resection for radiation colitis or stricture. The remaining 6 biopsies were obtained endoscopically. Mean age at time of

radiotherapy was 55 years, and the median interval following radiotherapy was 30 months (range 6 months - 20 years).

Normal mucosa was obtained from 10 control patients who presented with gastrointestinal symptoms in whom no abnormality was found on sigmoidoscopy and barium enema.

Methods

Assessment of Histological Sections

(a) Haematoxylin and Eosin: The diagnosis, degree of inflammation and extent of dysplasia in tissue sections was established by two Consultant Pathologists (TK - ulcerative colitis, diverticular disease, Crohn's disease and normal controls; SS - radiation injury) who were not aware of changes in mucin histochemistry. Dysplasia can be difficult to assess, particularly in the presence of inflammation (Riddell et al, 1983). Dysplasia in ulcerative colitis was diagnosed by the presence of 2 or more of the following features - hyperchromatic nuclei, prominent nucleoli, increased mitoses, villosity, stratified epithelial cells, altered cytoplasmic nuclear ratio and decreased mucin secretion (Plate 5).

Histological criteria of radiation injury were endarteritis, submucosal thickening and oedema, inflammatory cell infiltrate and the presence of bizzare radiation fibroblasts. Other changes included fibrinoid degeneration, epithelial

ulceration and varying degrees of cellular atypia (Ackerman, 1972, Shamsuddin and Elias, 1981). Cellular atypia in this group was diagnosed by epithelial cell morphology being more marked than that seen in inflamed or hyperplastic epithelium (Plate 6), following radiation injury. Transitional mucosa was also recognised by an increase in depth of colonic crypts and extensive branching of the colonic glands (Filipe, 1969).

(b) High-iron diamine alcian-blue: When assessing the slides for mucin staining, 3 patterns were recognised (Plates 1-3). A purely sulphomucin staining pattern and a mixed sulpho and sialomucin staining pattern were considered variants of normal (Filipe, 1969). Mucin patterns were assessed by the author, NAH and a pathologist, depending on the group of patients studied. Agreement about which sections showed a sialomucin pattern only was reached between the assessors in all cases.

Analysis of results

Sialomucin staining was correlated with the presence of morphological abnormalities, inflammatory or dysplastic changes. Comparisons between groups were by the Chi-square test or Fishers exact test of probability with the Yates correction for small numbers.

5.1.2 Results

Results are given in Table 28. All specimens from patients with diverticular disease and Crohn's colitis showed a brown mucin pattern on HID-AB stain, similar to that found in normal colonic mucosa. Nineteen of the 20 patients with ulcerative colitis but without dysplastic changes showed normal brown staining pattern. The remaining patient, a 70-year-old woman with a 20-year history of pancolitis showed an increase in blue staining with HID-AB demonstrating sialomucin change (Plate 4).

Table 28 Results of HID-AB staining from patients with diverticular disease, Crohn's disease, ulcerative colitis and radiation injury.

Patients (Group)	n	Mucin Pattern		
		Sialomucin	(%)	Sulphomucin
Normal	10	0	0	10
Diverticular	10	0	0	10
Crohn's	10	0	0	10
Ulcerative colitis:				
No dysplasia	20	1	5	19
With dysplasia	12	10	83	2
Radiation injury:				
No cellular atypia	23	8	35	15
Cellular atypia	3	1	33	2

The sections from 12 patients with ulcerative colitis with dysplasia demonstrated sialomucin staining with HID-AB in 10 cases (83%). Mucin production within dysplastic glands was scanty, while that adjacent to dysplastic areas appeared

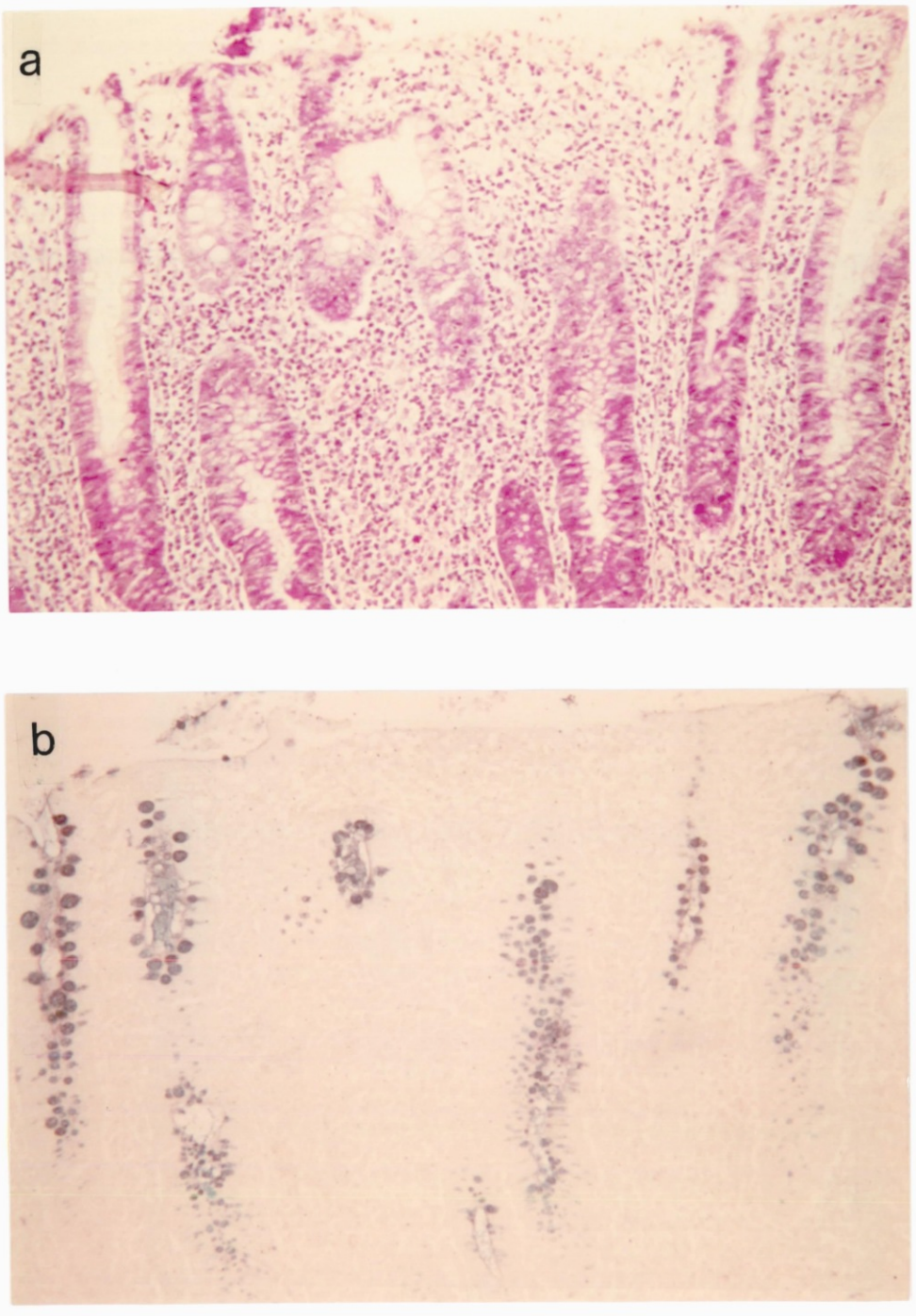


Plate 4 Human colonic mucosa -severe ulcerative colitis - no dysplasia.
(a)This section demonstrates severe ulcerative colitis. There is severe inflammation and ulceration of the mucosa, which is extensively infiltrated by inflammatory cells. There is decreased mucin secretion. The cells appear normal.(H and E x38) (b) The corresponding HID-AB section demonstrates sialomucin secretion by the colonic glands (x 38).

quantitatively normal but qualitatively was principally sialomucin (Plate 5).

All specimens from irradiated patients demonstrated radiation damage in varying degree. Eight out of 26 patients showed a normal brown (sulphomucin) staining reaction. A further 9 patients showed a mixed brown and blue pattern which is a normal variation of HID-AB staining. Nine specimens stained blue (sialomucin). An example of severe radiation damage with cellular atypia is shown in Plate 6.
Four patients underwent colonic resection for recurrent genito-urinary malignancy which had invaded bowel. Sialomucin secretion was not seen in these specimens. There was no relationship between sialomucin and age of the patient, dose of radiotherapy or interval from radiation.

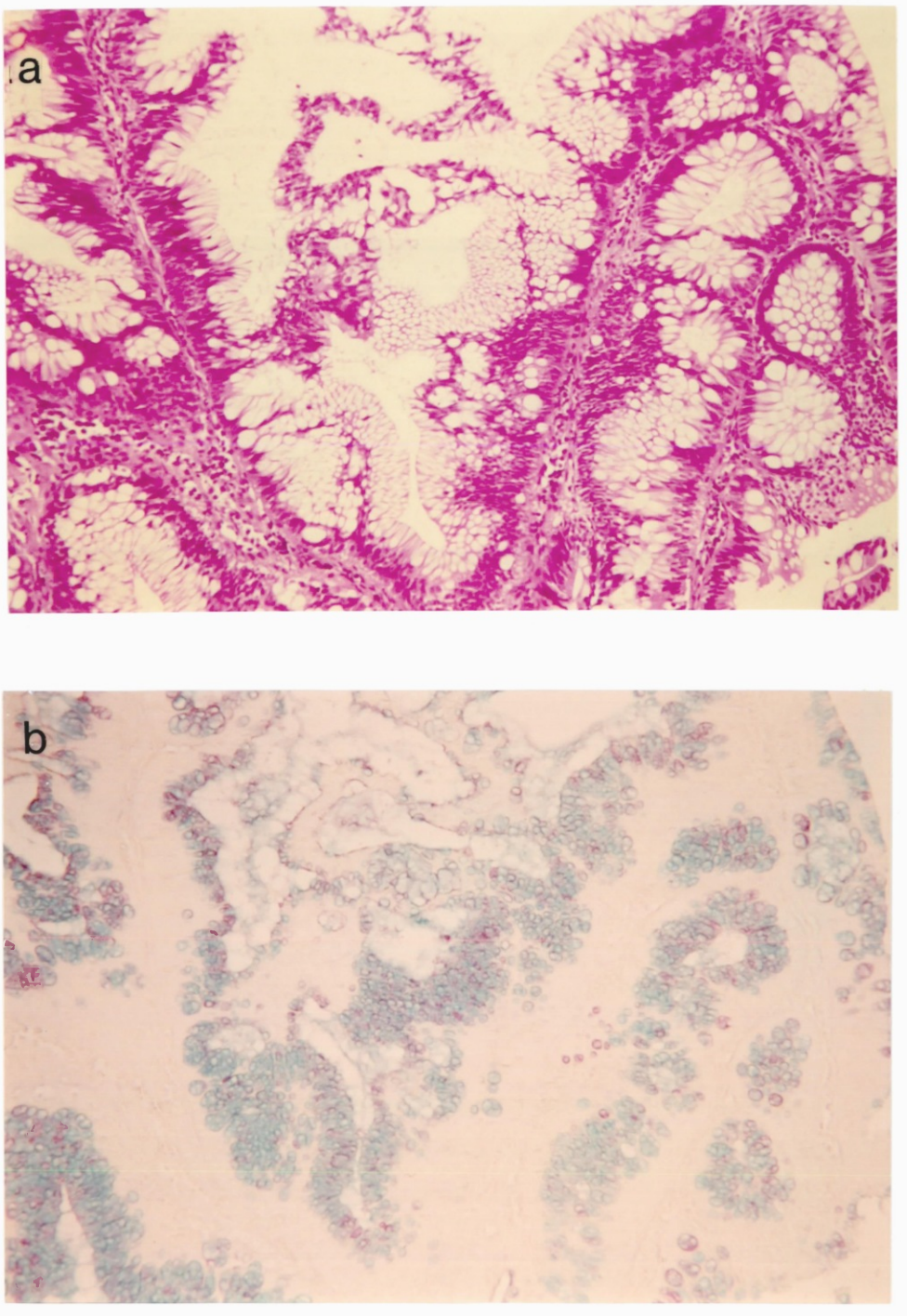


Plate 5 Human colonic mucosa -severe ulcerative colitis - moderate dysplasia.
(a) This section is from a patient with long-standing colonic ulcerative colitis in remission. There is moderate dysplastic change. Mucin secretion is abundant.(H and E x 38) (b) The corresponding HID-AB section demonstrates exclusive sialomucin secretion by the glands. (x38)

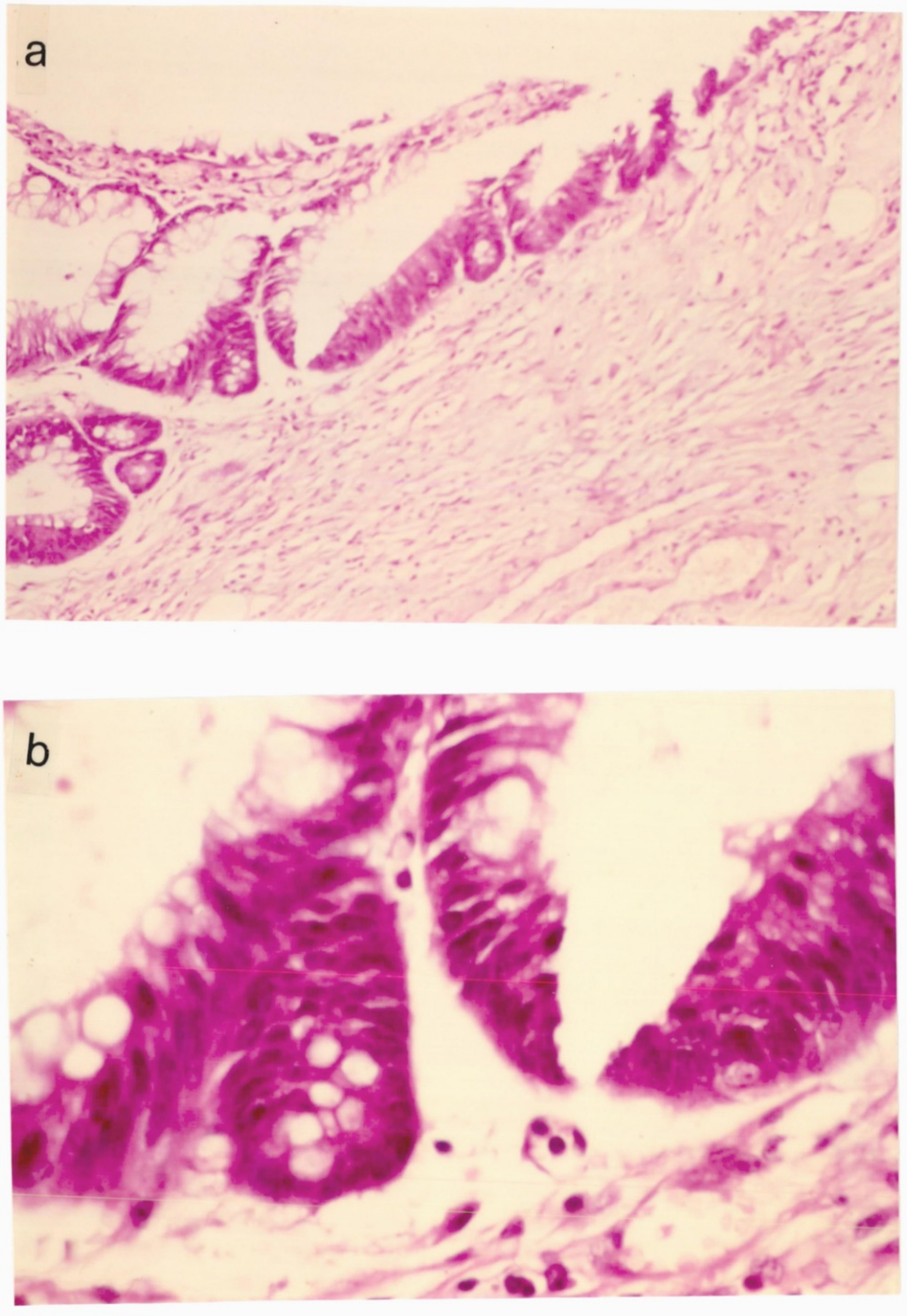


Plate 6 Human colonic mucosa -radiation damaged bowel. (a) There is mucosal ulceration and regeneration and repair of adjacent epithelium. There is decreased mucus secretion and cellular atypia with stratification of cells. In isolation, these findings could be interpreted as severe dysplasia(H and E x38). (b) A high power view (x60).

5.2 SIALOMUCIN CHANGES ADJACENT TO COLONIC POLYPS AND AFTER COLONIC RESECTION

5.2.1 Materials and Methods

5.2.1.1 Colonic Polyps

Eighty-two 82 colonic polyps taken from routine histopathology tissue blocks were examined. Specimens were taken from a variety of colonic and rectal sites, usually at endoscopy. There were 22 metaplastic and 60 tubulovillous adenomatous polyps. In this latter group, 24 polyps showed mild dysplasia, 9 moderate dysplasia, 13 severe dysplasia and 14 early carcinomatous invasion of the stalk.

The mucin slides were independantly examined by the author, NAH and one pathologist (GI). The pathologist independently assessed Haematoxylin and Eosin slides for degrees of dysplasia. Agreement was reached in every case regarding mucin staining according to previous criteria (Chapter 3). Mucin staining was characterised for the polyp itself and for the adjacent 'normal mucosa'(n=47).

5.2.1.2 Colonoscopy Study

Thirty patients who had undergone a curative resection of primary colon carcinoma were also studied. Colonoscopy was carried out by the author. Four quadrant biopsies were taken from the anastomosis. The remaining colon was biopsied at approximately 10 cm intervals on either side of the

anastomosis. The interval between primary resection and colonoscopy was categorised into three groups (Group A: 6-9 months, n=13; Group B: 10-15 months, n=11; Group C: 16-24 months, n=6). Biopsies were processed routinely and sections stained with haematoxylin and high-iron diamine alcian-blue (Chapter 3).

The sections were assessed independantly by a pathologist (SS) for morphological abnormalities and by the author and (SS) for sialomucin change. Presence or absence of sialomucin at the tumour resection margin was determined.

5.2.2 Results

5.2.2.1 Polyps

Table 29 demonstrates the different types of mucus production in the epithelium surrounding the different types of polyp.

All metaplastic polyps had a normal mucin pattern surrounding them. Of the 35 tubulovillous adenomas that had evidence of dysplasia (categorised mild in 10 cases, moderate in 8 cases, severe in 10 cases and in situ cancer in 7 cases) 28 (80%) had pure sialomucin secretion surrounding them (Plate 7). A mixed pattern was identified in 5 cases (14%) and a sulphomucin pattern seen in 2 cases (5%).

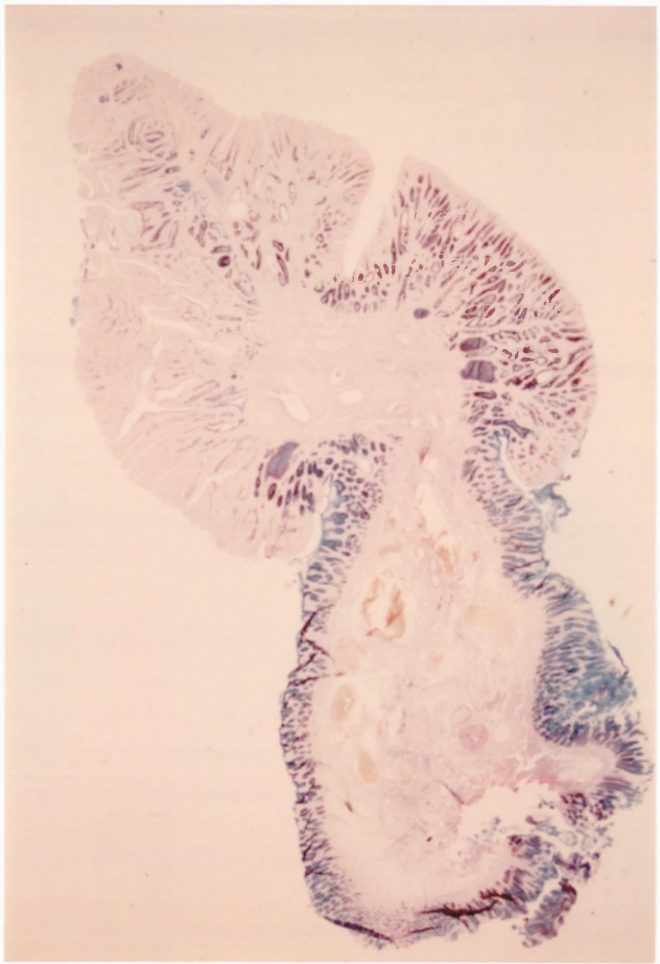


Plate 7 Human Colonic tubulovillous adenoma.
The sialomucin distribution in the stalk of the adenoma may be seen. The base of the pedicle shows a normal sulphomucin production. Mucus production within the adenoma itself is scanty and of a mixed type (HID-AB x 2).

Table 29 Mucin secreting characteristics surrounding colonic polyps (n=47).

Type of Polyp	Mucin Secretion					
	Sialomucin Pattern	(%)	Mixed Pattern	(%)	Sulphomucin Pattern	(%)
Metaplastic	0	(0)	5	(42)	7	(58)
Tubulovillous						
Mild dysplasia	6	(60)	3	(30)	1	(10)
Moderate dysplasia	8	(100)	0	(0)	0	(0)
Severe dysplasia	9	(90)	0	(0)	1	(10)
Severe + Invasion of the stalk	5	(71)	2	(29)	0	(0)
Total (tubulovillous)	28	(80)	5		2	

Table 30 Mucin secretion within the polyp (n=69)

Type of Polyp	Mucin Secretion Within The Polyp					
	Sialomucin Pattern	(%)	Mixed Pattern	(%)	Sulphomucin Pattern	(%)
Metaplastic	1	(10)	9	(90)	0	(0)
Tubulovillous						
Mild dysplasia	7	(30)	6	(26)	10	(44)
Moderate dysplasia	2	(22)	6	(66)	1	(12)
Severe dysplasia	4	(31)	9	(69)	0	(0)
Severe + Invasion	4	(28)	8	(57)	2	(15)
Total (tubulovillous)	17	(29)	29		13	

The remainder (n=13) showed no mucus secretion.

Mucus production within the polyp, rather than the surrounding mucosa, was decreased. Table 30 gives the different patterns observed in the various categories. There was no significant

trend or correlation between the mucin secretion and degree of dysplasia within the polyps.

5.2.2.2 Colonoscopy Study

There were 15 males (mean age 67.3 years) and 15 females (mean age 66 years). Details of sites and operations performed are shown in Table 31. One patient had two synchronous cancers. All patients were asymptomatic at the time of examination. The entire remaining colon was visualized in 93.3% of cases. The Dukes' staging and differentiation of the primary tumours is shown in Table 32.

Table 31 The sites of primary colorectal carcinoma and operation performed in 30 patients undergoing post-operative colonoscopy.

Site	n	%	Operation	n
Caecum	9	29	Right hemicolectomy	13
Ascending colon	4	13	Left hemicolectomy	1
Splenic flexure	1	3.2	Sigmoid colectomy	10
Sigmoid colon	11	35.4	Anterior resection	6
Rectum	6	19.3		
Total	31	100		30

(Includes 1 double primary)

Table 32 Dukes' stage and differentiation of primary carcinoma in 30 patients undergoing colonoscopy.

Dukes' Stage	n	%	Differentiation	n	%
A	1	3.2	Well	4	12.9
B	24	77.4	Moderate	18	58.0
C	6	19.3	Poor	7	22.5
			Variable	2	6.4
Total	31	100		31	100

(Includes 1 double primary)

Table 33 Histologic and mucin abnormalities at the anastomosis after resection for carcinoma.

Histological Abnormality	n	Sialomucin pattern	Prevalence of of sialomucin
Non-specific inflammation	5	1	20%
Mild Dysplasia	2	1	50%
Distorted glands	2	1	50%
Metaplastic polyp	1	0	0%
Normal	20	0	0%
Total	30	3	10%

Histology

One hundred and fifty-four biopsies were examined. The histological abnormalities observed at the anastomosis are shown in Table 33. These included non-specific inflammation (Plate 8), mild dysplasia (Plate 9), distorted glands and one hyperplastic polyp. No further adenomatous polyps or carcinomas were identified.

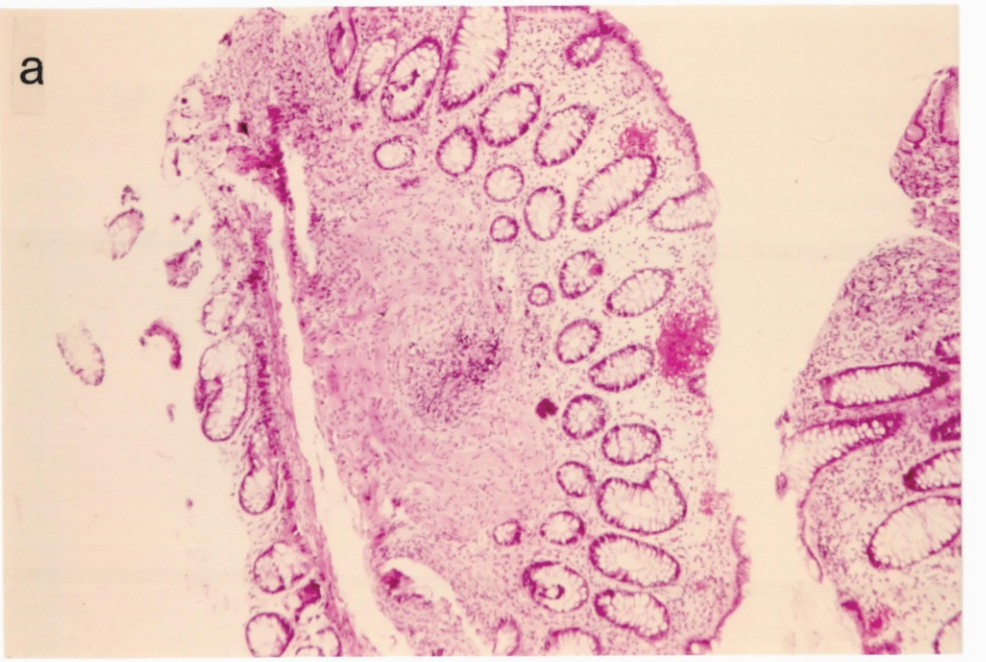




Plate 8 Human colonic mucosa.Anastomotic biopsy, 8 months after resection for carcinoma. (a) This section shows mild inflammation only (H and E x 60). The corresponding HID-AB stain demonstrates exclusive sialomucin secretion (x 60).

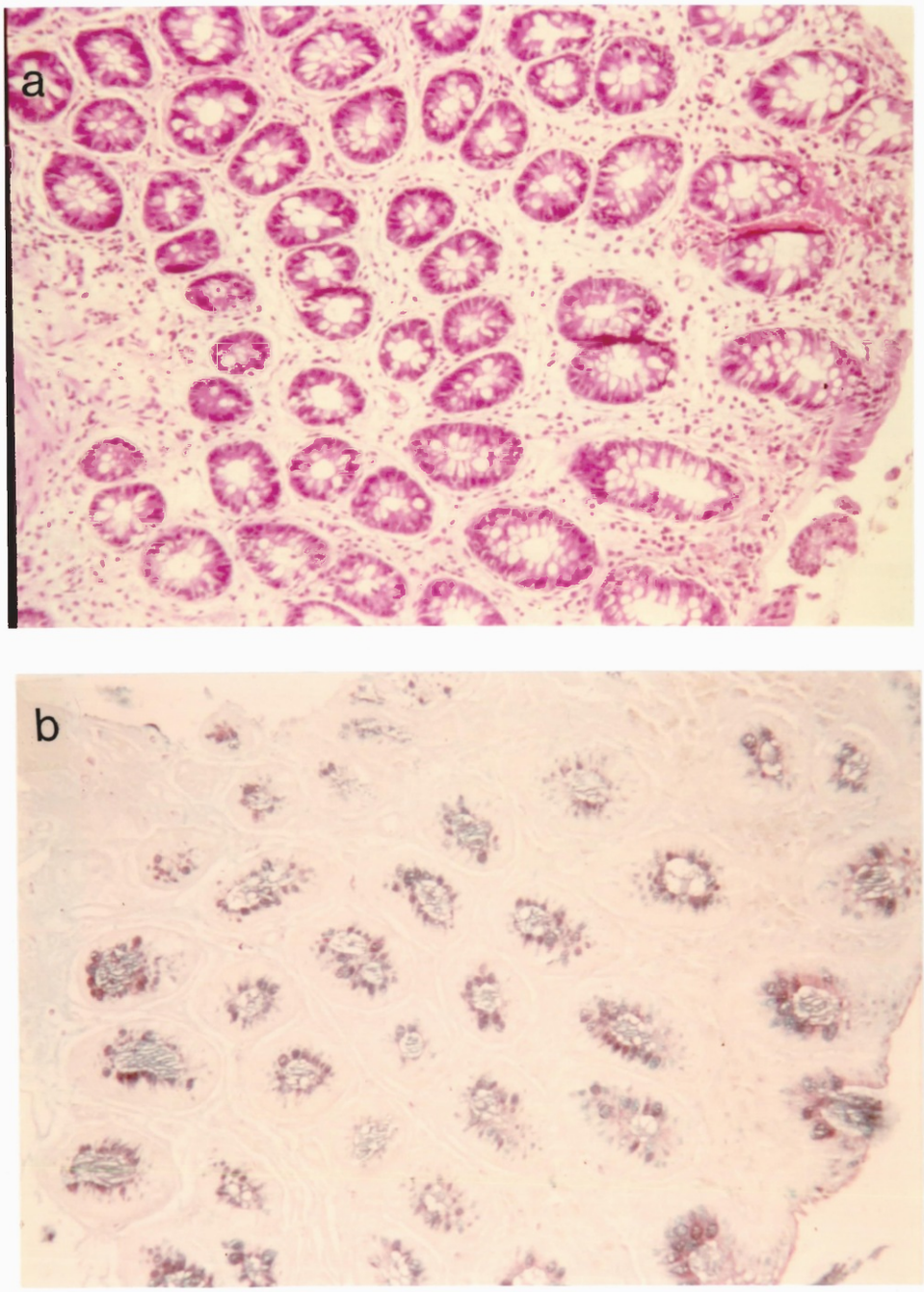


Plate 9 Human colonic mucosa.Colonoscopic biopsy of an anastomosis twelve months after curative resection for carcinoma(a). There is mild dysplastic change as demonstrated by crowded glands, decreased mucin secretion, enlarged hyperchromatic nuclei. There is no inflammation in this section(H and E x 38). (b) The corresponding HID-AB section demonstrates decreased mucus secretion which is predominantly sialomucin .

Mucin abnormalities

Sialomucin secreting mucosa at the anastomotic site was present in 3 (10%) patients. Sialomucin was also present at other sites in the colon in these 3 patients. The interval from resection was 11, 14 and 17 months respectively. A further five patients showed patches of sialomucin in different areas of the colon at 20, 24, 6, 10 and 7 months respectively (Table 34).

Original resection margins

Examination of the original proximal resection margin was possible in 20 patients and distal margin in 30 patients. The ileum constituted 10 resection margins. Results are given in Table 35. One patient was found to have sialomucin in the resection margin and at the anastomosis post-resection.

Table 34 Sites of Sialomucin production in the remaining bowel after colonoscopy

	Interval From Resection (months)			
	6-9	10-14	16-24	Total
Anastomosis	0	2	1	3
Caecum	0	1	1	2
Ascending	1	0	1	2
Transverse	0	0	1	1
Descending	0	1	0	1
Sigmoid	1	1	0	2
Rectum	1	0	1	2
Total	3	5	5	13

Table 35 Prevalence of sialomucin secretion at the original resection margin.

Site	n	Sialomucin		Sulphomucin	
Proximal resection margin	20	6	(30%)	14	(70%)
Distal resection margin	30	8	(26.6%)	22	(73.3%)

Clinical Follow-up

Patients were followed for a mean of 30.8 months (range 12-55 months). Three patients have developed local recurrence, of whom 2 had sialomucin in either proximal or distal resection margin. One patient with sialomucin in the anastomosis at colonoscopy has developed local recurrence. One of the 2 patients with dysplasia but no sialomucin at the anastomosis has developed local recurrence. One patient who has developed a metachronous tumour had no evidence of sialomucin at anastomosis.

5.3 SIALOMUCIN CHANGES IN AN ANIMAL MODEL OF NEOPLASIA AND INFLAMMATION

5.3.1 Colonic Trauma Model

5.3.1.1 Introduction

Attempts to produce an animal model of ulcerative colitis have included vascular impairment (Marston et al, 1969), bacterial infection (Brennan et al, 1965), allergy (Rosenburg and Fisher, 1964), immune manipulation (Ceredig et al, 1977), carrageena (Kirsner, 1961), irradiation (Brennan et al, 1965), diet (Rabin, 1977) and pharmaceutical agents (Katz et al, 1977). The main shortcomings of all these models are lack of reproducability and the absence of diffuse colonic involvement. The histopathologic features of human ulcerative colitis have not been produced in an animal. Thus, while models of colonic inflammation are available for study, there are no animal models of human ulcerative colitis.

Acetic acid has been used to produce chronic gastric ulcers (Takagi et al, 1970). This has been modified by MacPherson and Pfeiffer (1978) and Mann et al (1980) who applied dilute acetic acid onto the colonic mucosa via the rectum and produced colitis. The histopathological features of this colitis are diffuse ulceration of the distal colon, pseudopolyp formation, alteration in crypt depth, decreased mucus secretion and a transmural non-specific inflammatory

response. Serosal application of acetic acid causes similar lesions, but there is a higher incidence of fistula formation and a less well defined dose response. This appears to be a reliable colitis model, and was used in the following experiment which was designed to determine whether alterations in mucin chemistry occur in response to mucosal injury, and whether the prevalence of such change is similar to that seen adjacent to colon carcinoma.

5.3.1.2 Materials and Methods

Twenty-two female Sprague-Dawley rats, weighing 200-250 g each, were studied. A 2 cm length of fine polyethylene tubing was attached to a luer stub adaptor and lubricated for easy passage into the colon, via the rectum. A 2 ml syringe was fitted to the adaptor. The animals were lightly anaesthetised by a mixture of nitrous oxide, halothane and oxygen. The tubing was passed full length into the rectum with the luer stub resting on the anus. One ml of 10% acetic acid was injected slowly into the colonic lumen and left for 10 seconds after which the remaining acid was withdrawn and the colon irrigated with 2 ml normal saline. The animals were then allowed to recover from anaesthetic.

Animals were caged in groups of 4 and allowed free access to food and water. They were killed in groups of 5, 5 and 10 at 5, 10 and 15 days respectively. The entire colon was removed, opened longitudinally and strips of mucosa taken

from areas of obvious colonic inflammation and ulceration. These were then fixed in 10% formalin, processed and embedded in paraffin wax. Five micron thick sections were cut and stained with Haemotoxylin and Eosin. Corresponding sections were stained with the high-iron diamine alcian-blue sequence for mucins. The mucosal inflammatory change was assessed by a Consultant Histopathologist (SS). Mucin changes were assessed by the author and (SS).

5.3.1.3 Results

Six animals died prematurely. Four deaths occurred at 4 days and a further 2 at 8 days following installation of acid. In all cases, death was due to full thickness colonic perforation - most frequently at the splenic flexure. Some animals developed bloody diarrhoea on days 1 and 2 after instillation which resolved over the next week. Surviving animals remained fit for the remainder of the experiment.

Details of the macroscopic and microscopic changes are shown in Table 36. The areas of ulceration were patchy and varied from corrosive burns to mild inflammatory ulceration (Plate 10). Sialomucin change was seen in only one colon. It occurred at the edge of an inflammatory ulcer (Plate 11). Despite the presence of ulceration and inflammation in the remaining 19 colons, sialomucin change was not seen (Plate 12).

Table 36 Macroscopic, microscopic and histochemical features of colonic trauma model.

Rat No	Death (days)	Macroscopic Features	Microscopic Features	Histochemistry
1	5	Corrosive burn, dilated colon	Necrosis, ulceration, inflammation	Sulphomucin
2	5	Moderate colitis	Ulceration, inflammation	Sulphomucin
3	5	Moderate colitis	Ulceration, inflammation	Sulphomucin
4	4	Corrosive burn, perforation	Necrosis, ulceration, autolysis	Sulphomucin
5	10	Corrosive burn	Necrosis, diffuse inflammation	Sulphomucin
6	4	Necrosed rectum	Necrosis, ulceration	Sulphomucin
7	10	Moderate colitis	Ulceration, inflammation	Sulphomucin
8	10	Moderate colitis	Ulceration, inflammation	Sulphomucin
9	10	Severe colitis	Ulceration, inflammation, necrosis	Sulphomucin
10	10	Mild colitis	Ulceration inflammation	Sulphomucin
11	8	Perforated colon	Necrosis, ulceration	Sulphomucin
12	15	Mild colitis	Chronic inflammation only	Sulphomucin
13	15	Mild colitis	Ulceration, inflammation	Sialomucin at edge of ulcer
14	8	Corrosive burn, perforation	Necrosis, ulceration	Sulphomucin
15	15	NAD	Chronic inflammation	Sulphomucin
16	4	Ischaemic colon, corrosive	Necrosis, inflammation	Sulphomucin
17	15	NAD	Chronic inflammation	Sulphomucin
18	4	Perforated colon	Necrosis inflammation	Sulphomcuin
19	15	Corrosive burn	Inflammation ulceration	Sulphomucin
20	15	Colitis (mild)	Chronic inflammation	Sulphomucin

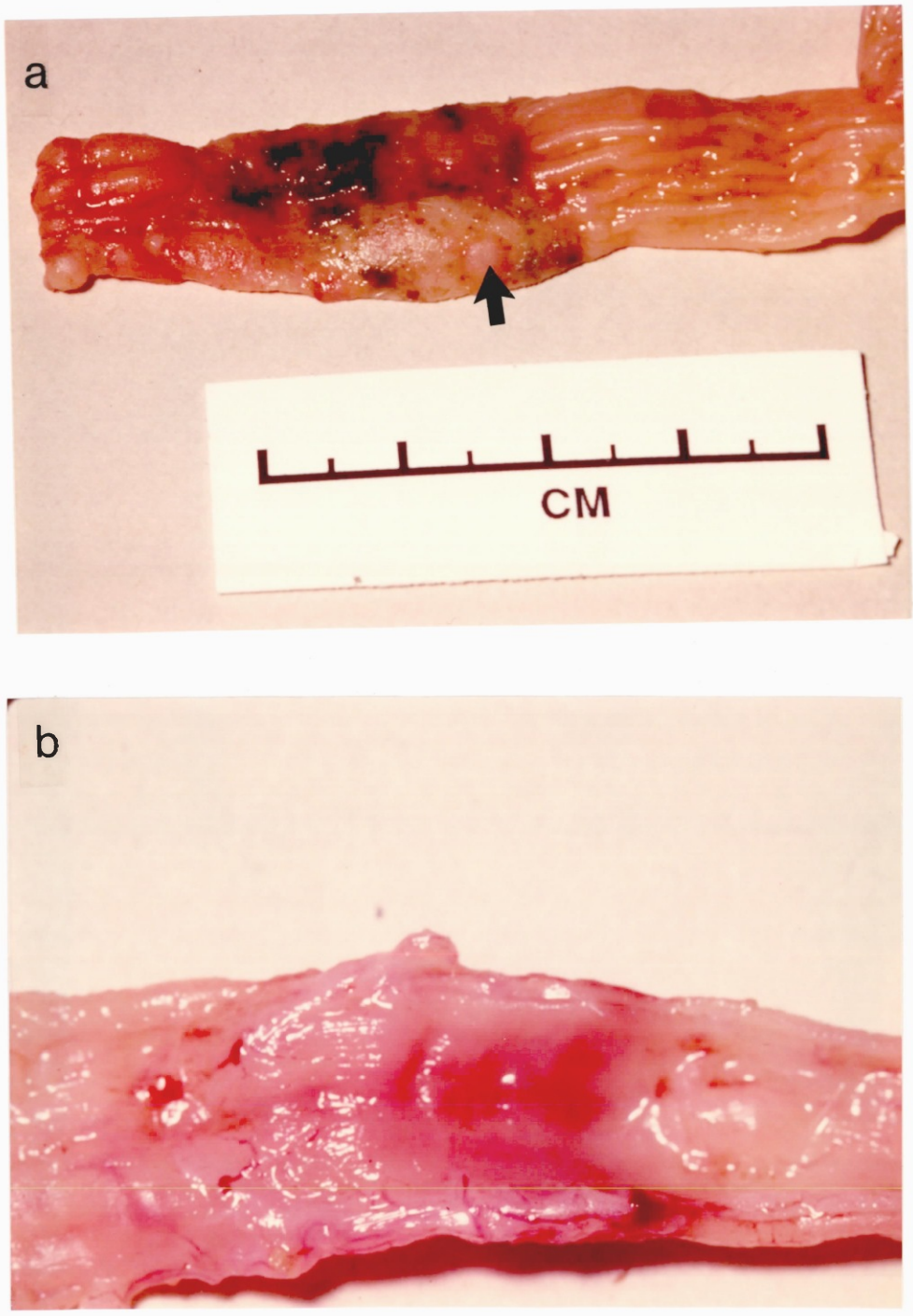


Plate 10 Rat colonic mucosa- macroscopic appearance of the rat colon ten days after instillation of 10% acetic acid .
(a) There is severe ulceration with areas of patchy necrosis. Islands of normal mucosa may be seen (arrow).
(b)The colon has healed in this animal, two weeks after the insult. An oedematous bowel wall may be seen with an intact epithelium.

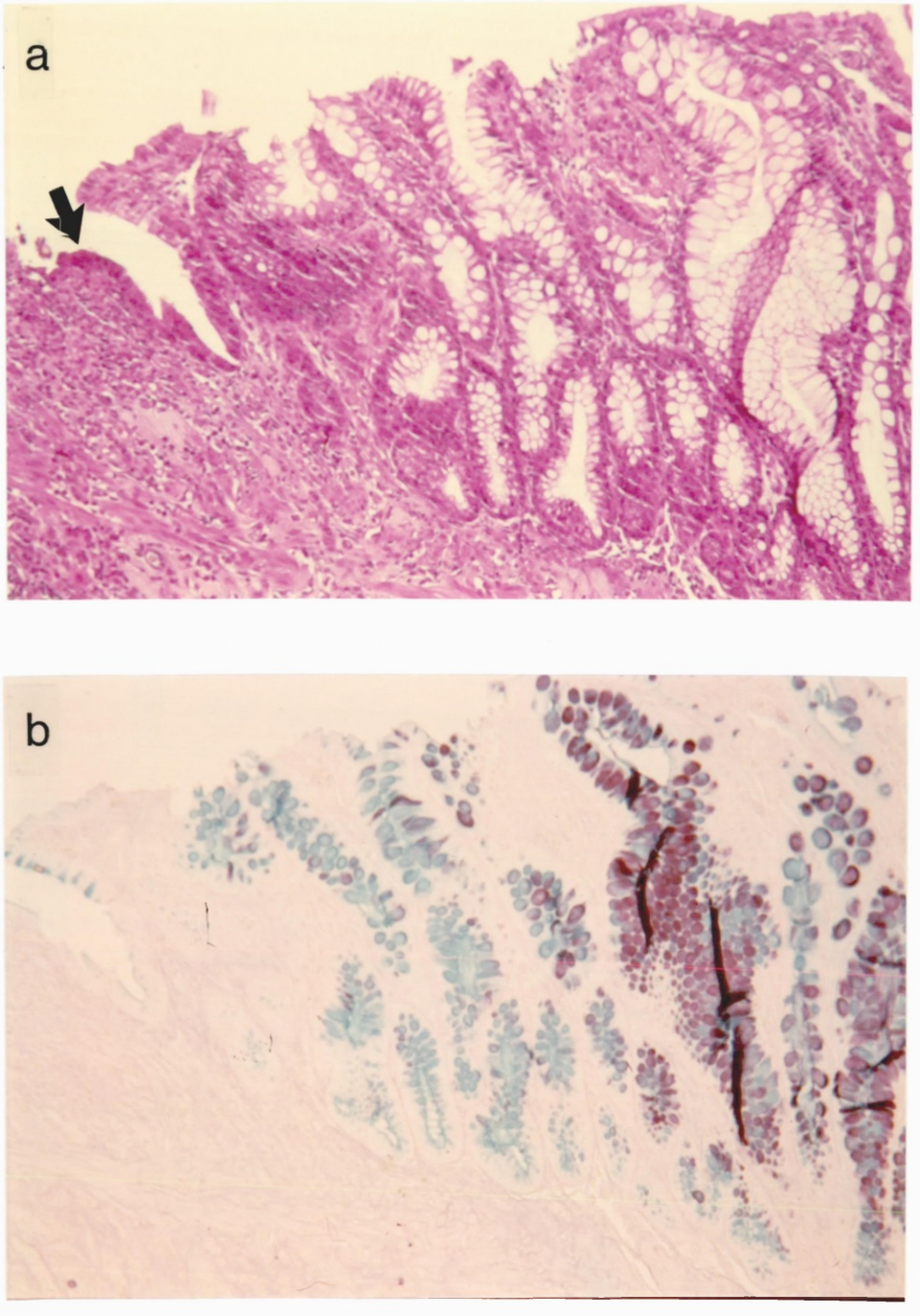


Plate 11 Rat colonic mucosa: Colonic trauma model. (a)There is severe inflammation and ulceration of the colonic mucosa. The base of the ulcer is composed of fibrous tissue and is infiltrated with inflammatory cells. The adjacent mucosa appears hyperplastic. Attempts at healing epithelium may be seen (arrow) (H and E x 60). (b)The corresponding HID-AB section demonstrates sialomucin secretion next to the ulcer(x60).

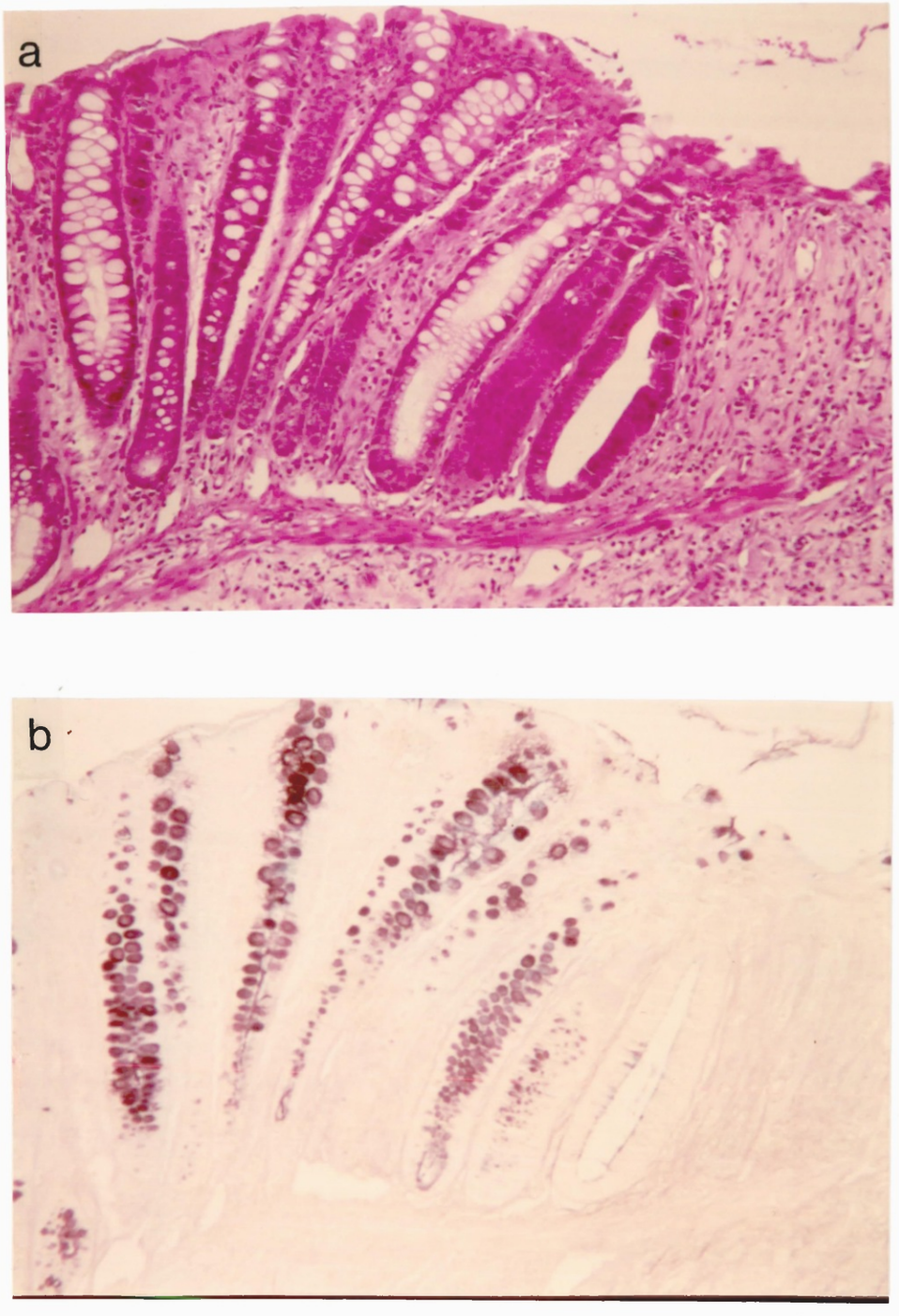


Plate 12 Rat colonic mucosa: Colonic trauma model.
(a) There is extensive inflammation and ulceration of the colonic mucosa, ten days after the instillation of 10% acetic acid. The ulcer is composed of fibrous tissue in the base and is infiltrated with inflammatory cells. The adjacent mucosa appears normal(H and E x60). The corresponding HID-AB stain demonstrates normal sulphomucin secretion (x 60).

5.3.2 Colonic Healing Model

5.3.2.1 Introduction

The aim of this experiment was to investigate whether sialomucin is produced in response to regeneration and repair by colonic mucosa. Increased cell proliferation in the vicinity of a colonic anastomosis has been demonstrated by Roe et al (1987). This area is also associated with a high incidence of tumours in rats receiving carcinogen (Williamson et al, 1982). This increase in cell proliferation and carcinogen susceptibility might be associated with an increase in sialomucin production in the absence of inflammatory change.

5.3.2.2 Materials and Methods

Twelve female (200 g) Sprague-Dawley Rats, were anaesthetised with Nitrous Oxide, Oxygen and Halothane. After laparotomy the descending colon 2 cm above the anus was isolated. The descending colon was transected and resutured with 5/0 Prolene. A 1 cm length of Prolene was left at the site of anastomosis to enable identification at post mortem. Rats were killed in groups of 3 at 1 week, 1, 2 and 3 months.

After autopsy the descending colon was removed, washed in saline and opened longitudinally. The area of anastomosis was removed, and routinely fixed, processed and embedded in paraffin. Four micron thick sections were cut and stained with Haemotoxylin and Eosin and the high iron diamine alcian

blue sequence. Mucin stains were interpreted according to previous criteria (Chapter 3).

5.3.2.3 Results

Histology

The changes described below occurred in all the specimens.

One week: The mucosa at the anastomosis was completely interrupted. The lamina propria showed evidence of acute and chronic inflammation. In addition regenerating epithelium was seen at the margin of transection overlying proliferating fibrous tissue.

One month: By one month a few chronic inflammatory cells remained in the fibrous tissue. The colonic glands appeared to contain an increased amount of mucin compared to normal. Healing was almost complete at this stage. Occasional distorted glands were seen (Plate 13).

Two and Three months: By two months complete healing had occurred, the colonic epithelium looking entirely normal, the serosa and muscular layers formed by mature fibrous tissue. Similar findings were seen at 3 months.

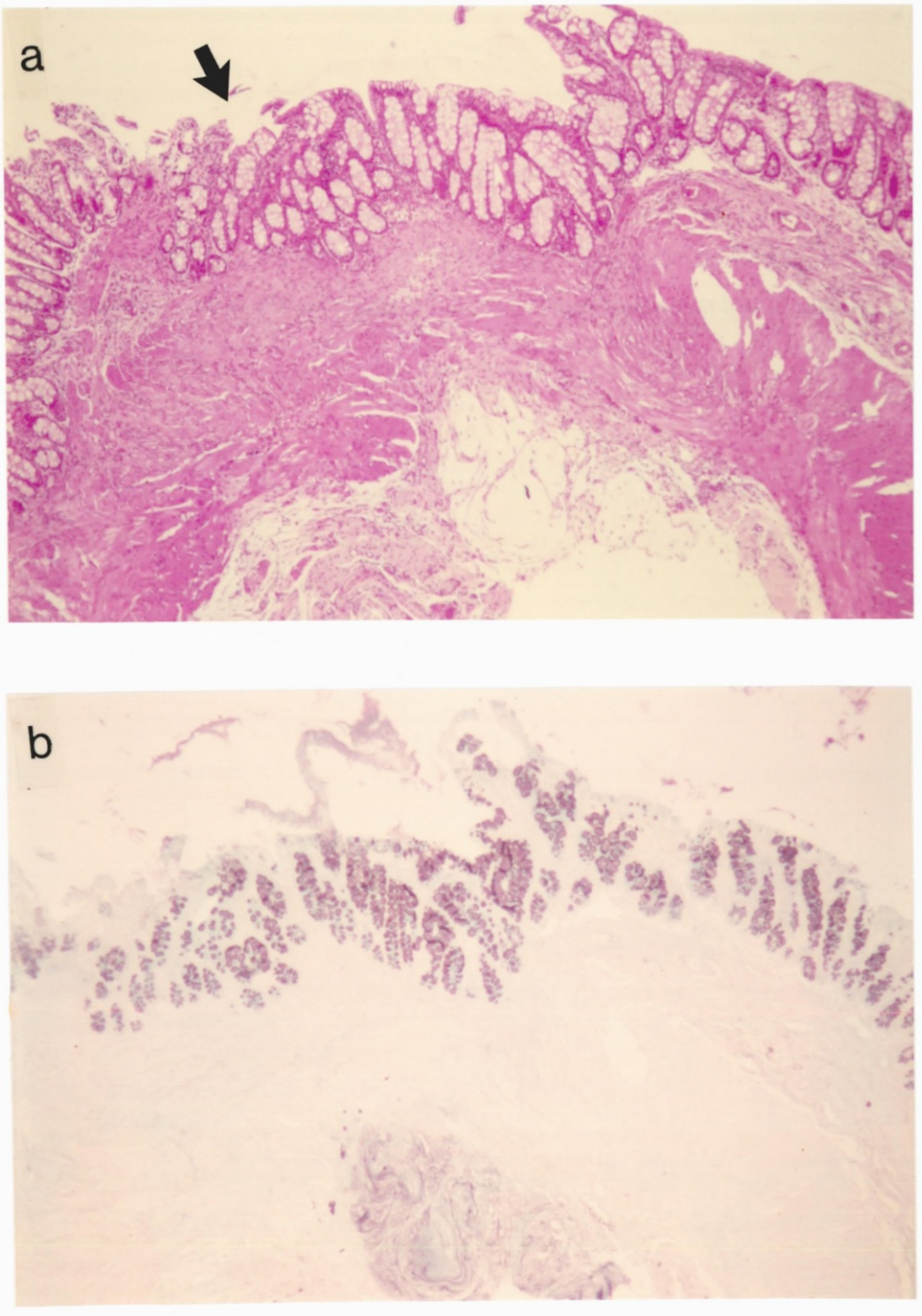


Plate 13 Rat colonic mucosa- 1 month after colonic anastomosis (a) almost complete healing has taken place (arrow) and the colonic glands appear normal (H and E x38).(b) The corresponding HID-AB section demonstrates normal sulphomucin secretion (x 38).

Mucin changes

The normal sulphomucin pattern found in the left colon was seen in every specimen throughout the study. Goblet cells appeared slightly more distended with mucin overlying the anastomsis compared to the surrounding mucosa (Plate 13).

5.3.3 Colonic Cancer Model

5.3.3.1 Introduction

A suitable animal model for colorectal cancer should fulfil the following criteria:

1. Tumours should be produced in a reliable and predictable fashion.
2. The tumours should be adenocarcinomas and should develop through a stage of adenoma formation.
3. The tumours should be distributed through the colon in the same pattern as in man.
4. The metastatic potential and biological behaviour should be similar to that which occurs in man.
5. Tumour induction should be specific for the colon.
6. For experimental convenience, there should be a reasonably short induction period.

The first report of experimental induction of large bowel cancer was by Walpole in 1952. This was achieved by using subcutaneous injections of the aromatic amine dimethylaminobiphenyl in rats. Since then, other agents have been used, eg cycasin (Laquer, 1964); 3 methylcholanthrene

(Homberger et al, 1972); and methylnitrosurea (Narisawa and Weisberger, 1975).

The most useful experimental colonic carcinogen is 1.2 dimethylhydrazine (DMH) discovered by Druckrey in 1967. This is a potent and specific synthetic analogue of the naturally occurring experimental colonic carcinogen cycasin. The DMH rat model satisfies most of the requirements listed above. DMH is not an active carcinogen. It is a procarcinogen that requires preliminary metabolic activation to exert its effect. It appears to act by a local effect in the bowel lumen and by bloodstream carriage to the colonic epithelial cells (Figure 11) (Fiala, 1975). The reason for colonic susceptibility to DMH carcinogenesis is not known. Colonic tumours can be produced in most rats within 30-36 weeks, (Druckrey, 1970; Thurnher et al, 1973). The tumours resemble the adenomas and adenocarcinomas found in man, both in histological characteristics and in distribution throughout the colon (Sunter et al, 1978; Ward et al, 1973). Tumours also occur in the duodenum, proximal jejunum, external ear and liver, but these can be reduced by alteration in dose and dose frequency.

DMH induced tumours spread to lymph nodes and to distant sites as in man. Unlike in man metastasis to the lung in contrast to the liver is more frequent. Thus, DMH induced

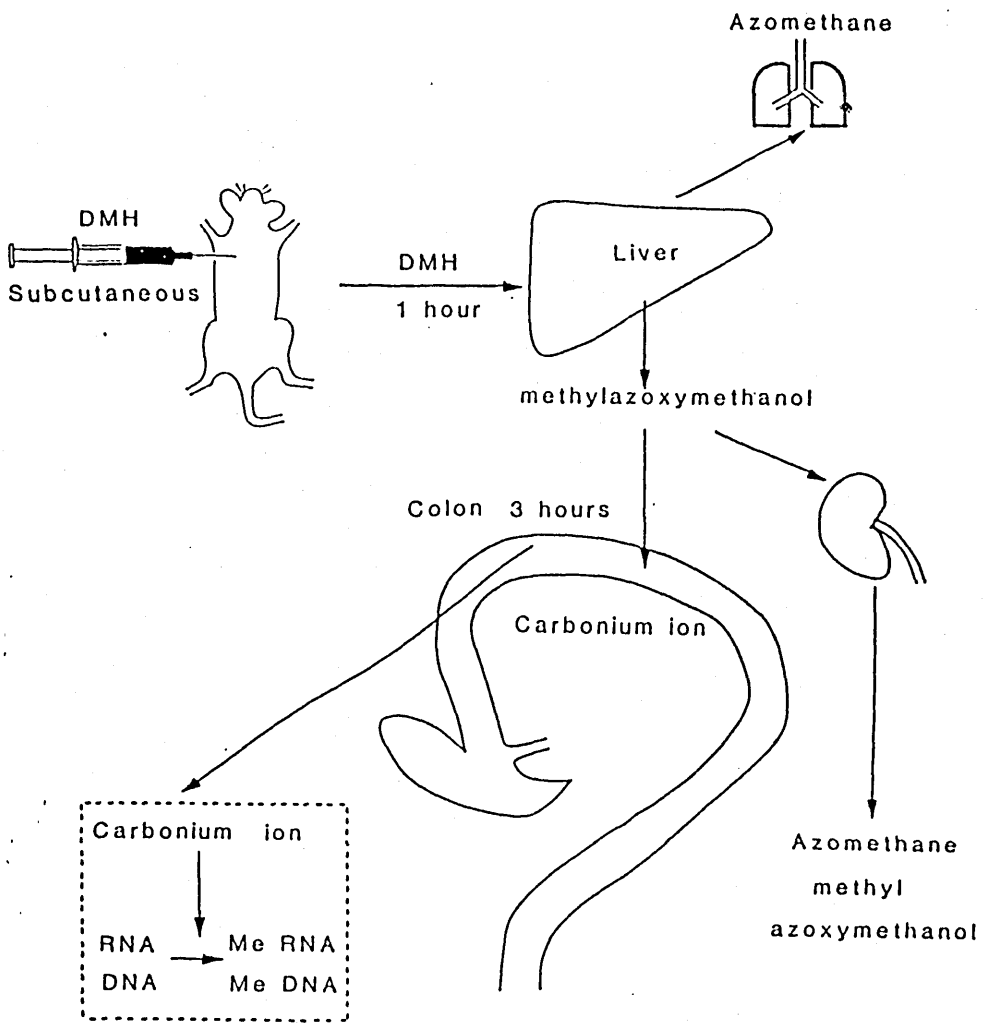


Figure 11 Metabolism of 1-2 dimethylhydrazine.
1-2 dimethylhydrazine is taken up by the liver and metabolised to azomethane, which is expired in air or converted to methyl azoxymethanol. This is either excreted in the kidneys or taken up by the colon where the active radical carbonium ion has the ability to methylate RNA and DNA.

colonic tumours in rats provide a good model for the study of colorectal adenocarcinoma.

5.3.3.2 Materials and Methods

All DMH experiments were performed in a purpose-build carcinogen facility within the Zoology department, Royal Postgraduate Medical School, Hammersmith Hospital, London. Procedures were in accordance with the Medical Research Council Guidelines for work with carcinogens in MRC establishments (1979). The principle safeguards were:

(i) The carcinogen facility was housed in a remote part of the building and a strict control on access maintained at all times.

(ii) Animal storage areas were separated from work areas and carcinogen-treated animals were housed separately from control animals.

(iii) All workers wore disposable double-layer protective aprons, masks, caps and double gloves.

(iv) Carcinogens were made up and injected in a high efficiency fume-extraction cupboard.

(v) Immediately after injection, animals were returned to the storage area to prevent contamination of the work area with expired azomethane (which is itself a potent carcinogen).

(vi) All surplus and waste materials were incinerated.

Twenty-four female Sprague-Dawley rats (aged 6-9 weeks, weight 200 g) were used. All rats were allowed free access to a standard laboratory diet and water. The rats were caged in groups of two and control rats were housed separately from carcinogenic-treated rats.

The 12 carcinogen-treated animals received weekly subcutaneous injections of 1.2 dimethylhydrazine dihydrochloride, 97 per cent symmetrical (purchased from Aldrich Chemical Co, Gillingham, Dorset) at a dose of 20 mg/kg body weight for 16 weeks. The stock solution of DMH was freshly prepared each week by dissolving 400 mg DMH in 100 ml normal saline containing 1.5 per cent EDTA as a buffer and the pH of the solution (generally 1.5-2.0) was titrated to pH 6.4 with 4 per cent sodium hydroxide. Rats were then weighed individually and placed in purpose-built cages during subcutaneous injection of the DMH solution into loose folds behind the neck.

Twelve controls received weekly EDTA injections subcutaneously. Rats were killed at intervals of 5, 10, 20 and 30 weeks in groups of 6 (3DMH, 3 control). The entire colon was removed immediately after killing, the mucosa removed and rolled up into a "swiss roll" (Filipe, 1975). The swiss roll was processed and embedded in paraffin wax. Four micron sections were cut and stained with Haematoxylin and Eosin and the high iron diamine alcian blue sequence

(Chapter 3). Sections were examined for abnormal mucosal morphology by a Consultant Histopathologist (PDC). The mucin stains were independantly assessed by the author and PDC.

5.3.3.3 Results

All animals survived until sacrifice. No significant difference in body weight between the control and test group of rats was observed.

Control rats

Goblet cells were abundant at all levels of the crypts and were present on the mucosal surface. They were uniformly distributed throughout the length of each crypt and those in the upper crypts were usually more distended with mucus. No histological epithelial abnormalities were identified. Mucin secretion appeared to vary according to the region of the colon. Sulphomucin (staining brown/black) was the predominant mucus detected at all crypt levels in the left colon and rectum, while in the caecum there was moderate production of sulphomucin in the upper two thirds of crypts, but sialomucin was secreted in the crypt base. Sialomucin was seen at all crypt levels in the proximal colon and some goblet cells contained a mixture of both types of mucin.

The remaining results are concerned with mucin changes in the distal colon.

DMH rats

During carcinogenesis a progressive increase in sialomucin production in the distal colon was seen.

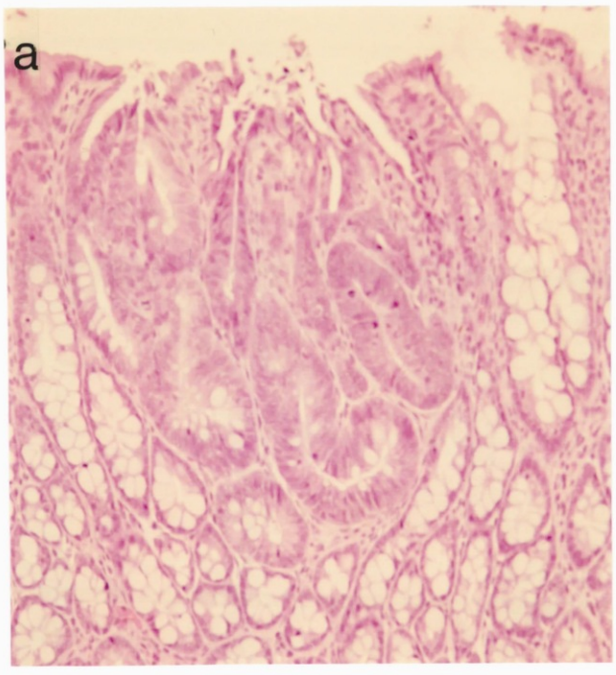
Five weeks: Areas of local hyperplasia were seen in one specimen, but not in association with sialomucin secretion. The remaining colons were normal, although the basal region goblet cells showed mild mucin depletion.

Ten weeks: Mucin depletion was marked. All three rats showed this change in addition to patches of sialomucin secretion in the basal goblet cells situated in the left colon. There were areas of mild focal dysplasia in the left colon in two cases.

Twenty weeks: There was a continued reduction in the number of goblet cells containing stainable mucin. Sialomucin occurred in association with areas of moderate dysplasia (Plate 14). One rat developed a benign tubular adenoma with surrounding sialomucin change (Plate 15).

Thirty weeks: Two colonic cancers were found. Both were invasive adenocarcinomas and both had surrounding sialomucin change (100%) (Plate 16 & 17).

Colonic mucosa separate from the tumours also demonstrated histochemical abnormalities. Goblet cells confined to the



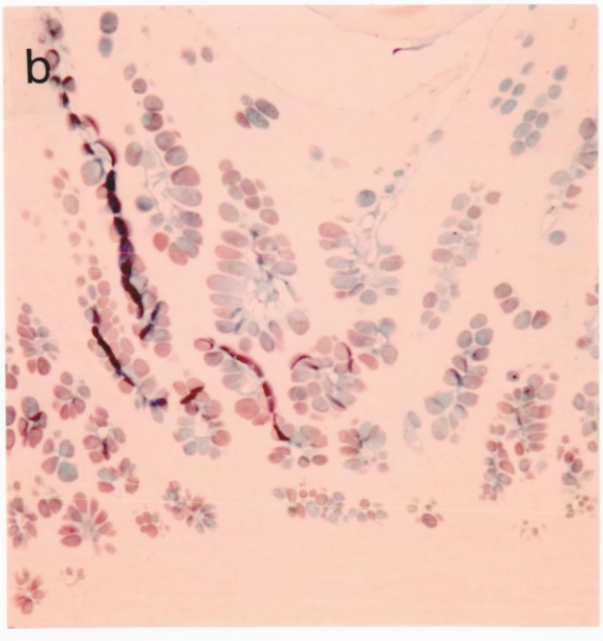


Plate 14 Rat colonic mucosa- an area of dysplasia within the colonic glands at Week 20, after injection of 1-2 dimethylhydrazine(H&E x38)(a). The corresponding HID-AB section (b) demonstrates that this area secretes mostly sialomucin, whilst the surrounding mucosa secretes the normal sulphomucin pattern (HID-AB x 30).

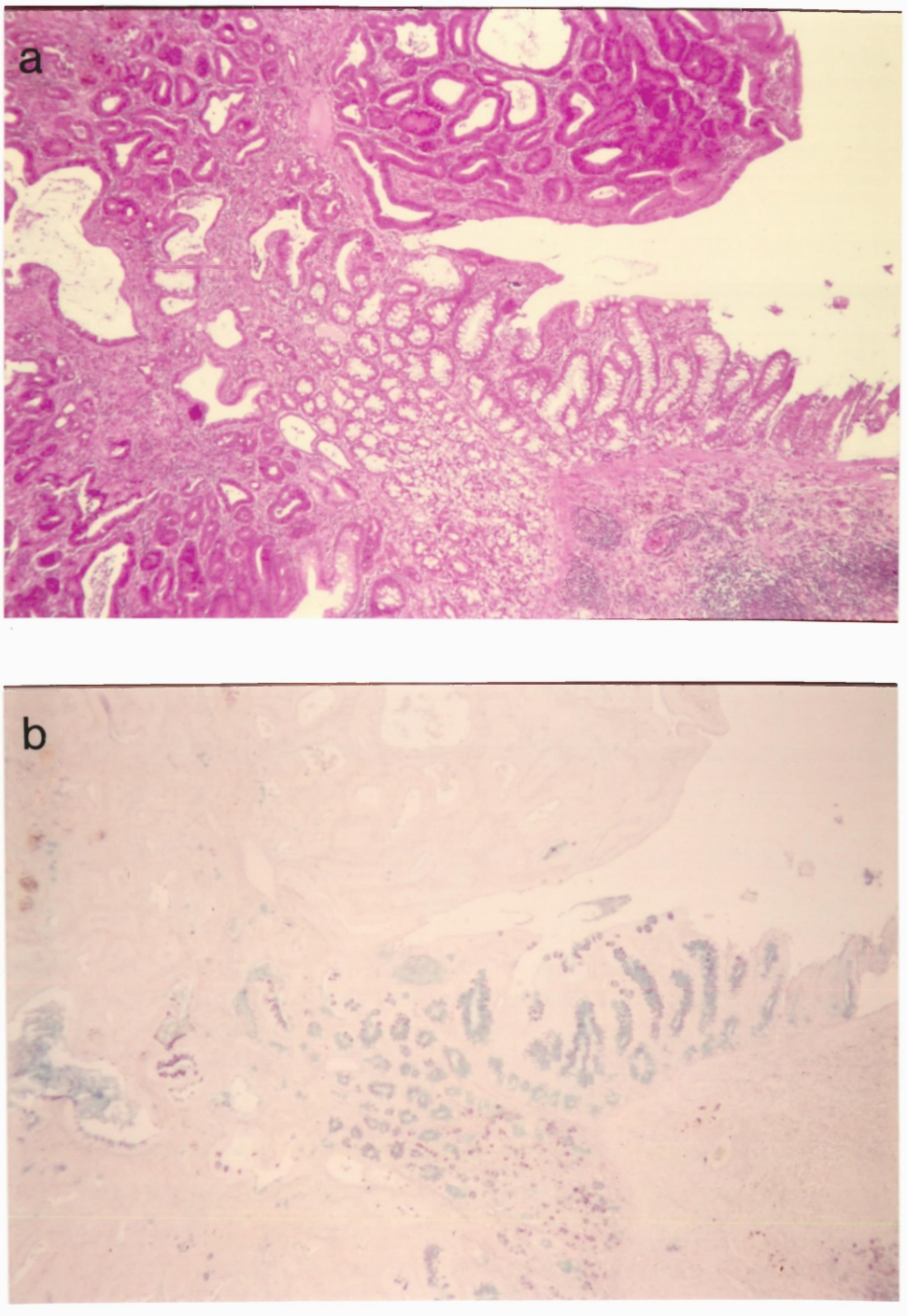


Plate 15 Rat colonic mucosa- benign tubular-adenoma. Normal colonic mucosa may be seen in (a)(H and E x38) which on the corresponding HID-AB section (b)shows transitional change(x38).



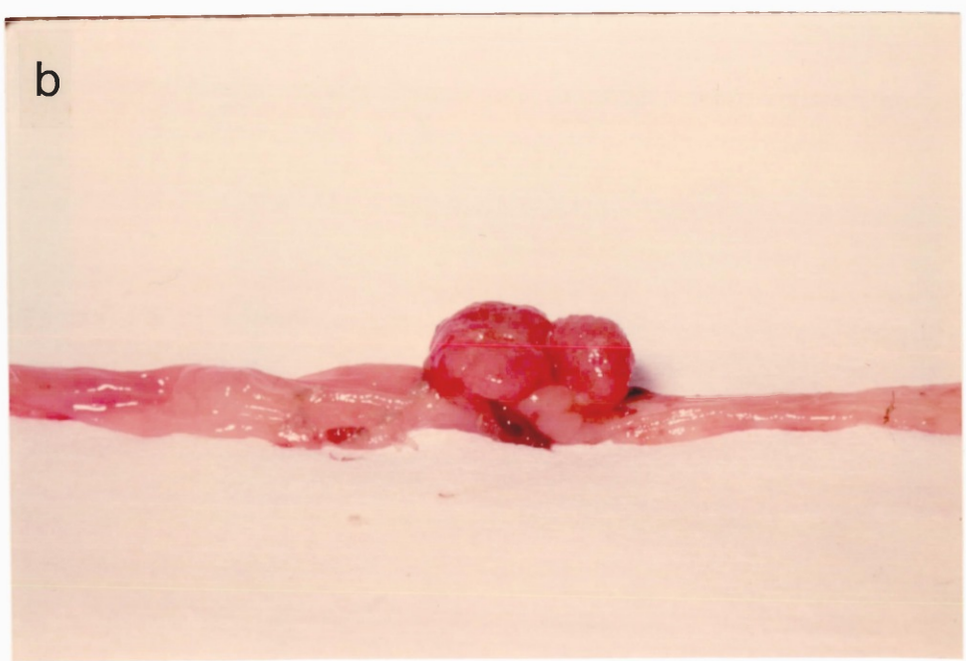


Plate 16 Rat colonic mucosa.Macroscopical views of a typical rat colonic adenocarcinoma induced by weekly injection of 1-2 dimethylhydrazine (week 30)(x1). The tumour has a typical polypoid appearance and occurred in the descending colon (x1).

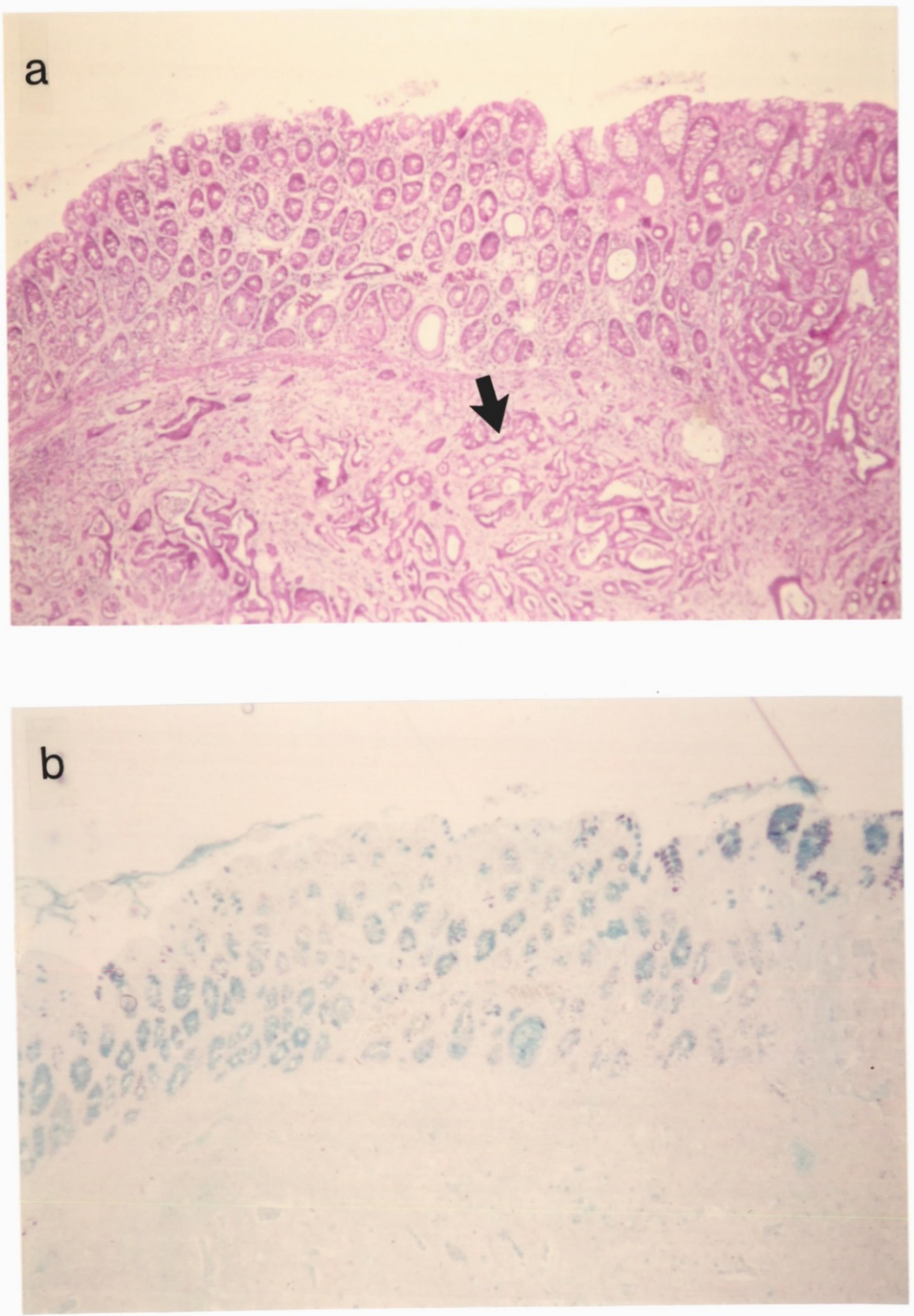


Plate 17 Rat colonic mucosa.Adenocarcinoma invading the submucosa (arrow)(H and E x 38)(a).
(b) The corresponding HID-AB section shows scanty mucus production within the cancer itself, whilst the colonic mucosa adjacent to it demonstrates pure sialomucin secretion. (x 38).

crypt depths secreted almost entirely sialomucin in a patchy field change. Mucin production within the tumour was scanty, showing a mixture of both sialomucin and sulphomucin.

5.4 Summary of the Findings

HUMAN STUDIES

Inflammatory Bowel Disease

* Sialomucin was not found in normal colonic mucosa.
* Sialomucin may occasionally (5%) be produced in response to inflammatory bowel disease.
* Sialomucin occurs more frequently (34%) in the radiation damaged bowel than in inflammatory bowel disease.
* The prevalence of sialomucin was increased in association with dysplasia in ulcerative colitis (83%).
* The presence of sialomucin does not signify pre-malignancy specifically.

Colonic Polyps and Cancer-Prone Colon

* Sialomucin was not found adjacent to human metaplastic polyps
* Sialomucin is found frequently (80%) surrounding human adenomatous polyps with varying degrees of dysplasia.
* Sialomucin may be present in the remaining colon for up to 2 years after resection of colorectal cancer in man

* Sialomucin found at the resection margin (28%) regresses after removal of the primary tumour (10%).
* Sialomucin occurs as a field change in human colorectal cancer (26% prevalence in colon from carcinoma patients).

ANIMAL STUDIES

* Sialomucin change was not seen in 15 animals in the healing model at the site of colonic anastomosis during a 3 month period.
* Sialomucin change was produced in 1 out of 20 (5%) animals with acetic acid induced colitis.
* Sialomucin change was seen colonic mucosa in 9 out of 12 (75%) of animals receiving DMH.
* Sialomucin was rarely produced in response to inflammation but more readily in response to carcinogen.

CHAPTER 6

DISCUSSION

DISCUSSION

6.1 What Is The Biological Significance Of Sialomucin?

The studies reviewed in Chapter 1 suggest that sialomucin change is a morphological response to a stimulus which affects cell proliferation. The nature of this proliferative stimulus could be either non-specific - for example inflammation adjacent to a carcinoma or other non-malignant conditions, or alternatively it could be part of the neoplastic process. In the former case the prevalence of sialomucin change would be greatest where inflammation was most marked, whereas in the latter it would be greatest in the presence of neoplasia.

In the study outlined in Chapter 5, sialomucin was frequently (80% prevalence) present adjacent to adenomatous polyps and as previously noted (Filipe et al 1981), this was independant of degree of dysplasia. Sialomucin was not found adjacent to metaplastic polyps which are not neoplastic (Muto et al 1975). Since the prevalence of change was so great adjacent to adenomatous polyps, there was little further increase in prevalence among carcinomas (86% prevalence).
Although the size of lesion has been suggested as important in sialomucin production (Jass et al 1984), a relationship with tumour size was not demonstrated in the carcinoma study in Chapter 3.

With the exception of one case of ulcerative colitis, patients with Crohn's disease, diverticular disease and ulcerative colitis (without dysplasia) did not show sialomucin change. The cause of sialomucin change in the one exception is not clear, possible reasons include extent and/or duration of disease (Ehsanullah et al 1982a).In this case the disease had involved the entire colon for over 20 years, and was the most long-standing case examined. Sialomucin has previously been reported in association with Crohn's disease and diverticular disease (Filipe, 1969, Ehsanullah et al 1982a). This divergence from the findings of the present study may be due to differences in staining technique. Comparison of prevalence of sialomucin change between different studies should be avoided (Cook, 1988).

Sialomucin change was identified in 34% of cases of radiation injury where it appeared to be associated with morphological abnormalities of colonic mucosa (regeneration, gland distortion and inflammation). This is a lower prevalence than that of sialomucin adjacent to dysplasia in ulcerative colitis (83%). The malignant potential in radiation injury is only X2-3.5 that of normal (Sandler and Sandler,1983), whereas that of ulcerative colitis is more than tenfold greater than this (Morson and Pang 1967).

These findings suggest a considerable increase in prevalence of sialomucin in association with either neoplasia or where

there is a significant risk of neoplasia as in dysplasia in ulcerative colitis. However, it is also clear that sialomucin change can less frequently develop in the absence of any significant risk of neoplasia. Irradiation increases the probability of sialomucin change, but not to the same extent as neoplasia. The reason for radiation damage stimulating sialomucin production is not clear. Perhaps radiation directly affects the cellular genome. Alternatively ischaemia may be responsible as is presumably the case with mucin changes which are seen in solitary ulcer syndrome (Ehsanullah et al 1982b).

The colonoscopy study (Chapter 5.2) suggested that sialomucin was more likely to occur in the presence of carcinomas or polyps than as a field change in a cancer-prone colon. Even though these patients were prone to colonic carcinoma, the prevalence of sialomucin in the remaining colon after resection was less (26%) than in association with adenomatous polyps (80%) or colorectal cancer (86%). Sialomucin secretion appeared to regress from the resection margin after resection of the primary tumour. However the prevalence was greater than in the normal colon. This may be a consequence of the hyperplasia which is said to occur after colon resection or by-pass (Williamson et al 1982, Olubuyide et al 1985). As with radiation-injury the sialomucin which was found in the cancer-prone colon was associated with

morphological abnormalities of the mucosa (distortion of glands - 50%; mild dysplasia - 50%; inflammation - 20%).

Isolated sialomucin change was found both in the human colorectal cancer study (Chapter 3) and in the residual colon study for up to 2 years after primary resection (Chapter 5.2). This suggests a stimulus extending throughout the colon since neither mucosal prolapse, ischaemia, nor inflammation could be implicated as the cause of sialomucin occuring in these isolated discontinous areas.

The animal studies (Chapter 5.3) confirmed these findings. Sialomucin could be produced in response to either trauma or inflammation. However the incidence of this response was low (5%). There was no evidence that sialomucin was produced by repair and proliferation at colonic anastomoses. This was despite the proliferative instability that is known to occur at this site for up to 3 months (Roe et al, 1987). By contrast in the rat colon cancer model, sialomucin was produced far more readily. Sialomucin developed in 75% of rats receiving 1,2-dimethylhydrazine as a field change throughout the left side of the colon. As previously reported (Filipe et al, 1975; Traynor et al, 1983) sialomucin appeared prior to the appearance of macroscopically-evident tumour. Therefore the appearance of sialomucin could not be explained as a secondary phenomenum resulting from the

presence of a large tumour or mucosal prolapse (Issacson and Attwood, 1979).

Thus both the human and animal experiments suggest that while sialomucin may occasionally be produced in response to inflammation (5% prevalence), it is much more commonly found adjacent to neoplasia, (80% polyps; 86% cancers) or where there is a risk of neoplasia (cancer-prone colon 26%, irradiated colon 34%, ulcerative colitis with dysplasia 83%). Sialomucin appears to be initiated by abnormal cell proliferation, arising either from injury or neoplasia, but more readily where the proliferation carries a risk of neoplasia. Sialomucin change, therefore, appears to be a cancer associated, but not cancer specific, abnormality.

6.2 What Does Sialomucin Tell Us About The Tumour?

It appears that in the presence of carcinoma sialomucin change can be assumed to be a consequence of the neoplastic process. In this study (Chapter 3) the sialomucin band was significantly longer in tumours which had penetrated the bowel wall to invade adjacent structures than in tumours which had not. Where the sialomucin band extended more than 3 cm from the tumour, there was a 70% chance that the primary tumour had extended to adjacent structures. Absence of a band of this length was associated with a 66% probability that the growth had not reached other structures. The high false negative rate (34%) presumably arises because there are

many other factors which affect local tumour invasion (in particular tumour position and host response). The length of sialomucin zone surrounding the tumour did not correlate with either Dukes' stage, degree of differentiation or size of tumour. These relationships held up both for right and left-sided colonic carcinomas and for rectal carcinomas. Thus it appears that length of sialomucin band does provide information about depth of invasion of the tumour. Sialomucin band was not a significant predictor of which tumours will metastasise (Chapter 4). Although local invasiveness and metastatic potential are frequently associated, it is likely that they are the result of different cell functions. The reason why sialomucin change did not predict metastatic potential but did predict ability to invade locally cannot be identified until more is known of factors controlling local invasiveness and metastasis. For example, if local invasion was dependant on tissue levels of capillary growth factors and if sialomucin change also reflected tissue levels of capillary growth factors, then the length of sialomucin might reflect local tumour invasiveness. The findings in this study suggest that length of sialomucin band does provide information about depth of invasion of the tumour, although the reasons for this are far from clear.

6.3 Can Sialomucin predict Local Recurrence?

As discussed in Chapter 1, there are other factors in addition to depth of invasion which affect local recurrence.

The most important of these are site of the tumour and experience of surgeon. In both of these cases the common factor is an inadequate resection - either because of the constraints of the pelvis or because of surgeon-inexperience. Where there is a locally invasive carcinoma there is a 70% probability of a >3cm sialomucin band. If the resection has been unsatisfactory it is likely that the distal margin of excision will be short. The result is that an inadequate excision of an invasive tumour should be characterised by the presence of sialomucin at the distal resection margin. An invasive tumour resected with a poor margin is the combination of circumstances which results in local recurrence. Thus, local recurrence can be expected to be associated with a primary tumour which contain sialomucin at the resection margin.

The study outlined in Chapter 4 demonstrated that several prognostic variables are associated with local recurrence and survival after potentially curative resection (Appendix C). The presence of a sialomucin band greater than 3 cm from the tumour identified significantly more patients with local recurrence ($X^2=5.5$, $p<0.02$) than if such a band was not present. This was confirmed by multivariate analysis which suggested that a band of >3cm was a significant independant predictor of local recurrence ($X2 = 4.59$, $p<0.006$) coming after Dukes stage and Hospital of operation in order of predictive power.

The predictive power was greater when sialomucin was present in the resection margin ($X^2=14.19$, $p<0.001$). Using multivariate analysis, sialomucin at the resection margin was a stronger predictor of local recurrence than any other variable including Dukes' stage. Dukes' stage remained the most important independant variable predicting overall survival including distant metastases. In cases where the ileal or anal resection margin was included, sialomucin band may be unreliable. However, patients who had colonic restorative procedures (therefore excluding this bias) also showed the same predictive power. The separate analysis for rectal carcinoma did not reflect this trend although sialomucin was a powerful predictor of subsequent survival.

As expected, histological differentiation, fixity and invasion of blood vessels by the primary tumour were significant determinants of survival (Appendix C). The height of rectal tumour from the anal verge influenced subsequent survival, but not local recurrence when analysed separately (Chapter 4). Size of tumour, operation performed or site of tumour made no difference either to recurrence or survival. In contrast to other studies (Williams et al, 1982; Pollet and Nicholls, 1982), distal margin of excision (<5cm) was an important variable for both local recurrence and survival.

Thus sialomucin change at a carcinoma resection margin was the strongest predictor of local recurrence which could be identified. Sialomucin appears to be a tumour associated abnormality whose presence can be used as a test to predict which tumours are at increased risk of local recurrence.

It is likely that most local recurrences are the result of inadequate excision (Quirke et al, 1986; Heald and Ryall, 1986). Although lateral spread has been shown to accurately predict local recurrence of rectal carcinoma, it is a time consuming test and the result is available after the operation. Sialomucin , however could be used as a pre-operative test of local recurrence - particularly for identifying patients at lower risk. If a >3cm sialomucin band is not found adjacent to a carcinoma on pre-operative colonoscopy, then there is only a 14% chance of local recurrence after standard resection (Chapter 4). Alternatively if a >3cm band is present, then there is a 50% chance that this is a tumour which will be locally aggressive. Operation by a more experienced surgeon and adjuvant radiotherapy should then be considered.

As a test of recurrence, if sialomucin is found at greater than 3 cm from the tumour then the sensitivity is 44%, the specificity 75% and overall accuracy 70% (Figure 12) . If sialomucin is found at the resection margin then the sensitivity is increased slightly to 48%, the specificity 75%

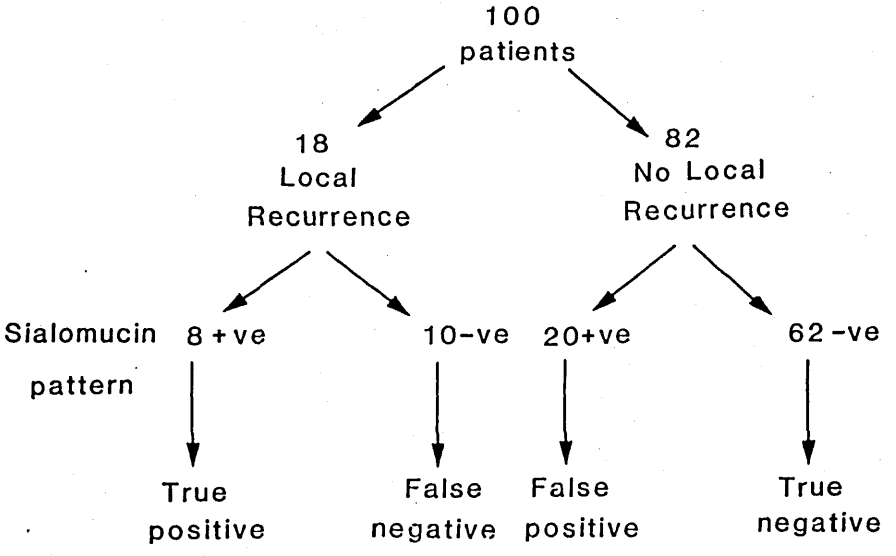


Figure 12 Predictive value of sialomucin >3cm for local recurrence.
The true and false positive and negative results are demonstrated in 100 tests. The corresponding sensitivity and specificity and overall accuracy of the test for local recurrence is demonstrated.

and overall accuracy 70%. If 100 tests were performed a positive sialomucin test would correctly identify half of those patients who developed local recurrence, and a negative sialomucin test would identify 75% of patients who would not develop local recurrence. This is a poor test when compared with pathological identification of lateral spread of tumour (95% accurate). Similarly when compared with CT of the pelvis (91% sensitive, 90% specific and 93% accurate) in determining local spread of rectal cancer (Williams et al,1985) sialomucin appears to be a poor test. Indeed it is marginally worse than digital examination of the rectum in the determination of tumour invasion (Nicholls et al 1982) . It is however of equal accuracy to serum CEA in the prediction of local recurrence (Northover, 1986). The advantage of the sialomucin test is that it can be used pre-operatively for all colorectal carcinomas. No other test has so far demonstrated this versatility. In addition, expensive and sophisticated apparatus is not required.

The high-iron diamine alcian-blue stain is a simple inexpensive technique which offers the prospect of identifying patients at risk of locally recurrent tumour at an earlier stage. Using this test pre-operatively to identify those patients who are at low risk of local recurrence, leaves a group who are sialomucin positive in whom there ia a high risk of local recurrence. Radical surgery by an experienced surgeon, with adjuvant radiotherapy

or chemotherapy may reduce local recurrence in this high risk group and result in improved survival in patients with colorectal carcinoma.

APPENDIX

(A) Participating surgeons and Pathologists

(B) Protocol (abstract)

(C) Examples of life table of survival for the whole series and colo-colic series

(D) Statistical appendix

APPENDIX A

The following consultant surgeons and pathologists consented to be in the multicentre trial:

Surgeons	Pathologists
Hammersmith Hospital	
Mr C B Wood	Professor N A Wright
Mr J Spencer	Dr H C Rees (HCR)
	Dr T Krausz (TK)
Windsor Hospitals	
Mr J W Blaxland	
Mr R J Luck	Dr K D Dempster
Mr G S Barrett	
Wexham Park, Slough	
Mr P G Cassell	Dr M H Ali
Mr S Knight	
Royal Berkshire and Battle Hospital	
Mr N G Rothnie	Dr L Horton
Dr D P Goodwin	Dr N Williams
Mr R G Faber	Dr P DaCosta (PDC)
Mr C S Kirkham	
Bristol Royal Infirmary	
Professor R C N Williamson	Professor J W Bradfield
Mr N J McMortensen	Dr G Igboaka (GI)
Mr D J Leaper	
St Peters, Chertsey	
Mr M Thomas	Dr N A Ratcliffe
Mr C J Anders	

APPENDIX B

PROTOCOL (abstract)

Any patient who is considered to have had a curative resection for colorectal carcinoma will be eligible for entry into the trial. Written permission from the consultants concerned to study their patients will be obtained.

Specimens from the proximal and distal resection margins will be obtained from the Histopathology Department by arrangement with the Consultant Histopathologist concerned. The specimens will be processed after fixation in 10% Formal saline at the Royal Postgraduate Medical School, London W12. All slides will be assessed by the authors and a qualified pathologist.

Access to the patients' case notes is required by arrangement with the Medical Records Department for prospective follow-up. Particular note will be made of the development of local recurrence. Patients' management according to the consultants preference will not be altered or added to in any way and no extra procedures will be performed as part of the trial.

Before starting the study, permission from the local Medical Ethical Committee will be obtained.

APPENDIX C

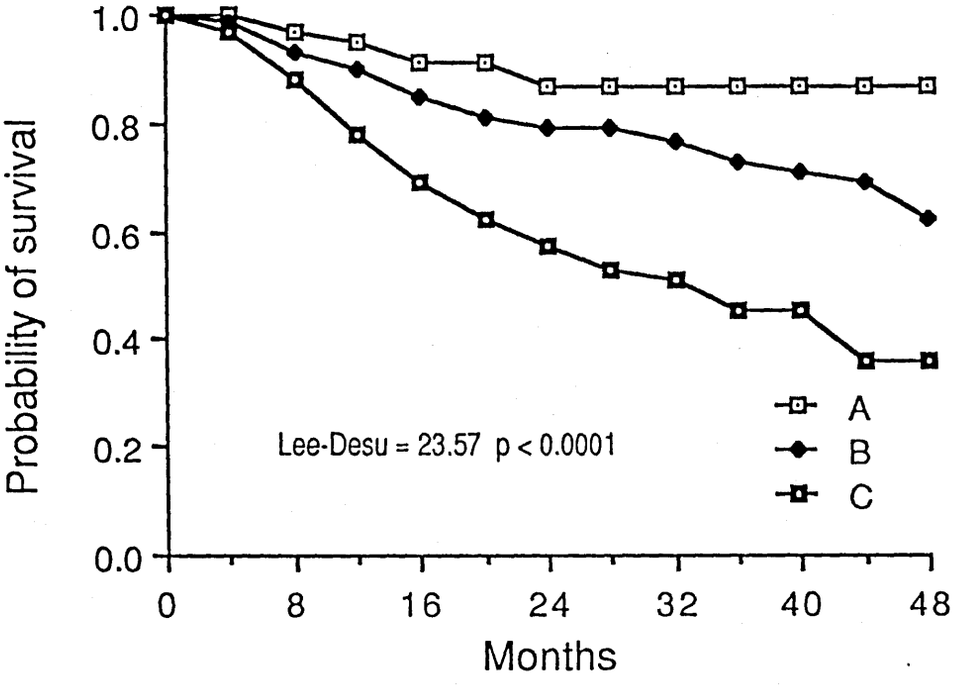
Examples of life tables of survival for the whole series and colo-colic series

The first series of table are from the whole series of patients

LIFE TABLE OF SURVIVAL

DUKES' STAGE

(n=358)



Dukes A	041	041	040	039	028	026	022	019	014	011	009	009	006
Dukes B	206	205	196	184	151	136	120	099	081	071	042	034	026
Dukes C	111	111	101	089	069	055	043	031	025	022	015	009	005

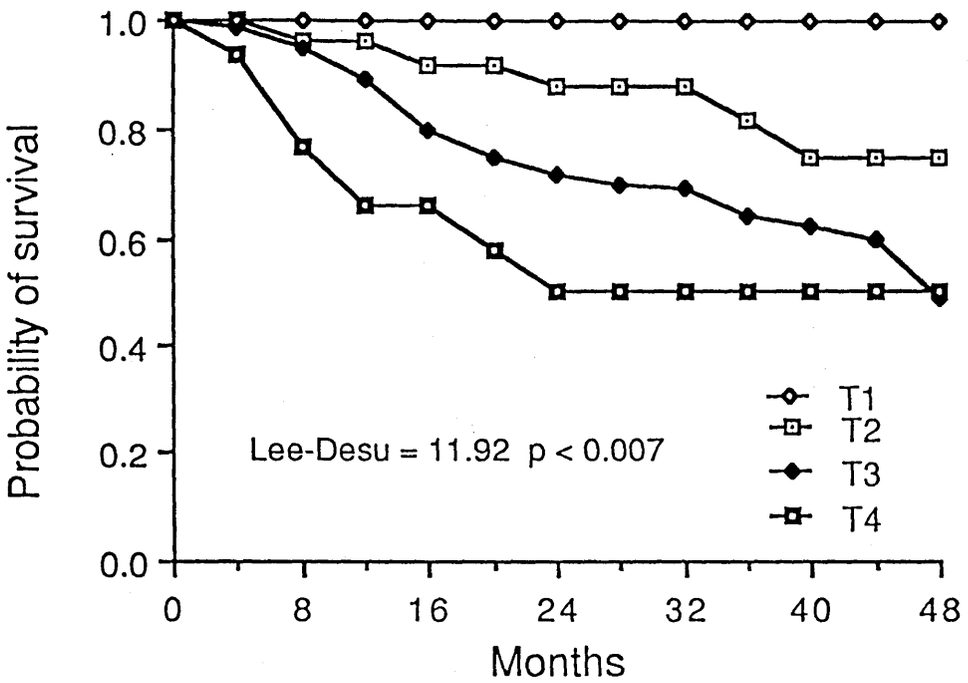
No of patients at risk

This life table for the whole series demonstrates as would be expected that patients with Dukes' B and C carcinomas have a worse prognosis than those patients with Dukes' A.

LIFE TABLE OF SURVIVAL

DEPTH OF INVASION

(T STAGE)



T1	008	008	008	008	006	005	004	003	002	002	002	002	002
T2	030	030	029	029	023	022	022	021	018	013	010	009	007
T3	206	205	198	185	137	112	097	079	063	053	031	023	016
T4	018	018	014	011	010	008	006	005	005	005	004	003	002

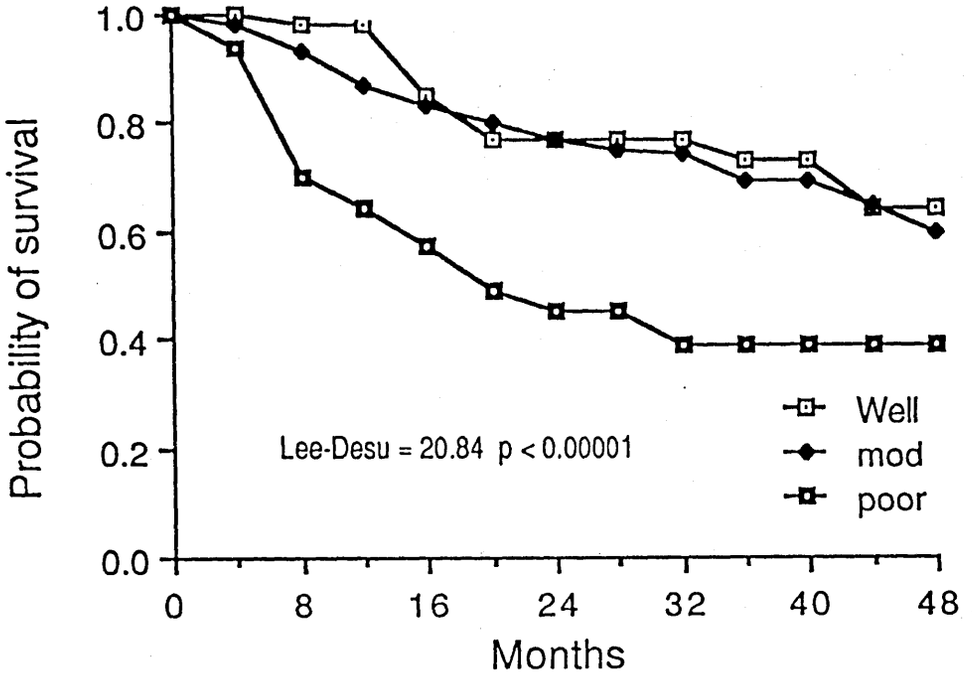
No of patients at risk

This life table demonstrates that there is a poorer prognosis for patients with stage T_4 carcinomas than stage T_1.

LIFE TABLE OF SURVIVAL

DIFFERENTIATION

(n=358)



Well	056	056	054	052	039	034	029	021	019	019	011	010	005
Moderate	257	256	247	229	186	166	141	115	092	078	051	039	030
Poor	034	034	026	021	017	012	010	009	005	004	003	002	001

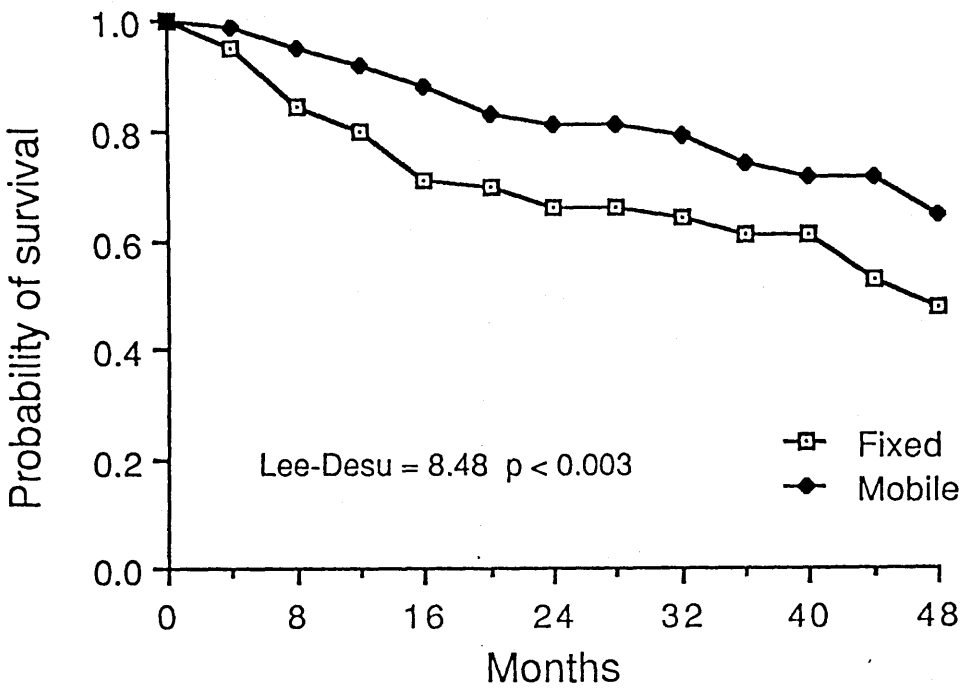
No of patients at risk

As would be expected patients with poorly differentiated tumours have a worse prognosis than those well and moderately differentiated tumours

LIFE TABLE OF SURVIVAL

FIXITY

(n =230)



Fixed	066	066	058	051	042	041	035	031	024	021	017	012	008
Mobile	164	163	157	151	132	118	103	086	068	059	032	028	021

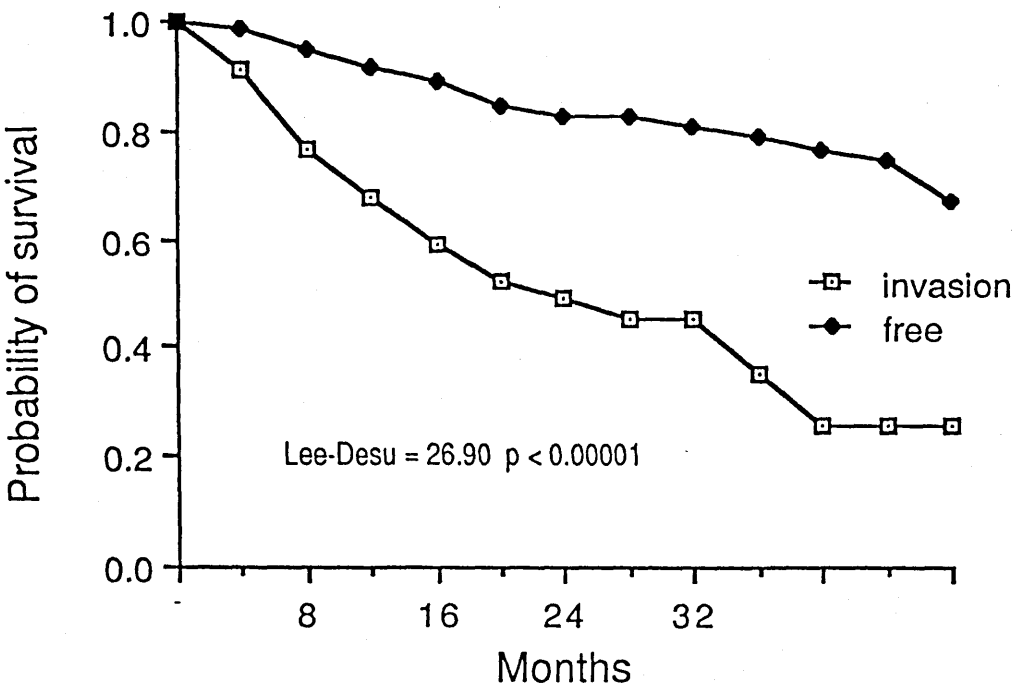
No of patients at risk

This life table demonstrates that fixed tumours have a worse prognosis than those which the surgeon considered mobile at operation.

LIFE TABLE OF SURVIVAL

INVASION OF BLOOD VESSELS

(n = 226)



None	191	190	184	176	153	138	123	103	081	072	045	038	027
Invasion	035	035	028	023	019	019	014	012	010	007	004	002	002

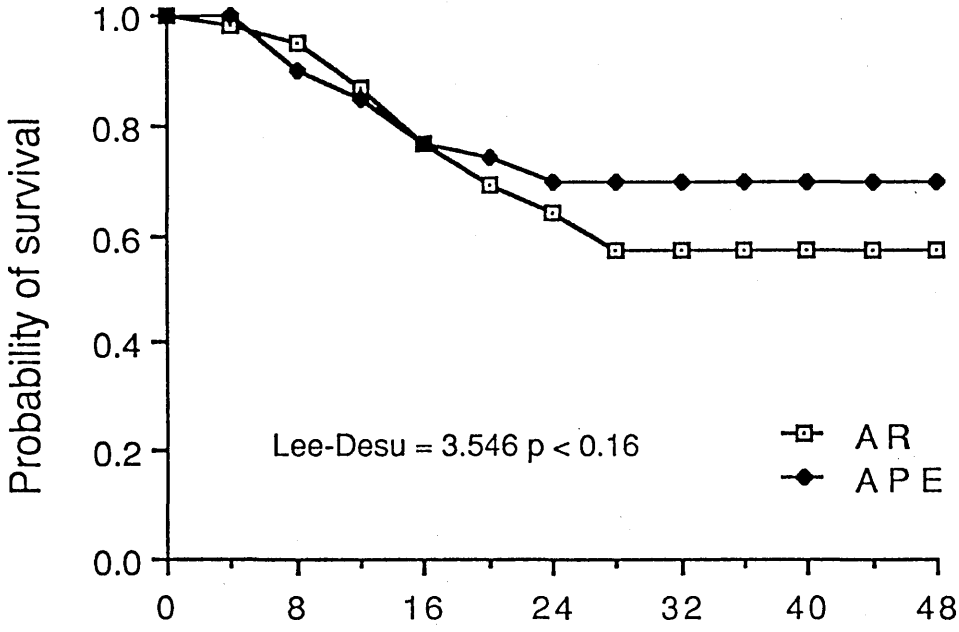
No of patients at risk

If there was histological proof that the tumour had invaded blood vessels then a worse prognosis ensued

LIFE TABLE OF SURVIVAL

ANTERIOR RESECTION VS ABDOMINOPERINEAL EXCISION

(n = 143)



Ant R	081	080	078	070	047	033	025	013	006	002	002	002	002
APE	042	042	039	037	027	021	018	009	003	001	000	000	000

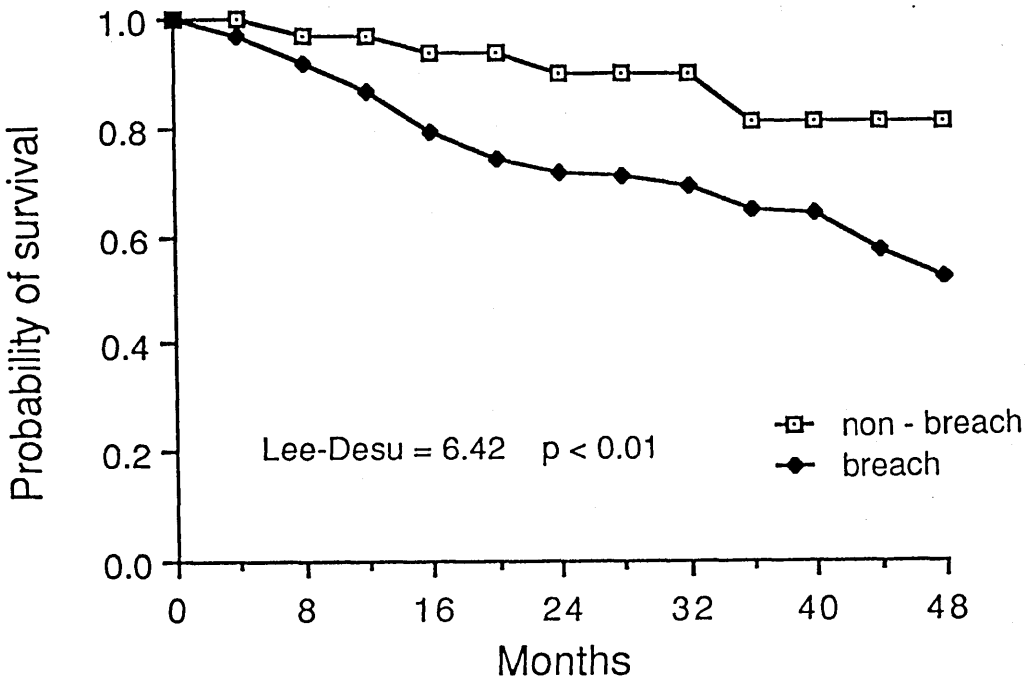
No of patients at risk

There was no significant difference between survival of patients treated by either abdominoperineal resection or anterior resection for rectal carcinoma

LIFE TABLE OF SURVIVAL

BREACH OF BOWEL WALL

(n=262)



Invasion	223	222	210	195	153	134	118	102	088	079	049	036	025
None	038	038	037	037	030	029	028	026	023	018	014	013	010

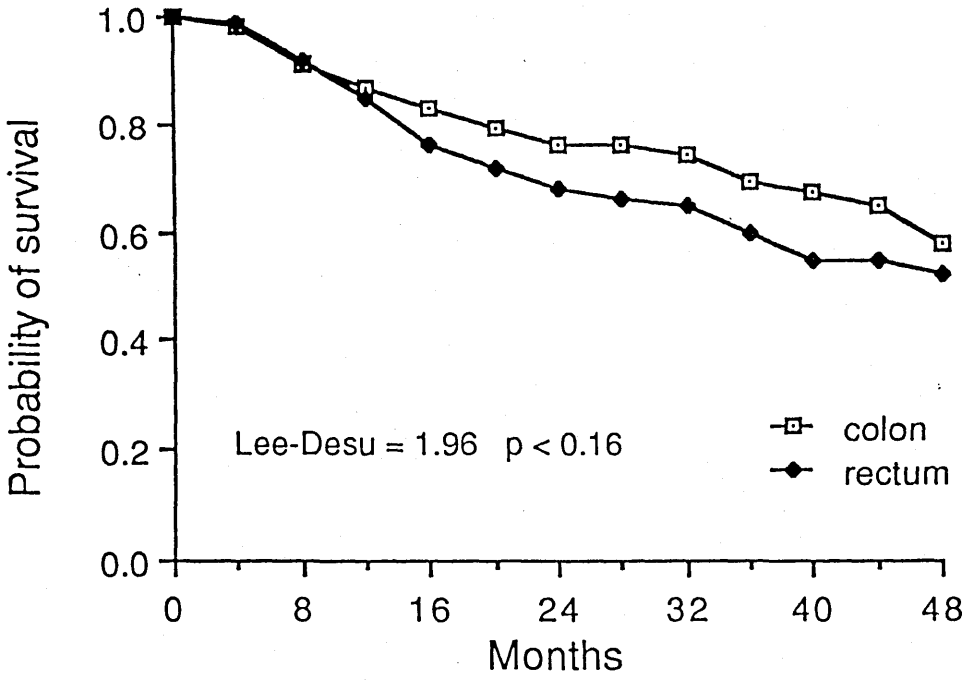
No of patients at risk

As would be expected, those patients with carcinomas that had extended through the bowel wall had a worse prognosis.

LIFE TABLE OF SURVIVAL

COLON VS RECTUM

(n=358)



Colon	225	225	211	117	156	134	112	086	069	061	039	031	019
Rectum	128	127	121	110	087	078	070	060	049	041	026	020	017

No of patients at risk

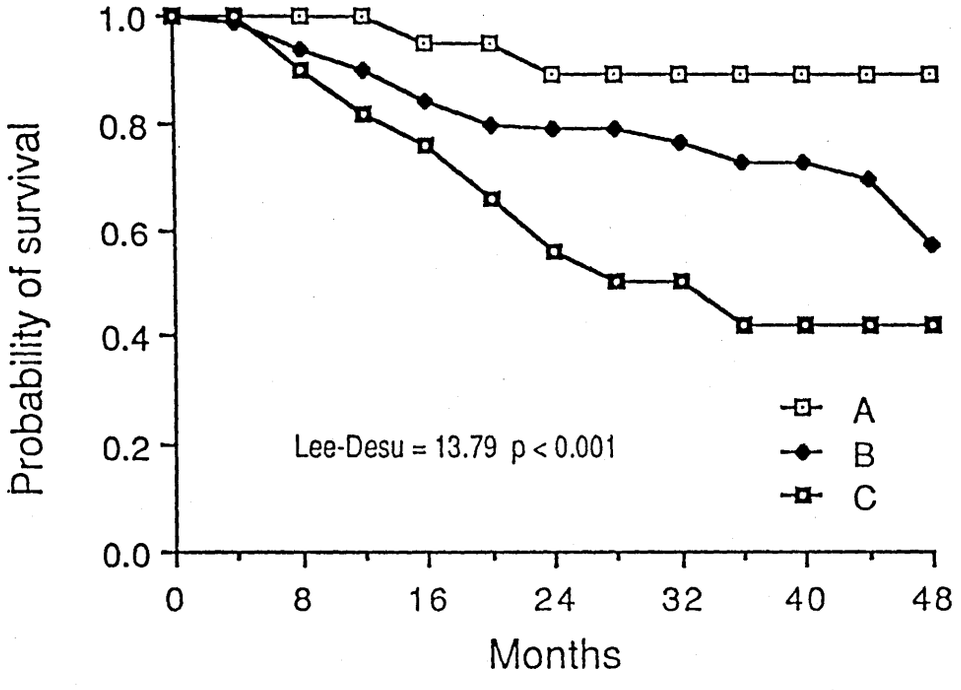
There was no overall difference in survival between colonic and rectal carcinomas

The following series of life tables are selected from the colo-colic/colorectal anastomosis series only

LIFE TABLE OF SURVIVAL

DUKES' STAGE

(COLO-COLIC)



Dukes A	029	029	029	029	020	018	016	014	010	009	008	008	006
Dukes B	117	116	114	104	085	076	068	058	044	039	023	021	014
Dukes C	062	062	058	051	041	032	023	015	013	012	008	006	005

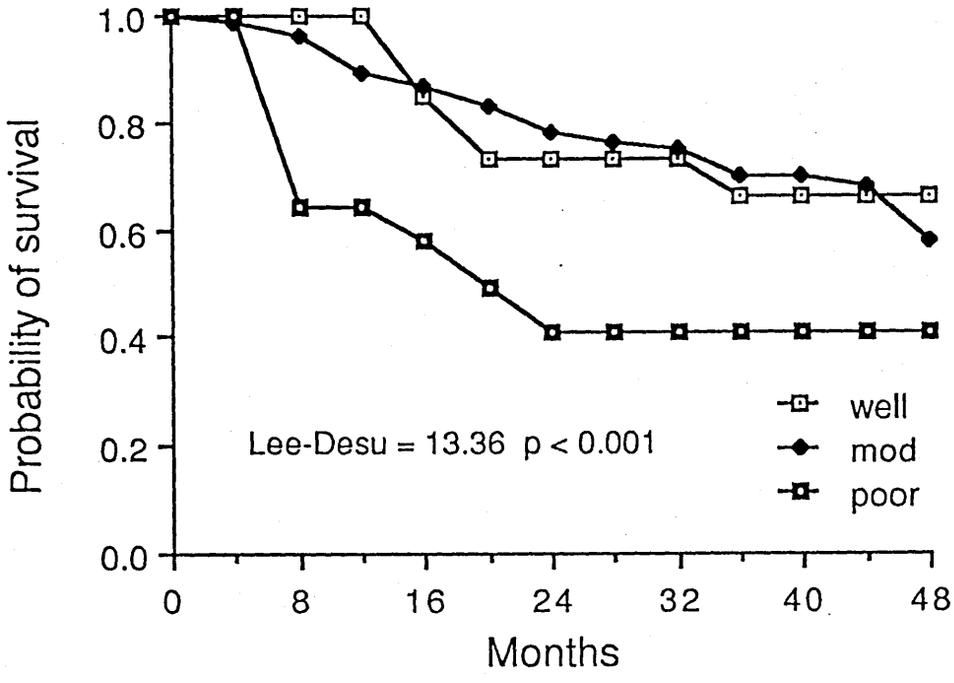
No of patients at risk

As would be expected, Dukes stage influences survival

LIFE TABLE OF SURVIVAL

DIFFERENTIATION

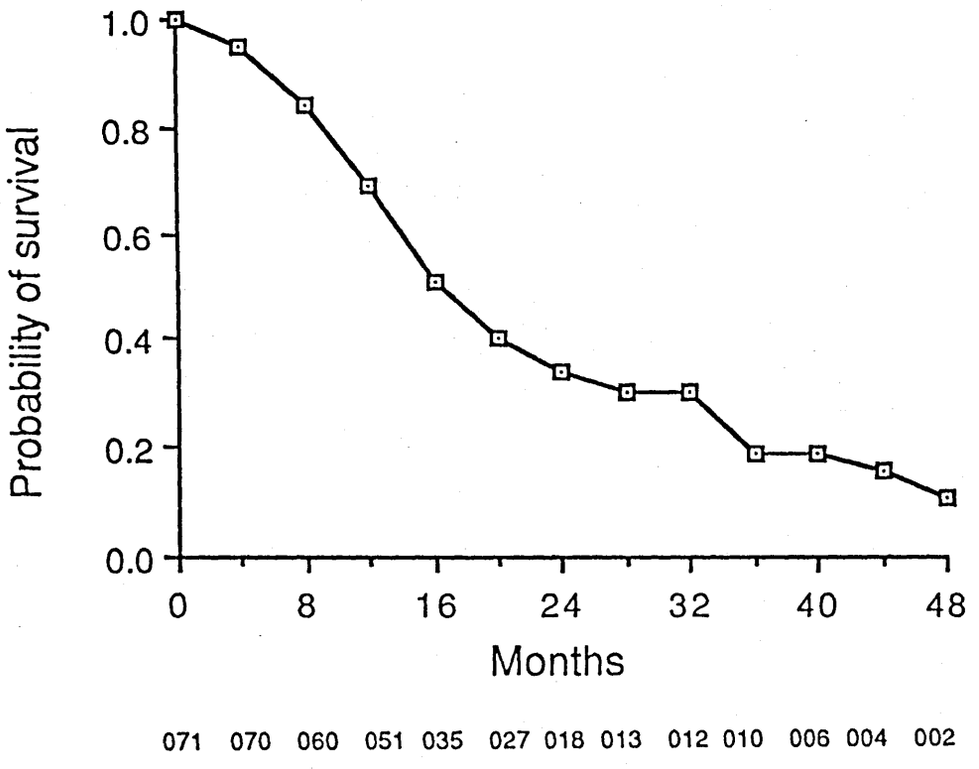
(COLO-COLIC)



Well	035	035	035	034	025	020	016	012	011	011	007	006	004
Moderate	152	151	149	136	110	099	085	069	051	044	028	026	019
Poor	017	017	013	010	009	006	005	005	004	004	003	002	001

No of patients at risk

Patients with poorly differentiated tumours have a worse prognosis



Life table of patients in whom local recurrence was identified

APPENDIX D

Statistical Appendix

The Cox regression model provides for an overall comparison of survival curves and permits adjustment for the effects of covariates (variables present at the time of operation) in the analysis of survival data. The model assumes that the death rate as a function of time depends on the covariates (denoted $\underset{\sim}{Z}$) by a simple multiplicative factor, ie

$$\lambda z(t) = \lambda_o(t)\ h(\underset{\sim}{Z})$$

where $\lambda_o(t)$ is the death rate (or "hazard" function when the covariates take a standard value specified by $\underset{\sim}{Z}_o$, and

$$h(\underset{\sim}{Z}) = e\beta^1 z^1 + \beta^2 z^2 + \ldots + \beta^p z^p$$

is a multiplier depending only on the covariate values and not on time. (For this reason the model is often referred to as the "proportional hazards" model). The survival function and death rate function are related by the following equation:

$$S(T) = e^{-\int_0^T \lambda(t)dt}$$

The covariates, Z_1, Z_2, ..., Z_p, may be continuous variables such as age at operation, tumour size, number of positive axillary nodes, etc, or they may correspond to categorical (0,1) variables that designate pathologic state, presence or absence of tumour in the infraclavicular nodes, etc. Treatment group is also expressed as a "covariate". In our analysis, for example:

Z_1 = (1 anterior resection

(0 abdomino-perineal resection

$\beta_1, \beta_2, \ldots, \beta_P$ are regression coefficiencts analogous to those given in ordinary multiple regression and subject to the same interpretations. Thus, each β_i measures the effect of the i^{th} covariate after allowing for the effects of the other covariates included in the model. For example, if treatment group is the only covariate specified (P=1), the β_1 estimates the difference in the logarithms of the death rates in the two treatment groups, so that $e\beta^1$ estimates the ratio of the two death rates. If other covariates are included in addition to treatment group (P>1), then β_1 reflects the difference in the (log) death rates adjusted for these other covariates. Positive regression coefficients from the Cox model correspond to higher death rates (ie, poorer survival) for larger covariate values, and negative coefficients correspond to lower death rates (ie, better survival). Significance tests for treatment and other covariates are performed by computing Z values given by $\hat{\beta}_i/SE(\hat{\beta}_i)$. The level of significance is determined by referring to the table of the normal distribution. For example, Z values greater than 1.96 in absolute value are judged statistically significant at the 5% level, and values greater than 2.58 are judged significant at the 1% level.

Statistic D. For use in sub-group comparisons, a statistic D is calculated from the survival scores using the algorithm of

Lee and Desu (1972). D is asymptotically distributed as Chi-square with g-1 degrees of freedom, where g equals the number of groups, under the null hypothesis that the subgroups are samples from the same survival distribution. The larger the D statistic, the more likely the subgroups come from different survival distributions.

Survival score. A survival score calculated for each observation by comparing its survival time to that of all other observations. The score starts at zero and is increased by one for each observation whose survival time is known to be lower and decreased by one for each observation that is known to be higher. If one of the observations is censored the censored observation is considered to have a greater survival time since it is known to have survived at least as long as its survival time.

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