A STUDY OF THE EFFECT OF URINARY DIVERSION ON COLONIC MUCOSA

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ABSTRACT

Patients with ureterosigmoidostomies have a high incidence of tumours, and this study clarifies the increased risk. The incidence of tumours with colonic diversions was 22%, whereas it was zero with ileal conduits, in exstrophy patients followed up for many years.

A rat model was developed, to mimic ureterosigmoidostomy with a low morbidity and mortality. Further animal groups were examined to determine the effect of a urine/faeces mix, urine and urothelium at the urothelial colonic anastomosis. The animals were studied using crypt cell production rate, cellular DNA content measurement, histochemistry and histology. The histology and crypt cell production rate confirmed the usefulness of measuring the cellular DNA content of colonic brushings. Urine caused hyperplasia of the colonic mucosa and the presence of faeces promoted these changes at the anastomosis, producing tumours. The urothelium within the colon also had the effect of producing tumours. Histochemical studies using the high iron diamine alcian blue stain were performed looking for "transitional mucosa" which is thought to be a pre-malignant marker. The animal study did not detect "transitional mucosa".

Histochemical staining and the measurement of cellular DNA content was performed on colonic biopsies from ureterosigmoidostomy patients. There was a change from

sulphomucin to sialomucin detected with the ureterosigmoidostomy patients but there were no other histological features of "transitional mucosa" seen. The cellular DNA content of the colonic mucosa away from the anastomosis showed no hyperplasia.

The animal model demonstrated hyperplasia of the colonic mucosa in the presence of urine, but this change was not seen in ureterosigmoidostomy patients. The latency of this tumour is approximately 30 years and any change could be too subtle to demonstrate. However, the animal studies would suggest that all patients with urine in contact with the colonic mucosa should have extended follow up because of the neoplastic risk.

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STATEMENT OF ORIGINALITY

The work performed for the presentation of this thesis has not been carried out before. It has used a novel animal model to examine the effect of urinary diversion on the colonic mucosa. The work on the anastomosis group of animals was performed in collaboration with Joyce Matthews (Chapter 9) as was the collection of data in the control patients (Chapter 12). Otherwise all the other contents within this thesis were collected and performed by me.

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SECTION

I

INTRODUCTION

CHAPTER 1

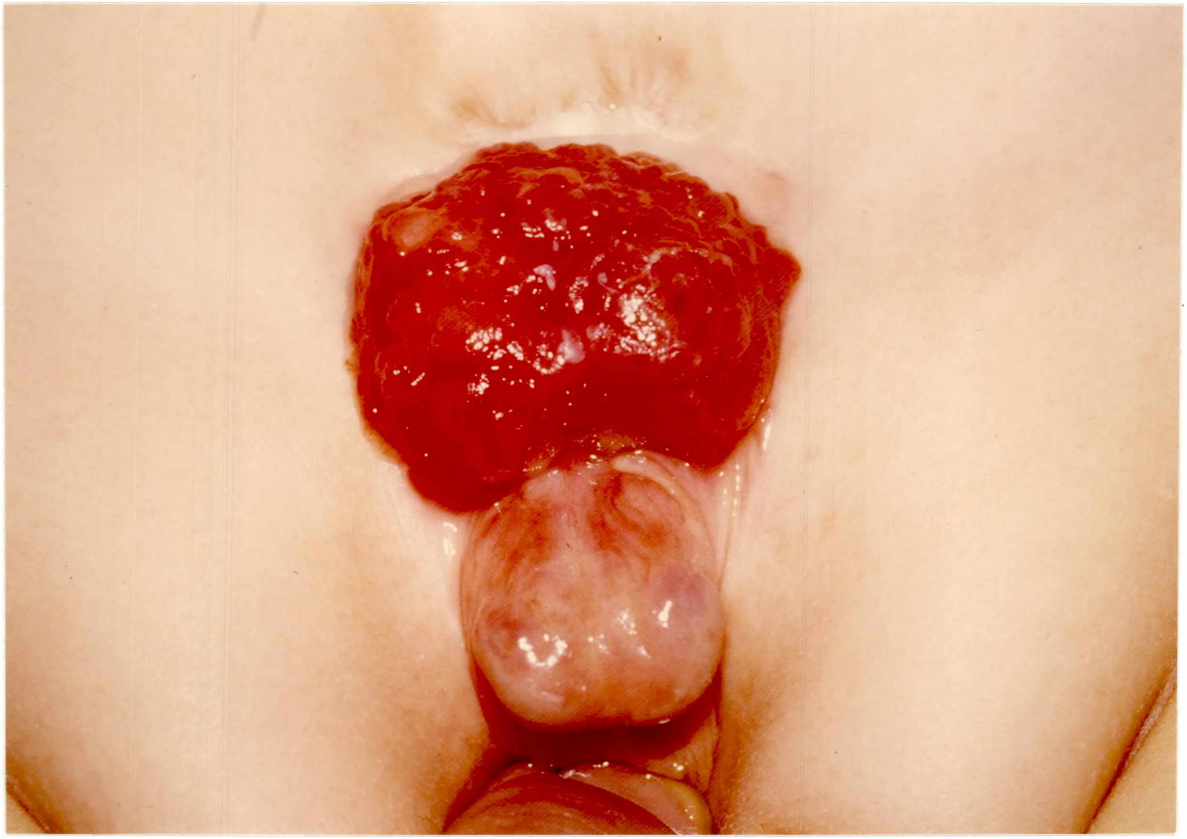
INTRODUCTION

The problem of urinary incontinence associated with ectopia vesicae prompted surgeons to look for a method of diverting urine away from the abdominal wall (Fig. 1). The first urinary diversion into the colon was attempted by Lloyd (1851). A fistula was created between the posterior bladder wall and the rectum in an adult with ectopia vesicae. This was performed by passing a seton between the posterior bladder wall and the rectum. The patient unfortunately died a few days later from peritonitis as the small bowel had been perforated during the procedure. A year later Simon (1852) created fistulae between the ureters and the rectum passing setons blindly up the ureter and into the rectum, as the instrument he devised for the operation failed to function. This patient developed upper urinary tract infections, stenosis at the ureterocolic fistulae and ureteric calculi before dying a year later. In 1894, Maydl developed the technique of excising an ellipse of bladder with the ureters and anastomosing this to the upper rectum, achieving some success at diverting the urine into the rectum. Urinary diversion into the colon was extended to total cystectomy (Kuster, 1891), ureteric fistulae (Chaput, 1894) and bladder tuberculosis.

Ureterosigmoidostomy

Sir Thomas Smith (1879) implanted the left ureter into the descending colon by an extraperitoneal route. Post

Fig. 1.



Photograph of child born with bladder exstrophy.

operatively a cutaneous fistula resulted which eventually closed spontaneously. The patient underwent a second operation, where the right ureter was implanted into the caecum, and the patient died a few days later. Post-mortem examination revealed infection of the kidneys and stenosis of both ureters. Modifications of this technique were performed by other surgeons, by anastomosing the distal ureter into the rectum.

It was not until the early twentieth century that Stiles (1911) and Coffey (1921) developed the ureterosigmoidostomy by a ureteric pull through method. These methods helped to popularise ureterosigmoidostomy as the diversion of choice. Stiles performed this by pulling the ureter through the colon and fixing it to the inner wall of the colon and muscularising the tunnel, while Coffey produced a submucosal tunnel. There were later modifications to this technique, Nesbit (1949) by a direct anastomosis to the colonic mucosa, Leadbetter (1951) by direct anastomosis and a submucosal tunnel, whilst Cordonnier (1950) extraperitoneally performed a mucosa to mucosa anastomosis potentially allowing reflux.

Complications

Ureterosigmoidostomy is not without its problems, as there is a high incidence of acute pyelonephritis, upper tract deterioration and electrolyte imbalance (Ferris and Odel, 1950, and Zabbo and Kay, 1986). Only about one third of

patients avoid all the major complications of this type of diversion. Acute pyelonephritis occurs in 20 to 57 percent of patients and upper tract dilation occurs in approximately 17 percent of patients. These problems can be reduced by the use of an anti-refluxing technique, antibiotics and electrolyte replacement (Zincke and Segura, 1975, and Wear and Barquin, 1973). Although it is hoped that the patient will be continent there is often some leakage, especially at night.

Colon Conduit

The number of ureterosigmoidostomies performed since the 1950's has fallen since the development of the ileal conduit (Bricker, 1950). At about the same time colon conduits were developed especially for use in children (Mogg, 1965). It was hoped that these diversions would not have the problems found with ureterosigmoidostomies although there was the disadvantage of a stoma. Although electrolyte imbalance did not cause as many complications, problems with acute pyelonephritis, reflux and stenosis were still a cause for concern (Elder et al., 1979, and Orr et al., 1981). The numbers of ureterosigmoidostomies performed in the West has fallen, but in countries where stoma care is unavailable this remains the diversion of choice. There is still a certain amount of stigma attached to an external diversion which sometimes demands that an internal diversion is performed.

Continent Perineal Stoma

Over the years there have been attempts to produce a continent perineal stoma to remove the stigma of a bag and yet control the urinary stream. The abdominal wall can not produce a sphincteric action but the rectal musculature can act as a sphincter to produce continence, and Gersuny (1898) developed a continent perineal urinary diversion. The ureters were attached to the isolated rectum and the sigmoid colon then pulled through between the internal and external anal sphincters. This operation often caused mixing of the urinary and faecal streams as there was retraction at the anastomosis site. This form of diversion is now rarely performed as there may be both faecal and urinary incontinence. Despite its theoretical advantage in reducing pyelonephritis, in practice this is not the case.

Colocystoplasty

Although in the late nineteenth century Mikulicz (1899) performed the first augmentation cystoplasty, this operation was not developed further until 1950 by Couvelaire.

Bladder augmentation was used initially for patients with a small contracted bladdder which caused intractable urinary symptoms. An isolated loop of bowel was used to augment the bladder, and from this developed the ileocaecocystoplasty (Couvelaire, 1950), and since then there have been many

variations of this type of operation. The caecum or sigmoid colon is anastomosed to the bladder remnant, either intact or detubularised and some are anastomosed end to end, while others side to end. There is debate over the need to detubularise the colon and whether to insert a small bowel segment to lower the intravesical pressure. The ureters are inserted either into the colon or small bowel or may be left in situ.

Bladder Substitution

Bladder replacement with bowel after a total cystectomy without the need for a stoma, takes augmentation one step further. The enterourethroplasty uses an isolated loop of large bowel anastomosed to the urethra (Deleveliotis and Macris, 1961, Gil-Vernet, 1965, and Turner-Warwick and Ashken, 1967). The techniques vary in the portion of bowel utilised and the manner of the anastomosis to the urethra.

Colonic Urinary Diversion

All the above procedures leave urine in contact with the colon at the urothelial-colonic anastomosis. The colon conduit, in theory has the least contact in that it should act as a conduit, however, it is well recognised that there is sometimes statis present and also urinary tract infection.

Bladder augmentation and substitution leave urine in the

colon for long periods at a time. There should be no infection if the segment empties completely but this does not always happen. The Gersuny operation has a mixing of the urinary and faecal stream at the anastomosis between the two segments of colon. The urine within the rectal pouch which contains the ureters then becomes infected.

The ureterosigmoidostomy has a combination of problems. There is urine statis within the colonic segment, and faeces give rise to infection of urine which is in contact with the ureterocolic anastomosis for a considerable length of time. It has been known for some time that patients with a ureterosigmoidostomy have a hyperchloraemic acidosis (Ferris and Odel, 1950). More recently Nurse and Mundy (1989) demonstrated that colon augmentation cystoplasties and sustitution ileocystoplasties have a metabolic acidosis with respiratory compensation.

Malignant Tumours and Colonic Urinary Diversion

The first case of adenocarcinoma complicating a colonic urinary diversion was reported by Hammer (1929). It was not clear whether it originated from the ectopia vesicae which had been exposed for 50 years or from the colon, which was anastomosed to the trigone as a Maydl operation for 10 years. Since then there have been another 76 malignant tumours reported (Table 1 and Appendix 1).

Table 1.

Ureterosigmoidostomy Tumours

Tumour	Number	Latency yrs. mean	(range)
Adenocarcinoma	73	27	3.5-54
Transitional Cell Carcinoma	3	6.7	1-15
Lymphoma	1	5	
Non-invasive Tumours	41	19	7-40

Transitional cell tumours at the ureterocolic anastomosis have been described in three papers. Two tumours were obviously spread to the ureter, as the histology was similar to that found in the original cystectomy specimen (Riches and Page, 1956, and Amar, 1961). However, a transitional cell tumour with areas of squamous metaplasia described by Sugg in 1962 was in a ureterosigmoidostomy with no previous history of malignancy. When the colon and ureter containing the tumour were resected the histological examination revealed an undifferentiated transitional cell carcinoma.

Lymphoma at the anastomosis has been described by Ghanem and Perry (1985). The above four tumours are atypical and have been excluded from the calculations below.

Tumours that were described as adenocarcinoma, anaplastic or scirrhous took an average 27 years (range 3.5-54 yrs.) (Fig. 2-4 and Table 1) to develop with the average age of patient presentation being 39 years (range 14-72 yrs.). In the six patients who were diverted after the age of 40 years the length of time to presentation was much shorter, 9.3 years (range 4-16 yrs.). The average age of presentation of these patients was 60 years (range 50-72 yrs.). Five of these patients were diverted for carcinoma of the bladder and one for exstrophy.

Fourteen patients who had a ureterosigmoidostomy which was

Fig. 2.



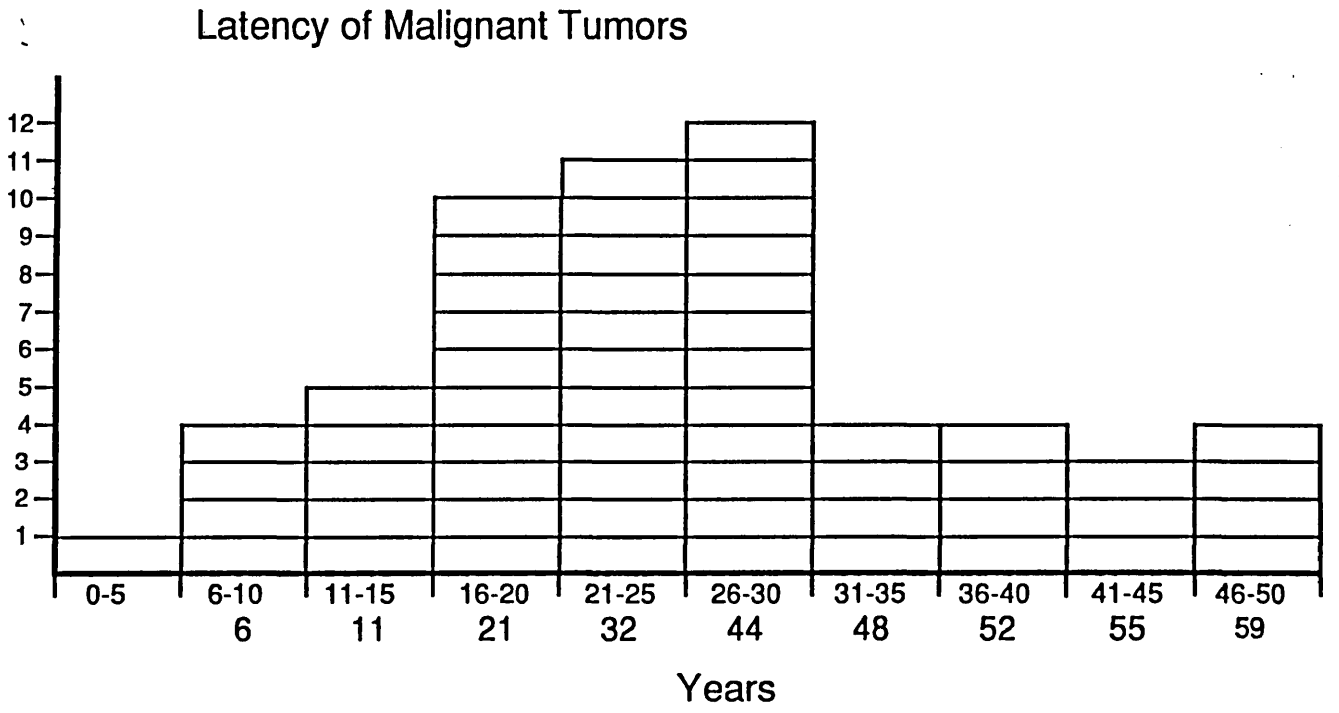
Photograph of ureterosigmoidostomy tumour seen via a colonoscope.

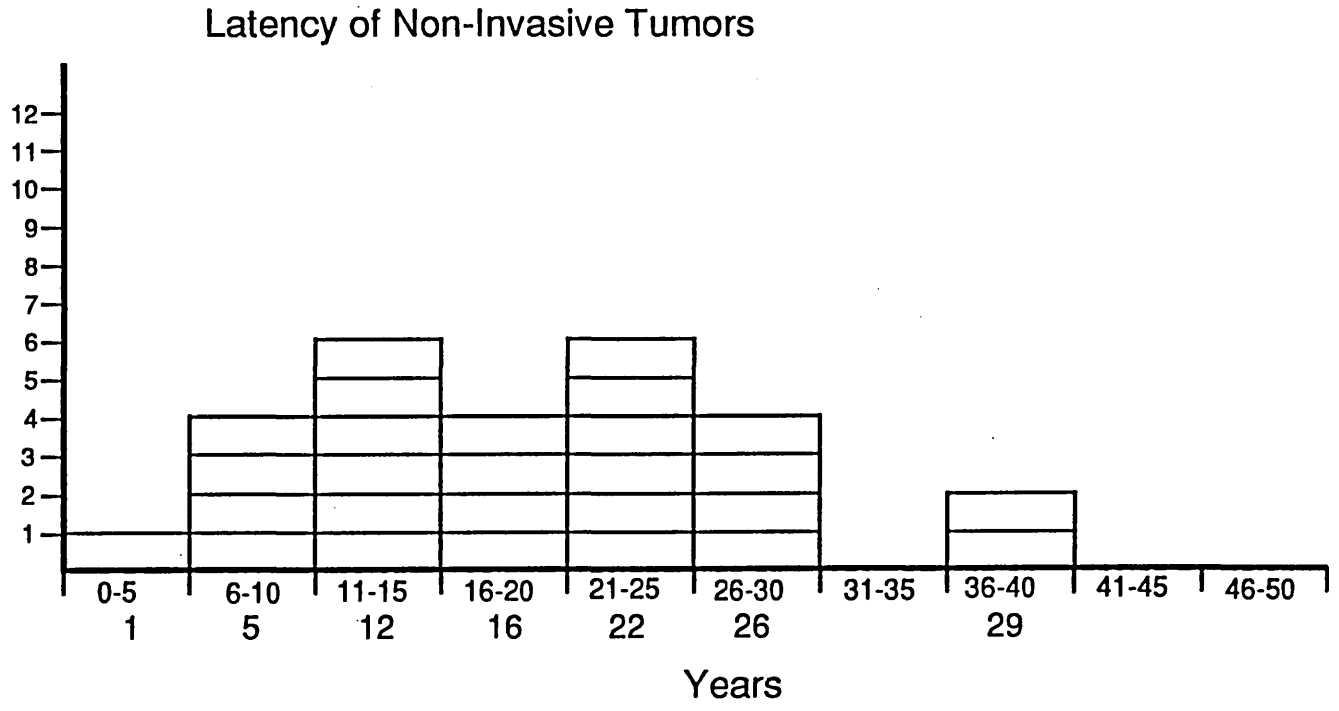
Fig. 3.



Photograph of ureterosigmoidostomy specimen demonstrating the ureter in continuity with a tumour.

Fig. 4





Graph demonstrating the latency of ureterosigmoidostomy tumours.

then diverted to another form of diversion also developed adenocarcinomas, which took 24.3 years (range 14-44 yrs.) to develop with an average age of presentation being 28.5 years (14-55 yrs.).

Risk of Malignant Change

There have been varying calculations of the increased risk of tumour formation after ureterosigmoidostomy from 90 to 7,000 times the expected incidence for the age group at risk (Table 2). The true incidence of this complication is very difficult to calculate as the data on which it is based is inadequate.

Stewart et al. (1982) made the most conservative estimate of the increased risk of tumour formation, at 90 times the expected incidence of adenocarcinoma. This study was open to many criticisms, as firstly they assumed that between 1938 and 1954 there were steady number of ureterosigmoidostomies performed each year and that there would have been 200 ureterosigmoidostomies performed for benign disease between 1938 and 1980. Secondly, the data collection was incomplete and thirdly the length of the follow up was not adequate, some patients with only a short follow up were included in the study. The mean age at cancer diagnosis was 42 years at that time and the degree of risk was calculated by comparing these patients with the cumulative incidence of rectosigmoid cancer for the

Table 2.

Ureterosigmoidostomy and Malignant Risk

Author	Increased risk of malignant tumour formation
Stewart et al., (1982)	90 x
Erkalis & Folkman (1978)	181 x gen. pop.
	7,000 x pop. under 25
Urdaneta et al., (1966)	500 x
Parsons et al., (1977)	280 x

Birmingham area.

Erkalis and Folkman (1978) also produced an incidence based on incomplete data. Ninety four ureterosigmoidostomies were followed up, but only 20 were seen at 30 years, with a mean latency of tumour formation of 26 years, at the time of publication. The incidence of ureterosigmoidostomy tumours was compared with both the general population and those under 25 years of age. This produced an increased risk of 181 times the general population and 7,000 times the population under 25 years, although these figures maybe an underestimate due to inadequate follow up.

In a North American series (Urdaneta et al. 1966) an increased risk of a 500 times was based on a review of 77 patients with ureterosigmoidostomies. This was a heterogeneous group of patients of whom 23 survived 10 years or more. Three of the 23 patients reviewed died at the average age of 45, two with proven adenocarcinoma at the ureterocolic anastomosis although the third case was not proven to have an adenocarcinoma. The incidence of colorectal carcinoma, at the age of 45, in North America was 24 per 100,000. The calculation again probably under estimated the true incidence of tumour formation as the follow up was on average 15 years. Also the incidence of 24 per 100,000 was taken for the whole colon rather than the rectosigmoid. Against this, the cumulative incidence should

have been used, which would reduce the increased risk calculation.

Parsons et al. (1977) in a review of the literature at that time concluded that the mean latency of tumourogenesis was 25 years, with a mean age at presentation of 34.3 years. Twenty nine patients with ureterosigmoidostomies were reviewed, two of those who had been followed up for greater than ten years were found to have an adenocarcinoma. The incidence was then calculated at 6,900 per 100,000, compared with expected incidence of adenocarcinoma of 24 per 100,000, (Urdaneta et al., 1966). Therefore, the increased risk of developing a carcinoma was approximately 280 times the normal population, but again this calculation was based on inadequate data and insufficient follow up. Also the data for the expected incidence of adenocarcinoma did not take into account whether this was for the whole population or for the cumulative incidence at the age of 35 years.

Leadbetter et al. (1979) reviewing the literature concluded there was a significant difference in latency for these tumours depending on the age of the patient when the operation was performed. In those who had the operation performed under the age of 40 years the latency was 21.4 years and in those performed over 40 years the latency was 8.7 years. This may have been related to increased susceptability of the colon in older patients to tumour

formation or that having had one tumour, most of these were diverted for tumour of the bladder, made them more at risk of developing another.

The average age at which patients with ureterosigmoidostomies presented with malignant tumours was 39 years which was two decades earlier than most colonic adenocarcinomas (Dukes and Bussey, 1958). The life time risk of tumour developing in these patients has been calculated at 5% (Leadbetter et al., 1979) although this was based on an estimated increased risk of adenocarcinoma of 500 (Urdaneta et al., 1966). They did not explain how the estimated life time risk of 5% was calculated but this was presumably compared with risk of spontaneous adenocarcinoma of the colon. A more recent study by Zabbo and Kay (1986) found that the risk in those patients who were followed for a minimum of 15 years was 11%.

Non-invasive Ureterosigmoidostomy Tumours

Non-invasive tumours take on average 19 years (range 7-40 yrs.) to develop (Fig. 4 and Table 1), and their average age at presentation was 28 years with a range of 14-69 years (Appendix 1). Forty one non-invasive tumours have been described, and there have been many variations in the terms used to describe them. Some have used a purely descriptive term such as polyp (Potet et al., 1977, and Dixon and Weissmann, 1948), while there have been various histological

descriptions used including, juvenile polyp (Markowitz and Koontz, 1966, and Ali et al., 1984), reactive polyp (Lasser and Acosta, 1975), inflammatory polyp (Leadbetter et al., 1979, and Dunn et al., 1979). More commonly they are described as adenomas or adenomatous polyps (Mogg, 1977, and Naryana et al., 1978), which are considered to be pre-malignant neoplastic polyps.

Many different terms have been used for these lesions, and they could all be the same type of tumour but the histological interpretation of them was difficult. Inflammatory and juvenile polyps are not usually regarded as pre-malignant, although this has been recently questioned (Rolles, 1987), while adenomas are (Muto et al., 1975). As they are normally excised, the natural history of these lesions is not completely known, but there is strong indirect evidence that the polyps are pre-malignant (Stewart et al., 1981).

Colon Conduit Tumours

Recently, two cases of adenocarcinoma have been reported in colon conduits (Chiang et al., 1982, and Wilson and Morales, 1982) where urine only comes in contact with the colonic mucosa. The patient reported by Chiang et al. (1982) had been diverted for exstrophy at the age of three years, he had repeated urinary tract infections over many years, and a moderately well differentiated adenocarcinoma presented 26

years later.

Wilson and Morales (1982) described an adenocarcinoma at the stoma of a colon conduit in a 63 year old woman with an ileal conduit following radiotherapy and pelvic exenteration for a squamous cell carcinoma. The ileal conduit broke down and was converted to a transverse colon conduit. One year later a painless mass presented at the stoma that was an adenocarcinoma not involving the ureterocoloic anastomosis. This was not typical of ureterosigmoidostomy tumours which are virtually always at the urothelial colonic anastomosis.

Colocystoplasty Tumours

Three colocystoplasties, which have developed an adenocarcinoma, have now been described. One caecocystoplasty only functioned with urine for one year before the ureters were diverted into a jejunal conduit because of upper tract dilation and infection (Kirby and Lloyd-Davies, 1985). The non-functioning caecocystoplasty was left in situ and an adenocarcinoma developed within its colonic mucosa eight years later.

The second case was a 38 year old man who presented with irritative bladder symptoms, 15 years earlier he had an ileocaecocystoplasty performed (Cado, 1988 Personal Communication). Cystoscopy revealed a mass and a cystectomy was performed. Histological examination showed a mucinous

papillary adenocarcinoma with foci of signet cells arising from the bladder remnant adjacent to the colonic mucosa.

The third case was a woman who had a caecocystoplasty performed for interstitial cystitis (Grainger et al., 1988). Three months later a cystoscopy showed a villous lesion in the caecum near the ileocaecal valve. This was resected and was staged histologically as a Dukes' A adenocarcinoma. The interval of three months suggested that the lesion was already present in the colon, rather than developing due the presence of urine.

As yet only a few colocystoplasty tumours have been described but this operation has been performed in relatively small numbers until fairly recently. It is predictable that if the operation continues to be used an increasing number of cancers are likely to be reporterd.

Because the reports of increased risk of tumour formation following ureterosigmoidostomy described above were inpart inadequate a further study has been conducted. The patients examined form a homogeneous group, with a long follow up and are described in Chapter 2.

CHAPTER 2

URETEROSIGMOIDOSTOMY AND EXSTROPHY

Previous studies had shown an increased risk of malignancy associated with ureterosigmoidostomy, although accurate assessment of this problem had not been possible because of inadequate data. This study was designed to minimise these inaccurancies.

Patients

One hundred and three patients born with exstrophy before 1964 were treated at the Hospital for Sick Children and the St. Peter's Group of Hospitals. There were 29 girls and 74 boys on whom operations were performed which are summarised in Table 3. Thirty four patients were initially diverted to a ureterosigmoidostomy, of whom 13 were subsquently converted to an ileal or colon conduit. The ureteric stumps would have been left in situ within the colon, as this was the practice at that time. Six patients initially had a Gersuny operation, of which two were later converted to a colon conduit. The Gersuny procedure diverts urine into the rectum by placing the ureters in the upper rectum. The detached sigmoid colon is then pulled through the anal spinchters to open onto the perineum. In theory the faecal and urinary streams should be separate but infact, because of retraction of the anastomosis there is mixing of the urine and faeces.

Two patients had a vesicocolic anastomosis fashioned, making

Table 3.

Follow up of Exstrophy Patients Operation

Original Operation			
"at risk" group			
Ureterosigmoidostomy	34	further diversion	13
Gersuny operation	6		
Vesicocolic anastomosis	2		
"non risk" group			
Ileal conduit	36		
Primary bladder closure	15		
Cutaneous ureterostomy	5		
Colon conduit	5		

a total of 42 patients were in the "at risk" group of developing a tumour as there was, or had been mixing of urine and faecal streams at the urothelial colonic anastamosis.

Sixty one patients were in the "non risk" group, 15 had a primary bladder closure, 36 had an ileal conduit, five a cutaneous ureterostomy and five a colon conduit.

Patients in the "at risk" group have had an annual colonoscopy performed since the risk of tumour formation was well recognised from 1980 onwards. Patients in the "non risk" group have had annual follow up for their diversions but this had not included a colonoscopy, unless symptoms justified this investigation. If a tumour was found at colonoscopy biopsy specimens were taken and an intravenous urogram carried out. Based on these results and the patients preference a colectomy, including both ureteric stumps was performed and the urine rediverted.

Results

Sixteen patients have been lost to follow up, twelve in the "non risk" group and four in the "at risk" group. There have been a total of eleven deaths, five in the "non risk" group and six in the "at risk" group (Table 4).

In the "non risk" group two died of renal failure, one of

Table 4.

Follow up of Exstrophy Patients	Outcome
42 "at risk" group	
Died carcinoma of bladder	2
Died carcinoma of colon	2
Died intestinal obstruction	1
Died inhalation of vomit	1
Colectomy, carcinoma of colon	2
Colectomy, non-invasive tumour	4
Lost to follow up	4
61 "non risk" group	
Died renal failure	2
Died intestinal obstruction	1
Died poliomyelitis	1
Died cause unknown	1
Lost to follow up	12

intestinal obstruction, one of poliomyelitis and one of an unknown cause.

In the "at risk" group one died of intestinal obstruction and one by inhalation of vomit. Two died with a carcinoma of the bladder remnant, aged 34 and 28 years, and two died with a carcinoma of the colon, aged 46 and 30 years (Table 5).

The first case of presumed carcinoma of the bladder was in a boy, who had his bladder exstrophy closed when two years old. At four years a ureterosigmoidostomy was constructed which was converted to an ileal conduit four years later. Also when he was four years old he underwent a mucosal cystectomy. This patient died at the age of 28 years, according to his general practitioner's records, of a carcinoma of the bladder remnant, but there was no post-mortem examination. The second case was a boy who had his bladder exstrophy closed at one year. At the age of two there was a further attempt at bladder closure, but at four years he had an excision of bladder and a ureterosigmoidostomy fashioned. Later that year a further excision of the bladder remnant was performed. The ureterosigmoidostomy was converted to an ileal conduit when he 20 years old. He presented at the age of 34 with a metastatic adenocarcinoma in the biceps muscle. He died three days after a laparotomy which revealed a necrotic mass

Table 5.

Tumours in the "at Risk" Group.

Age at ureterosigmoidostomy	Age at tumour presentation	Tumour
4 yr.	28 yr.	Bladder tumour
4 yr.	34 yr.	Adenocarcinoma of bladder
18 mth.	29 yr.	Adenocarcinoma of colon
?	40 yr.	Adenocarcinoma of colon
3 yr.	31 yr.	Adenocarcinoma of colon
2 yr.	35 yr.	Adenocarcinoma of colon
6 yr.	44 yr.	Non-invasive tumour
5 yr.	36 yr.	Non-invasive tumour
2 yr.	22 yr.	Non-invasive tumour
7 yr.	35 yr.	Non-invasive tumour

in the pelvis. Post-mortem examination showed the tumour to arise from the bladder remnant rather than the ureterosigmoidostomy anastomosis site.

Two patients died with a tumour at the ureterosigmoidostomy site. The first was a girl who had a cystectomy of her bladder exstrophy, and a ureterosigmoidostomy urinary diversion performed at eighteen months. She was converted to an ileal loop at 12 years, and at the age of 29 she developed a poorly differentiated Dukes' C adenocarcinoma at the ureterocolic anastomosis and died a year later. The second patient was diverted with a ureterosigmoidostomy, but the age at which this operation was performed was not recorded. At forty years of age he developed an adenocarcinoma of the colon at the ureterocolic anastomosis, dying 6 years later.

Of the 42 patients in the "at risk" group, mean age 36.1 years, two patients have died of carcinoma of the colon, 28 and 46 years after diversion into their colon. In addition two other patients have developed a carcinoma of the colon, 28 and 33 years following their diversion, but are still alive.

One boy born with exstrophy had a ureterosigmoidostomy performed when three years old, and 28 years later he developed rectal bleeding, and was colonoscoped revealing an

adenocarcinoma at the ureterocolic junction. He subsequently underwent a colectomy and reimplantation of the ureters into the colon more proximally. The second had his diversion when two years old and developed an adenocarcinoma at the ureterocolic anastomosis 33 years later.

Four other patients in this group have developed non-invasive tumours, two of whom had two or more polyps. All of the patients with non-invasive tumours had had their ureterosigmoidostomies converted to an ileal conduit many years previously. They had developed their non-invasive tumours 20,31,32 and 38 years after their initial ureterosigmoidostomy. All but one of these tumours developed at the ureterocolic anastomosis. Histologically they were given different descriptions, benign polyp, adenoma and reactive polyps.

In the 49 patients in the "non risk" group, none have developed colonic tumours, non-invasive or malignant, nor have they developed tumours of the bladder remnant.

Discussion

The mean age of the patients in the "at risk" group at the time of this study was 36.1 years (S.E.M 1.15), and the cumulative risk of developing a rectosigmoid adenocarcinoma at this age was 6.1 per 100,000 (Cancer statistics registrations, 1984). Four of the 38 "at risk" patients who

reached adulthood developed adenocarcinoma of the colon which produced an incidence of 10,526 per 100,000. Therefore, the increased risk of developing adenocarcinoma for this group was 1,726 times the expected incidence. Extensive studies on the incidence of adenomas were not available for comparison. Leadbetter et al. (1979) suggested that the life time risk of developing an adenocarcinoma with a ureterosigmoidostomy was 5%. In this study the risk of developing a malignant tumour at the ureterocolic anastomosis by 36 years was 11%, and of developing a tumour 22%.

Adenomas of the colon can develop, but not necessarily, into an adenocarcinoma of the colon (Muto et al., 1975). The time interval to develop an adenocarcinoma from an adenoma is thought to be about 5-10 years (Day, 1984). Four patients with adenomas which were untreated took 5-10 years to become malignant (Muto et al., 1975). Williams et al., (1982) in a prospective study examined the post-mortem colons in 82 patients for evidence of polyps. Fifteen colons were found to contain adenomas, but in the 19 patients under the age of 35 no adenomas were found. The non-invasive tumours may well have developed into adenocarcinomas if they had been left long enough to grow further.

This study of increased risk of tumour formation associated

with ureterosigmoidostomy was more complete than previous studies. The follow up has been carried out for a minimum of 20 years in a homogeneous population. There were very few patients lost for examination, but it was by necessity a retrospective study. It could not be determined from the clinical notes whether at the initial management there was any preference for a ureterosigmoidoistomy rather than another form of diversion. Having the ureterosigmoidostomy converted to another form of diversion was indicated on clinical grounds, and was either due to upper urinary tract dilation and infection, or inability to control continence.

The calculated increased risk of malignancy was greater than that found by most authors. For example Stewart et al. (1982) calculated the rate to be 90 times the expected incidence. However in their study too many assumptions have been made, firstly that the number of ureterosigmoidostomies performed each year were approximately equal, secondly the patients were not a heterogeneous group and finally the follow up was not adequate. The age at which the operation was performed alters the length of time to tumour formation (Leadbetter et al., 1979). They also suggested that the lifetime risk of developing a ureterosigmoidostomy tumour was approximately 5%. Again, in this study the follow up was too short to come to this conclusion. Zabbo and Kay (1986) found in patients who survived 15 years following their ureterosigmoidostomy operation there was an 11% chance

of developing a malignant tumour. They felt this was probably an underestimate as tumours may have been missed and there was a potential to develop more with time. The malignant tumour cumulative incidence by 20 years was 11% and another 11% for non-invasive tumours. If these patients had been followed for another 10 years the incidence may have been even higher, as there would be time for conversion from adenomas to adenocarcinomas (Muto et al., 1975 and Day, 1984). Any calculations involving a relatively small number of patients means that when calculating risk, marked amplification of the figures results from only minor changes. All series have suffered from this problem.

This study confirmed that the risk of tumour production was present in those converted to another form of diversion. There was little difference in the risk whether urine was present at the anastomosis or not. However, one could not know whether urine or the operation was required to initiate the neoplastic process. In Chapter 3 the proposed mechanisms of cancer formation following urinary diversion will be discussed.

CHAPTER 3

THEORIES OF TUMOUROGENESIS

Since it has been recognised that there is a relationship between ureterosigmoidostomy and neoplasia various theories have been put forward to explain its aetiology. The factors implicated have been trauma, urine, urine and faeces mix, electrolyte load, decreased immunity, and N-nitrosamines. Early colonic mucosal changes have been shown as well as alterations in the urothelium at the anastomosis.

Trauma

Aldis (1961) was the first to link ureterosigmoidostomy and neoplasia, during his report of a tumour in a 30 year old woman who was born with exstrophy. Her ureters had been implanted into the pelvic colon and 15 years later she developed a colonic neoplasm, histologically a scirrhous tumour. Three years earlier she had pyelonephritis and an intravenous urogram at that time had shown a dilated ureter down to the ureterocolic anastomosis. The association between ureterosigmoidostomy and neoplasia was linked to the fact that the tumour was at the site of the ureteric implant. The chances of a woman developing an adenocarcinoma of the pelvic colon at the age of 30 was 1 in 100,000, and therefore an uncommon tumour. Aldis said that it was well known that carcinoma of the colon could develop from both true and false polyps. The malignant transformation seen in his patient fitted with this concept. Futhermore he proposed that the pseudopolyp formed by the

anastomosis created a firmer area in the bowel which was then subjected to repeated microtrauma which then underwent malignant transformation.

Urine

Urdaneta et al. (1966) when reviewing the literature found three cases of adenomatous polyps adjacent to the ureteric orifice and a further eleven adenocarcinomas with a mean latency of 17 years. Four of the patients had ureterosigmoidostomies performed for malignant conditions, and as the latency of the tumour was greater than 5 years, the tumour was most unlikely to be due to a recurrence. The other seven diversions were performed for benign conditions. They concluded that a carcinogen in the urine was unlikely to have caused a primary bladder tumour and a colonic tumour. The findings suggested urine alone was sufficient stimulus to produce a colonic tumour. However, they could provide no direct evidence for this statement.

Leadbetter et al. (1979) also implicated urine as the carcinogen, despite the observation that cancer could be found in the ureteric stumps after the conversion of ureterosigmoidostomies to ileal conduits. Again, no plausible argument was submitted to support this view.

Urine/Faeces Mix

Charron and Delisle (1982) dismissed urine as a cause of

neoplasia because, as described above cancer could be found in ureteric stumps left in the colon. They also quoted, in support of their conclusion, an experimental study by Scott and Boyd (1953) who were unable to induce colonic neoplasia in dogs using beta-naphthylamine, a bladder carcinogen over a relatively short time. Their suggestion was that microtrauma to a nipple of ureter in the colon induced a neoplasia (Aldis, 1961) had no scientific basis. Their own speculation was that a mixture of urine and faeces could be the cause of tumour formation, but no evidence was submitted to support this view.

Electrolytes

Harguindey et al. (1975) measured the 24 hour loss of sodium and potassium ions through the rectum of a patient with a ureterosigmoidostomy. The normal daily stool loss was found to be 2-5 mEq of sodium ion and 10-15 mEq of potassium ion. The range of the sodium ion loss was 38-96 mEq daily (mean 49 mEq), and the potassium ion loss was 22-30 mEq daily, in the ureterosigmoidostomy patient. This loss was found at the rectum and the ion concentrations at the ureterocolic anastomosis could be higher. Therefore, the colon in which the tumours developed were subjected to an excess load of 7-48 times the normal sodium and 2-3 times the potassium load. In addition some patients with villous adenomas produce a sodium chloride loss of up to 100-160 mEq daily. Patients with villous adenomas have a high incidence of

tumours nearby (Wheat and Ackerman, 1958). It could, therefore, be the high electrolyte load which was stimulating tumour formation.

Mucosal Changes

Sommo and Traverso (1967) observed changes in the mucosa of patients who had undergone ureterosigmoidostomy, 2, 9, 12, 18 and 48 months earlier. An increase in the mucus production, as shown by histochemical studies, was followed by a proliferative phase, where there were increased mitoses observed in the colonic crypts. The proliferative phase was followed by an atrophic phase, from 12 months on, where the glandular crypts had the appearance of solid cores which they regarded as the oncogenic phase.

The early changes described above have not been demonstrated by others, but biopsies at this stage would not normally have been taken. The oncogenic phase which they also described has also not been observed by other groups carrying out similar studies. If the oncogenic phase occured from 12 months, yet malignant tumours occured about 27 years later the process is very prolonged.

Decreased Immunity

Haney and McGarity (1971) repeated that there was a relationship between colonic polyps and adenocarcinomas. There were two peaks in the incidence of tumours, one

between 5 and 20 years and another between 25 and 30 years. The data was confused by the fact that it did not differentiate between benign and malignant growths. The data showed that the finding of a tumour was related more to the length of time with the ureterosigmoidostomy anastomosis than to the age of presentation. Scott and Boyd (1953) at that time were unable to find any carcinogens that would stimulate colonic tumours. There was speculation that urine may decrease the natural tumour immune mechanism, but there was no evidence for this opinion (Haney and McGarity, 1971).

N-nitrosamines

Dietary nitrate, when diverted into the colon, via the ureters, comes into contact with endogenous secondary amines (Stewart et al., 1981). This combination in the presence of mixed bacteria leads to the formation and activation of N-nitroso compounds. N-nitrosamines have been shown to be carcinogenic in laboratory animals (Habs and Schmal, 1980), and may cause malignancy in man.

Rectal urine samples were analysed for total anaerobic bacteria, total aerobes and mean probable nitrate reducing organisms. Another part of the sample was assayed for nitrate, nitrite, and N-nitroso compounds, and the remainder examined for mutagens by Salmonella mutagenesis assay. In only seven of the 29 urines examined were the results complete. In those urines, high levels of nitrate, nitrite

and N-nitroso compounds were found and mutagens demonstrated. In the other 22 urines examined there were mixed bacteria, nitrates and nitrites found. It was assumed secondary amine production in the colon would go onto N-nitrosamine production, whereas N-nitroso compounds are normally found only as trace amounts.

N-nitroso compounds have been found in the bilharzia bladder (Hicks et al., 1982), a condition associated with local tumour which could be the cause of malignancy in ureterosigmoidostomy patients (Stewart et al., 1981). The same group have expanded this work further to include both ileal and colon conduits (Stewart, 1986). Urine from a conduit should be free of mixed bacteria and endogenous production of precursor amines would be unlikely. Nitrosable amines and amides, and secondary amines were excreted in the urine at the fairly constant rate of 20-30 mg per day. If mixed bacteria were present in the loop, N-nitrosation could take place.

Ureterosigmoidostomy and ileal conduit urines were found to be heavily contaminated with faecal type flora while only 37% of the colon conduits were contaminated. The urine of the uretersigmoidostomies and ileal conduits contained multiple organisms, while infected colon conduits contained only one type of bacterium. No results were produced separately for the small number of ileal and colon conduits

examined for nitrite and nitrate concentration. N-nitroso compounds found in the urine of ureterosigmoidostomies were 10 times more concentrated than the normal range.

Unfortunately the small number of tests performed on the conduits meant that valid conclusions could not be drawn. In two sterile urines from conduits, no N-nitroso compounds were found. Mixed bacterial infection of urine leads to N-nitroso compound production and possible malignant potential.

Urothelial Tumours

Chiarelli and Sandei (1984) suggested that the tumours at the anastomosis were tumours of the ureteric stumps. Non-invasive tumours found with ureterosigmoidostomies were variously described as "juvenile" (Markowitz and Koontz, 1966, and Ali et al., 1984), or "adenomatous" polyps (Lien, 1973, and Spence et al., 1979). Juvenile polyps are generally not regarded as pre-malignant, although this has been recently been questioned (Rolles, 1987). Six benign lesions were found in ureterosigmoidostomy patients at colonoscopy (Chiarelli and Sandei, 1984). The colon adjacent to the tumours showed mild lymphoplasmacytic inflammation and in one case squamous metaplasia was seen. In four patients polyps were present whose surface mucosa showed columnar mucinous epithelium with occasional areas of squamous metaplasia.

Chiarelli and Sandei (1984) argued that the colonic mucosa was not the source of the tumour for the following reasons; firstly there was only mild inflammation of the colon away from the tumour, secondly in one case the urothelium of the ureter was continuous with the areas of urothelium-like epithelium of the polyp, and finally the glands of the polyp were ectasic and lacked lining cells or had hyperplastic columnar epithelium as in juvenile polyps. The colonic mucosa was unlikely to develop the areas of squamous metaplasia and they postulated that the ureter undergoes glandular metaplasia, to polypoid hyperplasia and eventually to adenocarcinoma. Adenocarcinomas of the exstrophied bladder have been described and this may be due to the same process (O'Kane and Megaw, 1968).

Human studies have been speculative with little firm evidence on the cause of tumour formation and therefore the animal work is examined.

Animal Studies

Most experimental work has been carried out in small rodents but one early study was performed in dogs. Scott and Boyd (1953) studied the effect of beta-naphthylamine on the bladder and colon in dogs in varying experimental conditions.

Three control animals were given 300 mg of beta-naphthylamine and at 22 months they developed bladder tumours. There was no effect on the colon. The first dog developed squamous metaplasia and what was described as a well differentiated alveolar infiltrating tumour. The second animal had an anaplastic tumour and a well differentiated squamous carcinoma. The third dog showed neoplastic cells forming crypts and alveolar spaces lined with squamous epithelium.

Two dogs underwent unilateral ureterosigmoidostomy and the administration of beta-naphthylamine resulted in no histological change in the colon. Both dogs developed papillary tumours of the bladder that were neoplastic.

Four dogs had urine that was diverted into isolated sigmoid loop and were administered beta-naphthylamine. None of these animals developed tumours of the colon or the bladder, however two of these produced tumours of the ureter. One developed a squamous tumour and the other an undifferentiated tumour. These studies showed that urothelium may undergo metaplasia to papillary, squamous and adeno carcinoma under the influence of beta-naphthylamine. Urine and beta-naphthylamine did not produce bowel tumours in either ureterosigmoidostomies or isolated sigmoid loops. The faeces were not the cause of protection of the colonic mucosa from the beta-naphthylamine. They concluded that the

secretions by the mucous glands of the colon were protective, either by preventing contact or by neutralising its effect or that the beta-naphthylamine may not be carcinogenic to the colon.

Crissey et al. (1980) studied the effect of urinary and faecal carcinogens on controls, colon conduits and vesicocolic anastomoses, in male Wistar-Furth rats. Animals were divided into three surgical groups and then into a further four carcinogen groups. The three operative groups consisted of a control group, that had a vasectomy, a vesicocolic anastomosis group and a colon conduit group. There were four treatment groups, one had a urinary carcinogen, N-(4-(5-nitro-2-furyl)-2-thiazolyl) formamide (FANFT), a second a colonic carcinogen, dimethylhydrazine (DMH), a third a combination of the two carcinogens, and the fourth no carcinogen. The rats were autopsied if they died after 16 weeks, and the remaining rats were sacrificed at 33 weeks. In those animals where there was no carcinogen given, four out of six developed tumours at the anastomosis in the vesicocolic group. The animals in the other groups did not develop a tumour when no carcinogen was used. The macroscopic description of the tumour was of an exophytic tumour resembling tumours found in humans at the site of ureterosigmoidostomy. The tumours had a different appearance to those tumours induced with DMH. Histologically the tumours were of intestinal type, although

different from bowel polyps.

In the operative control animals stimulated with DMH ten out of 40 developed colonic tumours. Three out of five in the vesicocolic group developed tumours at the anastomosis but none were found elsewhere in the colon. In the colon conduit group none of the animals developed tumours at the anastomosis but four out of ten had tumours in the distal bowel.

In the animals stimulated with FANFT only one animal developed a tumour and this was at the vesicocolic anastomosis in the vesicocolic group. When a combination of DMH and FANFT was used colonic tumours were found at the vesicocolic anastomosis in four out of five and in the colon of 25 out of 35 controls but not in the colon conduit group.

Tumours were found at the anastomosis of the vesicocolic group but not at the anastomosis of the colon conduit. It was concluded that faeces were required at the anastomosis to induce neoplasia. The colon conduit group had tumours distal to the anastomosis which were caused by the carcinogen, DMH, being present in the urine. The tumours at the ureteric stump after rediversion of ureterosigmoidostomy in humans may be explained by the combination of two epithelia in the presence of a faecal stream. The conclusion, based on this study, was that enzymes in the

urine activate carcinogens in the faeces. Two other ideas were put forward, that urothelium was a local activator or acted as a mechanical irritant.

Anastomotic Tumours

Bristol and Williamson (1981) responded to the above paper and criticised the suggestion that urinary carcinogens activated the colonic tumours. Tumour formation occurred preferentially at an anastomosis in experimental animals (Williamson et al., 1980). The DMH acted partly through the vascular supply and would reach the distal colon, but the absence of tumours in those with a proximal colostomy reflected colonic atrophy of defunction (Terpstra et al., 1979). The chemical carcinogenesis in the distal colon was reduced but not abolished by the proximal colostomy (Campbell et al., 1975). It has been suggested that the resection is the cause of the tumour as hyperplasia was maximal at an anastomosis (Williamson, 1978, and Roe et al., 1987). Crissey et al. (1981) in reply quoted a then unpublished work in which vascularised bladder patches were found not to produce tumours at the anastomosis.

Further work performed by Gittes (1986) expanded the colonic urinary diversion model. It was necessary to give continuous antibiotic to counter the problems of chronic pyelonephritis. He did not state whether or not antibiotics were given to his control animals. Later he converted to

females to avoid the problems of scrotal ulceration. He found 80% of his rats developed tumours in the vesicocolic group, which were classified as either adenocarcinomas or polypoid adenomatous tumours. Tumours were not found before 8 months. The strain of rat was an important factor in the experiments. Wistar-Furth and Charles River C D rats developed tumours but Lewis rats did not. No tumours were found if the faeces were diverted away from the anastomosis but were if the colostomy was delayed for a few months. Sessile tumours were discovered in colostomies adjacent to the urothelial intestinal anastomsis, but none in animals where a simple suture line was closed.

Urothelial Patch

In animals with a vascular urothelial patch rotated from the bladder to the colon no tumours were found at the anastomosis (Gittes, 1986). However, there was squamous metaplasia of the urothelium and this was also observed in the urothelium at the ureterosigmoidostomy anastomosis.

Ileal Interposition

Ileal interpositions between the urothelium and the colon were also performed (Gittes, 1986). This experimental model produced no tumours at either the urothelial-ileal or ileal-colonic anastomosis.

The conclusion from this experimental work was that faeces

are required for tumour production at urothelial-colonic anastomosis. It was possible that the cause for tumours found in exstrophy and colonic conduits was chronic infection and irritation with enterobacteria. The tumours occurred at the anastomosis and this could be due to exogenous carcinogens acting at a rapidly dividing and healing anastomosis.

Conclusion

Human and animal studies had produced many theories as to the cause of carcinogenesis at ureterosigmoidostomy anastomosis. The aim of this study was to investigate by animal experimental model and human clinical studies the mechanism of the tumour formation.

CHAPTER 4

ANIMAL MODELS AND EXPERIMENTAL TECHNIQUES

In this section is described the animal model used to investigate urinary diversion as well as the cell kinetic and histochemical techniques that have been used to assess the effect of diverting the urine into the colon. No studies attempted to reproduce the clinical situation until Crissey et al. (1979) developed a rodent experimental model.

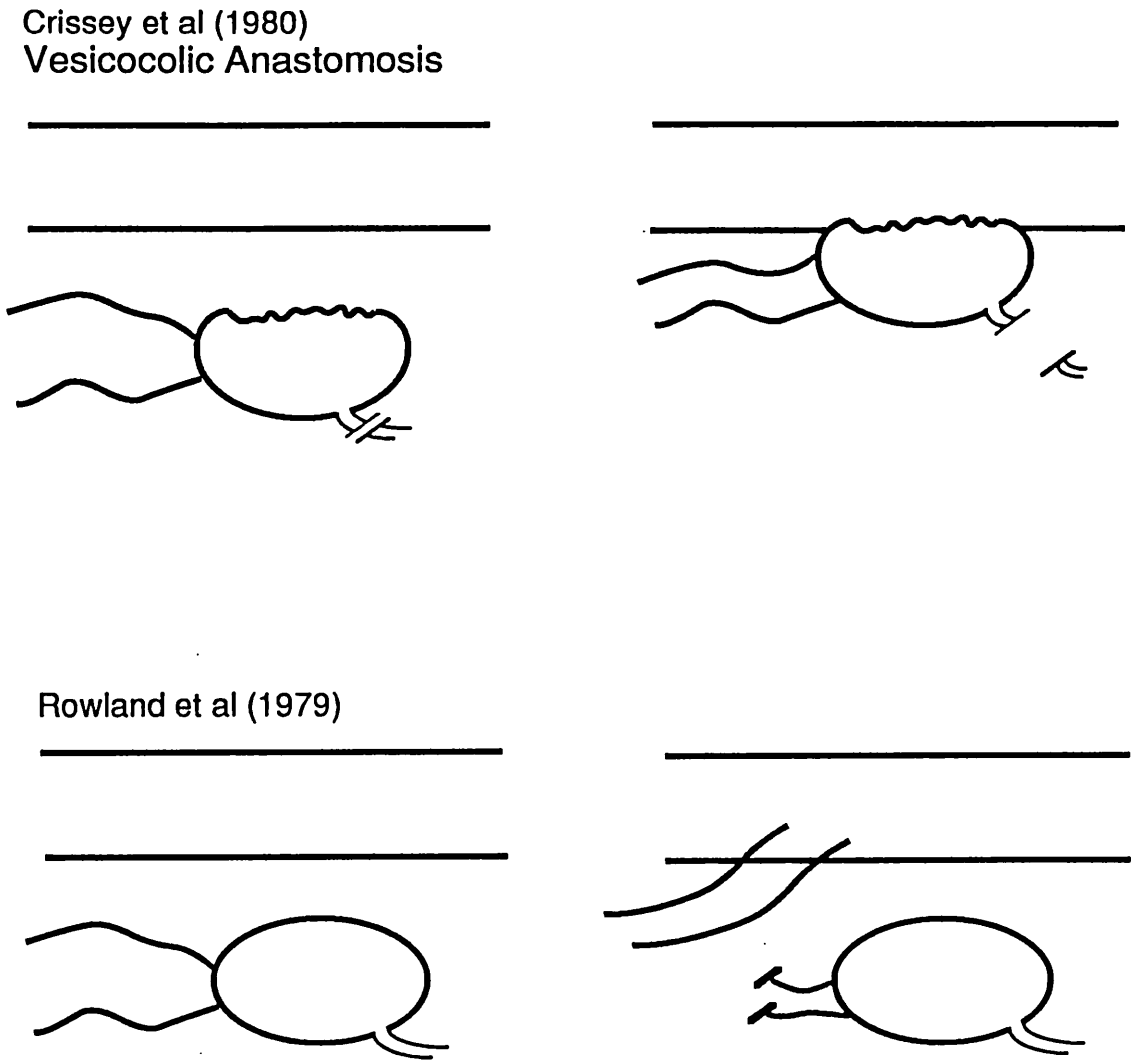
Animal Model

Crissey et al. (1980) showed that in Wistar-Furth rats, tumours could be induced at the urothelial colonic anastomosis when urine and faeces were mixed. They also developed the colon conduit to examine the effect of urine alone in the colon. Both these groups and control animals were in addition given colonic and urothelial carcinogens.

Vesicocolic Anastomosis

The technique for producing a vesicocolic anastomosis was as follows (Crissey et al., 1980). The urethra and vasa were divided to free the bladder base with its blood supply. The dome of the bladder was resected and anastomosed to the rectal wall with a continous 7/0 polyglycolic acid suture (Fig. 5). This techinque produced an overall mortality of 79% at 16 weeks. Only six of the 20 animals which underwent this operation without any additional carcinogen survived beyond 16 weeks, and of these, four produced a tumour at the

Fig. 5



Diagrammatic representation of vesicocolic anastomosis and ureterosigmoidostomy.

anastomosis (67%). The deaths were mainly due to upper urinary tract obstruction, pyelonephritis and perineal ulceration, deaths before 16 weeks were not subjected to a post mortem examination. Male rats were used in the initial study despite problems of scrotal ulceration as the surgery was technically easier. Rowland et al. (1979) recommended sawdust to minimise the scrotal ulceration produced by the urine faeces mix and this problem subsequently led Gittes (1986) to use female rats.

Ureterosigmoidostomy

Rowland et al. (1979) developed a model of ureterosigmoidostomy to study bladder tumour carcinogenesis in male C.D. Fischer rats. Through a midline incision, the ureters were freed from the retroperitoneum and the distal ureters were transected (Fig. 5). A colotomy was made and a 1cm submucosal tunnel created with fine forceps. The ureters were brought through the tunnel and the adventitia of the ureter sutured to the colonic mucosa with catgut. Twenty nine animals were operated on by this technique and only four (17%) died by 4 months. An intravenous urogram performed on the remaining animals showed mild to moderate hydronephrosis. Infection of the renal cortex was evident in one third of the animals. There were no abnormalities of the bowel found when the animals were sacrificed at 6 months. This method of urinary diversion was recommended in experimental animals because of the low morbidity and

mortality.

Colon Conduit

Simulation of the colon conduit was performed by mobilisation of the bladder in the same way as for the vesicocolic group (Crissey et al., 1980). The proximal colon was divided and anastomosed to the anterior abdominal wall (Fig. 6). The bladder was then anastomosed to the distal colon. This procedure had an overall mortality of 61% at 16 weeks, and in a sub group when no carcinogen was given this was reduced to 45%.

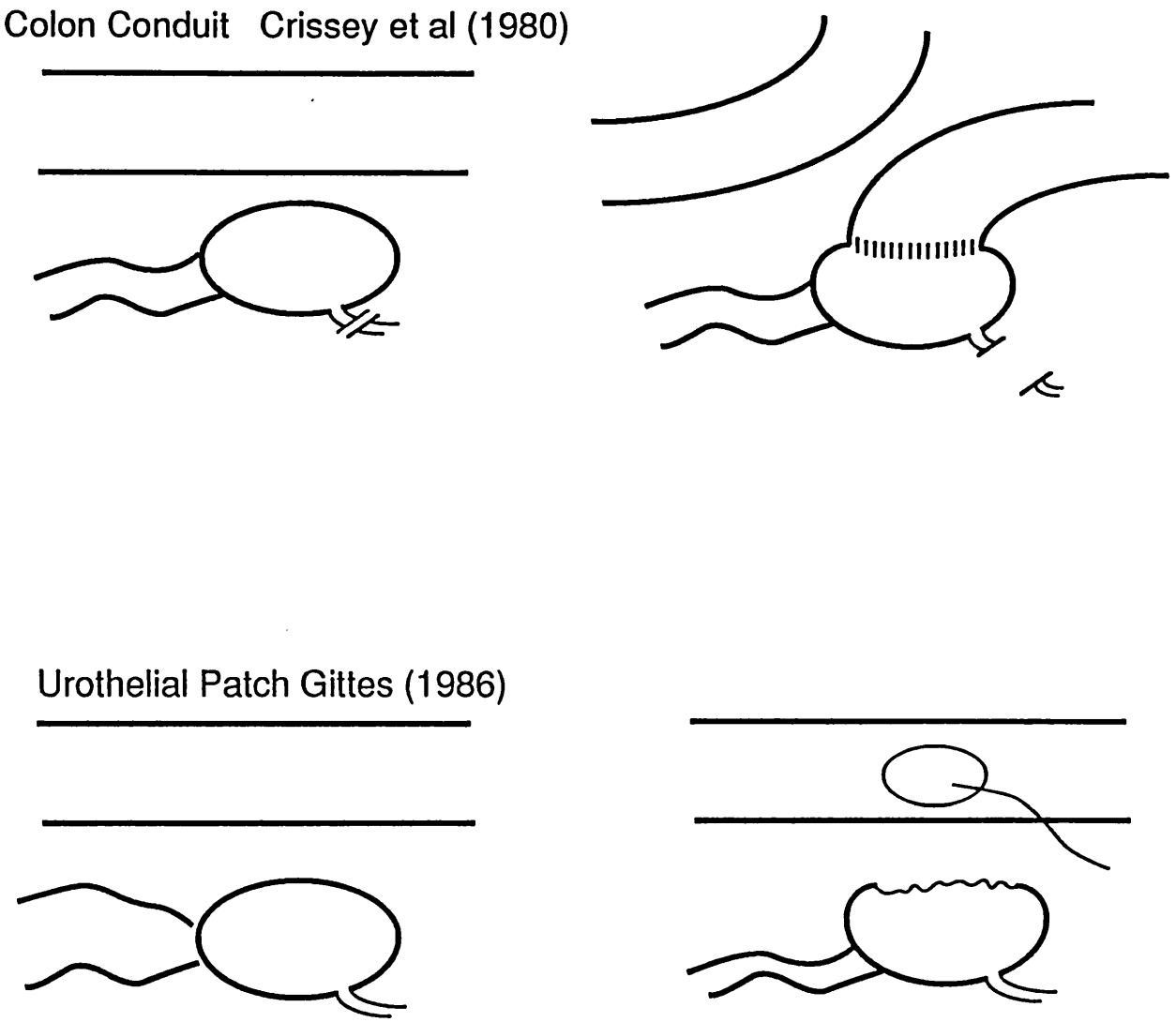
Urothelial Patch

A urothelial patch was created to demonstrate the effect of faeces alone at the urothelial colonic anastomosis. The dome of the bladder was mobilised with its vascular supply and inserted into the descending colon (Gittes, 1986)(Fig. 6). No experimental details of technique were provided, nor the numbers of animals used.

Ileal Interpositions

Gittes (1986) performed further experiments to help elucidate the cause of tumours at the urothelial colonic anastomosis. Ileal interpositions were performed between the urothelium and the colon, which allowed urine to interact at a urothelial ileal anastomosis, and urine/faeces to act at an ileal colonic anastomosis. Again no details of

Fig. 6



Diagrammatic representation of colon conduit and urothelial implant.

the experiments were given other than they were two large series with reproducible results.

Prophylactic Antibiotics

Antibiotics were not mentioned in the paper by Crissey et al. (1980). In a further publication antibiotics were used in the vesicocolic group (Gittes, 1986). It was found that without the use of antibiotics most rats were dead within 4 months. Survival was increased, most effectively, with the use of carbenicillin in the drinking water. It was not clear from the paper whether antibiotics were used in the control group.

Tumours

The histological description of the tumours produced at the vesicocolic anastomosis where urine and faeces were mixed was rather vague (Crissey et al., 1980). The tumours were described as exophytic tumours of the bowel mucosa at the urothelial junction. They resembled tumours produced in ureterosigmoidostomy patients at the anastomosis, and were different from those produced by dimethylhydrazine. It was said that histological examination showed that the tumours were intestinal in origin. There were no other features mentioned.

Gittes (1986) described adenocarcinoma and polypoid adenomatous tumours at the vesicocolic anastomosis. These

tumours were not found before 8 months. At 1 year there were often multiple tumours, some were sessile and locally invasive. The histological features were very vaguely described. The vesicocolic animals were the only group in which tumours were seen. Squamous metaplasia of the urothelium was present in animals with a urothelial patch in the colon. The ileal interposition animals did not develop tumours at either the urothelial ileal or ileal colonic anastomosis.

Species Susceptiblity

No tumours were found when Rowland et al. (1979) used C.D. Fischer rats. When Gittes (1986) used either Charles River C.D. or Wistar Furth rats tumours were found produced at the anastomosis, but none when Lewis rats were used.

Chemical Carcinogenesis

Tumours can be induced in female Wistar rats with 1,2 dimethylhydrazine HCL (DMH) Filipe (1975). The colonic mucosa underwent, hyperplasia, to dysplasia, to carcinoma in situ and eventually to invasive carcinoma. Histochemical changes were demonstrated with high iron diamine alcian blue (HID-AB) during this transition. Shamuddin and Trump (1981) used azoxymethane to induce tumours in Fischer 344 rats, and observed both histochemical and morphological changes. The histological changes ranged from dysplasia, to carcinoma in situ, to invasive carcinoma, but the adenoma to

adenocarcinoma sequence was not seen.

Ward (1974) examined the changes found in male Fischer (F344) rats using dimethylhydrazine to induce tumours. There were various descriptions of the tumours induced, polypoid adenocarcinomas, adenomas, adenocarcinoma in the mucosa and adenocarcinomas associated with polyps, 1mm lesions were located as either stalks or plaques. In the descending colon there were polypoid neoplasms and adenocarcinomas while there were mucinous adenocarcinomas in the ascending colon. It was concluded that malignant tumours did not arise from adenomas but de novo.

Cooke et al. (1984) used male Wistar rats and azoxymethane to induce colonic neoplasia to study crypt cell kinetics. Histological changes were demonstrated, as well as the crypt cell production rate prior to the production of tumours. Histologically they developed microadenoma with areas of dysplasia. These later became sessile adenomas and eventually invasive tumours, both tubular and mucinous adenocarcinomas.

The finding of adenoma, adenocarcinoma sequence by Cooke et al. (1984) was at variance with Filipe (1975), and Shamuddin and Trump (1981). It was not altogether clear why using the same carcinogen, or a variant of the same type of carcinogen, should produce a different result. The

difference could represent a species difference or variance in the interpretation of the results. Williamson and Rainey (1984) found using azoxymethane, although in Sprague-Dawley rats, that the tumours induced were adenomas and adenocarcinomas, but the histological features were variable.

Chang (1978) used 1,2 Dimethylhydrazine to induce neoplasms in female CF-1 mice. The colonic cells were examined using tritiated thymidine. Histologically there was early hyperplasia, at 9 weeks, with labelled nuclei found increasingly in the non-proliferative zone of the crypt. There were two patterns of tumour formation, either by downward growth forming invasive tumours, or upward growth forming polypoid lesions. This might explain why two different types of tumour formation were seen in the rat models.

Chang (1978) suggested that hyperplasia was the healing process related to the carcinogen which accompanied colon tumourogenesis, but was not a necessarily a precursor. Factors that promote hyperplasia may promote tumours. Two stages were recognised in the development of tumours, abnormal stem cells in the crypt which then populate it, followed by abnormal stem cells in the upper crypt which kept dividing causing cystic degeneration in the crypt below.

Conclusion

The studies described have shown the ureterosigmoidostomy in man can be simulated in rats. Tumours have been found in Wistar rats at the vesicocolic anastomosis, and histochemical and crypt cell kinetic techniques can be applied in this species.

CHAPTER 5

ASSESSMENT OF CARCINOGENESIS I

In this section the various techniques which have been used to assess carcinogenesis in experimental animals which include histochemistry, colonic crypt cell kinetics and the measurement of cellular deoxyribonucleic acid (DNA) content have been reviewed.

Histochemistry

The colonic mucosa contains glycoproteins which are conjugated mucopolysaccharides, sometimes known as epithelial mucosubstances. Acid mucosubstances comprise the largest group of which the epithelial group are divided into sulphomucins or sialomucins.

Spicer (1965) showed that N,N-Dimethyl-m-phenylenediamine under certain conditions could be used to distinguish types of mucopolysaccharides, one group whose staining was prevented by oxidation with periodate and another whose staining was unaltered. Impurities with the para isomer of N,n-Dimethyl-p-phenylenediamine altered the staining markedly. In a buffer at pH 6 a meta solution remained colourless and did not stain acid mucopolysaccharides, while the para solution developed a red purple colour and stained mucopolysaccharides grey-black. A mixture in the ratio 6:1, meta to para turned purple-blue and stained a purple colour which could be made selective for acid mucosubstances by differentiation in acid alcohol. When the pH was below 2.5

the solution became colourless. Staining with acidic mixed diamine solution was eliminated in some mucopolysaccharides with prior oxidation with periodate. The periodate induced aldehydes blocked the uptake of the mixed diamine reagent. Following this some mucopolysaccharides retained their affinity to base dyes while others did not. It was found that reducing agents inhibited the colour formation of mixed diamine while hydrogen peroxide increased colouration. The oxidation agent used was ferric chloride, and this could be used to stain acid mucopolysaccharides selectively for sialomucin and sulphomucin. The staining ability of various diamine stains were compared as was the use of a high concentration of ferric chloride against a low concentration. These were contrasted with other staining methods.

Sulphomucin

The high iron diamine method was found to visualise nearly all autoradiographically or histochemically identifiable sulphomucins (Dziewiatkowski, 1954), staining grey-black. It also stained sulphomucins demonstrated by staining with azure A at pH 1.5 or alcian blue at pH 1.0. The high iron diamine method stained less clearly sulphates, shown by autoradiography, whose acidity was masked. These could only be shown by aldehyde fuchsin. Non-sulphated mucosubstances lacked affinity to high iron diamine reagent. Prolonged staining, 18-24 hr, with high iron diamine was required when

there was a preponderance of sulphomucins. There were some autoradiographical proven sulphated mucins which stained weakly or not at all by the high iron diamine method in mouse and rabbit parotid alveoli.

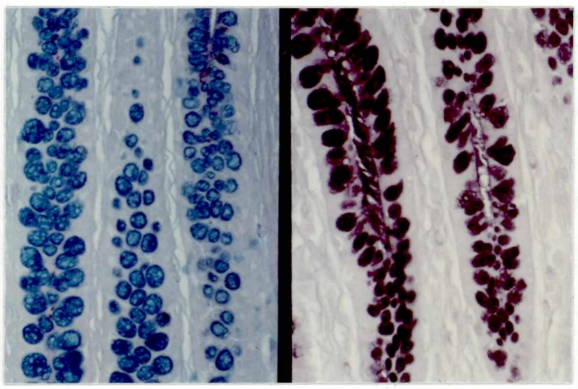
Sialomucin

Affinity for the high iron diamine reagent was lacking in acid mucopolysaccharide known to be non-sulphated, as shown by either failure to incorporate radiosulphate or by other histochemical means. Most of these acid mucopolysaccharides which did not stain with high iron diamine were sialomucins. Acid mucopolysaccharides which stained with low but not high iron diamine were sialomucins (Spicer, 1965).

The high iron diamine was used to stain the tissue and became fixed, and this did not alter when post-stained with alcian blue. The areas with autoradiographic evidence of sulphated mucins stained grey-black, while areas with enzymatically digestible sialomucin appeared blue (Fig. 7)(Spicer and Warren, 1960).

Preliminary studies in man had suggested the histochemical and histological changes of "transitional mucosa" adjacent to an adenocarcinoma were a pre-malignant indicator (Filipe, 1969). This was associated with a histochemical change to sialomucin throughout the crypt, and elongation and branching of the crypts histologically.

Fig. 7.



Photomicrograph of colon stained with high iron diamine alcian blue pH 2.5. The left demonstrates sialomucin and the right demonstrates sulphomucin.

Animal Studies

Filipe (1975) was the first to study mucin changes in animals that had tumours induced by carcinogens. Seventy five female Wistar rats weighing 140g were fed a normal diet with free access to water. The rats were injected subcutaneously with a weekly dose of, 20mg/kg of body weight, 1,2 dimethylhydrazine-2HCl (DMH). Two test and one control animal were sacrificed at weekly intervals. The colons were removed, placed in formal saline, sectioned and stained with haematoxylin and eosin (HE) and high iron diamine alcian blue, pH 2.5 (HID-AB).

Various morphological changes found within the colon were defined as follows. It was called hyperplasia when the crypts were longer than normal and the lumen dilated, with the nuclei aligned regularly on the basement membrane. Dysplasia was divided into grades 0-2. In grade 0 the crypts were not longer than normal but dilated and the goblet cells were more eosinophilic. In grade 1 the crypts were distorted, the nuclei were hyperchromatic and there was some pseudostratification. In grade 2 there were marked hyperchromatic nuclei, loss of nuclear orientation and mitotic figures. There was increasing tubular irregularity and loss of goblet cells. In carcinoma in situ, severe dysplasia grade 3, the crypts were tortuous, the nuclei were large and hyperchromatic and with mitotic figures at

differing heights.

Invasive carcinoma had the features of severe dysplasia with submucosal invasion. Both severe dysplasia and invasive carcinoma had surrounding hyperplasia and varying degrees of dysplasia.

Control animals throughout the time of the study showed no morphological or histochemical changes (Filipe, 1975). Control animals had straight crypts lined by mucus secreting goblet cells. The mucin pattern in the distal colon was predominately sulphomucin throughout the length of the crypt.

During the first three weeks there were no histological changes but during the following weeks there was hyperplasia with occasional dysplasia. From 7 to 13 weeks there was hyperplasia with all grades of dysplasia although rarely grade 2, and from 13 weeks onward there was severe dysplasia grade 2 and carcinoma in situ. At 19 weeks and thereafter there was invasive carcinoma seen in all but one animal in which there was carcinoma in situ. All the carcinomas found were well differentiated adenocarcinomas.

In the distal colon of the control animals there was mainly sulphomucin throughout the length of the crypt, although there was variation in the mucin pattern in different parts

of the colon (Filipe, 1975). During the period of hyperplasia, up to 6 weeks, there was a preponderance of sulphomucin. From 7 to 18 weeks, the hyperplastic glands contained mainly sulphomucin while goblet cells in areas of dysplasia, grades 0-1 sialomucin predominated. Secretion of mucins was reduced in grade 2 and carcinoma in situ and combinations of sialomucin and sulphomucin were detected. In the period 19 to 27 weeks, there was invasive carcinoma and carcinoma in situ, during this period there was a mixture of both acid mucopolysaccharides present.

Filipe (1975) suggested that the morphological changes in the colon when stimulated by this carcinogen were similar to the human large bowel cancer. This supported its use as a suitable animal model of human colorectal cancer, despite the lack of an adenoma to adenocarcinoma sequence as seen in man (Muto et al., 1975). During the period of hyperplasia there was no change in mucin pattern. As the animals progressed through dysplasia there was a change in mucin to sialomucin. Similar changes have been found in the surrouding "normal" colon of a carcinoma in humans as well as mucosa more proximal and distal from the lesion (Filipe and Branfoot, 1976).

Shamsuddin and Trump (1981) performed a similiar study with a few variations. Sixty male Fischer 344 rats, weighing 60g, were injected at weekly intervals with azoxymethane,

8mg/kg. The rats were divided into three groups, group 1 received 4 weekly injections and were sacrificed 4 weeks later. Group 2 received 6 weekly injections and were sacrificed 4 weeks later. Group 3 received 9-12 weekly injections and were then sacrificed 2,3,4,6,8 and 12 weeks after their last injection. No macroscopic tumours were found in groups 1 or 2. Fungating tumours were found in group 3, the first appeared 2 weeks after the completion of the injections. Most of the tumours were found in the distal colon. The first histological change was dilation and distortion of the crypt, and later there was the appearance of basophilic crypts and microcarcinomas, and this was followed by tumours. In group 1 there was dilation and distortion of the crypts with flattening of the lining cells, and in group 2 there were similar findings around microcarcinomas.

Histochemically sulphomucin was predominate in group 1, which had 4 weekly injections of carcinogen and this group showed no increased mitoses. However, in group 2, after 6 weeks there was an increase in mitoses which coincided with an increase in sialomucin present both in normal and distended crypts. In both the microcarcinomas and adenocarcinomas there was decreased uptake of stain but in those that took up the stain it was mainly sialomucin. In this animal model the adenoma to adenocarcinoma sequence was not seen, but in situ carcinoma was observed and the

polypoid tumours present were malignant. The change to sialomucin indicates that there is a alteration in the colonic epithelium which may then become malignant or revert to normal. (Shamsuddin and Trump, 1981).

Bowel Diversions

Filipe et al. (1982) developed a further animal model, using 110 female Wistar rats weighing 50g which were divided into 6 groups. In 20 rats the colon was divided and a short loop, 2cm long, which emptied into the colon was fashioned. In 20 rats a filling loop, 2cm long, was created into which the bowel contents would enter. A long empty loop, at least 10cm long, was created in 20 rats. A division of the colon and a re-anastomosis was performed in 10 animals and not subjected to carcinogen. Twenty animals were subjected to the administration of the carcinogen, 1,2 dimethylhydralazine 2HCl (DMH) only and another 20 were to act as a control.

When a steady weight had been attained at about 4 to 6 weeks all the animals, except the controls, were started on 20mg/kg of body weight of DMH at weekly intervals. Barium studies had shown that there was reflux into the short empty loops and 2cm into the long empty loops. Animals were then sacrificed at two weekly intervals in each group.

The control animals developed no mucosal changes (Filipe et

al., 1982). In the animals which had division and re-anastomosis, there were no changes except at the anastomosis, where hyperplasia was noted.

In all groups exposed to DMH there were marked mucosal changes. During first stage there was an increase in crypt length and dilation of the goblet cells, but no atypia. In the second stage dysplasia was seen with irregular crypts, a decrease in goblet cells, and an increase in immature cells and mitoses. During the transition from hyperplasia to dysplasia there was an increase in sialomucins. Carcinoma in situ and invasive tumour cells were non-secretory but the surrounding mucosa showed increased sialomucin.

In the group with DMH only, the tumours were found predominately in the distal colon. In the group with a short filling loop, tumours were observed mainly at the anastomosis and the distal colon was relatively spared. This pattern of tumour distribution was similar in those with the short emptying loop. In the rats with the long emptying loop, there were again tumours mainly at the anastomosis but others were found in the distal end of the loop where the barium studies had shown no reflux.

There was alteration in the proliferation and maturation associated with the change in histochemistry (Filipe et al., 1982), with an increased tumour incidence at the anastomosis

and with increased numbers of tumours proximal to the anastomosis. It was suggested that this was due to stasis secondary to an alteration in neuromuscular transmission or the effect in increasing volume of bile altering the faecal stream. Fewer tumours were found in the long loop, although this was not stated in the results, and the DMH may have reached this part of the colon via the blood stream or possibly by reflux. As in man tumours were observed more commonly at the anastomosis.

Conclusion

In animal experiments using chemical carcinogens an alteration in the mucin staining has been observed. The change to a sialomucin pattern was found during the transition from hyperplasia to dysplasia, and was thought to be a marker of malignant change.

CHAPTER 6

ASSESSMENT OF CARCINOGENESIS II

The previous chapter examined the histochemical changes associated with carcinogenesis. This chapter reviews colon crypt cell kinetics and its relation to carcinogenesis.

Cell Replication

The colonic crypt, on the basis of crypt cell kinetics, has been shown to consist of three zones, the stem cell zone, the proliferative zone and the functional zone (Eastwood, 1977)(Fig. 8). The stem cell zone consists of a few cells at the base of the crypt which have a notably longer cell cycle time than the cells in the proliferative zone. These proliferative cells provide a constant source of new cells which then migrate toward the lumen of the gut. As they migrate they lose the ablilty to divide and become more specialised. During the process of cell division the cells undergo the cell cycle (Fig. 9). The cells enter the cell cycle from interphase (G0), and proceed to the G1 phase. After G1 the cells enter the synthesis (S) phase during which deoxyribonucleic acid (DNA) replication occurs. The cells then enter the post-synthetic pre-mitotic gap, G2 phase before undergoing mitosis. After mitosis some cells enter a prolonged G1 phase, called G0 phase where synthesis is suspended but cell proliferative potential remains. Other cells enter a non-proliferative phase after mitosis and migrate out of the proliferative zone as differentiated cells. This is an over simplification of the cell cycle as

Fig. 8

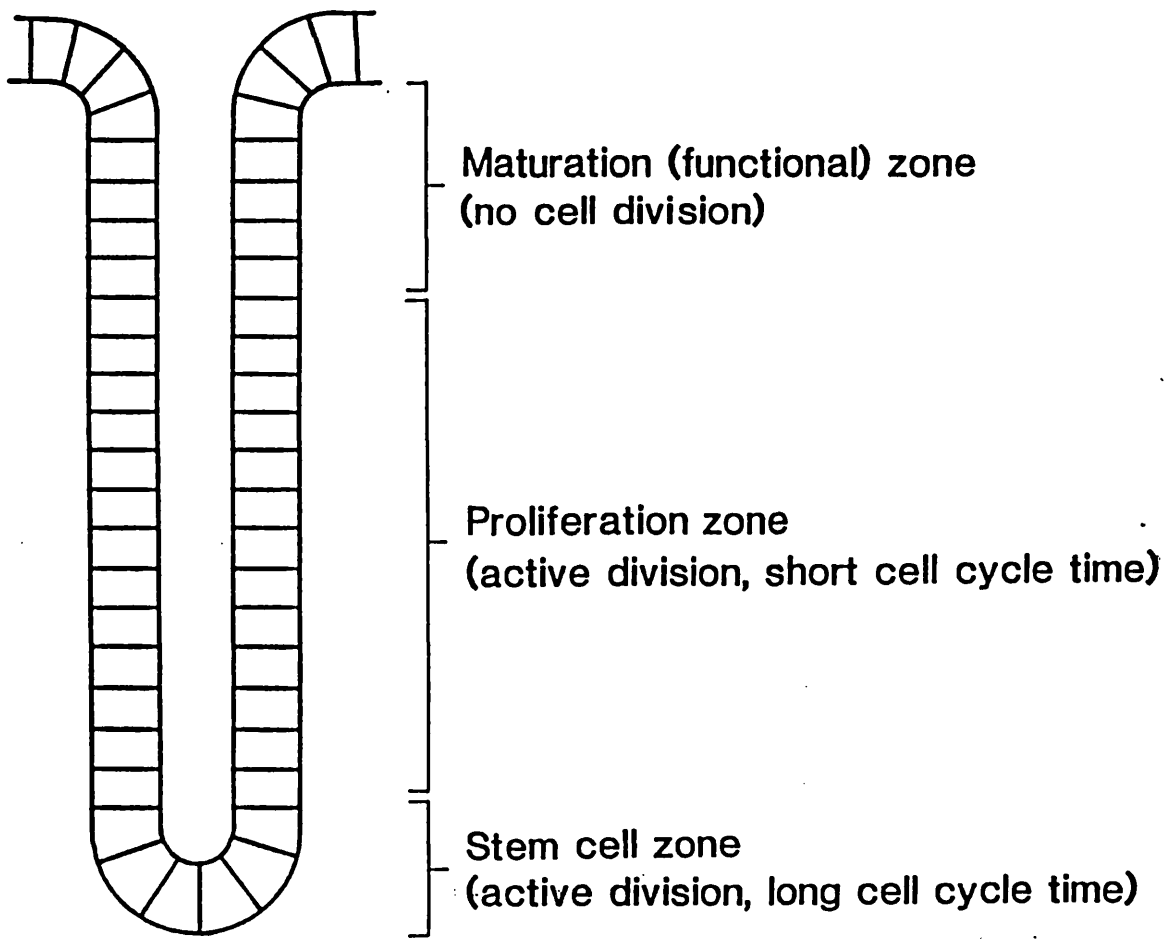
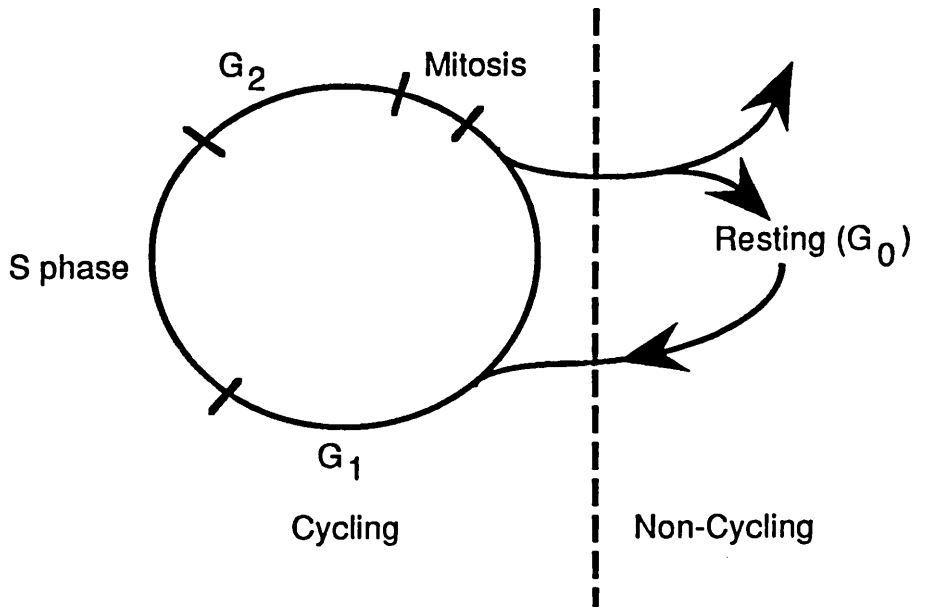


Diagram showing the structure of normal colonic crypts.

Fig. 9



Diagrammatic representation of the cell cycle.

there are many sub-groups (Pardee, 1978).

The cells migrating from the proliferative zone replace the surface epithelium every 2-3 days in rodents and 3-8 days in humans (Lipkin, 1974). In the surface epithelium of adenomatous polyps and carcinomas, nucleic acid metabolic enzymes have been found which are characteristic of immature proliferating cells (Peterson and Lipkin, 1974). The proliferative zone in polyps from patients with multiple polyposis was observed to extend to the upper crypt and mucosal surface (Deschner et al., 1963). This pattern was found at the periphery of tumours (Lieb and Lisco, 1966) and also in normal mucosa adjacent to benign polyps (Deschner et al., 1966). Lipkin (1974) suggested a model of 2 phases to explain neoplastic growth, in phase 1 there was an extension of the proliferative zone into the upper crypt, and during phase 2, there was abnormal cell proliferation with cell retention.

Assessment of Crypt Cell Turnover

The time taken for cell replication can be calculated from the number of dividing cells observed over a known time interval. Agents, such as colchecine or vicristine, which stop division in metaphase and allow the divided cells to accumulate will enable this calculation. Phase labelling techniques rely on the uptake of a marker which can then be observed through cell replication. The amount of

incorporation of the marker by the cell with time enables the cell turnover rate to be calculated.

Crypt Cell Production Rate

The crypt cell production rate (CCPR) is derived using a stathmokinetic technique. This method of calculating cell turnover involves the accumulation of divided cells when a chemical arrests metaphase. This is performed over a known time interval and the rate of turnover is calculated from this. Clarke (1970) first suggested counting the arrested crypts within a single crypt, using this as a constant reference point. He injected Colcemid 1.3mg/kg body weight in male albino rats to examine cells arrested in the small bowel. After fixation, the specimen was stained by the Feulgen technique and the crypts were microdissected and separated to allow measurement of arrested metaphases in an individual crypt. Seven animals, at 2 hours and 30 minutes and one at 30 minutes, showed cells in anaphase and telophase, which had escaped colchicine blockade. This would tend to underestimate crypt cell production rate. Clarke (1970) found that counts in a single crypt was reproducible (coefficent of variation = 3.9%). It was shown that the crypt cell production rate at the same site within the same animal gave a different mean although this was not statistically significant. The technique of microdissecting out individual crypts removed the problem of the thickness of the section and the angle of the section. The rate of

entry of cells into division was measured, if the cell population of the crypt remained a constant size and the cell loss was by migration into the colonic lumen.

Metaphase Arresting Agents

The ideal drug for producing cell arrest should be non-toxic, produce complete blockade without metaphase degeneration. Four drugs were compared by Tannock (1967), colchicine, colcemid, vinblastine and vincristine. The first part of the experiment was to establish the optimum dose for producing arrest. The effects of the drugs at different concentrations on small bowel and a tumour were compared. The concentration of drug injected intraperitoneally which produced a maximum number of arrests indicated the optimum concentration. In the experiments with tumour the optimum dose with all drugs was 1mg/kg body weight, which was the same for normal small bowel except vincristine whose optimum dose was 0.5mg/kg.

An additional study examined the increase in arrested metaphases with time (Clarke, 1970). The curve was not linear over the 4 hours of the study. The experiment showed there was tail off in arrested metaphases with time. This was due metaphase degeneration. The steepest part of the curve should be used to calculate the crypt cell producton rate, from 30 minutes to 2 hours 30 minutes. Also the curves did not pass through the origin, as there was delay

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to metaphase arrest.

Metaphase Degeneration

The time at which the arrested metaphases were measured was relevant to the calculation of the crypt cell production rate (Aherne and Camplejohn, 1972). Ideally, degenerating metaphases should be incorporated into the calculation to give an accurate calculation of the crypt cell producton rate. However, with readings less than 4 hours the number of degenerate metaphases was small enough to ignore. The durability of arrested metaphases was both species and tissue dependant.

Cell Turnover

Duffill et al. (1977) showed that the metaphase arrest technique could be used to calculate cell turnover times. The age distribution of the dividing cells had to be rectangular to make this calculation. This meant that dividing cells must remain in the same plane. Corrections to the calculation have to be incorporated if the age distribution of the cells was not rectangular. This did not apply to colonic crypts as they line the crypts as a single layer.

Diurnal Rhythm

When studying cell kinetics it is essential to standardise the experimental conditions as much as is practical. The

studies need to be performed at the same time of day as there was evidence of a diurnal rhythm (Scheving et al., 1978). Their experiment showed a marked increase in the crypt cell production rate during the transition from dark to light and a trough of activity between light to dark. It was not clear whether this change was due to the change in light or a response to food. To compare results between groups of the same species they must all be the same age as it was found that the crypt cell production falls with increasing age (Goodlad and Wright, 1982).

Changes in Crypt Cell Production Rate

There are three methods of increasing cell production within the reproductive compartment of the crypt. There can be an increase in the size of the proliferative zone within the crypt, an increase in the size of the crypt with a related increased size in the proliferative zone, or an increase in the speed at which the cell renewal cycle is taking place. The other technique for increasing overall cell production is to increase the number crypts in a given surface area.

Animal Crypt Cell Production Rate

Weekly injection of azoxymethane lead to tumour formation in Wistar rats which were histologically similar to human tumours (Cooke et al., 1984). Using this model there were increases in the crypt cell production rate as early as 5 weeks, after azoxymethane injections, but this did not reach

significance until 20 weeks. Histological lesions in the treated animals were not seen until 10 weeks. The alterations in the crypt cell production rate preceded histological changes.

Radioautographic Techniques

Radioautographic techniques use pulse labelled DNA analogues such as tritiated thymidine to estimate the time spent in each phase of the cell cycle. However, the time taken in each phase was not uniform for any given structure (Lipkin, 1973). Tritiated thymidine is a nucleoside precusor that is incorporated into DNA and can be used to study renewal and migration of cells. The gut was classified as a renewing cell population (Messier and Leblond, 1960). In a renewing population the cell division took place while developing into a more advanced stage of differentiation. The mature cell no longer divided and the cell population relied on input from the proliferative zone. The steady state was maintained by cells gained equaling cells lost. Tumour cells may divide without an increase in cells lost.

A pulse of tritiated thymidine was used to calculate the fraction of cells in the different stages of cell division. Initially there were a number of unlabelled mitoses, the duration of G2. The period from zero labelled mitoses to a maximum was equal to the time of mitosis. S phase duration was equal to the time with all mitoses labelled and the time

between the fall and subsequent rise in activity was equal to G1 and G2. However, as with any biological system there was soon desynchronisation of the phases with flattening out of the peaks of activity (Lipkin, 1973). Meisseier and Leblond (1960) also showed that if cross sections of fixed specimens were used, because the dividing cells were nearer the centre there would be an overestimate in the number of dividing cells.

In a steady state the mitotic index (MI) was Nm/Nc, number of cells in mitosis/number of cells in the prolifertive zone, = Tm/Tc duration of mitosis/duration of the cell cycle (Lipkin 1973). The thymidine labelling index (LI) was Ns/Nc, cells in S phase/cells in proliferative cycle. Ns/Nc = Tm/Tc and as Nm, Nc and Ns were known Tc can be calculated. The birth rate could also be calculated from this, kb = MI/Tm = LI/Ts, the duration of S phase = 1/Tc. A correction factor must be used with these calculations if non-proliferating cells were included.

The mitotic index measured the percentage of cells with mitotic figures to those in interphase within the proliferative zone. It, however would not detect any increase in cell production if there was an increase in numbers of proliferating cells. It cannot detect differences between a change in the proportion of cells in mitosis and changes in the duration of mitosis.

In the colon of man the tritiated thymidine was found in the basal three quarters of the crypt, 15-25% of cells in the proliferative zone were in DNA synthesis. The cell cycle in the rat is approximately 19 hours and in man 39 hours (Lipkin, 1973). G1 is not constant and is in a state of flux. Cells that differentiate can not return to the cell cycle. Cell kinetic techniques are used to detect and quantify changes in cell production (Wright, 1980).

Conclusion

The crypt cell production rate as calculated with a metaphase arresting agent has been shown to the most accurate method of calculating cell turnover. It also takes into account both an increase in the number of dividing cells and their increased speed of reproduction.

CHAPTER 7

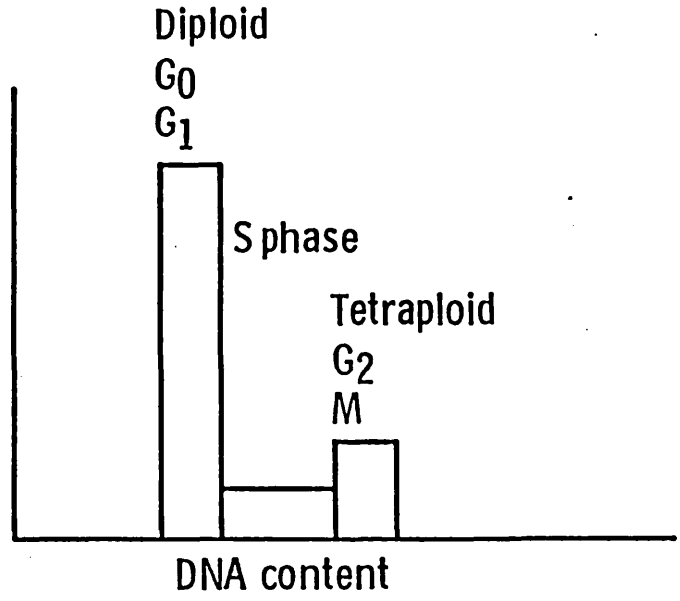
ASSESSMENT OF CARCINOGENESIS III

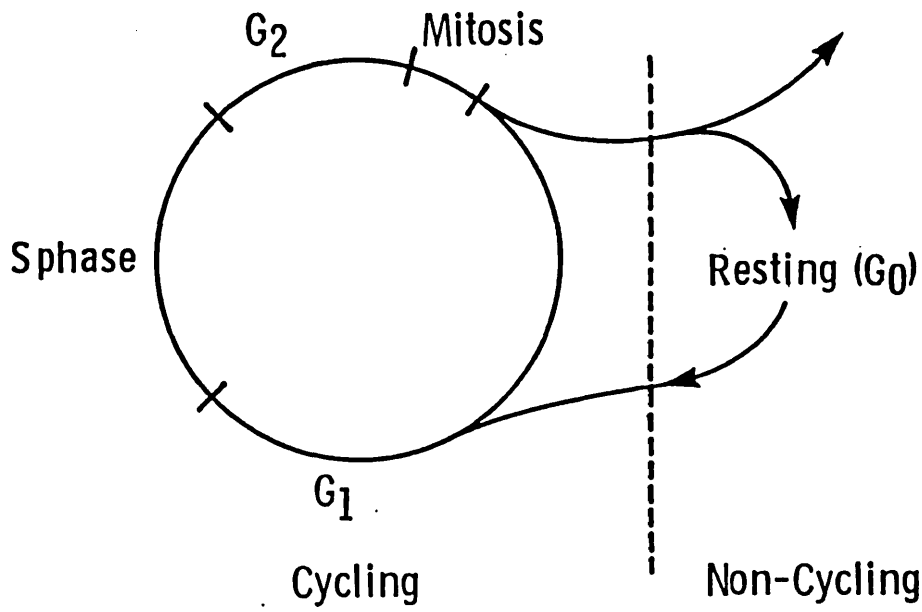
The previous chapter dealt with a direct measurement of cell turnover. In this chapter the cellular DNA content has been determined, which can be used to indirectly calculate cell kinetics.

Traditionally cell kinetic techniques employing the uptake of DNA with tritiated thymidine have been used, but in these studies an alternative approach has been used. As there is an increase in cell turnover the number of cells in each stage of cell duplication will change. Resting cells (G0) enter G1 phase (post mitotic gap), where they remain for a fairly constant period of time (Fig. 10). They then enter the S phase (synthesis). It is during this phase that the cell duplicates its DNA before entering G2 phase (pre mitotic gap). From G2 the cells undergo mitosis (M) and then either go into G0, resting, or back to G1 for further duplication. At this stage cells may differentiate and leave the cell cycle.

Cells in G0 and G1 phases of the cell cycle have a diploid amount of DNA (Fig. 10). In the S phase the DNA content is between that of diploid and tetraploid as the DNA being is synthesised. Cells in G2 or mitosis have a tetraploid DNA content. The percentage of cells in S, G2 and M phases can be calculated by measuring the DNA content of the cells within a population of cells. This is done by staining

Fig. 10





Diagramatic representation of the cell cycle and histogram plotting the number of cells in a proliferating population against their DNA content.

cells with a stain such as Feulgen's which binds solely and stoichiometrically to the DNA of the cell. The DNA content can be then measured by cytometric methods.

Feulgen Technique

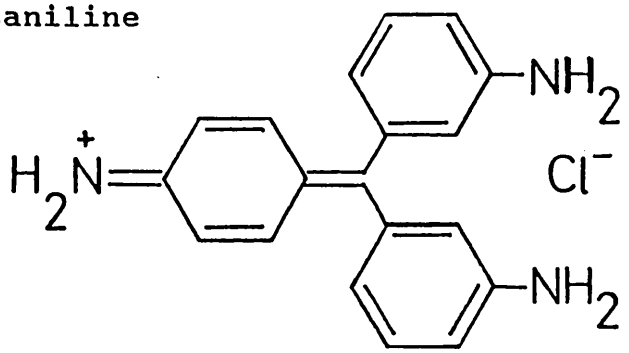
The Feulgen reaction (Feulgen and Rossenbeck, 1924) stains DNA stoichiometrically, so if properly controlled could be used to measure DNA content of cells. The Feulgen techinque is considered to be specific for DNA, as only nucleic acids containing deoxyribose show a reaction (Bancroft, 1975). Mild acid hydrolysis with warmed 1 molar hydrochloric acid unmasks aldehydes in the deoxyribose moiety of DNA but not the ribose moiety of RNA. During the process the RNA is removed from the cells and the acid also eliminates purines from the DNA. Hydrolysis also produces more aldehyde groups for staining, but if prolonged, hydrolysis splits internucleotide bonds and renders the DNA soluble. Fixation affects the binding of DNA to nuclear proteins and so could retard both loss of purines and the susceptibility of internucleotide bonds to hydrolysis.

Schiff's reagent is produced by a reaction of basic fuchsin with sulphurous acid which splits the quinoid structure to produce a colourless solution (Fig. 11). The aldehyde groups when exposed to Schiff's reagent become linked to them as a bisulphite bond to form a coloured complex by restoration of the quinoid structure. The unbound reagent

Fig. 11 The Feulgen reaction

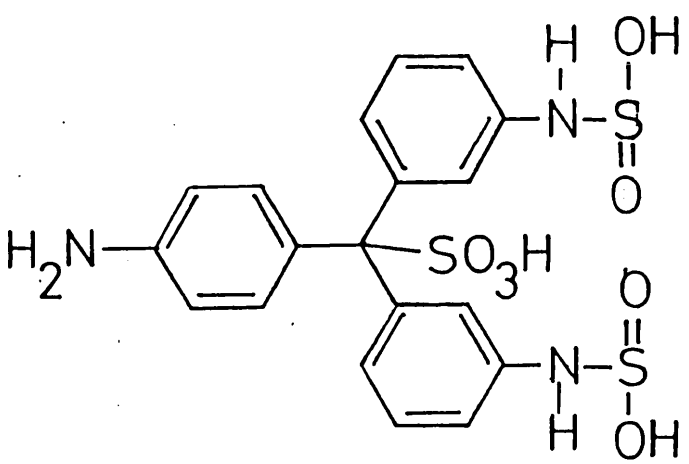
Basic fuchsin is a triphenylmethane dye and is usually a mixture of pararosaniline, rosaniline and magenta II. The chief constituent is pararosaniline.

Pararosaniline



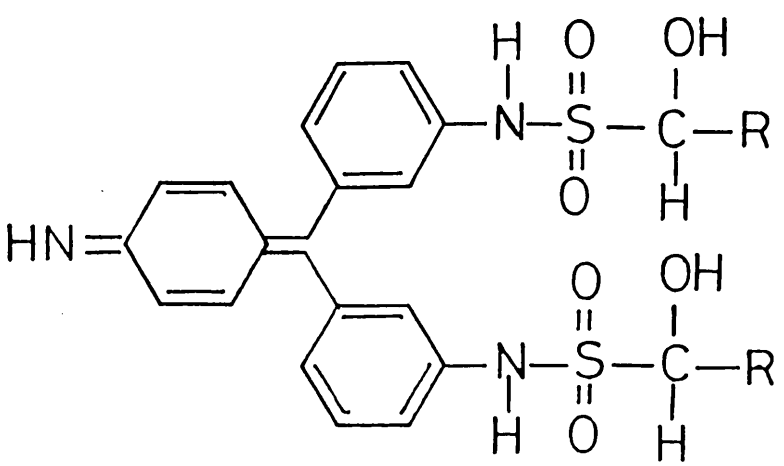
Coloured

When this dye is dissolved in sulphurous acid it forms a complex and becomes colourless.



Colourless

When this reacts with two aldehyde groups a coloured addition complex is formed which has an absorption at 560nm.



Coloured

is washed away using 2:1 by volume, potassium metabisulphite and 1 molar hydrochloric acid.

Cytometry

Once the cells have been stained stoichiometrically, the stain present in an individual cell requires the comparative DNA content to be measured. The first method used required ultra violet light and there were difficulties in the measurement. Pollister and Ris (1947) described the quantitation of stained cellular constituents using visible light. This required simpler instruments and from this developed static and flow cytometry. Only static cytometry has been used in this study, to avoid contamination of the specimen with non-epithelial cells.

Static Cytometry

The microspectrophometer is basically a spectrophotometer built around a microscope. The quantitation of the cellular DNA is based upon the specific interaction of photons from the densitometer and chromophores derived from the cytochemical method. The chromophores take up energy from photons from a suitable energy level, corresponding to a certain wavelength range, through reactions with unsaturated molecular bonds. The number of photons absorbed is directly proportional to the number of chromophores present. This relation was denoted by the Beer-Lambert law.

$$\text{Absorbance} = \log 10 \frac{(Io)}{(I)} = Kcl$$

where Io = incident light intensity; I = transmitted light intensity; K = absorptivity, i.e. the specific propensity of the chromophore studied to absorb light; c = concentration of chromophore; l = light path length. The concentration of the solute (c) could be related to the mass (m) and to the dimensions of the sample by the equation

$$c = \frac{m}{Al}$$

where A = projected surface area.

The most serious error arises as a result of the uneven distribution of the chemical substance being measured (Deeley, 1955). This difficulty can be overcome by dividing the sample optically into a large number of very small regions. These regions must be small enough to ensure that the individual areas contain optically homogenous material. Also it must not have a diameter greater than the resolution of the microscope. This was the method employed in the Deeling scanning and intergrating microdensitometer. There were 3 further types of error with this system, firstly glare error, secondly chromatic error and lastly diffraction error (Bjelkenkrantz, 1983). The sum of these errors may range from 3-15% and may be important when comparing DNA values for cells that differ in optical density, size or shape (Mares and van der Ploeg, 1980).

Vickers M85 Scanning and Integrating Microdensitometer

The instrument used in this study was the Vickers M85 Scanning and Integrating Microdensitometer. The stained specimen was first examined through the microscope using normal light, and then the area to be measured was marked optically. The sample was monitored by the instrument to ensure that measurements made were only within the area of the mask. Monochromatic light, forming a spot of area in the plane of the sample was then directed down through the objective and was scanned over the masked area of the sample (Fig. 12). The transmitted beam was collected by the microscope condenser and its intensity at each point was read by a multistage photomultiplier. The absorbance values at all points were then summed to yield a value of integrated absorbance.

The absolute values of a mass of chromophore could only be given if the value of the constant K was known. This was not essential for this study as the measurements of relative absorbance were proportional to the absolute values. More important was the fact that relative absorbance values could vary according to the voltage applied to the photomultiplier. This problem was overcome by checking the background absorbence at the beginning and end of reading each slide.

Fig. 12

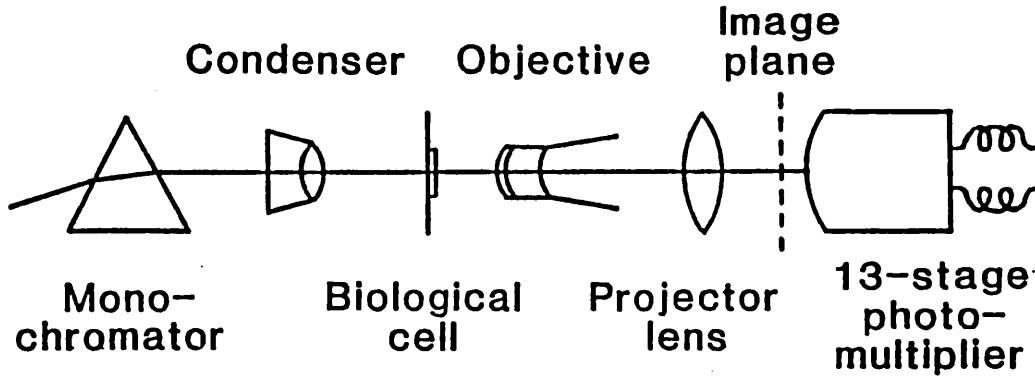


Diagram showing the principles of the Vickers M85 microdensitometer.

Comparison of Biochemical Techniques and Microdensitometry

The results obtained by microdensitometry need to be compared to standard biochemical methods before they can accepted for quantitative results. The DNA content of diploid nuclei of a species has been found to be constant (Ris and Mirsky, 1949), and therefore it is possible to compare the values obtained for a single Feulgen stained nucleus with the average value determined biochemically for a large number of nuclei. Ris and Mirksy (1949) used the Feulgen reaction to make measurements of the DNA content of liver nuclei from various animals. Their results were consistent, within 10%, with the biochemical determinations made on the same cell types. They did stress that the Feulgen technique should only be used to determine relative and not absolute cellular concentrations of DNA.

The inhomogenity of cellular material presented a problem for biochemical methods of analysis as it gave the overall value, rather than the individual cell value. Swift (1953) showed that rat liver cells when studied biochemically gave a DNA value that was between 2-4 times the DNA content of its spermatozoa. Ris and Mirsky (1949) demonstated that this result was due to rat's liver cells existing in the diploid, tetraploid and octoploid states. This showed the advantage of microdensitometry, compared with biochemical methods, in that it could calculate the DNA of individual cells rather than just the mean for a group of cells.

Conclusion

The technique of static cytometry has been shown to measure the DNA content of the cell. This can then be used to calculate the proportion of cells with increased amounts of DNA.

SECTION

II

ANIMAL EXPERIMENTS

CHAPTER 8

PRELIMINARY ANIMAL STUDIES

This chapter describes the preliminary experiments used in a feasability study to determine which animal model of urinary diversion should be used. The histochemical and cell kinetic techniques which were used to study the rodent colons will be described in later chapters. The overall experimental plan is shown in figure 13.

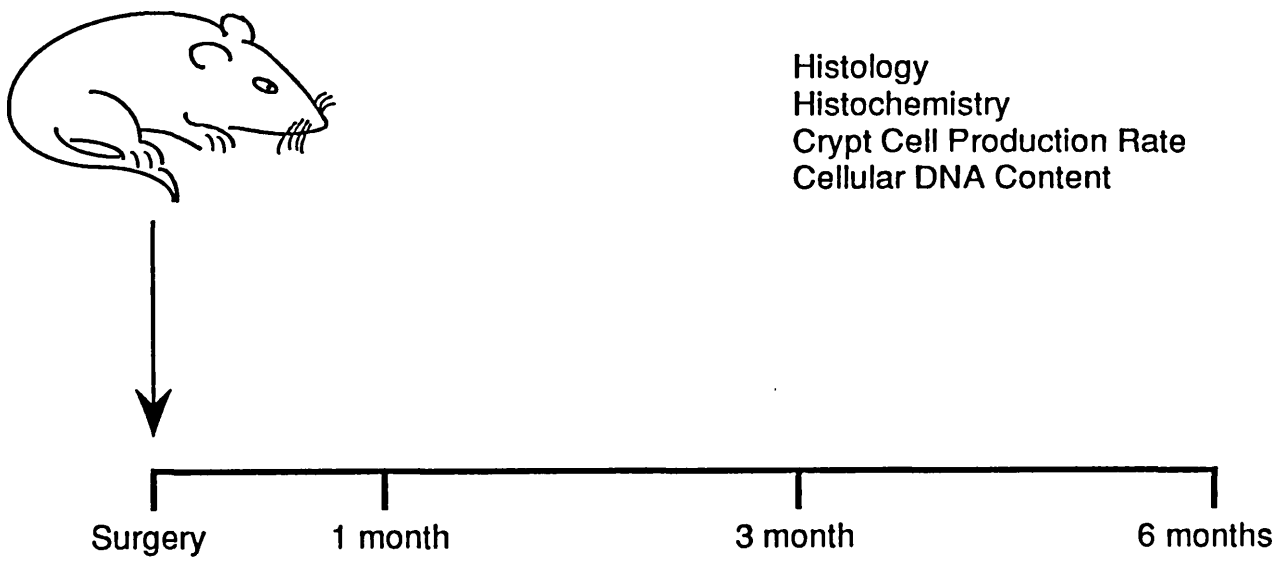
MATERIALS AND METHODS

Male Wistar rats were used for this study as they had been previously shown to produce tumours at a urothelial colonic anastomosis under certain conditions (Crissey et al., 1980). Histochemical as well as morphological changes occurred in Wistar rats when tumours were induced with a chemical carcinogen have been well described (Filipe, 1975), as well as the alterations in crypt cell kinetics (Cooke et al., 1984).

Caging of the animals, especially males, had been shown to be important for their well-being. Sawdust was changed on a daily basis to prevent perineal ulceration as observed by Crissey et al. (1980).

Animals weighing 200g were chosen as they had to be large enough to perform the surgery with minimal animal morbidity and mortality. They were fed a normal diet without additional medication and had unlimited access to water.

Fig. 13.



Plan of animal studies.

Hypnorm, a combination of Fentanylcitrate, a narcotic analgesic, and Fluanisone, a tranquilliser was used as a neuroleptic anaesthetic. Hypnorm was given subcutaneously, initially at a dose of 0.3ml, but this was found inadequate and was increased in some animals to 0.6ml. Animals later in the study were operated on under a general anaesthetic using halothane, nitrous oxide and oxygen. When Hypnorm was compared with a general anaesthetic, there were no advantages with Hypnorm and in several animals the level of anaesthesia was too light.

Ureterosigmoidostomy

Rowland's et al., (1979) technique with minor modifications was used to perform ureterosigmoidostomy on 20 animals. Access to the abdomen was gained through a midline incision, and the ureters were visualised and the distal end of the ureter ligated as distally as possible and divided. A colotomy was performed on the descending colon and the distal ureter attached to a suture brought through the colon in a submucosal tunnel to lie within the colon. Retraction of the ureter was prevented by a suture, 6/0 Ethibond or Prolene, which passed through the periureteric fat and was sutured to the colon. The colotomy was closed using 6/0 Ethibond or Prolene. The loose intraperitoneal fat was brought down to the anastomosis and the anterior abdominal wall was closed with chromic catgut. The skin was closed

with interrupted silk. In three animals unilateral rather than bilateral ureterosigmoidostomies were performed.

Vesicocolic Fistula

A modification of Crissey's et al. technique (1980) was performed as the original method they described had a high mortality rate. The reason for the high complication rate was probably the mobilisation of the bladder which could lead to bleeding and urinary fistulation and I modified this by leaving the bladder in situ for this investigation.

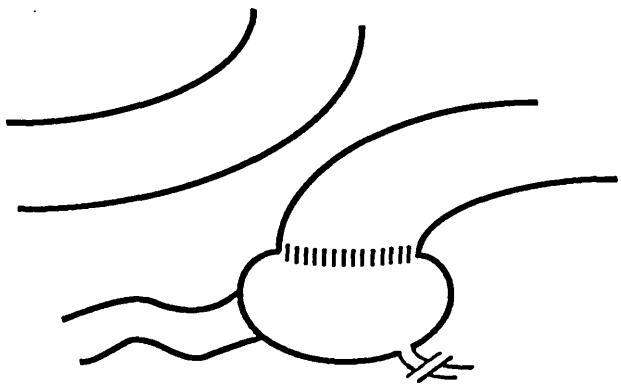
Male rats were used as the bladder neck was easier to see than in the female. Five animals were anaesthetised and a midline abdominal incision was made and the ureters observed entering the bladder. A 6/0 Prolene suture was passed under the ureters and around the distal sphincter mechanism and was tightened and the bladder obstructed (Fig. 14). A vesicotomy was performed and excess bladder removed. A colotomy was performed at the level of the vesicotomy and the bladder and the colon anastomosed with continous 6/0 Prolene.

Urothelial Patch

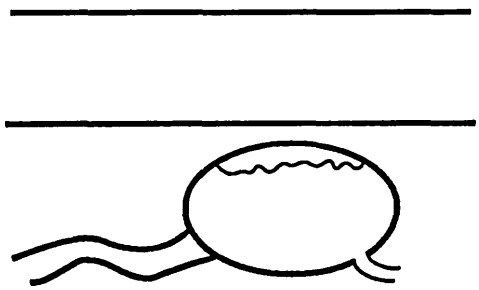
The effect of faeces alone at the colonic urothelial anastomosis was examined with a urothelial patch, this technique has not been previously described. As the patch was small a vascularised bladder patch was not required, and

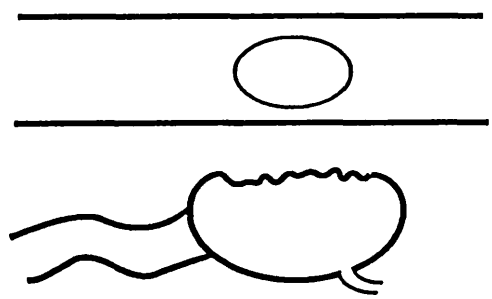
Fig. 14

Colon Conduit

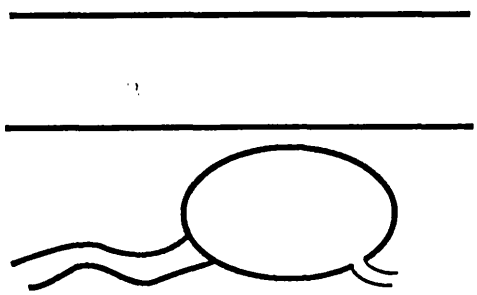


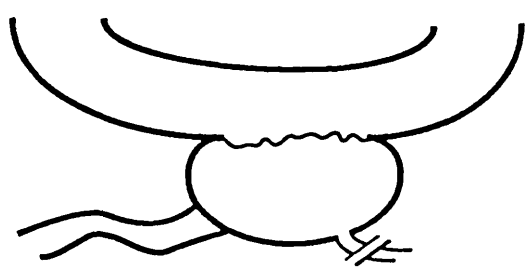
Urothelial patch





Vesicocolic anastomosis





was applied as a free graft (Fig. 14). Access to the abdomen was gained in five animals through a midline incision. The dome of the bladder was opened and a 4mm patch of bladder excised and the bladder was closed with 6/0 Prolene. A colotomy was made and the bladder patch inserted and sutured in position wth 6/0 Prolene.

RESULTS

Ureterosigmoidostomy

Two rats died during the procedure despite careful anaesthesia and another 15 animals died within the first week of the procedure. Four post mortems were performed and these showed that in all four, the ureter had come adrift from the colon and there was extravasation of urine into the abdominal cavity. Three animals survived to one month and then they were sacrificed. Two of these had a unilateral ureterosigmoidostomy. The side of the operation showed a grossly dilated ureter. There was evidence of renal scarring and one had stones lying within the ureter. In the rat with bilateral ureterosigmoidostomy both ureters were dilated.

Vesicocolic Fistula

All five rats survived to one month. The urine was shown to be in the colon by direct observation at post mortem and evidence of scrotal ulceration. Only one animal had gross evidence of renal scarring.

Urothelial Patch

Four animals survived to 6 months when they were sacrificed. The urothelial implants were still in position at that time. One animal died at 2 months, post mortem examination showed this to be due to bowel obstruction.

Discussion

The modification of Rowland's et al. (1979) method of urinary diversion had a high mortality. There were two areas where problems were encountered. Suturing the periureteric fat to the inner wall of the colon did not prevent the ureters from becoming detached and causing peritonitis. The second problem was of upper urinary tract dilation in those rats who survived the initial post-operative period due to ureteric scarring at the anastomosis. Two of the surviving animals, each had one kidney and ureter which was functioning normally.

The vesicocolic method of urinary diversion had by contrast a low mortality. This operation was much easier to perform and all animals were alive at one month. There was still the problem of scrotal ulceration but this could be kept under control by careful animal husbandry.

The free graft method of producing a urothelial patch was shown to be successful. Later, Gittes (1986) described a

vascularised bladder patch which was inserted into the colon wall, but this was proved to be unnecessary.

Conclusion

This preliminary study had shown that the most successful method of performing a urinary diversion into the colon was by the modified vesicocolic procedure described above. The novel method of producing a ureteric implant did not give rise to problems, and these methods were to be used in the definitive study using a general anaesthetic.

CHAPTER 9

ANIMAL STUDIES

The purpose of this study was to assess the effects of urine, either alone or mixed with faeces, at a urothelial colonic anastomosis. Another group was added later to examine the effect at a colonic transection and the distal bowel. Preliminary studies discussed in the previous chapter determined the techniques used.

MATERIALS AND METHODS

Male Wistar rats weighing 200g were divided into 5 groups (Fig. 15), with all operative procedures performed under general anaesthesia using halothane and nitrous oxide (Strachan et al., 1987). Animals were fed a normal diet with free access to water without additional medication. The floor of the cage was supplied with ample sawdust and changed on a daily basis to prevent scrotal ulceration.

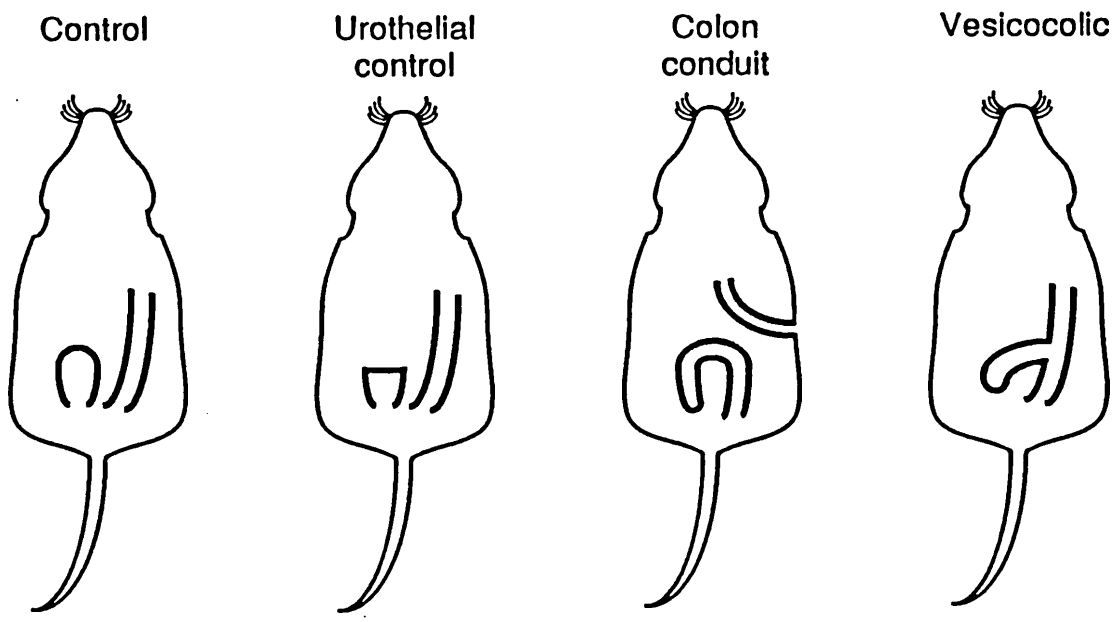
Group I Control

Twenty four rats were kept in three cages of eight. Eight rats were sacrificed at 1, 3 and 6 months. These animals acted as non-operative controls.

Group II Urothelial Implant

Twenty five rats had a urothelial implant placed in the colon as described in the previous chapter. A midline incision gave access to the bladder. The dome of the bladder was excised and implanted into the descending colon.

Fig. 15



Diagrammatic representation of the animal groups.

The vesicotomy and the colotomy were closed using 6/0 Prolene. There was one peri-operative death. Eight rats were sacrificed at one and six months, and six rats at three months.

Group III Colon Conduit

Twenty four rats had a colon conduit created in order to study the effect of urine alone at the urothelial colonic anastomosis. The bladder outflow was obstructed with a suture tightened around the distal sphincter, and the bladder anastomosed to the descending colon using 6/0 Prolene. The anastomosis site was defunctioned by a terminal colostomy brought onto the left flank. There were no peri-operative deaths. Eight rats were sacrificed at one month and seven rats were sacrificed at 3 and 6 months as there were two late post-operative deaths occurring after 1 month.

Group IV Vesicocolic

A urine faeces mix was achieved to mimic a ureterosigmoidostomy. In these animals the bladder was obstructed with a suture around the distal sphincter and a vescicolic fistula performed as in Group III without a defunctioning colostomy. Twenty eight rats had this procedure performed initially. There were four peri-operative deaths. Another seven rats had died by the 4th post-operative day. These rats were replaced by

performing another seven vesicocolic anastomoses. Eight animals were sacrificed at 1 month, and another seven at 3 months. Five animals died soon after 3 months and because of weight loss and perineal ulceration, the remaining four animals had their date of sacrifice brought forward to 4 months.

Group V Anastomosis

A later experiment was performed to examine the changes found at an anastomosis and to the distal colon. Through a midline incision the colon was cut tranversely and sutured with 6/0 Prolene. A marker suture was left in situ to identify the site of anastomosis. The animals in this group were sacrificed at 1 week, 1, 3 and 6 months. There were no peri-operative or late deaths.

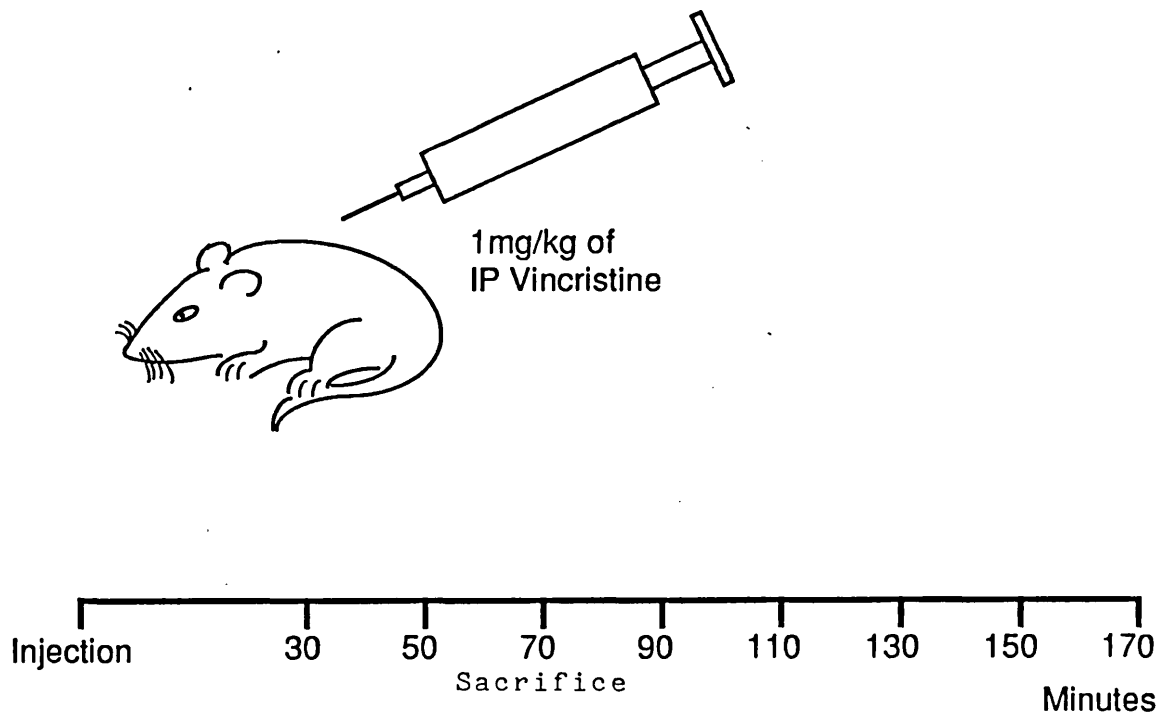
METHODS OF ASSESSMENT

Kinetic changes in the colonic mucosa were monitored by crypt cell production rate, colon cell DNA measurement and mucin changes by histochemistry.

Crypt Cell Production Rate

Groups of eight animals at each time interval were given Vincristine 1mg/kg intraperitoneally at 8.30 am. on the day of sacrifice and an animal was killed at 30 minutes and then every 20 minutes subsequently (Fig 16). Weight loss in the vesicocolic group meant that the final date of sacrifice was

Fig. 16



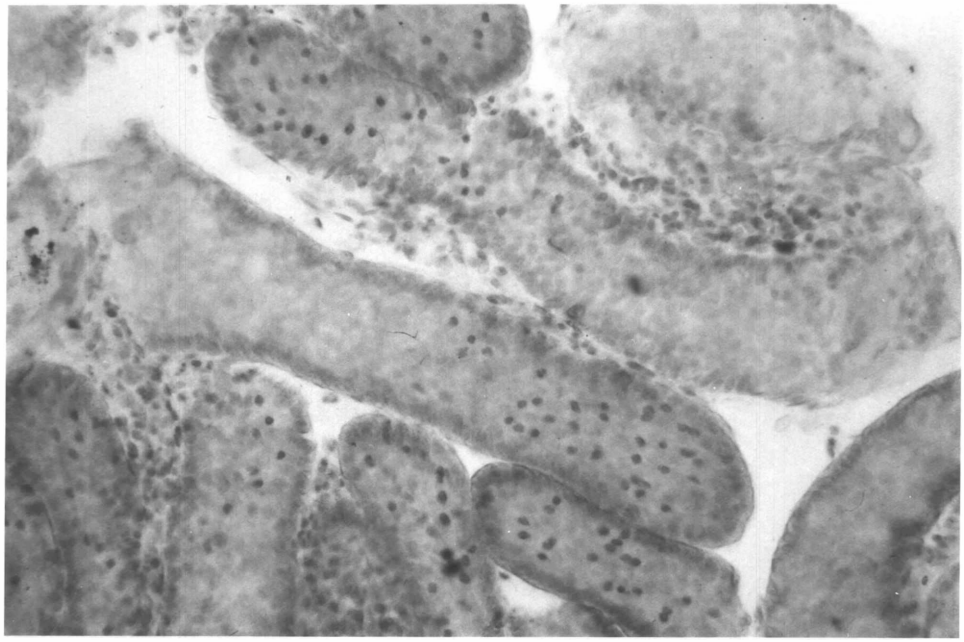
Diagrammatic representation of crypt cell production rate method.

brought forward to four months. In those incomplete groups where animals had died prematurely the timing of the sacrifice was adjusted to cover 30 minutes to 170 minutes.

The colon at the specified site was removed and placed in Carnoy's solution, (6 parts ethanol: 1 part glacial acetic acid: 3 parts chloroform,) for 4 hours and was then stored in 70% ethanol. Before being stained the specimens were placed in 50% alcohol for 5 minutes followed by 25% alcohol for 5 minutes. The specimens were then hydrolysed was then carried out in 1 molar hydrochloric acid for 6 minutes at 60 degrees C. before being stained in Schiff's reagent for 1 hour. After being rinsed in 45% acetic acid the specimens were ready for microdissection.

The crypts, using a dissecting microscope, were separated from one another with mounted needles. It was essential to keep the crypts moist with the acetic acid at this time. When there was adequate separation of the crypts they were squashed under a coverslip ready for counting (Fig. 17). The number of arrested metaphases were then counted in 10 crypts using a 40 times objective. The specimens were coded so that the count was "blind" in relation to the time of the sacrifice. If there were any anaphases or telophases present the count was discarded as the vincristine had not correctly arrested the metaphases. Once the specimens had been decoded the results were plotted and the slope of the

Fig. 17.



Photomicrograph of microdissected colonic crypt showing cells in metaphase arrest in the proliferative zone.

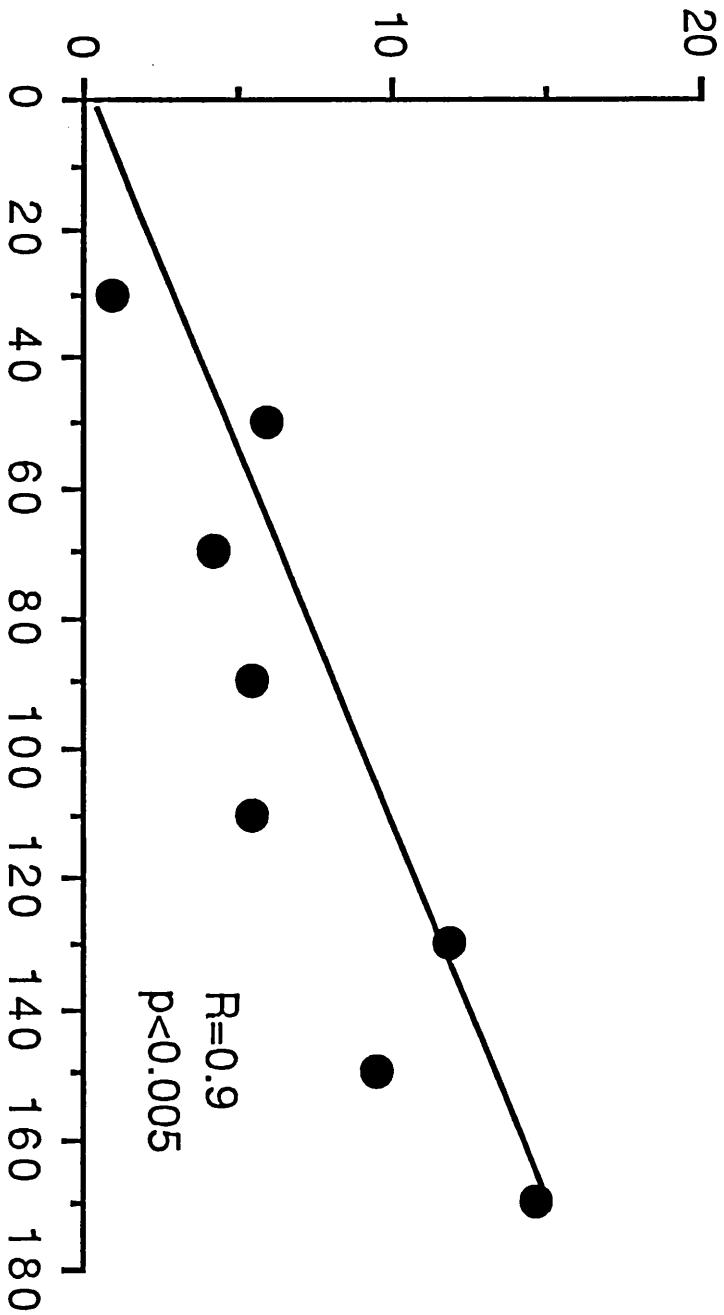
curve calculated by the linear regression of the least squares.

The slope for the accumulated metaphases gives the crypt cell production rate (Fig. 18). The line does not pass through the origin as there is a time delay to metaphase arrest, this is approximately 30 minutes. There is also a decay of the arrested metaphases with time. This time is variable in different animals and at different sites, although they are usually stable up to 3 hours. The slope was therefore calculated between the times 30 minutes to 2 hours 30 minutes. The crypt cell production rates were compared using a paired t-test. This test was used as the standard error of the mean of the linear regression meant that a type 1 statistical error could occur, when comparing two curves. The results were compared in this way as the study periods were the same in respect of time and conditions.

Cellular DNA Content

Colonic mucosa in the descending colon of the controls and anastomosis groups, and adjacent to the anastomosis in urothelial implant, colon conduit, vescicocolic and anastomosis groups were brushed to remove cells from the upper portions of the colonic crypts. The cells were stored in 5 ml of alcoholic Hank's solution and spun down by centrifugation. The cells were then syringed through a 25

Fig. 18



Graph of the number of arrested metaphases against time after vincristine ijection in the one month control animals.

gauge needle three times to break up clumps of cells. They were then washed three times in phosphate buffered saline solution pH 7.4. Cytopreparations were made with a Shandon cytopsin and post-fixed in Carbowax (ethanol, 1% glacial acetic acid and 0.3% polyethlene glycol). The cytopreparations were then dipped in 1 molar hydrochloric acid and then 5 molar hydrochloric acid for 50 minutes. The slides were then placed in Schiff's reagent for 1 hour, washed three times in 2:1 0.5% potassium metabisulphite: 1 molar hyrdrochloric acid, dehrydrated and then mounted.

The Feulgen technique binds to the DNA stoichiometrically, and the DNA content of one hundred epithelial cells was determined by microdensitometry. The microdensitometer mask was centred over the epithelial cells using a grid line system (Fig. 19) and the DNA content was assessed using the amount of light absorbed by the stain. The background reading was subtracted from the amount of light absorbed. The majority of readings were within a small band which contained diploid cells. The number of cells with more than one and a half times the diploid reading was expressed as a percentage and called the proliferative index. This included dividing cells, S, G2, and aneuploid cells. The cells in mitosis were not measured as the vincristine would cause the cells arrested in mitosis to accumulate with time. The results were compared using a Mann Whitney U test, which is a rank sum test for unpaired data.

Fig. 19

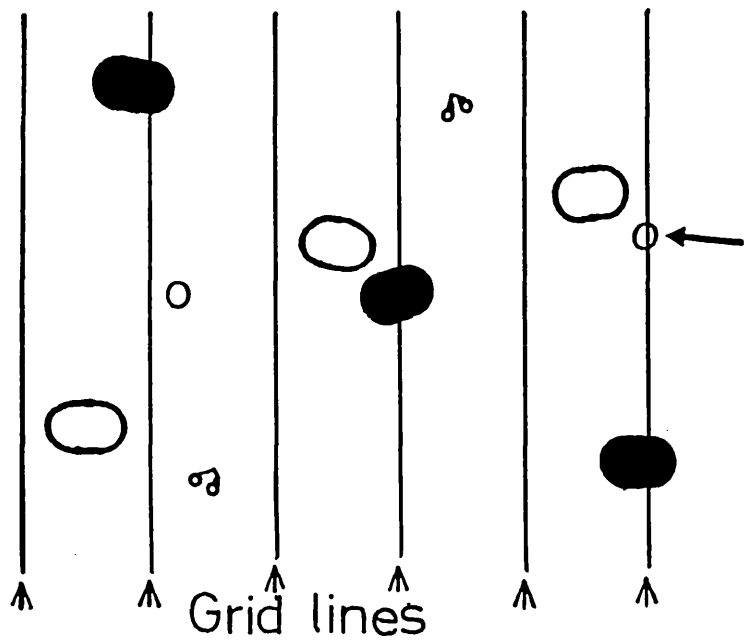


Diagram to show how nuclei are selected to be measured. Only the shaded nuclei are measured. The arrowed nucleus is not measured as it is not an epithelial cell.

Histology and Histochemistry

The histochemical study was not performed in the anastomosis group. In the rest of the groups the removed colon or anastomosis was fixed in formol saline solution and embedded in paraffin wax. Tissue blocks were then taken and embedded in wax. Sections, 5 microns thick, were cut and then stained with haematoxylin and eosin and examined for morphological changes. The sections were also stained with high iron diamine alcian blue pH 2.5 (HID-AB)(Spicer, 1965) to examine for histochemical changes. A modification of the histological classification used by Filipe (1975) in the assessment of the colon mucosa, by calling carcinoma in situ, severe dysplasia was employed.

Technique for High Iron Diamine Alcian Blue Stain

The sections were deparaffinised using graded alcohols and rinsed in running water. The high iron diamine consisted of 120mg of N,N-dimethyl-m-phenylenediamine and 20mg of N,N-dimethyl-p-phenylenediamine dissolved in 50ml of distilled water with 1.4 ml of 40% ferric chloride, approximately 10% ferric ion, added. The sections were incubated for 18 hours in the high iron diamine, rinsed in water and stained for 30 minutes in alcian blue (1g in 100ml of 3% glacial acetic acid). The sections were then rehydrated, cleared and mounted in DPX.

This technique stains sialomucin blue and sulphomucin brown/black. The sections were judged by the system used by Marcheggiano et al. (1984). This method divides the crypts into upper, middle and lower zones. The mucins were graded as zero, if there was no sialomucin present, one, if one third of the cells in that zone were sialomucin positive, two, if half were positive, three, if two thirds were positive and four if all the cells were sialomucin positive. The sections were examined and cross checked by Dr. Rees and there was no inter observer variation in the animal study.

RESULTS

CRYPT CELL PRODUCTION RATE

Full details of the mitotic counts seen are given in appendix 2, and from these the crypt cell production rates were calculated which are summarised in figures 20-23 and table 6.

Control

The crypt cell production rate in the control animals was 4.9 cells/crypt/h at one month. At three months the crypt cell production rate had fallen to 3.4 cells/crypt/h, and by six months was 2.4 cells/crypt/h. None of these results were significantly different.

Table 6.

Results of Crypt Cell Production Rate

	1 Month	3 Months	6 Months
Control	4.9	3.4	2.4
Urothelial Implant	7.4	5.9	9.5
Colon Conduit	27.6**	21.1**	34.3*
			4 Months
Vesicocolic Anastomosis	23.5**	22.9**	20.9*

Compared with the Control Group * $p<0.05$

** $p<0.005$

1 month.

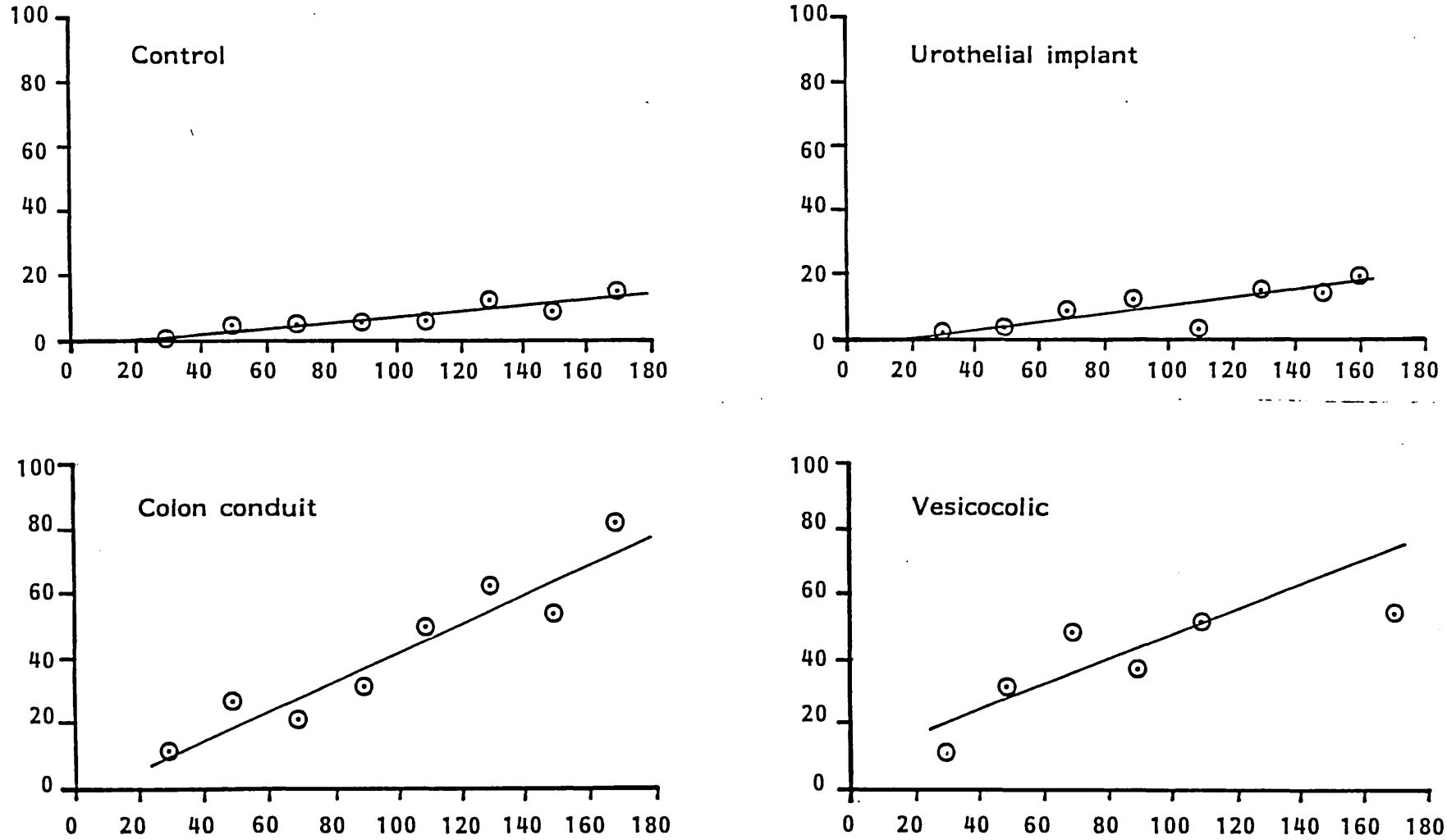
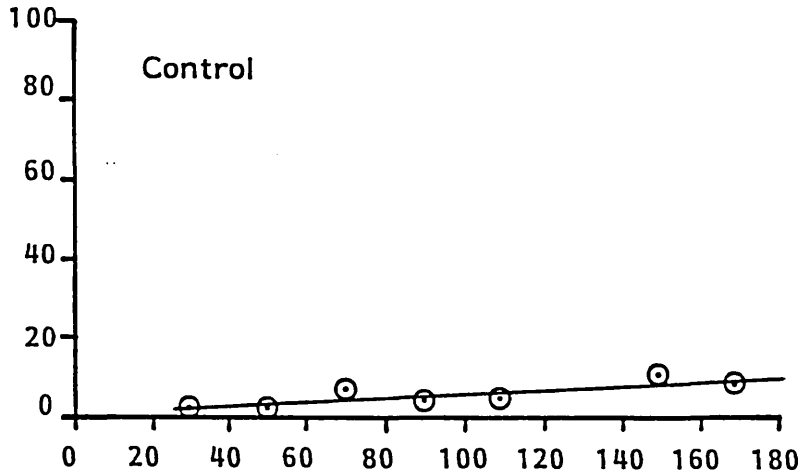
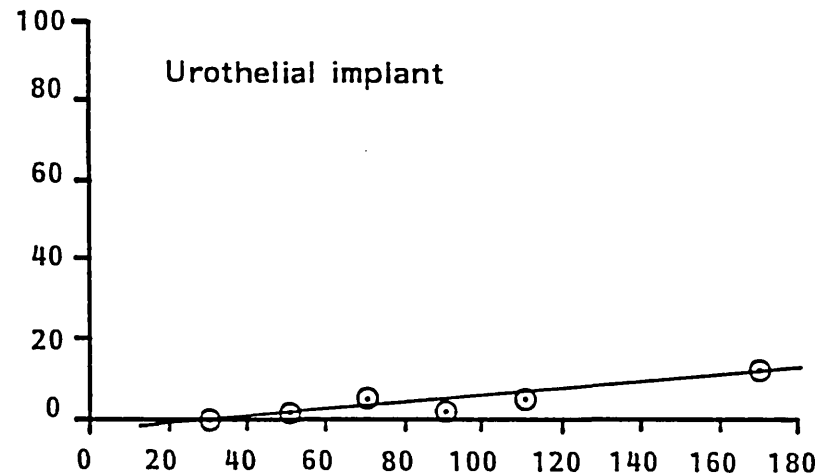
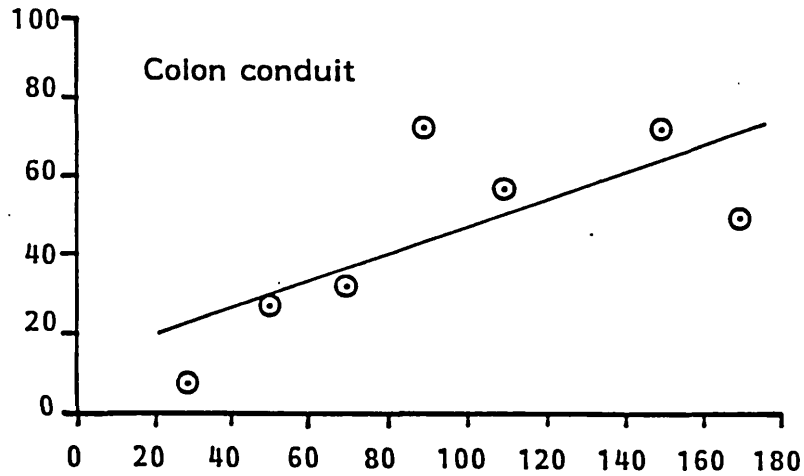


Fig. 20

3 months.







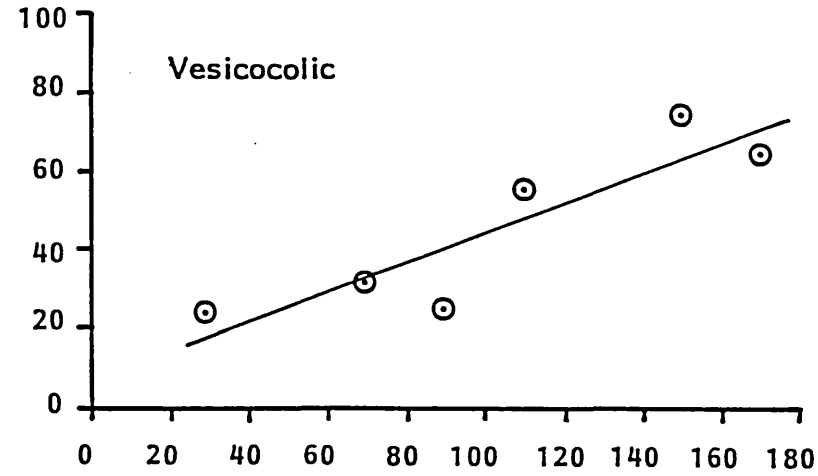


Fig. 21

6 months.

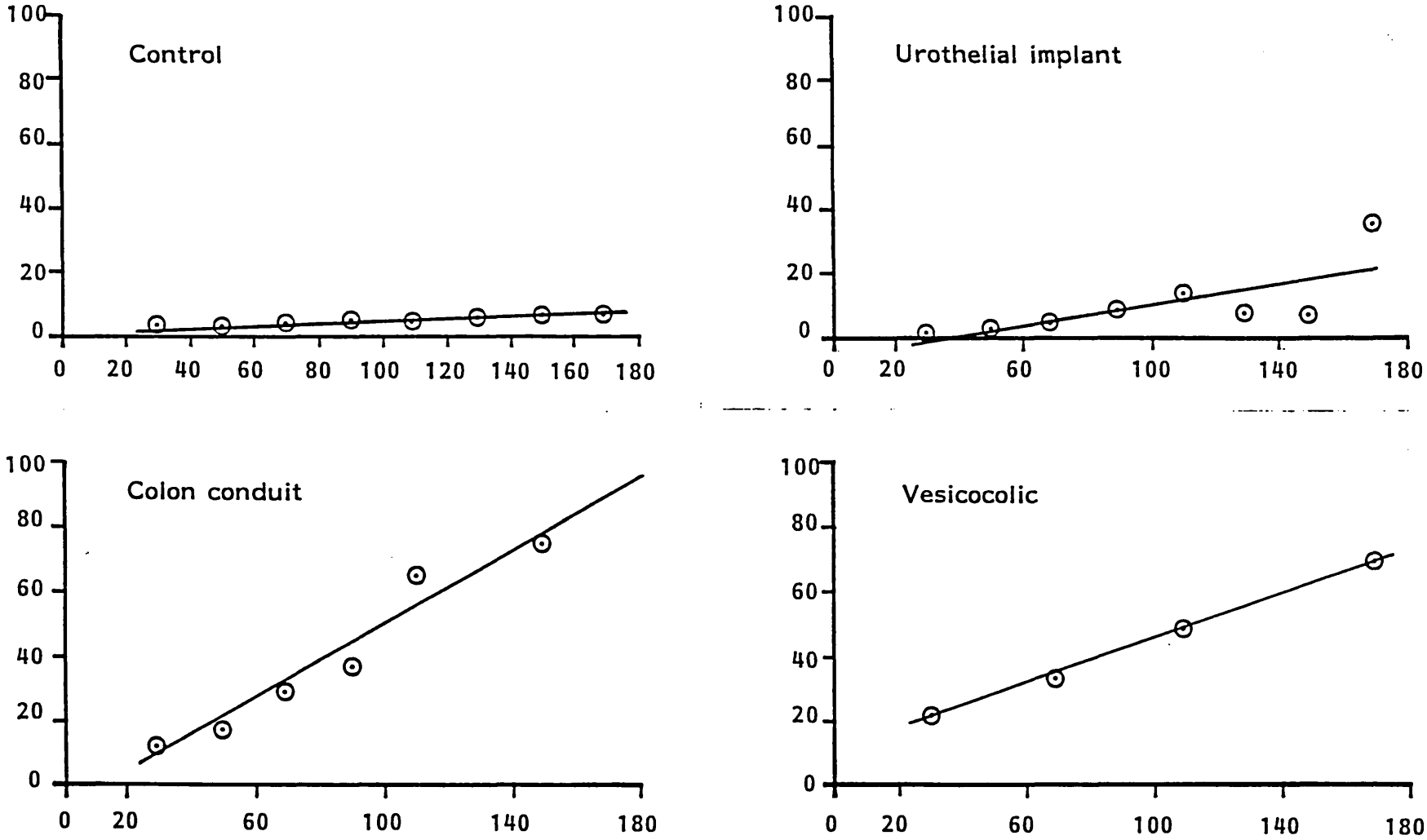
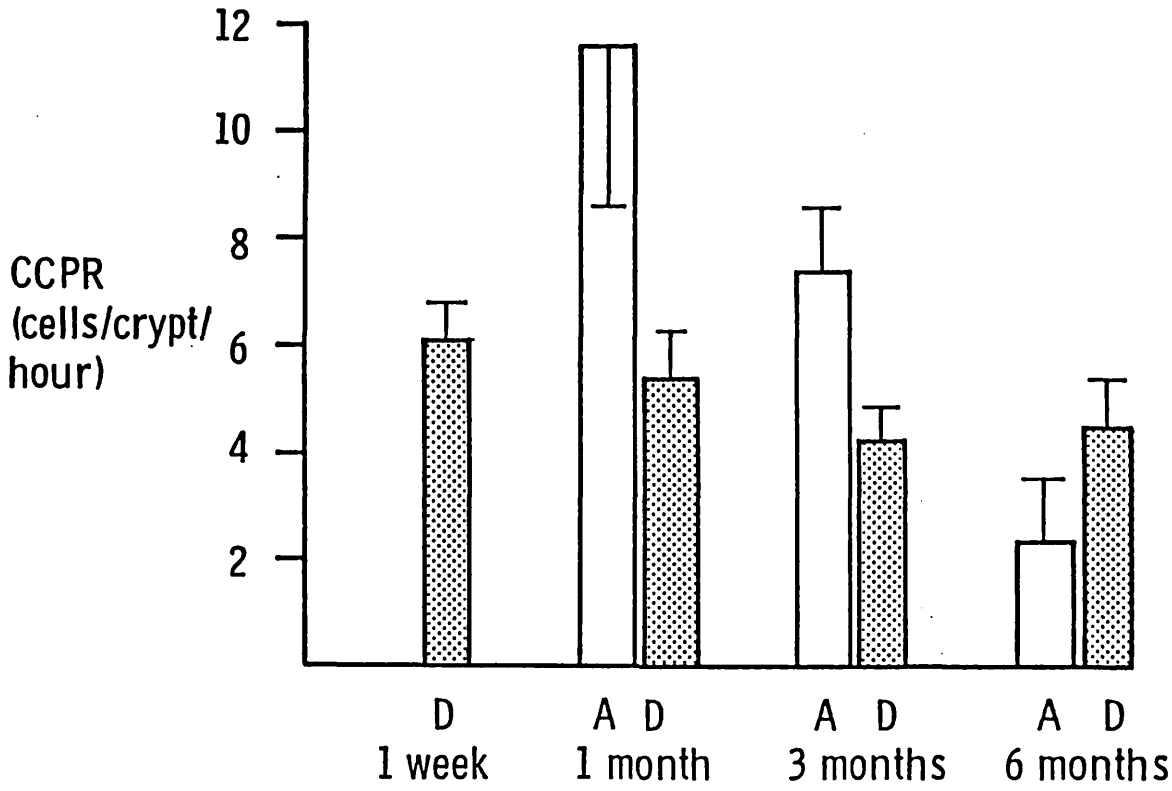


Fig. 22

Fig. 23



Histogram of the mean crypt cell production rates (CCPR) of mucosa adjacent to (A) and distal from (D) the anastomosic site at different times after transection.

T=SEM

Urothelial Implant

The crypt cell production rate at 1 month was 7.4 cells/crypt/h, at 3 months the value was 5.9 cells/crypt/h, and at 6 months, 9.5 cells/crypt/h which was not significantly different. When compared with the control values there was no statistical difference at any time interval.

Colon Conduit

At 1 month the colon conduit's crypt cell production rate was 27.6 cells/crypt/h and this was maintained at 3 months, (21.1) and 6 months, (34.3). At 3 months the slope of the curve did not reach $p<0.05$ level of significance. This was probably a type 1 error as all the other slopes reached statistical significance. However, there was no significant difference between the animals with time but there was a significant difference between this group and the control at 1 month, $p<0.005$, 3 months $p<0.005$ and 6 months $p<0.05$.

Vesicocolic

The vesicocolic group showed a four fold increase over the control at 1 month with a crypt cell production rate of 23.5 cells/crypt/h. At 3 months it was 22.9 cells/crypt/h and by 4 months 20.9 cells/crypt/h. The level of significant difference compared with control a 1 and 3 months was $p<0.005$ and at 4 months $p<0.05$. Strictly this should not be compared with the control as it was not paired data as the

time interval was different.

Anastomosis

The crypt cell production rate at one week could not be calculated at the site of anastomosis as there was a wide variation in the arrested metaphases within individual crypts. The crypt cell production rate in the distal colon was 6.1 cells/crypt/h which remained constant with time. The crypt cell production rate at the anastomosis at one month was 12.4 which at 3 months fell to 7.5 and by 6 months 2.4 cells/crypt/h.

CELLULAR DNA CONTENT

Full details of the DNA count values are found in Appendix 3, and a summary of the results are given in figure 24 and tables 7 and 8.

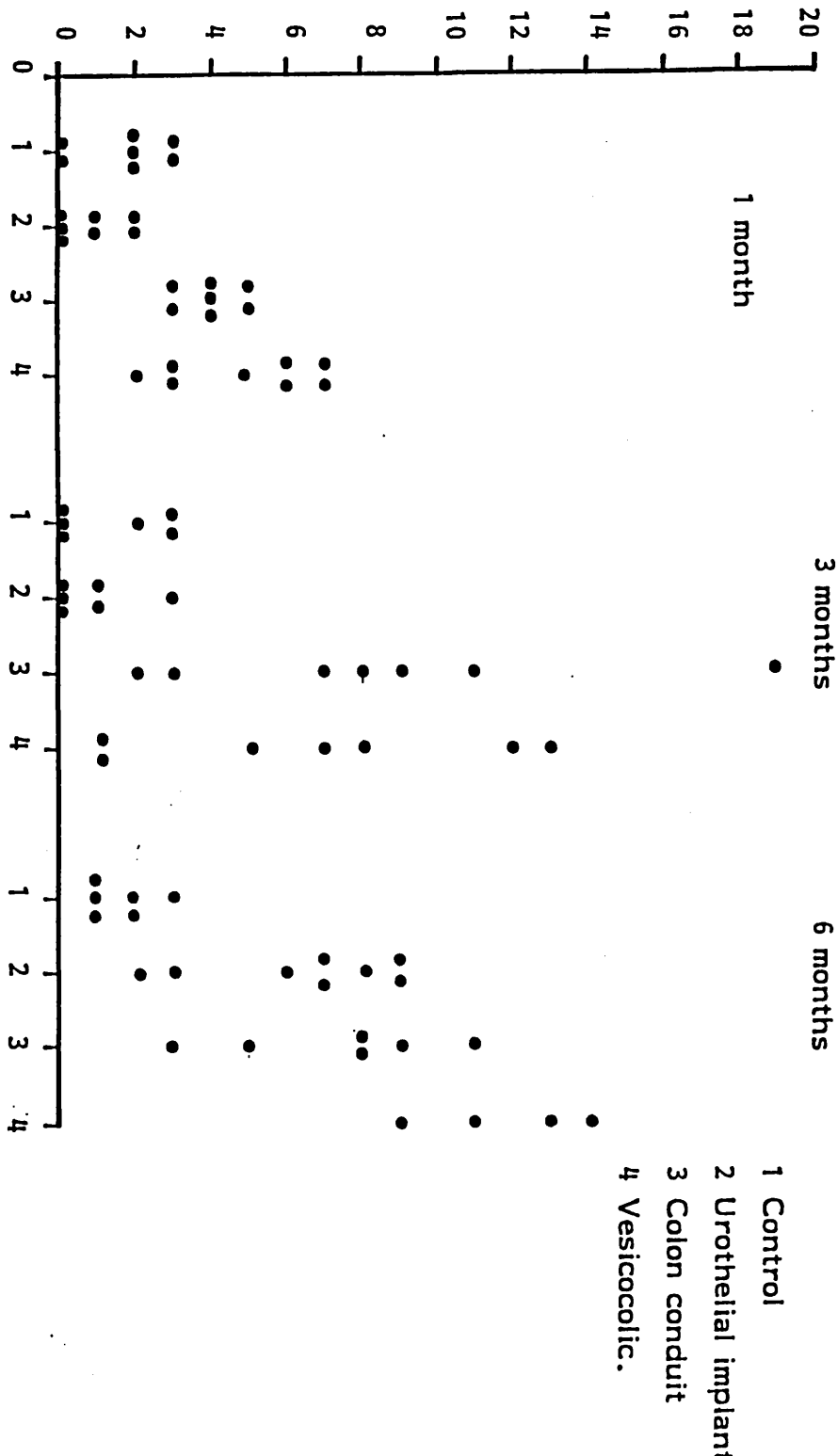
Control

The median value for the proliferative indices in the control animals at 1 month was 2 (range 0-3). This did not alter significantly with time and was 1 (range 0-3) at 3 months and 1.5 (range 0-3) at 6 months.

Urothelial Implant

The urothelial implant group's proliferative index was 1 (range 0-2) at 1 month and 0.5 (range 0-3) at 3 months. At 6 months there was a significant increase to 7 (range 2-13),

Fig. 24



Graph of proliferative index results.

Table 7.

Results of the Poliferative Index

	1 Month	3 Months	6 Months
Control	2	1	1.5
Urothelial Implant	1	0.5	7*
Colon Conduit	4*	8*	8*
			4 Months
Vesicocolic Anastomosis	5.5*	7*	12*

Compared with Control Group * $p<0.001$

Table 8.

Change in Proliferative Index with Time

Groups	p<		p<
Control			
1 month v 3 months	N.S.	v 6 months	N.S.
Urothelial Implant			
1 month v 3 months	N.S.	v 6 months	0.001
Colon Conduit			
1 month v 3 months	0.005	v 6 months	0.005
Vesicocolic			
1 month v 3 months	N.S.	v 4 months	0.005

$p<0.001$. Against control there was no significant difference at 1 and 3 months, but at 6 months the difference was significant, $p<0.001$.

Colon Conduit

The colon conduit at 1 month had a proliferative index of 4 (range 3-8). At 3 months this had increased to 8 (range 2-19), $p<0.005$, and remained at 8 (range 3-11) at 6 months, $p<0.05$ compared to 1 month. There was a significant increase over the controls at all time intervals of $p<0.001$.

Vesicocolic

There was a significant increase in the proliferative index at all time intervals compared with the control animals of $p<0.001$. There was no significant difference between the proliferative index of 5.5 (range 2-7) at 1 month, and 7 (range 2-13) at 3 months, however the increase to 12 (range 9-14) at 6 months was significant, $p<0.005$.

Anastomosis

The proliferative index at both the anastomosis and the distal colon at one week was 1.5. This value did not vary significantly with time at either the anastomosis or the distal colon.

MACROSCOPIC APPEARANCE

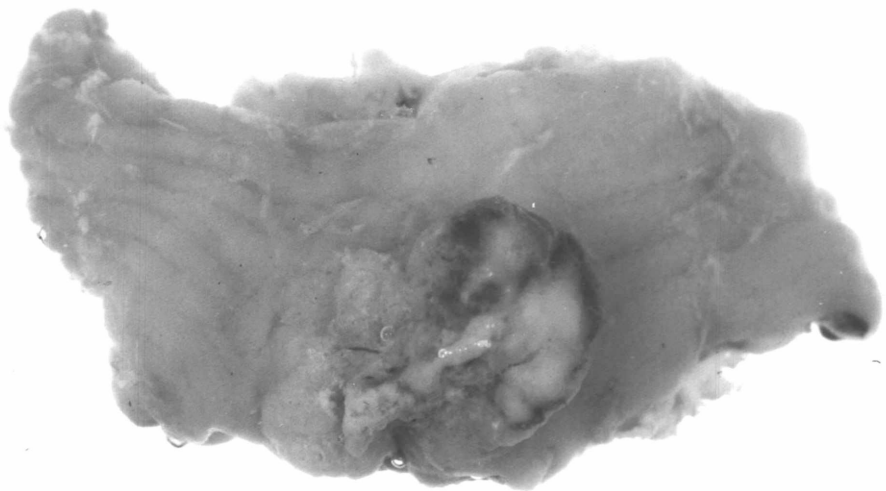
There were no macroscopic changes in the control rats. In

the urothelial implant group there were no changes until 6 months. At that time the urothelium patch formed a small diverticulum at the anastomosis and small tumours (0.1cm) were present at the suture line. The colonic conduit group also had small tumours (0.1cm) at the anastomosis at 6 months. In the rats with colon conduits there was evidence of hydronephrosis and pyelonephritis, although this was far more marked in the vesicocolic group of animals. In this group there were no changes at 1 month, but at 3 months small tumours had developed at the anastomosis one of which was 0.2 cm across. At 4 months all the rats in this group had developed tumours that were 0.4cm across and filled the lumen of the bowel (Fig. 25). There were no macroscopic changes found in the anastomosis group.

MICROSCOPIC APEARANCE

The microscopic assessment of the specimens was complicated by the use of vincristine in the study of crypt cell production rate. This technique arrested cells in metaphase and therefore the number of mitoses seen could not be used to determine the degree of dysplasia, and one of the indicies for assessing this was lost. Therefore, the assessment was more dependant on other citeria such as architectral distortion and cytological atypia. The latter can include nuclear enlargement, an increase in the nuclear cytoplasmic ratio, altered chromatin pattern and prominent nucleoli, which may be mimicked by inflammatory changes and

Fig. 25.



Photograph of tumour at the vesicocolic anastomosis at 4 months.

therefore further complicate the assessment. The results of individual animals' histological finding are shown in appendix 4.

Control

The colonic mucosa of the control remained normal throughout the experiment, with short, straight crypts with well ordered architecture and normal cellullar morphology (Fig. 26). There was occasional lymphoid tissue in the submucosa.

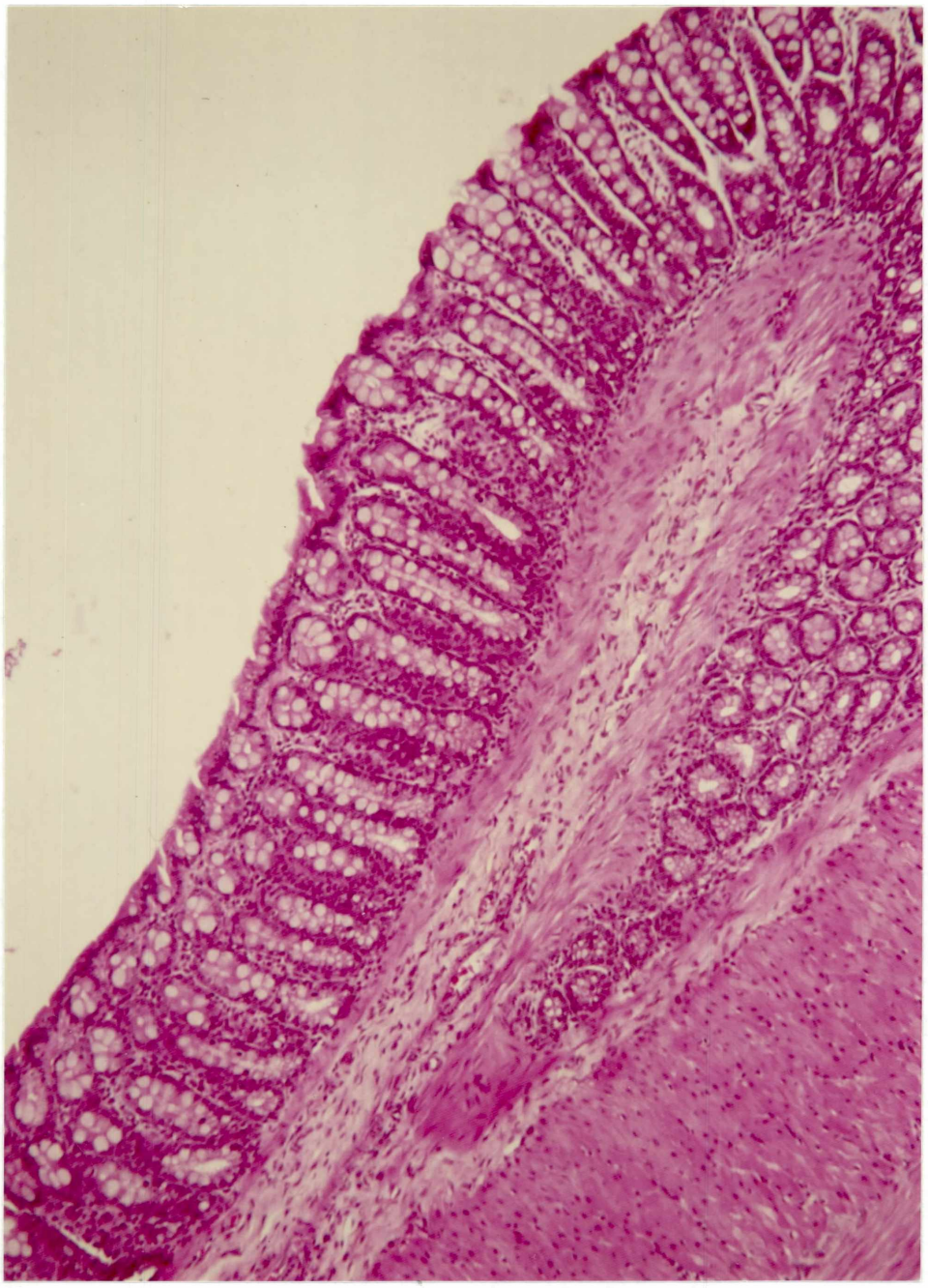
Urothelial Implant

At one month the colon was normal distal to the anastomosis. In the four animals in which there was urothelium to study the urothelium was normal. The urothelium was not found in four animals. This may have been due to problems with sectioning or alternatively, it may have become necrotic and lost. The histology at the anastomosis was normal but in one animal there was evidence of inflammation.

At three months the colon remained normal in all the animals. In the five animals with urothelium to study, three were normal. One animal showed squamous metaplasia with keratinisation and another had glandular metaplasia. In three there was inflammation at the site of anastomosis.

At six months the colon was normal and three had normal urothelium. One had glandular metaplasia and another

Fig. 26.



Photomicrograph (H&E x 40) of control rat colonic mucosa.

squamous metaplasia with keratinisation. At the anastomosis four had inflammatory changes, one showed dysplasia and another an abscess.

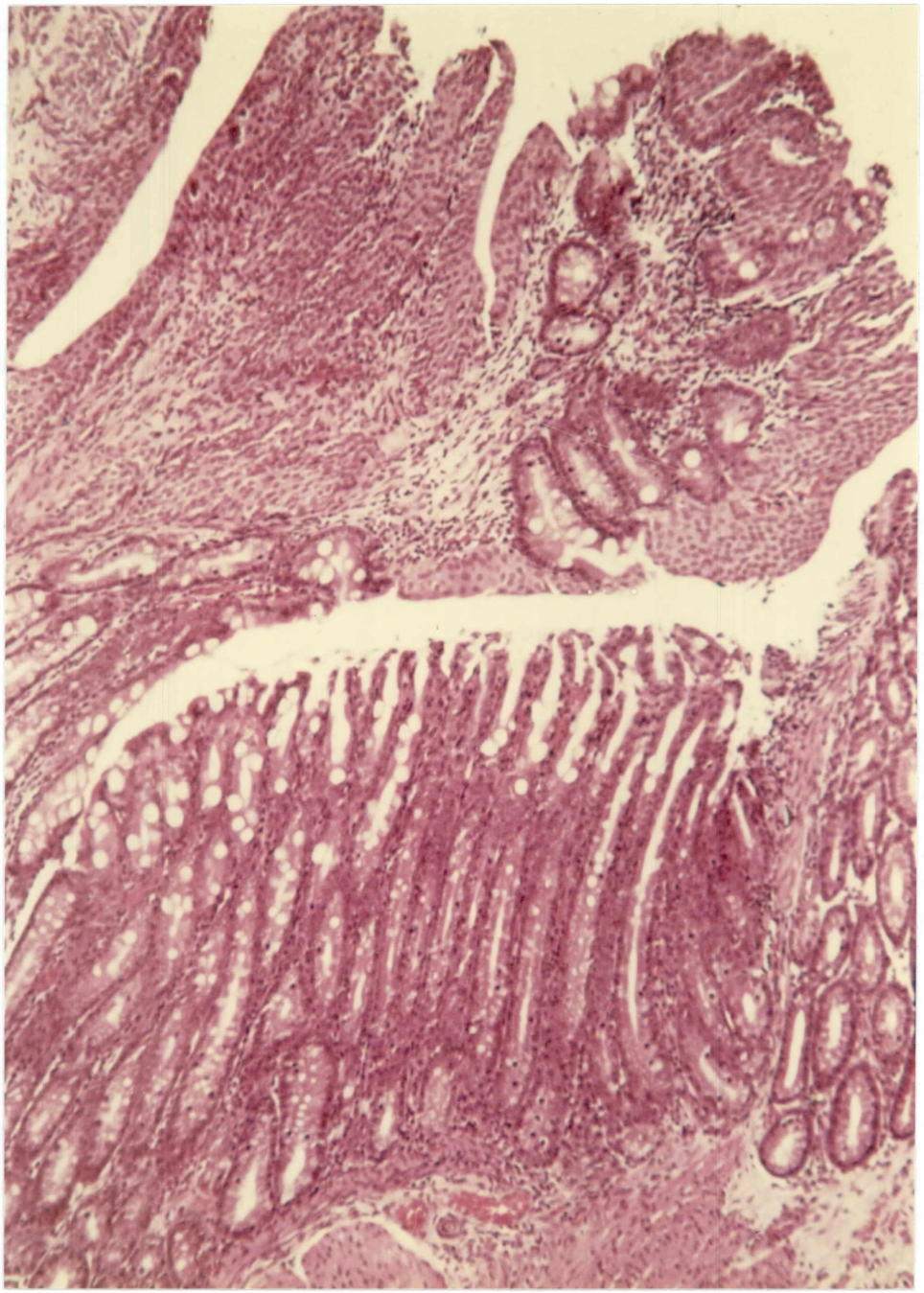
Colon Conduit

The colonic mucosa in the colon conduit group showed hyperplasia in seven animals and mild dysplasia in one (Fig. 27). The urothelium was normal in two, two had proliferative changes, two showed glandular metaplasia and another showed squamous metaplasia with keratinisation (Fig. 28) The anastomosis appeared normal except in the one where there was squamous metaplasia associated with inflammation.

At three months six animals had hyperplasia of the colonic mucosa and one showed mild dysplasia. The urothelium was normal in two, proliferative changes were present in four and one had both squamous and glandular metaplasia. The anastomoses in these animals remained normal.

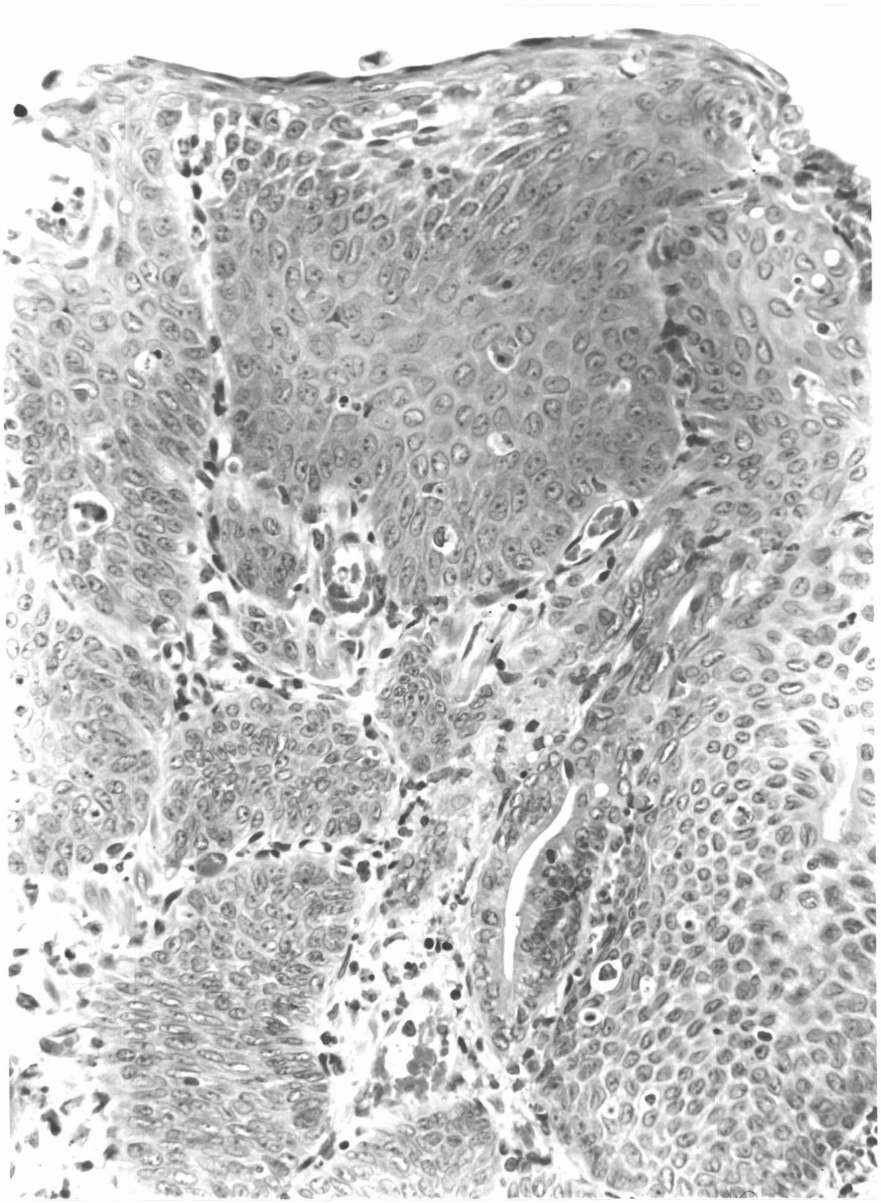
At six months three had hyperplasia of the colon, four mild dysplasia and one moderate dysplasia. The urothelium studied was normal in three, had proliferative changes in one and glandular metaplasia in three. The anastomosis in two showed severe dysplastic changes associated with inflammation.

Fig. 27.



Photomicrograph (H&E x 40) of colonic hyperplasia adjacent to urothelium in colon conduit at one month.

Fig. 28.



Photomicrograph (H&E x 250) of urothelial implant showing proliferating urothelium with areas of squamous metaplasia at 6 months.

Vesicocolic

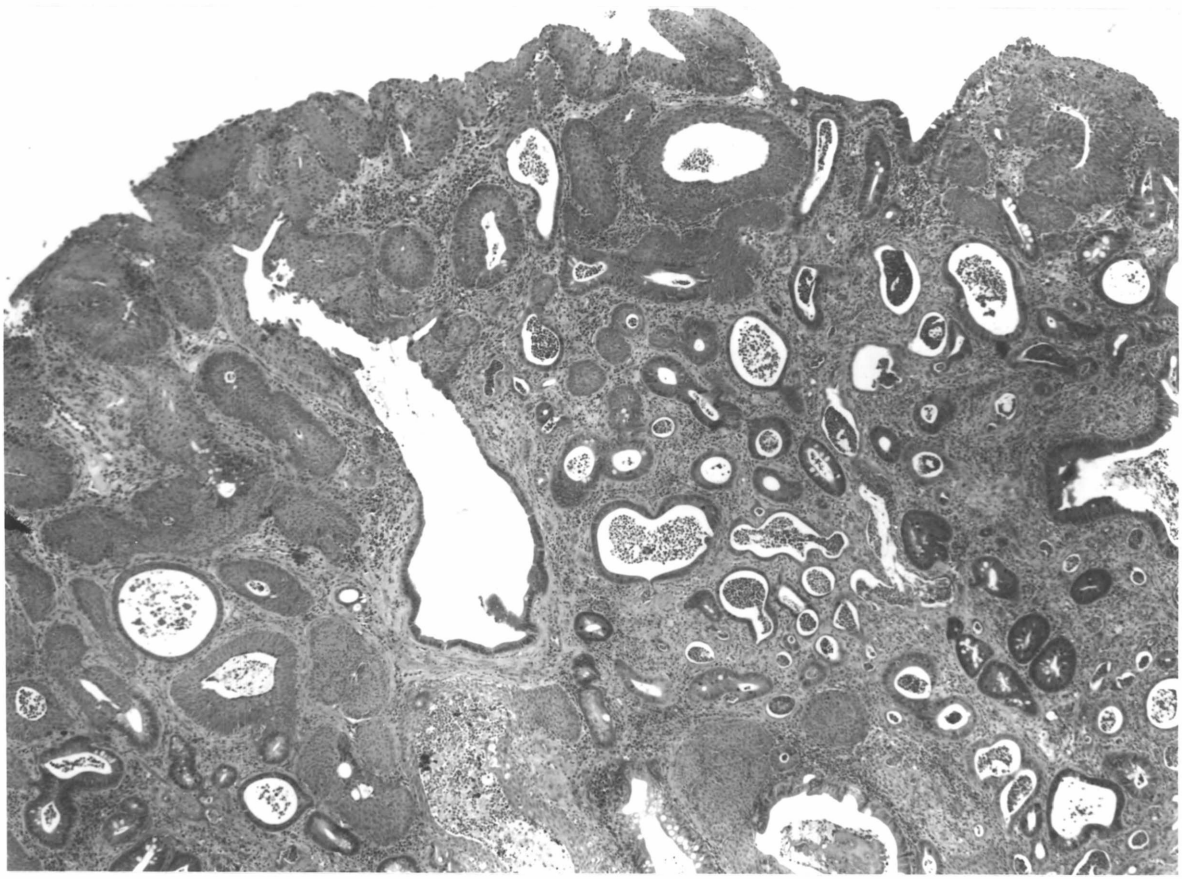
At one month six animals showed hyperplasia of the colonic mucosa, one mild dysplasia and another had a normal appearance. Four had normal urothelial appearances and one proliferative changes in the urothelium. The anastomoses in this group of animals appeared normal.

At three months the colonic mucosa was hyperplastic in four animals and mildly dysplastic in two. The urothelium was normal in two animals, proliferative in one and in three there were squamous metaplastic changes, one associated with keratinisation and another with glandular metaplasia. The anastomosis in the animal with squamous metaplasia had dysplastic changes.

At four months four animals had hyperplasia and two mild dysplasia of the colonic mucosa. Proliferative changes were present in urothelium of three rats and glandular metaplasia in one, and in another it was normal. At the anastomosis there were dysplastic changes and these were in the presence of inflammatory changes (Fig. 29 and 30).

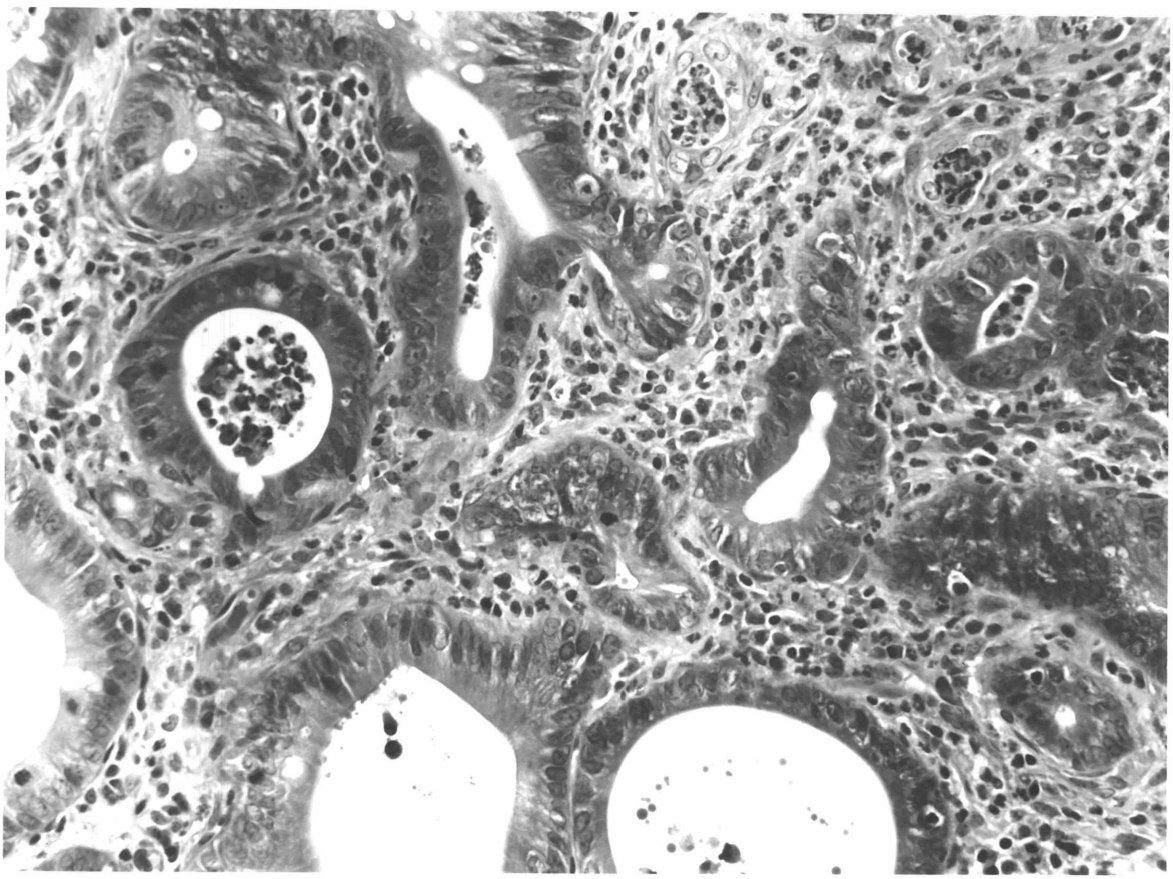
Glandular metaplasia in those animals with a urothelial colonic anastomosis could be due to several reasons. This finding may be due to the way the sections were cut, or

Fig. 29.



Photomicrograph (H&E x 40) of anastomotic site of a colonic conduit at 6 months showing marked proliferation of the urothelium with underlying and adjacent proliferation of glandular elements.

Fig. 30.



Photomicrograph (H&E x 250) of anastomotic site of a vesicocolic anastomosis at 3 months showing proliferating glandular elements with architectural distortion and marked cellular atypia.

entrapped colonic crypts beneath the proliferative urothelium or due to true metaplasia of the urothelium to the colonic type. In the animals where there was severe dysplasia present at the anastomosis it was not possible to tell whether it orginated from the urothelium or the colonic mucosa.

Anastomosis

One week post operatively the site of transection showed the mucosa had broken down with evidence of an inflammatory reaction in the lamina propria. At one month the anastomosis site had re-epithelised but there was some distortion of the crypts, and at three months the epithelium looked normal.

HISTOCHEMISTRY

The control animals maintained sulphomucins through out the study. The only position that sialomucin was seen in these animals was in the more proximal colon. The urothelial implanted group had a normal mucin pattern throughout the time of the experiment. The colonic conduit group had sulphomucin throughout but there appeared to be mucin depletion, and this pattern of mucin was found in the vesicocolic group.

DISCUSSION

In these experiments an animal model of colonic urinary diversion in male Wistar-Furth rats has been used to compare and contrast, histological, histochemical and cell kinetic techniques.

Urothelial Implant

The colonic mucosa away from the anastomosis remained histologically normal, similar to the control animals. The urothelium in this group showed metaplasia to both squamous and glandular epithelium. Inflammation was present at the anastomosis, which may have caused some of the proliferative changes observed. Four of the seven animals showed evidence of inflammation at the anastomosis in this group at 6 months. In addition one had evidence of dysplasia. There was a small but insignificant increase in the crypt cell production rate at 6 months. This compared with a insignificant difference of the proliferative index which became significant at 6 months. This may reflect that in order to measure the proliferative index cell brushings were taken across the anastomosis whereas those used to study the crypt cell production rate were taken slightly further away.

The crypt cell production rate away from the anastomosis in the urothelial implant animals was similar to the control animals'. The increase in the proliferative index in the

urothelial implant group indicated an alteration in the cell proliferation associated with dysplasia at the anastomosis.

There were no histochemical changes found at any of the time intervals. Gittes (1986) found no histological changes at the anastomosis using female Wistar-Furth rats, even though he used longer term experiments. It was possible that the sex of the animal was important, but the difference was most likely due to the use of a vascularised graft, rather than a free graft (Gittes, 1986). The relative ischaemia in a free graft could obviously influence the healing at the anastomosis. Other groups of animals in Gittes' study received antibiotic therapy and it was not clear whether these were used in the control or urothelial graft groups. This would alter the bowel flora and possibly the carcinogenic activity within the bowel.

Colon Conduit

There were tumours found in this group, although they took longer to form and were smaller in size than those found in the vesicocolic group. Histologically there was marked hyperplasia of the colonic mucosa at the anastomosis and also in the distal colon. The crypts were longer with mitoses seen outside the proliferative zone toward the mouth of the crypt. These changes were seen with a significant increase in the crypt cell production rate and the proliferative index. When the colon is defunctioned there

is normally atrophy of the colonic mucosa (Terpstra et al., 1979 and Wiiliamson and Rainey, 1984). However, presence of the urine not only prevented atrophy but produced hyperplasia.

There was good agreement between the cell kinetic methods of examining the colonic mucosa and histology. The histochemical study of the colonic mucosa showed no change from the sulphomucin pattern, although the stain was taken up with less intensity than in the controls.

There were proliferative changes present within the urothelium, with metaplastic changes to squamous and glandular epithelium. The degree of dysplasia, in this study, relied on the distortion and orientation of the crypt architecture and the cellular changes. There was marked inflammation present at the anastomosis which also made interpretation difficult.

It was not clear why tumours should have been found in this study when others found no such changes (Crissey et al., 1980, and Gittes, 1986). It is possible that use of antibiotics could influence this and it is not apparent whether they were used in the other studies. Neither of the previously reported studies made comments on the presence of colonic mucosal hyperplasia which was easily recognisable in this investigation. Gittes (1986) commented that the

urothelium adjacent to the anastomosis underwent some squamous metaplasia. Marked inflammation of the urothelium can give the appearance of dysplasia and this should be taken into account when interpretating the urothelial histology.

Vesicocolic

There was good correlation in the results found in this group of animals, between the cell kinetic techniques and the histology. An increase in the crypt cell production rate and the proliferative index was reflected by an increase in the length of the colonic crypts. The histochemical study showed no alteration from sulphomucin throughout the study although subjectively there was less intensity of staining than in controls. There was also the appearance of dysplasia at the anastomosis, in animals at 6 months.

Anastomosis

There was a local hyperplastic response at the transection site which settled by three months. The absence of change in the proliferative index shows there was no shift in activity to the upper part of the colonic crypt. It is unlikely that a temporary hyperplastic response in the abscence of a carcinogen will lead to tumour formation. In the urothelial group of animals, the increase in proliferative index was seen at six months. The presence of

urothelium at the anastomosis has a different effect to that of a simple transection. In animals with urothelium there was a continued inflammatory reaction and this may have been the cause of the sustained hyperplastic response.

Histochemistry

The histochemical techniques were disappointing in not being able to detect any changes throughout the study. In those aninamls with urine in the colon there seemed to be a depletion in the staining ability. The work of Filipe (1975) and Shamsuddin and Trump (1981) showed that most of the changes to the sialomucin were associated with mild dysplasia. This study showed no change to sialomucin at any stage. This may be due to difficulties with the staining technique (Williams, 1985), although this seemed unlikely as there were no problems encountered with other studies carried out (vide infra). The observed changes in the colonic mucosa from hyperplasia to severe dysplasia may have progressed without intermediate dysplastic changes, which are associated with the maximal changes to the sialomucin pattern Filipe (1975). Other possiblities include the method of tumour induction which may influence mucin changes or urine within the colon may interfer with the staining ablitiy in some way.

Crypt Cell Production Rate

The vesicocolic and colon conduit groups demonstrated that

urine induced an increase in the crypt cell production rate, whereas no changes were seen in the other groups. There was no significant difference between the control and the urothelial implant groups although the urothelial implant values were consistently higher. This may reflect the manipulation and healing associated with surgery and the presence of an implant acting as a foreign body, although a similar change was seen at the site of transection in the anastomosis group but not distal to the suture line.

There was a marked increase in the crypt cell production rate in the vesicocolic and colon conduit groups compared with the controls although the difference between the operative groups was not significant. This showed that urine, or a substance within urine has a marked proliferative effect on the colonic mucosa.

Cooke et al. (1984) showed that in rats with tumours induced by azoxymethane there was an increase in the crypt cell production rate before tumour formation. The presence of urine produced a far greater increase in the crypt cell production rate, although the two studies are not directly comparable. Chang (1978) in a murine model studied the effect of 1,2 Dimethylhydrazine on the colon using tritiated thymidine. Initially there was hyperplasia, with dividing cells later seen outside the proliferative zone. Tumours then either developed downward into the submucosa as an

invasive growth or upward as a polypoid growth. Hyperplasia was also seen as a reaction to healing, and hyperplasia may accompany colon tumourogenesis. Urine, or a substance within urine is therefore capable of producing a marked proliferation of the colonic mucosa.

This study showed that in a rat, urine caused colonic hyperplasia and rendered the colon more susceptible to tumour formation. This would seem to rule out trauma as the primary cause of tumour formation. Urine as a cause of tumour in man has often been discounted as tumours are found in undiverted ureterosigmoidostomies. Further studies would need to be performed to see whether the increased crypt cell production rate is reversible when urine is diverted away from the the colon previously bathed in urine. Harguindey et al. (1975) suggested the electrolyte load in the urine could be a cause of the tumours. The colon may need to undergo hyperplasia to remove the excess potassium and sodium ions.

Urine is known to contain a high concentration of epidermal growth factor (Dailey et al., 1978) and this may stimulate the colonic mucosa . Goodlad et al. (1987) showed that intravenous epidermal growth factor stimulated the colonic mucosa. However, when it was given enterally there were no such changes in the colon although these were relatively short term studies.

The presence of faeces, as shown by the vesicocolic group promotes the changes to produce tumours. This could be a mechanical effect (Aldis, 1961) or N-nitrosamines (Stewart et al., 1981). Stewart observed that urinary nitrates in the presence of mixed bacteria would undergo change to N-nitrosamines and as previously discuued these have been reported to be carcinogenic. The combination of a hyperplastic colonic mucosa and carcinogen may produce tumours. Colon conduits were not immune from this problem as the urine within conduits were often contaminated with the N-nitrosamine producing organisms (Stewart, 1986). The local hyperplasia within the urothelial implant group may be sufficient to allow the carcinogens to act at the anastomosis.

Anastomotic Tumours

The tumours within the colon were found only at the urothelial colonic anastomosis. The anastomosis group of animals showed a temporary increase in the crypt cell production rate at the transection site. There were no tumours produced in the six months of the study. Tumours have been shown to occur preferentiently at anastomotic junctions in the presence of a carcinogen (Williamson et al., 1980 and Williamson et al., 1982). Presumably the proliferation associated with the healing process, allowed the carcinogen to act more readily at that site. Sunter et

al. (1985), in man, observed chronic inflammation at a resection anastomosis. It may be that the presence of this chronic foreign body reaction caused by the urothelium and therefore hyperplastic healing reponse predisposed to tumour formation. This feature was not present in the simple transection group.

The time interval to tumour formation was much shorter than that found by Crissey et al. (1980). Post-mortem examinations were not performed on the early deaths and some early tumours may have been missed. Gittes (1986) may well have altered the carcinogenic potential with the use of antibiotics, effecting the bacteria that produce N-nitrosamines. There could be a different effect because of the use of female animals, and there may also be intraspecies variation. There is interspecies variation, Charles River C.D. and Wistar Furth rat developing tumours at a urothelial colonic anastomosis, while Lewis rats do not (Gittes, 1986). The size of the animals used may effect the result of any carcinogen and the animals susceptiblity. The description of the tumours found was vague (Gittes, 1986) and it was therefore impossible to compare with this study.

As well as the colonic changes there were changes within the urothelium. There were metaplastic changes to both glandular and squamous epithelium, the squamous epithelium

occasionally being keratinised. The intermingling of the epithelia at the anastomosis meant the tumour type was difficult to assess. The tumour was an adenocarcinoma but it was not clear if it had developed from colonic mucosa or from glandular metaplasia of the urothelium. The urothelium overgrew the colonic mucosa and in some animals the urothelium was continous with the colonic mucosa. This study did not confirm whether the colonic mucosa or urothelium was the source of the neoplastic change.

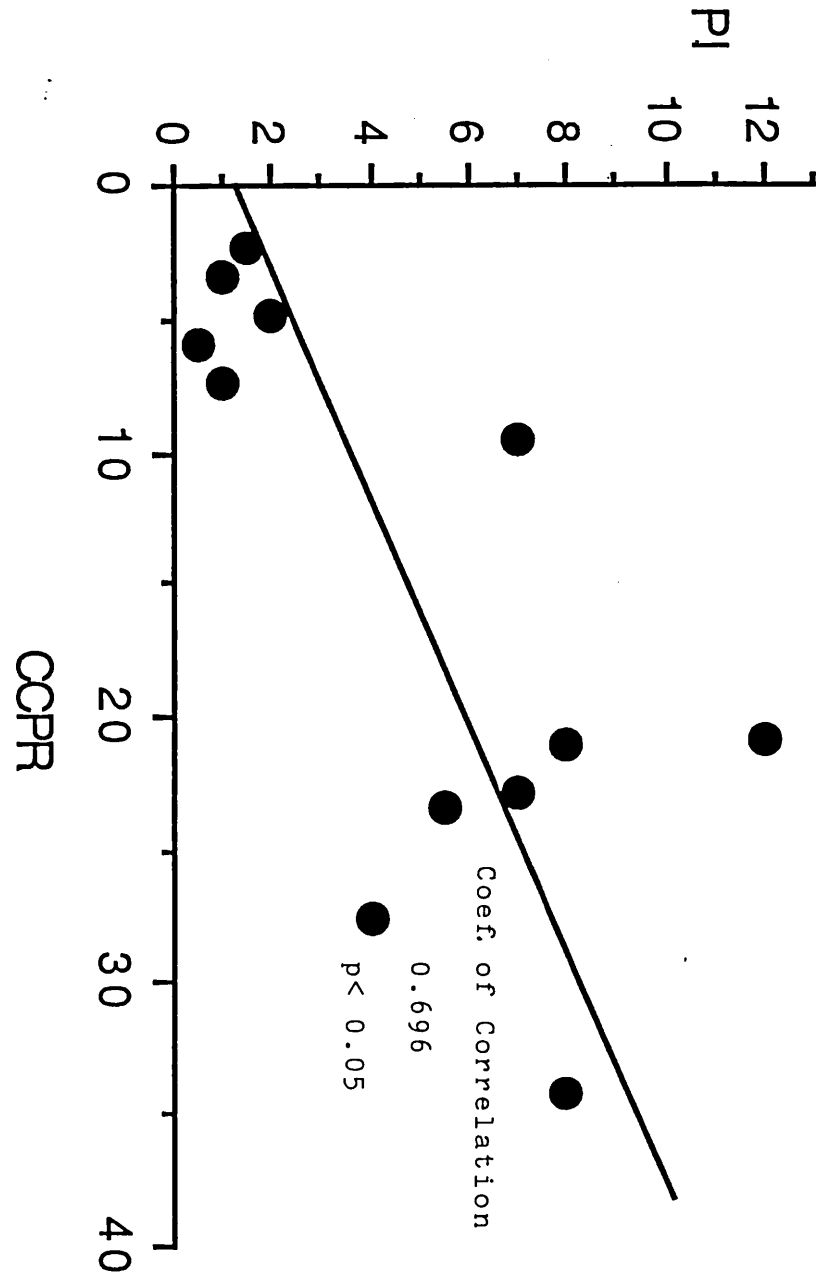
Proliferative Index

The brushings taken from the controls, and the urothelial implant group up to 6 months were normal. In the urothelial implant at 6 months, the colon conduit and vesicocolic there was a significant increase compared with the controls. This change was consistent with the changes in the crypt cell production rate and the histology. The proliferative index was shown in these rats to reflect the crypt cell production rate , coefficent of correlation 0.696, $p<0.05$ (Fig. 31). This suggests that the proliferative index is a valid technique for investigating colonic mucosal proliferation in man where the stathmokinetic or thymidine labelling methods are not applicable.

Summary

Urine produced an increase in the crypt cell production rate and the proliferative index, which corresponded to the

Fig. 31



Graph showing the correlation between the proliferative index and the crypt cell production rate.

histological appearence of hyperplasia. This study validated the use of proliferative index as an indirect measure of cell turnover. The presence of a foreign body, urothelium, at an anastomosis developed a chronic inflammatory reaction associated with dysplasia. There was a failure to demonstrate histochemical changes in this experimental model.

SECTION

III

CLINICAL STUDIES

CHAPTER 10

HISTOCHEMICAL TECHNIQUE IN MAN

Although the animal study described in Chapter 9 failed to demonstrate any histochemical change associated with hyperplasia and dysplasia, which was at variance with other published reports, the explanation of this may be the animal model chosen for these experiments.

Adenocarcinoma

Filipe (1969) had shown a change to sialomucin associated with adenocarcinomas of the colon in man and it was for this reason a histochemical study in patients after urinary diversion was carried out and is described in this section. For her study Filipe (1969) examined surgical specimens from cases of ulcerative colitis, Crohn's disease, non-specific inflammation, diverticulitis, and non-invasive and malignant tumours of the colon and rectum. If possible, tissue throughout the colon was examined in ulcerative colitis and Crohn's disease. In patients with carcinoma of the colon specimens were taken 5cm from the tumour, "normal" mucosa near the tumour and from the tumour itself. These were stained with haemotoxylin and eosin, and with high iron diamine alcian blue pH 2.5, as well as other stains. The normal colonic mucin pattern of the sigmoid colon was sulphomucin in the bottom two thirds of the crypt with a mixture of sialomucin and sulphomucin in the upper third.

The term "transitional mucosa" was applied to the area

adjacent to a malignant tumour but not showing microscopic features of malignancy, although there were histological changes present (Filipe, 1969). In tissue close to the tumour there was replacement of the sulphated mucin with sialomucin. The sialomucin change started at the bottom of the crypt and worked toward the surface until the crypt contained mainly sialomucin. This pattern was found in all six cases of carcinoma of the colon, and 11 of the rectum. Two of these showed some areas of a sulphated pattern close to the tumour. There were two zones within "transitional mucosa", one more peripheral with elongation and branching of the crypts containing sialomucin. The second zone closer to the tumour had short narrow crypts with virtually no goblet cells and weak secretory activity. The adenocarcinoma cells of the malignant tumour showed minimal secretory activity. In the few cells that were secreting acid mucins, sialomucins were predominate.

Non-invasive Tumours

Non-invasive epithelial tumours produced a varied pattern of mucin production (Filipe, 1969). Metaplastic polyps were found to have a normal pattern of mucin production, but there was quantitativeley more mucus produced. Well differentiated adenomatous polyps showed sulphated mucin predominate in the base of the crypt which produced weaker secretory activity. More dedifferentiated adenomatous polyps showed very little mucin production. The meagre

mucin produced was mainly sulphated, in the three cases described.

Inflammatory Disease

Filipe (1969) found in non specific inflammatary conditions there was an overall reduction in mucin production with an irregular mixture of mucins. In nine cases of inflammation of the rectum there was generally an increase in mucin production. In nine out of 15 cases of ulcerative colitis the surface mucins were decreased. In six cases of chronic ulcerative colitis with mild inflammation there was pseudopolyp formation with large pools of mucin. Crohn's disease had some variation in the mucin pattern, but mainly sulphomucin, and diverticular disease had a normal pattern.

Most goblet cells on the surface epithelium of the normal colonic mucosa contained sialomucin (Filipe, 1969). This may be explained by either the formation of two mucopolysaccharides, or one which has varying sialic acid and ester sulphates, which may differentiate to either. The ageing process as the epithelial cells migrated from the bottom of the crypt could be responsible. In the "transitional mucosa" the cells at the base of the crypt changed to sialomucin while those in the mid-zone of the crypt were still sulphated. As cells migrated from the base this would seem unlikely.

It was postulated that the failure of sulphation could be due to extracellular factors, whereby sulphate was not available to the cells (Filipe, 1969). There could be an intracellular change which prevented sulphation, perhaps enzymatically. It was assumed that this was an enzymatic change which maybe specific or non-specific. Malignant cells may lose the capacity for sulphation or the loss of sulphation was due to non-specific factors which induced proliferation.

Cells at the bottom of the colonic crypt are undifferentiated and immature, capable of DNA synthesis. As they migrate to the surface through the proliferative zone they lose their capacity for DNA synthesis and mature (Filipe, 1969). When the cells are on the surface they lose their mucin content and die. There is variation in the mucin pattern within the colon both in time and distribution throughout the colon. In the fetal colon there is only weak staining for sulphomucin and there is a predominance in the sialomucins (Lev, 1968) and in adults the sialomucins predominate as far as the splenic flexure, while in the left colon the sulphomucins are in the majority. It is not clear why there should be a difference in the mucosal pattern in these different areas and age groups. The functions of the mucins within the colon are not known.

Carcinoma of the colon should not be regarded as an isolated focal lesion, rather a field change of the colon (Filipe, 1969). Normal colonic mucosa in the left colon has straight crypts with no branching and histochemically the goblet cells contain predominately sulphomucins. Adenomatous polyps and villous adenomas are pre-malignant and in familial polyposis coli this change could be traced back to hyperplasia affecting a single crypt (Filipe et al., 1980) and the mucosa between polyps shows the features of "transitional mucosa". "Transitional mucosa" is thicker due to the elongation of the crypts and some branching. Histochemically this is characterised by a preponderance of sialomucin, and these changes are seen adjacent to the tumours but can also be found in areas remote from the tumour.

"Transitional mucosa" has certain characteristics which are intermediate between normal and carcinomas in terms of mucus secreted (Filipe, 1969). There are mucin changes found unrelated to the tumour, which do not occur in non tumourous colons. This suggests that the presence of "transitional mucosa" is not just a local feature secondary to the tumour but a primary effect showing abnormal mucin production. The overall prognosis seems to be related to the anatomical content of "transitional mucosa" in the orginal resected specimen (Filipe and Branfoot, 1976).

Light microscopy and histochemistry were used to study 100 cases of malignant bowel tumours (Greaves et al., 1980). Ninety three of these had an adenocarcinoma, either Dukes' B or C. All but two cases had changes of "transitional mucosa" with an increase in crypt length, greater than 0.8mm, and stained sialomucin positive. It was classified as "transitional mucosa" if both criteria were present. Minor abnormalities were ignored if there was normal crypt architecture as there were variations in the HID-AB stain between batches and in the controls. The distance of the "transitional mucosa" from the tumour varied from 0 to 19.5cm, with a mean of 3.4cm. "Transitional mucosa" was seen in relation to a malignant melanoma of the colon. In Dukes' B tumours there was a good correlation to survival and the length of "transitional mucosa", although this was not seen in association with Dukes' C tumours. It was suggested that because two patients did not have "transitional mucosa" that the change is primary rather than secondary to the tumour, which would always be present. Riddell and Levin (1977) thought the "transitional mucosa" was not always demonstrated because the tumour may destroy the change, or was a sampling error.

Shamsuddin et al. (1981) compared 15 cases of carcinoma of the colon, without any predisposing disease with 15 control specimens taken from immediate post-mortems. In the 15 cases with carcinoma they found dilation of the lumen of the

crypts, overcrowding within the crypts and no mature goblet cells. Atypia of the cells was present with nuclear stratification. Staining with HID-AB in the controls was normal. In the "transitional mucosa" close to the tumour there was an increase in the mucous with increased sialomucin. In areas remote from the tumour there was hyperplasia in 70% and sialomucin in 100% of the specimens. The changes were thought to be primary, as "transitional mucosa" changes were found remote from the tumour. Further evidence for "transitional mucosa" being a primary event was not finding "transitional mucosa" in non-cancerous controls. This increase in sialomucin was similar to that found by Dawson and Filipe (1976). Shamsuddin et al., (1981) dismissed the arguments of Isaacson and Attwood (1979) that these changes were non-specific as they were found at colostomies and anal ulcers. The natural history of colostomies and anal ulcers was not fully documented and may become malignant. Dawson and Filipe (1976) favoured the view that change to sialomucin was specific to a carcinogenic stimulus rather than being non-specific or secondary to a cancer. The conclusion was that HID-AB could be used for screening of colorectal tumours, although there were no good arguments advanced for this.

Ureterosigmoidostomy

Marcheggiano et al. (1984) examined the rectal mucosa from nine patients who had ureterosigmoidostomies for a median

time of 6 months. The mucosa in 10 cases of rectal carcinoma and 10 normal controls was examined. The crypts were divided into three equal segments and the proportion of sialomucin present split into four unequal groups. Inaccurate statistical analysis was applied which showed a significant increase in the amount of sialomucin in the lower and middle segments of the crypt, in both rectal carcinoma and ureterosigmoidostomy. The colons examined were only a median 6 months post-operative and the mean time to ureterosigmoidostomy tumours was approximately 30 years. However, it was suggested that an operation which was known to produce malignancy has early histochemical changes, therefore these changes may represent pre-malignancy.

Anastomotic Recurrence

Habib et al. (1984) examined the question of whether sialomucins were predictive of recurrence in 48 Dukes' B adenocarcinoma of the colon. The patients were sex, age, stage and tumour matched, 24 had local recurrence and 24 were free of recurrence at 5 years. In those with local recurrence 20 of 24 had normal haematoxylin and eosin appearance at initial presentation but a marked change to a sialomucin pattern, while 22 of the 24 with no recurrence showed a sulphomucin pattern. It was suggested that "transitional mucosa" was unstable and more likely to become malignant rather than tumour implantation at the time of surgery. The strict definition of "transitional mucosa" was

not used as there were no histological changes present. Tumours treated by anterior resection have higher local recurrence than those treated by anterior perineal resection despite clear resection margins (Sunter et al., 1985). Patients following anterior resection were colonoscoped and 11 of 28 had evidence, at the anastomosis, of inflammation with associated ulceration and granulation often with suture material present. Some of those with inflammation had disorders of crypt structure. Seven cases showed histochemical changes of "transitional mucosa" and when the original specimen was reviewed these changes were not present. The ulceration present up to 2 years after the operation may stimulate cell proliferation and act as a promoting influence in the process of neoplastic development.

Isaacson and Attwood (1979) used the HID-AB staining method in mucosa adjacent to adenocarcinoma of the rectum, melanoma, squamous tumours, lymphomas as well as normal mucosa. The mucosa adjacent to solitary ulcers and colostomies was also stained with HID-AB. The normal mucosa was found to contain predominately sulphated mucin while that adjacent to adenocarcinoma was sialomucin. The other tumours and benign conditions were also found to have a change to sialomucin with a corresponding increase in the depth of the crypts. The prolapsing of the tumour, colostomy, or ulcer may alter the blood supply to the

tumour, colostomy and ulcer and therefore the mucin change was a secondary regenerative process rather than a pre-malignant condition as suggested by Dawson and Filipe (1976).

Franzin et al. (1981) examined 27 cases of solitary ulcer of the rectum, 20 of which were stained with HID-AB. Sixteen cases had deeper crypts with branching, and an increase in the number and size of goblet cells resembling "transitional mucosa". This demonstrated a corresponding change to the sialomucin pattern. Twenty three cases showed surface hyperplastic changes with an elongation and increase in the surface epithelial cells. Ischaemia may be the cause of these changes with a secondary regenerative process.

Later Franzin et al. (1983) examined further tissue for "transitional mucosa" change. Forty cases of quiescent ulcerative colitis specimens showed an increase in the crypt length and the uninvolved mucosa showed a mixed mucin pattern. The mucosa of colostomies and Crohn's colitis showed a similar pattern, although ischaemic colitis showed variable changes. Hyperplastic polyps both pedunculated and sessile demonstated dilated branched crypts and were positive for sialomucin, while metaplastic polyps were predominatly stained sulphomucin. It was concluded from this study that some colonic disease produced a sialomucin pattern that fitted the criteria of "transitional mucosa".

Others, however, showed mucin changes without crypt changes and therefore alteration in mucin was independant of the microscopic findings. As "transitional mucosa" had been found in various conditions it was regarded as non-specific and not pre-malignant.

Saffos and Rhatigan (1977) examined twenty cases of adenocarcinoma of the colon and found "transitional mucosa". They felt that its presence could be a non-specific change secondary to an irriating factor, specific to carcinoma or a response from which carcinomas develop. Later Rhatigan and Saffos (1979) looked at eight cases of diverticulosis, six with gross thickening of the muscle wall and three with diverticulitis with fibrous or abscess formation. Five cases showed non-polypoid mucosal hypertrophy and had thickening of the bowel associated with hypertrophy of the muscle layer plus or minus inflammatory change. Therefore, thickening of the mucosa in relation to tumours was non-specific. This gave further evidence that "transitional mucosa" was not a pre-cancerous condition but its presence distant from tumour in familial colorectal cancer did suggest an association. Some groups (Greaves et al., 1980, and Shamsuddin et al., 1981) found no "transitional mucosa" in normal control colon, while others (Listinsky and Riddell, 1981) have found "transitional mucosa" in normal colons resected for trauma.

Idiopathic Bowel Disease

Filipe and Branfoot (1976) looked at ulcerative colitis to examine the mucin changes largely ignoring the morphological changes. An excess of sialomucin in inactive ulcerative colitis was uncommon while it occurred in cases of long-standing colitis or those with dysplasia. Further studies confirmed the link between dysplasia and predominance of sialomucin, although inflammation was often present (Ehsanullah et al., 1985). In some cases mucin changes preceded the dysplastic changes. Franzin et al. (1983) found a predominance of sialomucin in virtually every case of ulcerative colitis whatever the disease activity. The detection of mucin change, therefore, was felt not to be helpful in the identification of high risk patients with ulcerative colitis.

Problems with the Technique

Differences in results between centres may be due to modification of the histochemical technique (Filipe, 1984). Among the factors that may have influenced the results, are the water rinse, pH and the source of the chemicals. The original paper by Spicer (1965) recommended 10% ferric chloride, but in fact it should have read 10% ferric ion, i.e. 37.5% ferric chloride (Sheahan et al., 1970). Barr showed that in control specimen slides sent to 16 different hospital laboratories the sialomucin to sulphomucin ratio could be markedly different (Williams, 1985), which raised

questions regarding the validity of this staining technique. The ferric chloride concentration can also change the histochemical result by lowering the pH, preventing the carboxyl groups reacting with the diamine complex molecules (Sorvari, 1972). The N,N-dimethyl-p-phenylenediamine used, depends on how many hydrochloric molecules it was linked to, and may alter its staining ablitiy. Diamine salts are also unstable and fresh solutions must be used.

Conclusion

This technique of staining requires further development before being recommended for routine histochemical assessment. If this can be developed further with consistent results then it could possibly become a useful staining method although its role for determining pre-malignancy has not been shown to be conclusive (Williams, 1985).

CHAPTER 11

HUMAN MUCIN STUDIES

Despite reservations regarding the histochemical technique discussed in the previous chapter, I have used the same methodology as part of an assessment of the colonic mucosa in patients who have had urinary diversion.

PATIENTS AND METHODS

Colonic biopsy specimens were obtained from forty patients who had undergone urinary diversion. Some of these patients who had had a ureterosigmoidostomy for a benign condition, which was later converted to another form of urinary diversion had biopsies of the colon performed either St. Mark's or Shaftesbury Hospitals. Specimens from ureterosigmoidostomies performed for malignant conditions or from colon conduits were obtained from Ealing or Hammersmith Hospitals. These specimens were stained by haematoxylin and eosin (HE), and the high iron diamine alcian blue (HID-AB) methods (Strachan et al., 1985).

Group A. Ureterosigmoidostomy for Benign Aetiology

Nineteen patients with ureterosigmoidostomies performed for benign conditions at a mean age of 16.6 years (range 1-57) were examined with a flexible colonoscope and biopsied adjacent to the ureteric orificies, which were identified using intravenous methylene blue, if necessary. Their diversions had been present for a mean of 22 years (range 5-38)(Table 9).

Table 9.

Patients in Mucin Study

	Number	Range years
Group A Ureterosigmoidostomy for Benign Aetiology	19	5-38
Group B Ureterosigmoidostomy for Malignant Aetiology	9	1-26
Group C Undiverted Ureterosigmoidostomy	8	12-37
Group D Colon Conduit	4	2-14

Group B. Ureterosigmoidosotmy for Malignant Aetiology

Nine patients with ureterosigmoidostomies performed for malignant conditions were examined with a rigid sigmoidoscope and random biopsies were taken 13 cm from the anal margin. The mean age at operation was 64.7 years (range 57-74). The mean duration of the ureterosigmoidostomy was 9.4 years (range 1-26).

Group C. Undiverted Ureterosigmoidostomy

Eight patients had ureterosigmoidostomies which were later converted to another form of diversion. Biopsies were taken via a colonoscope near the site of the ureteric orifices. The average time before conversion was 10.4 years (range 8-18) and after conversion 12.6 years (range 8-18). The average age at initial operation was 14.8 years (range 3-47) and the overall time to biopsy was 24.5 years (range 12-37) after the first operation.

Group D. Colon Conduit

Four patients had urinary diversion into a colon conduit. Biopsies were taken both from the colon conduit and the sigmoid colon. The mean age at operation was 9.3 years (range 2-14), and the average length of time with the conduit was 17.5 years (range 5-24).

The colonic biopsy specimens were placed in 10% formol

saline solution and were later stained with haemotoxylin and eosin (HE) and also with high iron diamine alcian blue pH 2.5 (HID-AB) (Spicer, 1965). The same method has been described in detail in Chapter 9.

The mucins in the colonic mucosa were differentiated by staining with HID-AB. This technique stained sialomucin blue and sulphomucin brown/black. The sections were judged by the system used by Marcheggiano et al. (1984). This method divided the crypt into upper, middle and lower zones. The mucins were graded as 0, if there was no sialomucin present (Fig. 32), 1, if one third of the cells in that zone were sialomucin positive, 2, if half were positive, 3, if two thirds were positive and 4 if all the cells were sialomucin positive (Fig. 33).

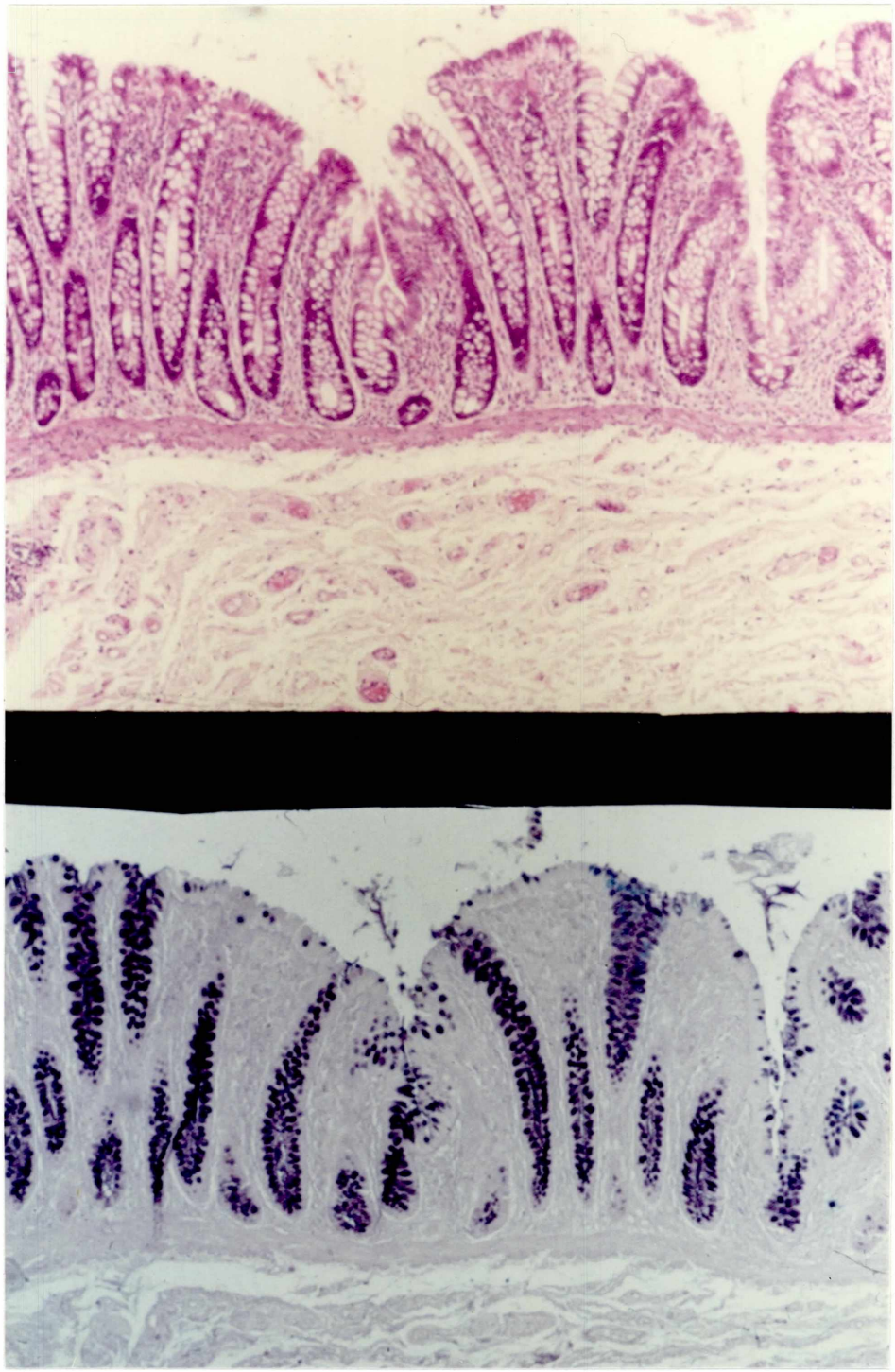
The biopsy specimens were examined by Dr. Rees and myself and the histochemical staining graded 1 to 4. There was 5% initial inter-observer variation with a maximum variation of one grade. In those specimens where there was disagreement consensus was reached and appear in Appendix 5.

RESULTS

Group A. Ureterosigmoidostomy for Benign Aetiology

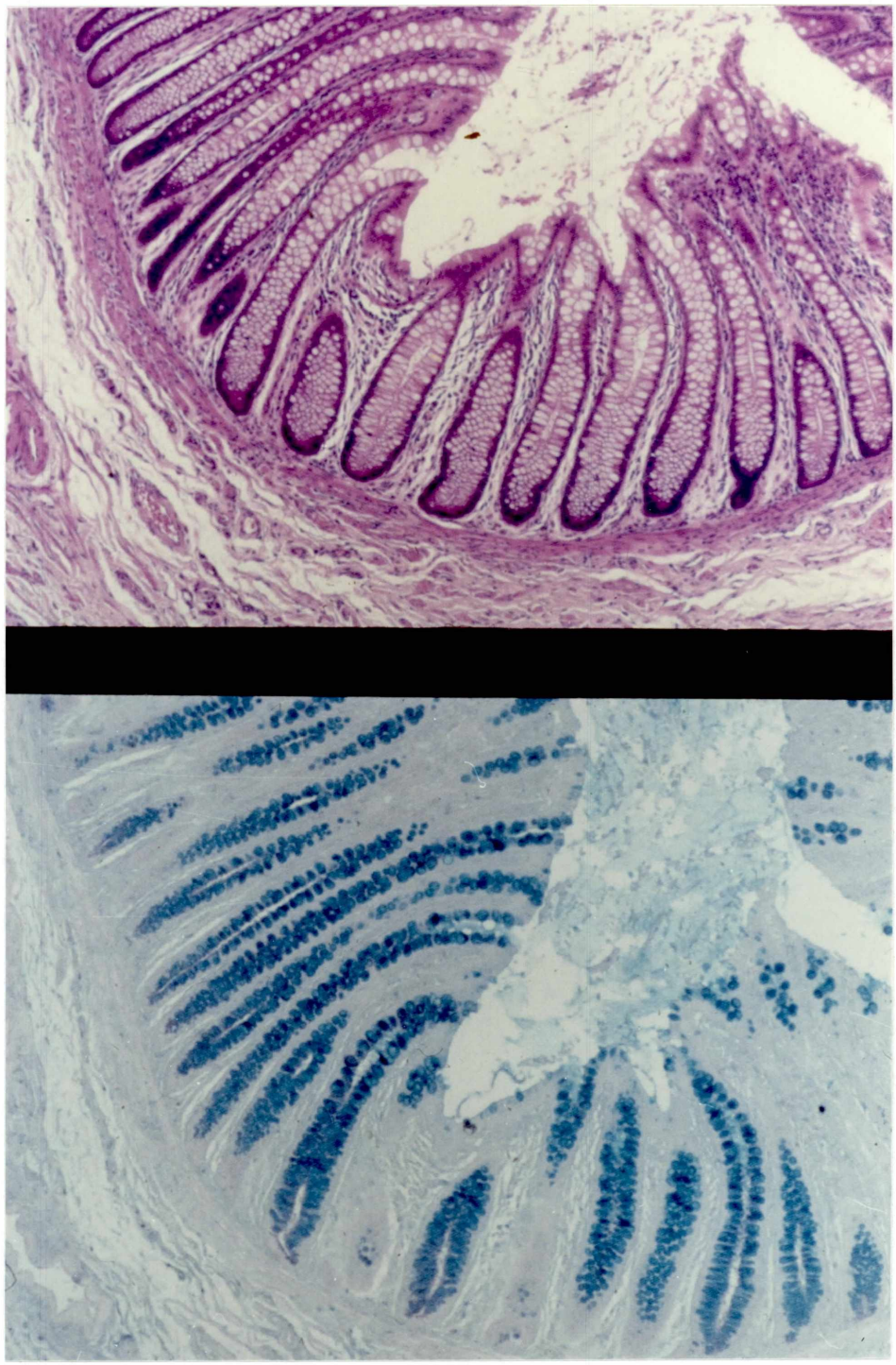
In this group who originally had an operation for a benign condition all of the slides examined with HE were morphologically normal. When examined with HID-AB, 17 out

Fig. 32.



Photomicrograph of human colonic mucosa stained with H&E (top) and high iron diamine alcian blue pH 2.5 showing sialomucin.

Fig. 33.



Photomicrograph of human colonic mucosa stained with H&E (top) and high iron diamine alcian blue pH 2.5 showing sialomucin.

of 19 were sialomucin positive (Table 10). Two patients had normal mucins, one had had a previous bowel resection for adenocarcinoma at the ureteric orifice after 18 years with the biopsy taken 11 years later, and the other had had a previous local resection for an adenoma.

Group B. Ureterosigmoidostomy for Malignant Aetiology
These patients, who originally had neoplastic disease of the bladder, whose diversions were still intact, the H+E staining revealed one tubular adenoma, while the other eight were morphologically normal. HID-AB staining showed positive sialomucins in four patients, including the patient with the tubuloadenoma. One patient had positive sialomucins within a year of diversion. The other five patients had a normal mucin pattern.

Group C. Undiverted Ureterosigmoidostomy
This sub-set of patients no longer had a colonic urinary diversion, the biopsies of the colon revealed an adenocarcinoma discovered 26 years after the original operation and 22 years since rediversion, while the other seven had normal morphology. HID-AB showed positive sialomucins in three patients, including the patient who had the tumour discovered at biopsy at 26 years. The second of these had had an adenocarcinoma and colectomy at 26 years with the biopsy 11 years later, and the third had the biopsy at 12 years without a morphological abnormality. One of the

Table 10.

Results of Mucin Stain

		Sialomucin	Sulphomucin
Group A Ureterosigmoidostomy for Benign Aetiology		17	2
Group B Ureterosigmoidostomy for Benign Aetiology		4	5
Group C Undiverted Ureterosigmoidosotomy		3	5
Group D Colon Conduit	Colon Conduit	3	1
Colon Conduit	Colon	0	4

five patients with normal mucins had had a previously resected adenocarcinoma.

Group D. Colon Conduit

In the four patients with colon conduits both the conduits and the intact colon were histologically normal. The HID-AB showed three of the four to have sialomucins predominating in the conduit and all with a normal mucin pattern within the colon.

Discussion

In those patients in whom a biopsy was taken at the anastomosis (Group A), 17 out of the 19 patients had changed the mucin pattern from sulphomucin to sialomucin. The two patients without this change had had a previous colonic resection for tumour formation at the anastomosis. It was not clear why these two patients' mucins should be different from the others, but having removed the rectosigmoid previously for tumour the bowel being biopsied for this study was the original splenic flexure, which has previously been observed to have a predominately sialomucin positive pattern (Filipe and Branfoot, 1976).

In Group B there were mucin changes to the sialomucin pattern in four out nine patients some distance from the ureterosigmoidostomy anastomosis which suggested that the change to sialomucin was related to the presence the

ureterosigmostomy anastomosis. In the one patient with a morphological abnormality there was a change to sialomucin.

In Group C, in those patients whose ureterosigmoidostomy was diverted to another form of diversion, usually an ileal conduit, three of the eight were sialomucin positive. One had an adenocarcinoma at the time of biopsy, and one had a previous tumour and colectomy, while the third had never had any morphological changes. In this case the patient who had the colectomy remained sialomucin positive whereas the two patients in Group A who underwent colonic resection had no change in sialomucin pattern. In this group the number of patients with change to sialomucin was less than that in Group A, which suggests that urine is required in some patients to maintain the change to sialomucin, or that once induced, the change is reversible. Biopsies are more difficult to target accurately after the urinary anastomosis has been taken down as there is no urine to act as a landmark. The biopsies in this group could therefore be further from the anastomosis than in Group A.

In Group D the biopsies of the intact colon have acted as controls for the staining method. At no time have they had urine present in the colon and there was no evidence of a change to the sialomucin pattern. However, in the colon conduits from these patients, usually derived from the sigmoid colon, three of the four showed change to

sialomucin. This seems to indicate that urine stimulates the change to sialomucin in the colon, as there were no changes in the colon from approximately the same site that had not been exposed to urine. It was not clear why one patient should be different.

In this study the morphological changes associated with "transitional mucosa", i.e. lengthening and widening of the crypts (Filipe, 1969) were not seen. There was, however, a change from the sulphomucins to sialomucin in those patients in whom urine was present in the colon. Similar results to those of patients in Group A have been described by Marcheggiano et al. (1984), although in their study patients were examined with a very much shorter follow up, a median interval of 6 months. They felt that the change to sialomucin after urinary diversion confirmed that it was evidence of pre-malignant change. However, because the change was present at a very early stage and the average time to tumour production is 29 years the latency between pre-malignant change and malignant tumour formation was very long, it could could be equally well argued that the relationship was coincidental.

In only a few patients in the recorded literature was tumour formation distant from the ureterosigmoidostomy anastomosis (Stewart et al., 1982, and Sooriyaarachchi et al., 1977). In ureterosigmoidostomy patients after sucessful resection

of the tumour there was not a problem of local recurrence. This did not correlate with the observation of Habib et al. (1984), who found in patients undergoing colectomy that sialomucin present at the resection margin indicated a high incidence of local recurrence. If sialomucin was a pre-malignant indicator then one would expect more tumours away from the anastomosis and more commonly reported local recurrence.

It is possible that the tumour is predominately at the anastomosis because it is urothelial in origin (Chiarelli and Sandei, 1984), or because of local proliferative changes associated with healing means that any carcinogen acting within the colon occurs predominately at that site (Sunter et al., 1985, and Williamson and Rainey, 1984).

Conclusion

This study has shown that there was a change to sialomucin in the presence of urine without the morphological changes of "transitional mucosa". However, I have not been able to confirm that these changes were pre-malignant. It is well known that these tumours take many years to form, and it is conceivable that this is the earliest manifestation of a malignant change. That the mucin changes were also found away from the site of tumour formation makes this unlikely. Changes in the mucin pattern are therefore unlikely to be of benefit in screening these patients for risk of developing

malignant tumours.

CHAPTER 12

CHANGES IN COLONIC CELL PROLIFERATION AFTER URINARY DIVERSION

Following the changes described in Chapter 9 that proliferative changes occurred in the colons of animals exposed to urine, the study was extended to patients with urinary diversions. Previously described stathmokinetic experiments in man (Bottomley and Cooper, 1973) have limited or no application in man. It was for that reason the technique of cellular DNA measurement to produce a proliferative index, which in the animal study correlated well with other established cell kinetic techniques, was applied.

Patients and Methods

Thirty three control patients were colonoscoped following referal to a gastrointestinal clinic and were found to have either a normal colon or diverticular disease. The mean age of the patients was 62, range 24-88.

Eleven patients, aged 19-69 years, 12-49 years post ureterosigmoidostomy were studied. Two of the patients originally had a ureterosigmoidostomy for 10 and 6 years that was then converted to an ileal conduit for 14 years. As was the practice at that time the ureteric stumps were left in situ in the colonic wall. One patient had a ureterosigmoidostomy for 5 years and then developed a tumour at the ureterocolic anastomosis. This was treated by an anterior resection and the ureters were re-anastomosed to

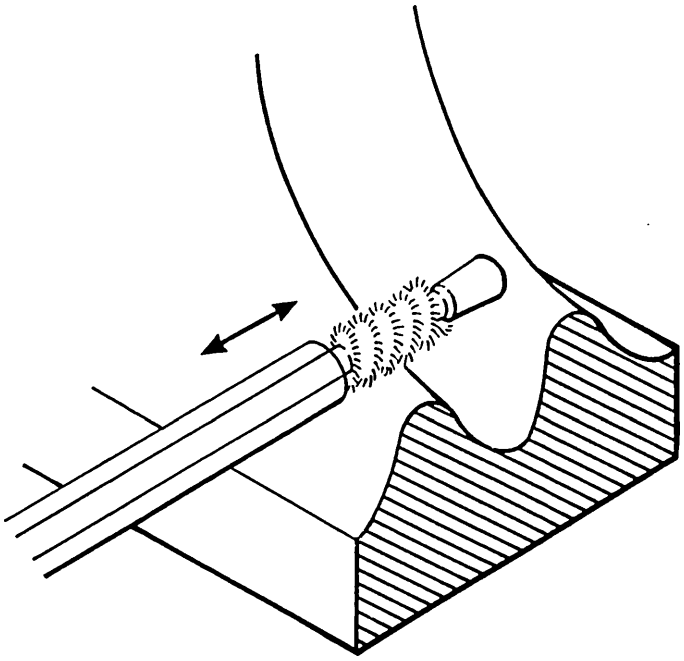
the colon more proximally and this was examined six years later.

All patients were examined using a 60cm flexible sigmoidoscope, as part of an annual follow up. This was used to examine the colon for any mucosal lesions and for observation of the ureterocolic anastomosis. A cytological brush was passed down the side arm and brushings were taken adjacent to the anastomosis (Fig. 34). The brush was placed in alcoholic Hank's solution and the epithelial cells shaken into the solution. The suspension was syringed 3 times through a 25 gauge needle to disperse clumps of cells. Cytopreparations were prepared with a Shandon cytopsin and post-fixed in Carbowax. The cells were then stained by the Feulgen technique and the DNA content of 100 individual epithelial cells were measured by microdensitometry. The percentage of cells with a hyperdiploid DNA content was calculated and this was termed the proliferative index (PI).

Biopsies were taken from the anastomosis only if indicated by the colonic examination. Previous biopsies taken from these patients were reveiwed.

Three patients who had colocystoplasties for small contracted bladders had urine taken for microdensitometry to see if it was feasible to study the shed cells from colocystoplasties. In this situation urine alone is in

Fig. 34



Diagrammatic representation of brushing taken from the colonic mucosa.

contact with the colonic mucosa. A voided urine was spun down and the cells collected. The cells were transfered to Hank's solution and treated in the same way as colonic brushings. The patients had had their colocystoplasties for 1, 1.5 and 13 years.

RESULTS

Control Patients

All the values for the poliferative indicies in the control group of patients were within the range 0-7.

Ureterosigmoidostomy Patients

Patient 1.

This man had a ureterosigmoidostomy performed when he was three years old. A colonoscopy performed 28 years later revealed a tumour, 4.5cm in diameter, at the ureterocolic anastomosis. The histology of the tumour was of a poorly differientiated adenocarcinoma with adjacent mild dysplasia and inflammation. The colon away from the tumour appeared normal. The PI could not be calculated because of the presence of aneuploid cells, (Table 11).

Patient 2.

This woman had a ureterosigmoidostomy performed when 6 years old. Only one biopsy had been performed which showed mild chronic inflammation with some cystic

Table 11.

Proliferative Index in Ureterosigmoidostomy Patients

Patient	Age yrs.	Time with ureterosigmoidostomy yrs.		>2N
1.	31	28		Aneuploid
2.	18/19	12/13		6/Faeces
3.	26/27	22/23		18/0
4.	73	19		2
5.	66	19		3
6.	19	15		0
7.	30/31	27/28		0/0
8.	66	27		Faeces
9.	53	49		3
10.	24	18		Aneuploid
11.	69	12		3
		ureterosigmoidostomy	ileal conduit	
12.	35	10	14	0
13.	22	6	14	3

metaplastic glands within the colon. Her first colonoscopy was performed 12 years post-operatively and she had a PI of six. The following year the specimen was too heavily contaminated with faeces to give a proliferative index result.

Patient 3.

The ureterosigmoidostomy was performed in this man when 4 years old. Two biopsies had been performed 2 years apart, both of which showed chronic inflammation. The first colonoscopy, 22 years post surgery gave a PI of 18 while one year later it was zero.

Patient 4.

This woman had her ureterosigmoidostomy performed when 54 years old. The colonoscopy was performed 19 years later when she was 73. Two biopsies had been performed 7 years apart. The first showed non specific inflammation with some colonic architectural distortion. The second demonstrated inflammation within the colon with some granulation. The PI was two.

Patient 5.

This woman aged 66 years had her ureterosigmoidostomy for 19 years at the time of colonoscopy. There had been no previous biopsy. The PI was three.

Patient 6.

This man had a ureterosigmoidostomy at 4 years and colonoscopy at 19 years. There had been no previous biopsies. The PI was zero.

Patient 7.

This man had a ureterosigmoidostomy performed at the age of 3 years. No biopsy was thought to be indicated. Colonoscopy was performed at 27 years and one year later and the PI on both occasions was zero.

Patient 8.

This 66 year old man had been diverted 27 years prior to colonoscopy. In the past he had had a normal colonic biopsy. The measurement of the PI was rendered impossible because of the presence of faeces.

Patient 9.

This man had a ureterosigmoidostomy for 49 years, performed when 4 years old. A previous biopsy had shown the appearance of a small colonic adenoma. The PI was three at colonoscopy.

Patient 10.

This man had his diversion when 4 years old. Two biopsies had been performed four years apart. The first was normal and the second had shown inflammation to be

present. There were a few aneuploid cells present but no evidence of tumour.

Patient 11.

This man aged 69 initially had a ureterosigmoidostomy performed at the age 57 years and 5 years later he developed a tumour at the ureterocolic anastomosis. The histology of the tumour showed a polyp covered with transitional epithelium. There was metaplasia of the urothelium to the squamous type and down growths of von Brunn nests. There was also inflammation present and the appearance of a superfically invasive carcinoma. A biopsy performed 6 years later at the new ureterocolic anastomosis showed inflamed colonic mucosa. The PI at the time of the colonoscopy of this study was three.

Patient 12.

This 35 year old man had a ureterosigmoidostomy performed in 2 stages. The right ureter was anastomosed to the colon when he was one year old and the left when 6 years old. The diversion was then converted to an ileal conduit, but leaving the ureteric stumps at the ananstomosis as was the practice at that time. The colonoscopy was performed 14 years after the conversion to an ileal conduit. There was a tumour at the site ofone ureterocolic anastomosis measuring 6x4x3cm. The ureter within the mass showed benign inflammatory

changes. The colonic mucosa was hyperplastic with only a minor degree of dysplasia. These changes were thought to be secondary to stomal prolapse. The PI taking a brushing from the tumour was zero.

Patient 13.

This man had his ureterosigmoidostomy at the age 2 years. This diversion was then converted to an ileal conduit 6 years later. 14 years later at his routine colonoscopy two tumours were found, one at each ureterocolic anastomosis. One tumour measured 2x1.5x0.8cm. and the other 5x4x3cm. There was a hyperplastic colonic mucosa with foci of dysplasia. There was a mixed inflammatory infiltrate within the submucosa. These tumours were also thought to be secondary to stomal prolapse. The PI taken directly from the tumours was three.

Colocystoplasty Patients

Only in one patient, with a colocytoplasty, was it possible to identify epithelial cells for proliferative index measurements. This was in the patient who had her augmentation for 13 years. This produced a proliferative index of three. In all patients there was heavy contamination with inflammatory cells and mucous which made this figure of doubtful significance. The other two patients had so many inflammatory cells present that the

epithelial cells could not be clearly identified.

Discussion

Aneuploid cells were present in the brushing of patient 1 who at the time of the colonscopy had an adenocarcinoma present at the ureterocolic anastomosis. Patient 3 had an abnormally high number of hyperdiploid cells which a year later returned to the normal range. The histology at the anastomosis previously had only shown chronic inflammation without any evidence of dysplasia. The question arose as to why there was such variation in the results. One explanation was that there was a fault in the technique of the DNA readings. There could be inclusion of non epithelial cells which would distort the results. Brushings were taken at the anastomosis and it may be that there was a difference when the brush passed near the anastomosis as opposed to directly over it.

In patient 10 a few aneuploid cells were seen even though no tumour was seen. This finding of a quantitative change in the DNA would strongly suggest that evidence of malignant transformation had been detected. The biopsy in this case showed evidence of inflammation but no other morphological change.

In contrast patients 12 and 13 both had obvious tumours and the brushings were taken from the tumour. The proliferative

indices were both within normal limits, without an aneuploid profile, and the histology of these polyps showed minimal dysplastic features.

The histology of the tumour or colon was comparable with the DNA microdensitometry in all but patients 3 and 10, with no histological evidence of hyperplasia or dysplasia of the colon there was no increase in the proliferative indices. Matthews et al. (1987) have shown previously that the proliferative index in normal colons should be no greater than seven, while those with multiple colonic tumours the proliferative index was greater than seven. It therefore appears that the proliferative pattern in the colon of those patients with ureterosigmoidostomies away from the anastomosis falls within the normal range.

There was only one result for colocystoplasties which was because of the difficulty in interpretating the proliferative index in the presence of inflammatory cells and it lay within the normal range. A greater number of patients would be required to study this further. Epithelial cells can be distinguished from polymorphs and lympocytes, but not from macrophages. The overwhelming numbers of inflammatory cells present prevented clear indentification of the epithelial cells.

CHAPTER 13

GENERAL DISCUSSION

Risk

In these studies of ureterosigmoidostomy I have used a homogeneous population with a long follow up which essential as the latency of the this tumour is approximately 30 years. As discussed earlier, the follow up in most previous studies has been limited and they were also deficient in that the group observed comprised of a number of different pathologies.

At the follow up in this study four patients had developed an adenocarcinoma and another four patients had developed a tumour that with time might progress to an adenocarcinoma. The twenty year risk of developing a tumour was about 22%. If the follow up was longer the risk of tumour formation might have be greater and therefore the previous estimate of a 5% lifetime risk would seem to be an underestimate (Leadbetter et al., 1979), and is in broad agreement with Zabbo an Kay (1986).

Tumour Origin

The tumours found at ureterosigmoidostomy anastomosis were usually regarded as colonic in origin as they were adenocarcinomas in continuity with the colonic mucosa. Non-invasive tumours at the anastomosis were usually classified as juvenile or adenomatous. Chiarelli and Sandei (1984) on examination of four polyps came to the conclusion

that they were juvenile which were not normally regarded as pre-malignant, although this has been questioned (Rolles, 1987). MacGregor (1968) suggested that the tumours may be urothelial in origin, having undergone glandular metaplasia. The tumours nearly always occurred at the anastomosis and if urine was carcinogenic tumours could be expected in other areas of the colon, although an anastomosis is more at risk of tumour formation (Williamson et al., 1980). Also, tumours still developed at the anastomosis even after patients had been converted to an alternative form of urinary diversion (Urdaneta et al., 1966, Schipper and Decter, 1981, Spence et al., 1979, and Strachan et al., 1987a). In one study a patient developed tumour that spread from the ureterocolic anastomosis to the ipsilateral renal pelvis (Shapiro et al., 1979). The environment of the exposed ureter is similar to that of the exposed urothelium of the exstrophied bladder which is well known to develop adenocarcinoma (Chiarelli and Sandei, 1984).

Urothelium is capable of changing to other cell types and may develop an adenocarcinoma when provoked by the appropriate stimulus. In a review of tumours associated with bladder exstrophy, 47 of the 54 tumours were adenocarcinomas (O'Kane and Megaw, 1968). This incidence of adenocarcinoma was far greater than the usual transitional cell tumours of the bladder. Two explanations have been put forward to account for these changes. Culp (1964)

postulated that the exstrophied bladder was abnormal and that enteric nest cells were present within the bladder and these subsequently developed into adenocarcinomas. These abnormal nest cells have been found within the exstrophied bladder from a very young age. O'Kane and Megaw (1968) examined the histology of the neonatal exstrophied bladder and found no evidence of abnormal histology. The change in histology presumably was due to exposure of the bladder. Glandular metaplasia has been found in the renal pelvis and ureter secondary to inflammation where an embryological factor would be difficult to envisage. Schistosomiasis of the bladder is also associated with a high incidence of squamous and adenocarcinoma in which N-nitrosamines have been implicated (Hicks et al., 1982).

If the urothelium within the ureterosigmoidostomy was at risk of malignant transformation one might see malignancy in ileal and colon conduits, colocystoplasties and ileocystoplasties where urine, urothelium and bowel mucosa have an opportunity to interact. The commonest urinary diversion is the ileal conduit which has been performed in great numbers since the original report by Bricker (1950). Since that time there has been only one case report of a tumour developing at the uretero-ileal anastomosis (Shousa et al., 1978). If the tumours were urothelial in origin far greater numbers of tumours ought to have been reported by now. In ileocystoplasties there have been case reports of

tumour developing in the ileal part of the augmentation (Kamidono et al., 1985, Smith and Hardy, 1971, and Takasaki et al., 1983), and an adenocarcinoma has been reported to develop in the bladder remnant (Hasegawa et al., 1988). The greater incidence in the augmentation group may be due to it acting as a storage organ rather than a conduit.

The colon conduit has been performed in far fewer numbers over the last 25 years Mogg (1965) and there have been two case reports of associated malignancy, and only one of these at the anastomosis (Chiang et al., 1982). In colocystoplasties there has only been two cases where a tumour developed (Kirby and Lloyd-Davies, 1985, and Cado, 1988, personal communication).

Histology

In the present series the colonic mucosa away from the urothelial colonic anastomosis remained normal in all but a 74 year old with a tubular adenoma, and there were none of the morphological features of "transitional mucosa" seen. Sommo and Traverso (1967) demonstrated initial hyperplasia of the colonic mucosa followed by atrophy from which they felt the tumour developed. There were only five patients in their study with the maximum follow up of 48 months while none of the patients in this study were changes of hyperplasia or atrophy observed.

Biopsies, or resection specimens of the urothelial colonic anastomosis show variable histology. In one the tumour showed a polyp with a covering of transitional epithelium which had undergone squamous and glandular metaplasia. In this specimen there was evidence of a superfical invasive tumour. A second patient in whom the urothelium could be examined, there were inflammatory changes only.

Von Brunn developed the concept of glandular metaplasia secondary to the proliferation of the irritated bladder. The urothelium proliferates downward to produce von Brunn nests. These may develop further to form cystitis cystica and eventually adenocarcinoma. Chronic inflammation is an important factor in these changes (Mostofi et al., 1955).

The majority of the malignant tumours associated with ureterosigmoidostomies in the literature were described as adenocarcinomas, although other types are described (Appendix 1). There have been three transitional cell tumours, one of which was not a recurrence of the original bladder pathology. There has also been a description of a lymphoma (Ghamen and Perry, 1985). There have been many descriptions of non-invasive tumours, juvenile (Ali et al., 1984, and Markowitz and Koontz, 1966), adenoma (Mogg, 1977, and Spence et al., 1979), reactive (Lasser and Acosta, 1975) and inflammatory (Leadbetter et al., 1979, and Dunn et al., 1979). The non-invasive tumours were described in various

terms which then go to form an adenocarcinoma. Present at all the tumours in my study but one, there was evidence of chronic inflammation. Chronic inflammation when present for many years may lead onto tumour formation and this change has been found at resection margins where tumours reccur (Sunter et al., 1985). Chronic inflammation has also been associated with juvenile polyps which are generally regarded as benign. Moorcraft et al. (1983) demonstrated chronic inflammation in the colon of colon conduits which became more marked with increasing years and could lead to tumour formation.

Most authors when discussing ureterosigmoidostomy tumours felt that the tumours were colonic adenomas, which then became adenocarcinomas. One reason for this opinion was that the non-invasive tumours, associated with ureterosigmoidostomies present approximately 5 years before malignant tumours, supporting the colonic adenoma, adenocarcinoma sequence theory of the genesis of colonic carcinoma (Muto et al., 1975 and Day, 1984). However, on balance I feel that the tumours appearing at the urothelial colonic anastomosis are more likely to be urothelial in origin, because of the site of tumour formation, and other than the histochemical change the colonic mucosa appears normal.

Histochemistry

Seventeen of the 19 (89%) patients with biopsies taken at the ureterosigmoidostomy anastomosis demonstrated a sialomucin pattern. In biopsies that were taken further away this figure dropped to 44% and to 36% in those who had a subsequent rediversion. In the patients who had urine only in the colon 75% were positive. However, this was just a mucin change and there was no morphological change to "transitional mucosa". This study does not give further evidence for the prognostic implication of mucin change.

Microdensitometry

The results of the microdensitometry were variable. There was no correlation between the histology results of two patients and the proliferative indices but it reflected the morphological changes present in 85% of the patients.

It would seem that microdensitometry reflects the morphological appearance of the colon far better than the mucin changes. The time to tumour formation is very long in this tumour and therefore one might not expect there to be any changes in the proliferative index until late into follow up and there may be some value in serial measurement.

Animal Model

The animals produced tumours at the urothelial colonic

anastomosis where urine, faeces, and a urine faeces mix were in contact. This was similar to man where tumours were described in colonic conduits, undiverted ureterosigmoidostomies and ureterosigmoidostomies. The tumours appeared at the suture line in both cases associated with chronic inflammation. There was also difficulty in differentiating the cells of origin.

The effect of urine in the rat colon appeared to be different to that of the human. In rats the crypt cell production rate, DNA microdensitometery and histological changes were very marked with an increase in cell turnover. In the human colon no such changes were seen, although there were some histochemical variation which may reflect more subtle changes.

The urothelium at the anastomosis undergoes proliferation, and both squamous and glandular metaplasia were seen, in both rats and man although these changes were not consistent. This form of metaplasia can go onto malignant change.

Conclusion

If this animal model can be regarded as a fair reflection of the human situation are there any lessons for the future? It is well known that tumours are found at ureterosigmoidostomy junctions, and a few are reported in

colon conduits and colocystoplasties. The colon conduit and colocystoplasty has been use for about 20 years, but only in the last 5 years in large numbers. Tumours following ureterosigmoidostomy take approximately 30 years to become malignant. Therefore, patients with these operations will be increasingly at risk in the 1990's and beyond. The risk of urine or faeces alone as a cause of tumour formation, based on these animal experiments would seem to be less than those with a urine faeces mix. Patients with urothelial colonic anastomosis should be followed up to safeguard against this potential risk.

Future Studies

The animal model described has shown that urine produces a hyperplastic response of the colonic mucosa. However, from these studies it is not possible to tell whether this response is reversible. It would be feasible to perform a vesicocolic fistula and then take down the anastomosis, closing the colon and bringing out the trigone onto the skin. The timing of these operations and the date of sacrifice could altered to determine if the hyperplastic process is reversible and if so how quickly it occurs.

Urine has produced a hyperplastic reponse, but the component within urine that has this effect has not been determined. It may be possible to cannulate the colon with a fine catheter and run in solutions with different constituents of

urine and then determine the rate of cell turnover, and from this one part in the neoplastic process.

This study has not shown conclusively whether the tumours have originated from the urothelium or the colonic mucosa in animals. Further studies using specific markers to the different surface epithelia may help find the cell line of the tumours although this may only indicate what the cells are developing into, rather than their origin.

It is now well known that tumours develop over a long period of time in ureterosigmoidostomy patients. These animal studies suggest that patients with colocystoplasties and colon conduits may have an increased risk of tumour formation. Most ureterosigmoidostomy patients are now under surveillance and I feel that colocystoplasties and colon conduits should have a close follow up. The tumours will presumably have a long latency and a national registry of those patients having these operations should be kept, as the number being performed in any one centre is small, to help discover if this risk is theoretical or real.

APPENDICES

Appendix 1

Tumours Associated with Ureterosigmoidostomy

Author	Age at Operation yrs.	Latency of Undiverted Tumour yrs.	Tumour yrs.
MALIGNANT TUMOURS			
Hammer	50	10	Adenocarcinoma
Thompson et al.	3	27	Adenocarcinoma
Wilson	16	31	Adenocarcinoma
Aldis	18	15	Scirrhous
Kozak et al.	2	15	Adenocarcinoma
Parsons et al.	1.5	33.5	Adenocarcinoma
Richter et al.	2	23	Adenocarcinoma
Whitaker et al.	7	15	Adenocarcinoma
''	31	27	Adenocarcinoma
''	19	20	2 x Anaplastic
Sacre et al.	14	28	Adenocarcinoma
Mueller et al.	9	46	2 x Adenocarcinoma
Preissig et al.	8	26	Adenocarcinoma
Harguindey et al.	2	24	No histology
Carswell et al.	2.5	23.5	Adenocarcinoma
Besancenez et al.	4	25	Adenocarcinoma
Urdaneta et al.	21	17	Undifferentiated
Charron et al.	38	20	Adenocarcinoma
Brekkan et al.	3	20	Anaplastic
''	6	38	Adenocarcinoma

Brekkan et al.	6	41	Adenocarcinoma
Stewart et al.	mean 17	mean 25	7 x Adenocarcinoma
Kille et al.	10	16	Adenocarcinoma
Dixon et al.	3	30	Adenocarcinoma
Mogg	20	15	Adenocarcinoma
''	6	22	No histology
''	7	38	2 x Adenocarcinoma
Labow et al.	30	41	Adenocarcinoma
Leadbetter et al.	2	50	Adenocarcinoma
''	12	19	Colloid Carcinoma
Lindsey	?	?	Adenocarcinoma
Sooriyaarachchi et al.	6	37	Adenocarcinoma
Urwin et al.	3 mths	33	Adenocarcinoma
Potet et al.	7	25	Adenocarcinoma
Eraklis et al.	4	28	Adenocarcinoma
''	2	29	Adenocarcinoma
Ellis	?	?	Adenocarcinoma
Harford et al.	3.5	54	2 x Adenocarcinoma
''	21	40	Adenocarcinoma
Labow et al.	56	16	Adenocarcinoma
Urdaneta et al.	55	9	Adenocarcinoma
Amar	58	3.5	Adenocarcinoma
Massachusetts	55	8	Adenocarcinoma
Scheinman	42	9	Adenocarinoma
Warren et al.	2	49	2 x Adenocarcinoma
Shapiro et al.	16	21	Adenocarcinoma

Shapiro et al.	2	34		Adenocarcinoma
Oetjen et al.	3 mths	23	8	Adenocacinoma
Tank et al.	9	46	3	2 x Adenocarcinoma
Lien	7	26	14	Adenocarcinoma
Shapiro et al.	4.5	17	14	Adenocarcinoma
Rivard et al.	15	23	8	Adenocarcinoma
Eraklis et al.	6	27	18	Adenocarcinoma
''	4	17	14	Adenocarcinoma
Spence et al.	3 mths	14	9 mths	Adenocarcinoma
Recht et al.	6 mths	23	15	Adenocarcinoma
Dube et al.	4	20	7	Adenocarcinoma
Schipper et al.	1.5	22	1	Adenocarcinoma
Strachan et al.	4	27	3.5	Adenocarcinoma
Parsons et al.	3	26	16	Adenocarcinoma
Urdaneta et al.	4	29	6 mths	Not known
Sugg	5	15		Transitional
Riches et al.	61	1		Transitional
Amar	46	4		Transitional
Ghanem et al.	40	5		Lymphoma

NON-INVASIVE TUMOURS

Mogg	20	15		Adenoma
Leadbetter et al.	12	19		Inflammatory adenoma
Ali et al.	1	22		Juvenile polyp
Qvist et al.	13	10		Polyp dysplasia
Markowitz et al.	1	14		Juvenile polyp
''	2	15		Juvenile polyp

Markowitz et al.	1	18		2 x Juvenile polyp
Lasser et al.	10	27		2 x Reactive polyp
Dunn et al.	12	7		Inflammatory polyp
''	1	13		Adenoma
Chiarelli et al.	1	26		Polyp
''	4	22		2 x Polyps
''	2	22		Polyp
''	31	8		Polyp
Harford et al.	21	40		Tubular adenoma
Naryana et al.	6	14		2 x Adenoma
Spence et al.	1	26		Adenoma
Dixon et al.	3	30		Polyp
Brekkan et al.	3	20		Not known
Lien	1.5	23.5		2 x adenomas
Potet et al.	9	25		2 x polyps
Kille et al.	4	15		Adenoma
Stewart		mean 18		7 x Adenomas
MacGregor	57	5		Mucus adenoma
Dube et al.	4	20	7	Adenoma
Haney et al.	6	38	13	Adenoma
Gillman	75	7	6	Mucus adenoma
Ansell et al.	47	22	20	Adenoma
Spence et al.	3 mths	14	13	Adenoma

Appendix 2

Crypt Cell Production Rate Counts

Control 1 month.

Code No.	Counts in Individual Crypts										Mean
A	4	6	3	1	1	7	8	2	6	4	4.2
B	13	13	11	19	12	7	16	7	11	9	11.8
C	8	2	5	6	6	6	11	5	5	6	6.0
D	8	7	6	10	10	9	11	18	8	9	9.6
E	1	1	1	1	1	1	1	1	1	1	1.0
F	15	18	15	14	17	13	16	14	9	16	14.7
G	8	5	6	3	8	4	4	4	4	9	5.5
H	8	7	1	7	6	4	4	6	5	7	5.5

Decode

Code No.	E	C	A	H	G	B	D	F
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	1.0	6.0	4.2	5.5	5.5	11.8	9.6	14.7

Coefficient of Correlation = 0.9

$p<0.005$

Crypt Cell Production Rate = 4.9 cells/cyrpt/h

Control 3 months

Code No.	Counts in Individual Crypts										Mean
A	2	1	3	3	2	5	2	2	2	4	2.6
B	9	7	5	7	7	11	9	10	8	10	8.3
C	4	6	9	9	6	2	5	4	4	3	5.2
D	2	2	0	0	1	0	0	1	0	2	0.8
E	4	4	3	6	4	5	4	5	5	3	4.3
F	8	13	11	11	13	11	13	10	7	7	10.4
G	8	10	9	6	6	4	5	6	7	6	6.7

Decode

Code No.	D	A	G	E	C		F	B
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	0.8	2.6	6.7	4.3	5.2		10.4	8.3

Coefficient of Correlation = 0.87

$p<0.05$

Crypt Cell Production Rate = 3.4 cells/crypt/h

Control 6 months

Code No.	Counts in Individual Crypts										Mean
A	3	2	2	3	4	3	4	5	3	4	3.3
B	2	14	3	9	5	10	6	6	7	9	7.1
C	10	8	4	3	5	9	4	14	8	9	7.4
D	2	3	1	2	3	2	2	5	1	2	2.3
E	3	4	2	5	1	5	5	5	3	2	3.5
F	7	9	8	6	8	5	9	6	10	6	7.4
G	5	1	5	4	0	7	2	5	6	9	4.4
H	2	0	7	6	2	2	4	4	6	3	3.6

Decode

Code No.	D	A	H	G	E	B	C	F
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	2.3	3.3	3.6	4.4	3.5	7.1	7.4	7.4

Coefficient of Correlation = 0.92

$p<0.005$

Crypt Cell production Rate = 2.4 cells/crypt/h

Urothelial implant 1 month

Code No.	Counts in Individual Crypts										Mean
A	4	7	12	4	7	8	9	6	13	11	8.1
B	12	13	17	18	16	17	18	10	21	15	15.7
C	0	1	2	0	0	2	1	0	1	2	0.9
D	7	10	9	15	11	18	16	6	19	10	12.1
E	22	22	15	20	17	18	24	14	27	14	19.3
F	5	2	9	3	2	3	3	2	5	5	3.9
G	25	13	9	16	15	13	13	19	20	15	15.8
H	2	3	3	4	3	3	5	2	2	4	3.1

Decode

Code No.	C	H	A	D	F	B	G	E
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	0.9	3.1	8.1	12.1	3.9	15.7	15.8	19.3

Coefficient of Correlation = 0.88

$p<0.005$

Crypt cell Production Rate = 7.4 cells/crypt/h

Urothelial implant 3 months

Code No.	Counts in Individual Crypts										Mean
A	3	6	3	4	2	3	3	2	1	2	2.9
B	5	8	5	6	2	6	6	5	9	5	5.7
D	2	1	2	0	0	0	2	0	0	0	0.7
E	25	16	25	17	8	8	10	8	16	13	14.6
F	0	0	1	2	0	1	0	0	0	1	0.5
G	6	7	4	12	5	3	6	7	6	6	6.2

Decode

Code No.	F	D	B	A	G			E
Time min	30	50	70	90	110	130	150	170
Mean cells/ crypt	0.5	0.7	5.7	2.9	6.2			14.6

Coefficient of Correlation = 0.94

$p<0.01$

Crypt Cell Production Rate = 5.9 cells/crypt/h

Urothelial implant 6 months

Code No.	Counts in Individual Crypts										Mean
A	5	6	11	5	4	2	4	3	1	3	4.4
B	2	2	0	2	0	0	0	0	1	3	1.0
C	19	28	27	42	34	43	34	44	31	47	34.9
D	5	5	3	1	4	3	5	5	4	4	3.9
E	4	5	4	7	7	6	7	6	7	13	6.6
F	5	11	12	15	16	13	20	14	13	21	14.0
G	7	10	8	10	5	12	6	9	7	5	7.9
H	6	5	9	13	8	9	11	9	12	6	8.8

Decode

Code No.	B	D	A	H	F	G	E	C
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	1.0	3.9	4.4	8.8	14.0	7.9	6.6	34.9

Coefficient of Correlation = 0.73

$p<0.05$

Crypt Cell Production Rate = 9.5 cells/crypt/h

Colon Conduit 1 month

Code No.	Counts in Individual Crypts										Mean
A	22	27	10	22	23	26	20	24	12	25	21.1
B	33	75	47	74	34	57	53	52	39	36	50.0
C	14	16	8	20	19	11	11	5	9	4	11.7
D	86	47	64	47	77	63	64	74	57	42	62.1
E	70	62	28	60	44	46	77	55	60	42	54.4
F	33	12	13	38	30	38	41	32	32	44	31.3
G	16	25	18	35	34	31	26	30	14	34	26.3
H	93	100	92	67	70	80	102	70	77	67	81.8

Decode

Code No.	C	G	A	F	B	D	E	H
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	11.7	26.3	21.1	31.3	50.0	62.1	54.4	81.8

Coefficient of Correlation = 0.95

$p<0.001$

Crypt Cell Production Rate = 27.6 cells/crypt/h

Colon Conduit 3 months

Code No.	Counts in Individual Crypts										Mean
A	26	51	31	65	63	30	49	50	44	76	48.5
B	97	82	83	58	63	61	34	70	105	63	71.6
C	27	19	40	26	42	40	40	38	24	25	32.1
D	80	57	98	58	67	67	70	64	63	89	71.3
E	54	68	48	65	57	41	75	57	55	52	57.2
F	15	8	10	8	4	9	10	6	3	6	7.9
G	27	34	28	19	24	31	11	35	33	23	26.5

Decode

Code No.	F	G	C	B	E		D	A
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	7.9	26.5	32.1	71.6	57.2		71.3	48.5

Coefficient of Correlation = 0.72

p N.S.

Crypt Cell Production Rate = 21.1 cells/crypt/h

Colon Conduit 6 months

Code No.	Counts in Individual Crypts										Mean
A	24	38	35	22	32	27	23	35	32	22	29.0
B	31	17	44	45	30	44	35	48	37	31	36.2
D	51	89	54	71	61	43	78	78	37	85	64.7
E	96	74	62	54	69	64	66	79	59	95	74.9
F	17	13	15	17	24	17	14	25	18	17	17.7
H	20	11	5	7	7	16	18	16	14	8	12.2

Decode

Code No.	H	F	A	B	D		E	
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	12.2	17.7	29.0	36.2	64.7		74.9	

Coefficient of Correlation = 0.97

$p<0.005$

Crypt Cell Production Rate = 34.3 cells/crypt/h

Vesicocolic fistula 1 month

Code No.	Counts in Individual Crypts										Mean
A	65	47	61	63	60	51	46	8	58	42	50.1
B	81	90	79	96	82	78	99	99	111	103	91.8
C	37	40	32	27	28	42	27	26	22	28	30.9
D	26	30	25	33	31	36	42	55	54	43	37.9
E	60	33	43	49	60	40	40	43	61	53	48.2
F	16	4	9	7	16	18	13	7	16	9	11.5
G	59	47	52	40	54	58	61	69	46	67	55.3

Decode

Code No.	F	C	E	D	A		B	G
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	11.5	30.9	48.2	37.5	50.1		91.8	55.3

Coefficient of Correlation = 0.81

$p<0.05$

Crypt Cell Production Rate = 23.5 cells/crypt/h

Vesicocolic fistula 3 months

Code No.	Counts in Individual Crypts										Mean
A	32	34	32	29	46	30	21	34	32	39	32.9
B	56	41	76	50	53	53	60	51	58	65	56.3
C	70	80	68	95	63	66	63	63	42	59	66.9
D	29	37	19	17	25	22	12	31	36	21	24.9
E	108	103	81	70	53	64	66	80	44	83	75.2
F	25	36	18	21	17	20	27	33	20	35	25.2

Decode

Code No.	D		A	F	B		E	C
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	24.9		32.9	25.2	56.3		75.2	66.9

Coefficient of Correlation = 0.89

$p<0.05$

Crypt Cell Production Rate = 22.9 cells/crypt/h

Vesicocolic fistula 4 months

Code No.	Counts in Individual Crypts										Mean
A	75	59	46	36	39	45	41	53	50	40	48.4
B	22	16	18	25	39	12	25	18	23	16	21.4
C	71	61	76	68	74	65	60	59	82	75	69.4
D	40	27	29	29	35	22	33	41	21	50	32.7

Decode

Code No.	B		D		A			C
Time min.	30	50	70	90	110	130	150	170
Mean cells/ crypt	21.4		32.7		48.4			69.4

Coefficient of Correlation = 1.0

$p<0.005$

Crypt Cell Production Rate = 20.9 cells/crypt/h

Appendix 3.

Proliferative Index of Each Rat

1 Month

Control	Urothelial Implant	Colon Conduit	Vesicocolic
0	0	3	2
0	0	3	3
2	0	4	3
2	1	4	5
2	1	4	6
3	2	5	6
3	2	5	7
		8	7
Median 2	1	4	5.5
p< v Control	N.S	0.001	0.001

3 Months

Control	Urothelial Implant	Colon Conduit	Vesicocolic
0	0	2	2
0	0	3	2
0	0	7	5
2	1	8	7
3	1	9	8
3	3	11	12
		19	13
Median 1	0.5	8	7
p< v Control	N.S.	0.001	0.001

6 Months			4 Months
Control	Urothelial Implant	Colon Conduit	Vesicocolic
1	2	3	9
1	3	5	11
1	6	8	13
2	7	8	14
2	7	9	
3	8	11	
	9		
	9		
Median 1.5	7	8	12
p< v Control	0.001	0.001	0.001

Appendix 4
Histological Changes in Rat Models

Urothelial Implant

Animal	Colon	Urothelium	Anastomosis
1 Month			
1	Normal	Normal	Normal
2	Normal	-	-
3	Normal	-	-
4	Normal	-	-
5	Normal	Normal	Normal
6	Normal	Normal	Normal
7	Normal	-	-
8	Normal	Normal	Inflammation
3 Months			
1	Normal	Normal	Inflammation
2	Normal	Normal	Normal
3	Normal	-	-
4	Normal	Glandular metaplasia	Inflammation
5	Normal	Squamous metaplasia & keratin	Normal
6	Normal	-	-
7	Normal	Normal	Inflammation
6 Months			
1	Normal	-	-
2	Normal	Glandular metaplasia	Dysplasia
3	Normal	Normal	Inflammation
4	Normal	Squamous metaplasia & keratin	Inflammation
5	Normal	-	-
6	Normal	Normal	Inflammation
7	Normal	Normal	Abscess

Colon Conduit

Animal	Colon	Urothelium	Anastomosis
1 Month			
1	Hyperplasia	Proliferation	Normal
2	Hyperplasia	Normal	Normal
3	Hyperplasia	Normal	Normal
4	Hyperplasia	Glandular metaplasia	Normal
5	Mild dysplasia	Squamous metaplasia & keratin	Inflammation
6	Hyperplasia	Proliferation	Normal
7	Hyperplasia	Glandular metaplasia	Normal
8	Hyperplasia	-	-
3 Months			
1	Hyperplasia	Proliferation	Normal
2	Hyperplasia	Glandular & squamous metaplasia	Normal
3	Hyperplasia	Normal	Normal
4	Hyperplasia	Proliferation	Normal
5	Hyperplasia	Proliferation	Normal
6	Hyperplasia	Normal	Normal
7	Mild dysplasia	Proliferation	Normal
6 Months			
1	Hyperplasia	Normal	Normal
2	Hyperplasia	Normal	Normal
3	Hyperplasia	Normal	Normal
4	Mild dysplasia	Glandular metaplasia	Dysplasia
5	Moderate dysplasia	-	-
6	Mild dysplasia	Glandular metaplasia	Dysplasia
7	Mild dysplasia	Glandular metaplasia	-
8	Mild dysplasia	Proliferation	Normal

Vesicocolic Fistula

Animal	Colon	Urothelium	Anastomosis
1 Month			
1	Hyperplasia	Normal	Normal
2	Hyperplasia	Normal	Normal
3	Hyperplasia	-	
4	Mild dysplasia	Normal	Normal
5	Hyperplasia	Normal	Normal
6	Normal	Proliferation	Normal
7	Hyperplasia	-	-
8	Hyperplasia	-	-
3 Months			
1	Hyperplasia	Normal	Normal
2	Hyperplasia	Glandular & squamous metaplasia	Normal
3	Hyperplasia	Squamous metaplasia & keratin	Normal
4	Mild dysplasia	Proliferation	Normal
5	Hyperplasia	Squamous metaplasia	Dysplasia
6	Mild dysplasia	Normal	Normal
4 Months			
1	Hyperplasia	Proliferation	Dysplasia
2	Hyperplasia	Normal	Dysplasia
3	Mild dysplasia	Proliferation	Dysplasia
4	Hyperplasia	Normal	Dysplasia
5	Hyperplasia	Glandular metaplasia	Dysplasia
6	Mild dysplasia	Proliferation	Dysplasia

Appendix 5

Assessment of Mucin Grade

Sex	Ureterosigmoidostomy years	Crypt U	Crypt M	Crypt L	Crypt U	Crypt M	Crypt L	Sialomucin
Group A.								
F.	5	4	4	4	4	4	4	Positive
M.	7	4	4	3				Positive
F.	9	4	4	4	4	4	3	Positive
M.	9	4	4	4				Positive
F.	9	4	4	4	3	3	2	Positive
F.	13	4	4	4	4	4	4	Positive
M.	13	3	3	3	4	4	3	Positive
M.	14	4	4	4	4	4	4	Positive
F.	17	4	4	4	4	4	4	Positive
F.	25	4	4	4	4	4	4	Positive
M.	25	2	2	2	4	4	4	Positive
F.	25	3	3	3	3	4	4	Positive
F.	27	3	4	4	4	4	4	Positive
F.	27	0	0	1	1	1	0	Negative
M.	28	2	2	1	4	3	4	Positive
M.	28	2	3	2				Positive
M.	33	4	4	4				Positive
M.	38	0	1	0	2	2	2	Positive
F.	38	0	0	0	0	1	0	Negative

Group B.

Sex	Ureterosigmoidostomy years	Crypt U M L	Crypt U M L	
M.	1	2 2 2		Positive
M.	1	1 3 3		Positive
M.	2	2 3 2		Positive
M.	3	0 0 0		Negative
M.	3	0 0 0		Negative
M.	4	0 0 0		Negative
F.	21	0 0 0		Negative
M.	24	2 2 2		Positive
M.	26	0 0 0		Negative

Group C.

	Ureterosig. years	Conduit years			
F.	3	9	2 3 3	4 4 4	Positive
M.	5	9	0 0 0	1 0 0	Negative
M.	7	8	0 0 0	0 0 0	Negative
F.	13	9	0 0 0	1 0 0	Negative
M.	7	16	0 0 0	0 0 0	Negative
M.	12	18	4 3 3	4 4 4	Positive
M.	12	23	0 0 0	0 0 0	Negative
M.	27	10	0 0 0	4 4 4	Positive

Group D.

Sex	Diversion years	loop crypt U M L	Sialomucin	colon crypt U M L	Sialomucin
M.	25	2 3 3	Positive	0 0 0	Negative
M.	20	4 4 4	Positive	0 0 0	Negative
F.	14	0 0 0	Negative	0 0 0	Negative
M.	18	4 4 4	Positive	0 0 0	Negative

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