Intestinal adaptation and experimental carcinogenesis after partial colectomy

Increased tumour yields are confined to the anastomosis

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SUMMARY Small-bowel resection enhances experimental colorectal carcinogenesis, probably by stimulating epithelial cell proliferation. The possibility that similar mechanisms might explain metachronous large-bowel cancers in man was tested in Sprague-Dawley rats submitted to partial colectomy before or after a five-week course of azoxymethane (total dose 50 mg/kg). The timing of operation did not affect tumour yields at 40 weeks. Caecal resection augmented mucosal mass in the ileum and right colon but did not affect carcinogenesis. Right hemicolectomy only increased ileal segmental weight (by 22%); left hemicolectomy increased the protein and DNA contents of the residual right colon by 18-42%. Large-bowel tumours in 84 rats were distributed as follows: proximal colon 36, colonic anastomosis 51, distal colon 87, rectum 43. Consistent with this left-sided predominance, left hemicolectomy reduced the number of large-bowel tumours. A twofold increase in distal tumours after both transection and right hemicolectomy simply reflected the high incidence of anastomotic tumours. Furthermore, one rat given vehicle as opposed to carcinogen developed an invasive mucinous adenocarcinoma at the colorectal anastomosis, after left hemicolectomy. The large bowel shows limited adaptation to partial resection and is not at increased risk of carcinogenesis, except in the region of the suture line.

Epithelial neoplasia of the large bowel is a multifocal disease. Adenomas are multiple in 28-35% of patients at the time of diagnosis;^{1 2} carcinomas are multiple in 3-5%, occurring either at the same time or later.^{1 3} Although synchronous cancers outnumber metachronous cancers, between 1 and 2% of patients surviving resection of one carcinoma will subsequently develop a second growth.^{1 3} This figure would undoubtedly be higher if patients lived longer: colon cancer affects an elderly population, crude five year survival rates seldom exceed 50%, and metachronous carcinomas take an average of 13.5 years to develop.⁴ The cumulative risk of the second carcinoma may reach 5% at 25 years.⁴

Previous investigations have shown that a number of different operations on the small bowel can promote experimental carcinogenesis in the large bowel. These procedures include small-bowel resection, whether proximal⁵ or distal,⁶⁻⁸ end-to-side je-

junoileal bypass⁹ and distal diversion of bile and pancreatic juice.^{10 11} The common factor may be the ability of these operative procedures to stimulate cell proliferation in the colonic mucosa,¹² though increased delivery of cocarcinogens such as bile salts could also play a part.

The adaptive response of the colon to partial colectomy is poorly documented. Right hemicolectomy caused compensatory growth of the left colon in one experiment, but the reverse did not apply.¹³ We have found a modest increase in chemically-induced tumours in the rectum after subtotal colectomy¹⁴ and in the distal colon after closure of a proximal defunctioning colostomy.¹⁵ The present study explored the possibility that metachronous colorectal neoplasia might occur in response to adaptive hyperplasia after partial colonic resection.

Methods

Two hundred young male Sprague-Dawley rats were used, weighing 159 ± 14 g (SD) at the start of the experiment. Animals were allocated to undergo oper-

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Fig. 1 Experimental design. The mean weight of each group is given at the time of operation and when killed. Unoperated controls were starved for 24 hours while corresponding groups were submitted to operation.

ation either immediately before starting a course of carcinogen injections ($n=53$), or one week after completing a similar course of injections of carcinogen ($n=92$) or vehicle ($n=55$). The carcinogen, azoxymethane, was diluted with sterile water (pyrogen-free) on receipt from the manufacturers (Ash Stevens Inc., Detroit, Michigan, USA); solutions were stored at -20°C until required. Rats were given five weekly subcutaneous injections of azoxymethane (10 mg/kg/wk) or vehicle (sterile water). The experimental design is summarised in Fig. 1, including the number of rats in each group and their mean body weight at operation and when they were killed.

Surgical operations (Fig. 2) were performed under ether anaesthesia. In each case food (but not water) was withdrawn for 24 hours postoperatively, and anastomoses were performed using a single continuous layer of 6-0 silk suture. Caecal resection was followed by end-to-end anastomosis between the terminal ileum and the ascending colon. In right hemicolectomy the proximal colon was excised together with the caecum, and the terminal ileum was anastomosed to the transverse colon at the site of midcolon transection. Left hemicolectomy comprised resection of the distal transverse and descending colons

with colorectal anastomosis at the pelvic brim. As a control for the effects of partial colectomy, colonic transection and immediate resuture was carried out just to the left of the middle colic vessels—that is, near the midpoint of the colorectum. Other animals received splenectomy. Controls had no operation but were starved for 24 hours.

Rats were weighed daily for 10 days after operation and weekly thereafter until they were killed at 40 weeks. Animals showing evidence of intestinal tumours, such as progressive weight loss or rectal bleeding, were killed when moribund, according to criteria similar to those described elsewhere.¹⁶ In carcinogen-treated rats a full necropsy was performed, and the entire intestinal tract was scrutinised for macroscopic tumours. All possible tumours and metastases were stored in 10% formalin for subsequent histological classification, as previously described.⁷ Apart from two tumours in which frankly malignant cells were limited to the mucosa, the term 'carcinoma' was applied only to lesions in which there was obvious penetration of the muscularis mucosae or the stalk of a polyp. In borderline cases several sections were obtained at different levels to determine the presence or absence of invasion.

Vehicle-treated rats were used to assess intestinal

Fig. 2 Operations performed. A, colonic transection; B, caecal resection; C, right hemicolectomy; D, left hemicolectomy. Other animals had no operation or splenectomy. The numbers give the distance in cm proximal to the anus, as measured when killed (40 weeks). Shaded areas represent 5 cm segments of terminal ileum, right colon and left colon that were used for estimation of mucosal DNA and protein contents.

adaptation. Immediately after death by cervical dislocation, the small bowel, caecum, and colorectum were excised and flushed clean with ice-cold saline. The last 20 cm of small bowel (terminal ileum) and the remaining small bowel were considered as separate segments. Segments of bowel were opened longitudinally, blotted dry, weighed and measured for length. The surface area of the caecum was estimated as before.⁷ Mucosal scrapings were obtained from duplicate 5 cm segments of terminal ileum, measured under gentle stretch, and from 5 cm segments of right and left colon, where present (Fig. 2). Specimens were frozen in liquid nitrogen within 10 minutes of death and were stored at -20°C .

Analysis of the DNA and protein content of mucosal samples was subsequently performed in triplicate. Specimens were weighed and homogenised, and aliquots were treated with ice-cold 25% trichloroacetic acid. The precipitate was washed with ice-cold alcoholic 0.1 M potassium acetate and then with absolute alcohol for 30 minutes at 60°C . After overnight drying at 45°C , pellets were dissolved in 1.0 M potassium hydroxide at 85°C . Protein was estimated by the method of Lowry *et al.*,¹⁷ and DNA was measured fluorimetrically by a modification of the method of Kissane *et al.*,¹⁸ which is specific for this compound. Mean values were obtained for each sample from the three aliquots taken for analysis.

Statistical analyses were as follows: Student's *t* test to compare indices of mucosal mass, and the Mann-Whitney U test to compare tumour yields.

Results

SURVIVAL AND WEIGHT GAIN

Operative mortality rates were 0/43 animals receiving operation before carcinogen, 18/82 (22%) animals receiving operation after carcinogen, and 12/55

Table 1 Intestinal length and segmental weight eight months after operation (means $\pm$ SEM)

	Controls (n=9)	Caecal resection (n=8)	Hemicolectomy	
			Right (n=7)	Left (n=9)
Small bowel (less terminal ileum)				
Length (cm)	124.6 $\pm$ 3.6	129.8 $\pm$ 3.4	128.0 $\pm$ 2.9	130.0 $\pm$ 3.6
Segmental weight (mg/cm)	94.3 $\pm$ 2.8	99.1 $\pm$ 4.2	103.0 $\pm$ 2.0*	97.7 $\pm$ 3.1
Terminal ileum				
Length (cm)	20.0 $\pm$ 0.4	19.6 $\pm$ 0.3	20.1 $\pm$ 0.3	20.1 $\pm$ 0.3
Segmental weight (mg/cm)	72.0 $\pm$ 2.1	103.4 $\pm$ 7.0†	87.8 $\pm$ 3.2‡	79.1 $\pm$ 3.0
Caecum				
Surface area (cm ²)	15.2 $\pm$ 0.8	—	—	15.8 $\pm$ 0.9
Segmental weight (mg/cm ²)	115.1 $\pm$ 5.7	—	—	112.0 $\pm$ 4.3
Colorectum				
Length (cm)	26.9 $\pm$ 0.5	30.5 $\pm$ 0.8‡	17.8 $\pm$ 1.3	19.7 $\pm$ 0.3
Segmental weight (mg/cm)	140.4 $\pm$ 3.9	116.0 $\pm$ 2.3‡	153.3 $\pm$ 9.5	154.0 $\pm$ 3.3‡
Total weight (g)	3.8 $\pm$ 0.1	3.6 $\pm$ 0.2	2.7 $\pm$ 0.2	3.0 $\pm$ 0.1

Significance versus controls (Student's *t* test): *, $P<0.05$; †, $P<0.01$; ‡, $P<0.005$

(22%) rats receiving operation after vehicle. Most deaths arose from gastrointestinal haemorrhage, and we have subsequently confirmed that older Sprague-Dawley rats are more susceptible to this complication than their younger counterparts (unpublished data). Operated rats lost 3–13% of their body weight during the next 24 hours. Weight loss in unoperated controls starved for 24 hours was 8%. Subsequent weight gain was unaffected either by azoxymethane or by any of the operative procedures. Mean body weights for the various groups at 40 weeks ranged between 630–748 g (Fig. 1); vehicle-treated rats receiving left hemicolectomy were at least 60 g heavier than any other group.

INTESTINAL ADAPTATION

Splenectomy did not affect the length, wet weight, or mucosal mass of any intestinal segment. Values obtained from the other groups receiving vehicle are shown in Table 1 and Fig. 3. In the terminal ileum caecal resection increased segmental weight by 44% (Table 1) and protein content by 37% (Fig. 3). As

Fig. 3 Mucosal contents of protein and DNA (mg/5 cm) in the ileum, right colon, and left colon of vehicle-treated rats. Mean values are given $\pm$ SEM. Ileal values represent the mean of duplicate samples. Significance versus controls (Student's *t* test): *, $P < 0.05$, **, $P < 0.005$, ***, $P < 0.001$.

DNA was also increased by 47%, this was a true hyperplastic response. Right hemicolectomy increased only segmental weight (by 22%), whereas left hemicolectomy increased only DNA content (by 65%). There was a 9% increase in the segmental weight of the remaining small bowel after right hemicolectomy. In the caecum no adaptive changes were detected eight months postoperatively.

Evidence of lasting colonic adaptation after partial resection of the large bowel was limited to the remaining right colon. A 13% increase in colorectal length after caecal resection was balanced by a 17% reduction in segmental weight (Table 1). Measurements of the length and weight of the remaining large bowel after right and left hemicolectomy are difficult to assess, because of uncertainty regarding the precise extent of the original resection. The length of the remnants was 66–73% of that of the entire colorectum in control rats, and their total weight was 71–79%, but segmental weight was unchanged after right hemicolectomy and was only 10% greater after left hemicolectomy. Nevertheless, mucosal contents of DNA and protein in the right colon increased by 31–38% after caecal resection and by 18–42% after left hemicolectomy (Fig. 3). No significant changes were observed in the left colon.

CARCINOGENESIS

Although one or two rats in most groups died prematurely from bleeding or obstructing tumours (Fig. 4), the large majority survived for 40 weeks. As the timing of surgery in relation to azoxymethane did not affect subsequent carcinogenesis in any way, data from animals receiving operation before and after carcinogen have been combined. Table 2 shows the number of rats in each (combined) group with intestinal neoplasms and metastases. Secondary deposits occurred mostly in mesenteric and mesocolic lymph nodes, but mucinous carcinomas metastasised to the lungs and mediastinum in three cases and throughout the peritoneum in one case. Tumours of other organs included squamous carcinoma of the ear canal (12), transitional cell carcinoma of the kidney (two), and sarcoma of the anterior abdominal wall (one).

Twenty-nine rats had a total of 34 small-bowel tumours, distributed mainly in the distal duodenum and upper jejunum (Fig. 5). Not a single tumour arose within the ileum. The predominant histological type in the small bowel was mucinous adenocarcinoma (26), but there were five tubular adenocarcinomas and three adenomas. Operation did not affect enteric carcinogenesis; in particular, no tumours were found in ileum rendered hyperplastic by caecal resection.

Eighty-four rats had a total of 217 large-bowel tumours, of which 113 (52%) were benign and 104

Fig. 4 Intussuscepting carcinoma of the ascending colon in an unoperated rat. Death has resulted from intestinal obstruction.

(48%) were malignant. There was a tendency for tumours to cluster in the lower descending colon, with relative sparing of the right colon and especially the caecum (Fig. 6). The distribution was as follows: proximal colon 16.5%, colonic anastomosis 23.5%, distal colon 40%, rectum 20%. Adenomatous polyps predominated, and most adenocarcinomas were of papillary or tubular type. Only a third of large-bowel carcinomas were mucinous, and these mostly occurred in the right colon or at sites of surgical anastomosis. Mucinous carcinomas were particularly likely to invade deeply and to metastasise.

There were rather fewer tumours after splenectomy, but differences from controls did not reach statistical significance (Table 3). Caecal resection had no effect on colorectal carcinogenesis, but in

Fig. 5 Distribution of small-bowel tumours. Each circle represents one tumour, and data are collected from every group. Open circles denote mucinous adenocarcinomas and closed circles other histological types. The numbers describe the distance in cm from the pylorus.

contrast to the other operations no tumours developed at the site of (ileocolic) anastomosis. Both mid-colon transection and right hemicolectomy doubled the total yield of tumours arising at and distal to the anastomosis (Table 3, Fig. 6), but in each case the increment merely reflected the large number of tumours immediately adjacent to the suture line in the transverse colon (Fig. 7). Right hemicolectomy did not significantly enhance carcinogenesis in the

Table 2 Number of rats with tumors

Group	No.	Intestinal tract	Small bowel	Large bowel	Metastases	Extra-intestinal
Control	19	13	2	13	0	1
Splenectomy	10	6	2	4	1	1
Colonic transection	27	24	9	22	2	2
Caecal resection	10	8	3	6	2	2
Right hemicolectomy	26	21	5	21	2	4
Left hemicolectomy	28	22	8	18	2	5
Total	120	94	29	84	9	15

Fig. 6 Distribution of large-bowel tumours in rats without operation (A) and in rats with mid-colon transection (B), right hemicolectomy (C) or left hemicolectomy (D). Each circle represents one tumour. ○ Mucinous adenocarcinomas. ● Other histological types. The numbers describe the distance in cm from the anus.

left colon, and left hemicolectomy made no difference whatsoever to the development of tumours in the right colon. Left hemicolectomy reduced the number of tumours overall by 52–58%, compared with transection and right hemicolectomy. Outside the colorectal anastomosis, it also decreased the number of colorectal tumours compared with controls.

In three groups—transection, right and left hemi-

colectomy—anastomotic tumours comprised nearly a third of the total yield (51 ex 162). It made no difference whether the suture line was created before or after exposure of the rat to azoxymethane. Moreover, one rat receiving vehicle, *not* carcinogen, developed a 'spontaneous' tumour at the site of colorectal anastomosis after left hemicolectomy. This tumour was an invasive mucinous adenocarcinoma with osseous metaplasia (Fig. 8).

Table 3 Number of large-bowel tumours per rat

Group	No.	Proximal colon	Anastomosis*	Distal colon	Rectum	Total excl. anast.	Anastomosis + distal bowel	Overall total
Control (no operation)	19	0.53	—	0.79	0.32	1.63	1.11	1.63
Splenectomy	10	0.50	—	0.50	0.00	1.00	0.50	1.00
Colonic transection	27	0.30	0.85	0.93	0.37	1.59	2.15†	2.44
Caecal resection	10	0.70	0.00	0.50	0.10	1.30	0.60	1.30‡
Right hemicolectomy	26	—	0.54	1.38	0.50	1.88	2.42†	2.42
Left hemicolectomy	28	0.21	0.50	—	0.46	0.68*	0.96	1.18§

*Includes tumours arising within 5 mm of the suture line.

Significance differences (Mann-Whitney U test): *, $P < 0.05$ vs control; $P < 0.005$ vs transection; †, $P < 0.05$ vs control; ‡, $P = 0.05$ vs transection; §, $P = 0.05$ vs right hemicolectomy, $P < 0.005$ vs transection.

Fig. 7 Remaining colorectum in an animal with previous right hemicolectomy. A large mucinous carcinoma (arrowed) has arisen at the site of ileocolic anastomosis. Several synchronous tumours can be seen in the left colon and especially the rectum.

Fig. 8 Well-differentiated mucinous adenocarcinoma arising 'spontaneously' at the site of colorectal anastomosis, following left hemicolectomy in a rat that had received vehicle but not carcinogen. Minor foci of osseous metaplasia are seen within the tumour. Haematoxylin and eosin $\times 9.25$ (original magnification).

Discussion

Beyond the immediate vicinity of the surgical anastomosis there was no enhancement of carcinogenesis by any type of partial colectomy. Indeed, by removing the most susceptible portion of colon, left hemicolectomy actually reduced the tumour yield. We conclude that metachronous carcinomas probably do not result from the stimulus of compensatory growth, but rather that multiple neoplasia reflects a field change throughout the colonic mucosa. In support, the presence of synchronous adenomas at the time of original resection for carcinoma doubles the risk of developing a second malignancy in the remaining colon.⁴ Sixty per cent of patients with metachronous carcinomas and 75% of those with synchronous carcinomas have associated adenomas in the large bowel.^{19, 20} This concept of an unstable epithelium reaches its ultimate expression in familial polyposis coli, in which 48% of patients have multiple carcinomas,¹ and to a slightly lesser extent in long-standing ulcerative proctocolitis, in which the corresponding figure is 25%.²¹

The absence of any increased neoplastic tendency in the remaining colon agrees with the results of two other experiments using the related carcinogen dimethylhydrazine. In one of these, the number of tumours in the left colon was unaffected by right hemicolectomy, which included 5 cm of terminal ileum.²² In the other, left hemicolectomy was accompanied by end transverse colostomy and right hemicolectomy again by ileotransversostomy;²³ carcinogenesis was unaltered in the residual left colon and probably reduced in the residual right colon. These results contrast with the ability of extensive enterectomies or ileal transplantation of the bile duct to affect colon carcinogenesis,^{5, 7, 11} an ability shared by diversion and later restoration of the faecal stream.^{15, 24} The difference may lie in the relatively minor physiological derangement that accompanies partial colectomy.

Adaptive changes in the remaining colon eight months after partial resection were relatively slight. Elongation of the remnant followed caecal resection and may have occurred after hemicolectomy, but increases in segmental weight and mucosal mass were limited to the right colon. By contrast, Masesa and Forrester (1977) found a marked increase in the weight of the descending colon three months after right hemicolectomy;¹³ indeed, the distal remnant weighed 37% more than the entire colon of control animals. No such response followed left hemicolectomy. In the present study left hemicolectomy tended to cause greater adaptation than right hemicolectomy, but mean body weight when killed happened to differ by over 100 g in favour of the left

hemicolectomy group. This weight differential did not occur in the carcinogen-treated groups.

The relative lack of lasting adaptation in the shortened colon contrasts with the vigorous and prolonged response of the small bowel to partial enterectomy.²⁵ The colon is quite capable of responding to other adaptive stimuli, such as fasting and refeeding²⁶ or defunction and restoration of colonic continuity.¹⁵ The main factor limiting its response to partial colectomy may be the presence of an intact distal small bowel. Colonic weight and mucosal thickness may not increase greatly after caecectomy in rats, unless the terminal 50 cm of ileum are also excised.²⁷ Perhaps ileal hyperplasia can partially compensate for colonic loss. In our study the small bowel was preserved to within 1 cm of the ileocaecal valve, but in right hemicolectomy for cancer in man it is customary to resect a relatively greater segment of ileum. Analysis of 47 patients with metachronous colorectal carcinoma in Chicago revealed that the first lesion had arisen in the left colon in 28, the right colon in 13, and the transverse colon in six;³ these figures approximately reproduce the normal distribution of large-bowel carcinoma. As more left hemicolectomies than right hemicolectomies are performed for cancer, post-colectomy adaptation is probably not an important factor in the development of a second growth.

There was some evidence of prolonged adaptation of the ileum to partial colectomy. Caecal resection caused ileal hyperplasia at eight months and in another study at eight weeks.²⁷ As a corollary, we have recently found caecal hyperplasia seven months after ileal resection.⁷ After right hemicolectomy, however, when both caecum and proximal colon were removed, ileal adaptation was much less marked.

Failure of the distal small bowel to undergo neoplastic change despite both azoxymethane and post-operative hyperplasia contrasts with the susceptibility of other intestinal segments to this combined stimulus.¹² Other operations causing ileal adaptation, such as jejunectomy and subtotal colectomy, are equally unable to render the distal small bowel susceptible to cancer.^{5, 15, 28} Only chronic Crohn's disease in man²⁹ and direct irradiation in rats³⁰ appear to overcome ileal resistance to carcinogenesis.

There was a striking propensity for neoplasms to develop at and immediately adjacent to anastomotic sites. We and others have previously observed this phenomenon at a wide variety of intestinal anastomoses: duodenal,¹¹ duodenoileal,¹¹ jejunoileal,^{5, 14} ileocolic,²³ colocolic,^{15, 31, 32} jejunorectal,³³ and ileorectal,¹⁴ and also at the site of descending colotomy.³⁴ The present study showed several

Table 4 Reports of spontaneous colorectal carcinomas in rat

Strain	Sex/age (m)	Site	Metastases	Reference no.
1 Fischer	M/13	Caecum	+	37
2 Stock albino	M/9	Asc. colon	—	38
3 Stock albino	M/9	Asc. colon	+	38
4 Rochester	F/18	Colon	—	39
5 Wistar	M/ca. 32	Caecum	—	40
6 ACI	M/—	Rectum	—	41
7 Wistar	F/6	Caecum/asc. colon	—	42
8 Wistar	F/6	Caecum/asc. colon	+	42
9 Wistar	M/4	Asc. colon	+	43
10 Chester Beatty	M/24	Colon	—	44
11 Wistar	F/5	Asc. colon	—	45

tumours at ileocolic, colocolic, and colorectal anastomoses, whether these were established before or after exposure to azoxymethane. But no tumours arose at the anastomosis between terminal ileum and ascending colon (after caecal resection). Colocolic anastomoses close to the caecum are similarly resistant to neoplasia,^{22, 31} and so are ileoileal anastomoses in our experience.^{6, 7} However, one report has described anastomotic tumours after ileal resection but not after ileal transection.³⁵ For experimental suture-line cancers to develop, it generally seems necessary for at least one bowel end to be a segment commonly affected by the carcinogen. Neither ileum nor lower ascending colon fulfils this role.

The one invasive mucinous adenocarcinoma at the site of colorectal anastomosis in a rat not exposed to carcinogen is an exciting phenomenon, which does not appear to have been previously reported. 'Spontaneous' carcinomas may occur at the site of colostomy in Wistar rats,³⁶ but they are otherwise extraordinarily rare. Eleven previously-reported cases (Table 4) have mostly been confined to the caecum and ascending colon.³⁷⁻⁴⁵ Self-limiting outbreaks of proximal colon cancer in two laboratories were probably caused by epizootics,^{46, 47} and bacterial infection can promote mucosal dysplasia in mice exposed to dimethylhydrazine.⁴⁸

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References

- Bussey HJR. Multiple adenomas and carcinomas. In: Morson BC, ed. *The pathogenesis of colorectal cancer. Major problems in pathology 10*. London: Saunders, 1978:72-80.
- Gillespie PE, Chambers TJ, Chan KW, Doronzo F, Morson BC, Williams CB. Colonic adenomas—a colonoscopy survey. *Gut* 1979; **20**:240-5.
- Enker WE, Dragacevic S. Multiple carcinomas of the large bowel: a natural experiment in etiology and pathogenesis. *Ann Surg* 1978; **187**:8-11.
- Morson BC. The polyp-cancer sequence in the large bowel. *Proc R Soc Med* 1974; **67**:451-7.
- Williamson RCN, Bauer FLR, Ross JS, Oscarson JEA, Malt RA. Promotion of azoxymethane-induced colonic neoplasia by resection of the proximal small bowel. *Cancer Res* 1978; **38**:3212-7.
- Oscarson JEA, Veen HF, Ross JS, Malt RA. Ileal resection potentiates 1, 2-dimethylhydrazine-induced colonic carcinogenesis. *Ann Surg* 1979; **189**:503-8.
- Williamson RCN, Lyndon PJ, Tudway AJC. Effects of anticoagulation and ileal resection on the development and spread of experimental intestinal carcinomas. *Br J Cancer* 1980; **42**:85-94.
- Tilson MD. Colonic carcinogenesis after partial resection of small bowel and a single dose of dimethylhydrazine in rats. *Surg Forum* 1980; **31**:413-5.
- Bristol JB, Williamson RCN. Increased colonic carcinogenesis after jejunoileal bypass in rats is prevented by extreme reduction in body weight. (Abstract.) *Gut*, 1981; **22**:440-1.
- Chomchai C, Bhadrachari N, Nigro ND. The effect of bile on the induction of experimental intestinal tumors in rats. *Dis Colon Rectum* 1974; **17**:310-2.
- Williamson RCN, Bauer FLR, Ross JS, Watkins JB, Malt RA. Enhanced colonic carcinogenesis with azoxymethane in rats after pancreaticobiliary diversion to mid small bowel. *Gastroenterology* 1979; **76**:1386-92.
- Williamson RCN, Malt RA. Promotion of intestinal carcinogenesis by adaptive mucosal hyperplasia. In: Appleton DR, Sunter JP, Watson AJ, eds. *Cell proliferation in the gastrointestinal tract*. Tunbridge Wells: Pitman, 1980:303-15.
- Masesa PC, Forrester JM. Consequences of partial and subtotal colectomy in the rat. *Gut* 1977; **18**:37-44.
- Williamson RCN, Bauer FLR, Terpstra OT, Ross JS, Malt RA. Contrasting effects of subtotal enteric bypass, enterectomy and colectomy on azoxymethane-induced intestinal carcinogenesis. *Cancer Res* 1980; **40**:538-43.
- Terpstra OT, Peterson Dahl E, Williamson RCN, Ross JS, Malt RA. Colostomy closure promotes cell proliferation and experimental carcinogenesis in the distal colon. *Gastroenterology* 1981; **81**:475-80.
- Cruse JP, Lewin MR, Clarke CG. *Corynebacterium parvum* enhances colonic cancer in dimethylhydrazine-treated rats. *Br J Cancer* 1978; **37**:639-43.

- 17 Lowry OH, Rosebrough NH, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**:265–75.
- 18 Kissane JM, Robbins E. The fluorimetric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *J Biol Chem* 1958; **233**:184–8.
- 19 Heald RJ, Lockhart-Mummery HE. The lesion of the second cancer of the large bowel. *Br J Surg* 1972; **59**: 16–9.
- 20 Heald RJ, Bussey HJR. Clinical experiences at St Mark's Hospital with multiple synchronous cancers of the colon and rectum. *Dis Colon Rectum* 1975; **18**:6–10.
- 21 Riddell RH, Shove DC, Ritchie JK, Lennard-Jones JE, Morson BC. Precancer in ulcerative colitis. In: Morson BC, ed. *The pathogenesis of colorectal cancer. Major problems in pathology 10*. London: Saunders, 1978: 95–118.
- 22 Werner B, de Heer K, Mitschke H, Knipper A, Thoran P, Dahm K. Experimental carcinogenesis in the resected colon of the rat. *Z Krebsforsch* 1977; **89**:53–60.
- 23 Loth R. Der Einfluss der Hemicolectomie auf die Ausbildung von Dickdarmtumoren nach subkutaner Injektion von 1,2-Dimethylhydrazin. *Langenbecks Arch Chir Suppl Chir Forum* 1974; 123–5.
- 24 Campbell RL, Singh DV, Nigro ND. Importance of the fecal stream on the induction of colon tumours by azoxymethane in rats. *Cancer Res* 1975; **35**:1369–71.
- 25 Williamson RCN. Intestinal adaptation. 1. Structural, functional and cytokinetic aspects. *N Engl J Med* 1978; **298**:1393–402.
- 26 Stragand JJ, Hagemann RF. Effect of luminal contents on colonic cell replacement. *Am J Physiol* 1977; **233**:E208–11.
- 27 Scarpello JHB, Cary BA, Sladen GE. Effects of ileal and caecal resection on the colon of the rat. *Clin Sci Mol Med* 1978; **54**:241–9.
- 28 Celik C, Mittelman A, Lewis D, Paolini NS Jr, Holyoke ED. Effect of colectomy on carcinogenicity of symmetrical dimethylhydrazine in rats. *Surg Forum* 1980; **31**:415–7.
- 29 Hoffman JP, Taft DA, Wheelis RF, Walker JH. Adenocarcinoma in regional enteritis of the small intestine. *Arch Surg* 1977; **112**:606–11.
- 30 Coop KL, Sharp JG, Osborne JW, Zimmerman GR. An animal model for the study of small-bowel tumors. *Cancer Res* 1974; **34**:1487–94.
- 31 Gennaro AR, Villanueva R, Sukonthaman Y, Vathanophas V, Rosemond GP. Chemical carcinogenesis in transposed intestinal segments. *Cancer Res* 1973; **33**:536–41.
- 32 Kanazawa K, Yamamoto T, Sato S. Experimental induction of colonic carcinomas in rats. Analysis of factors influencing upon the incidence. *Japan J Exp Med* 1975; **45**:439–56.
- 33 Celik C, Mittelman A, Paolini NS Jr, Lewis D, Evans JT. Effects of 1,2-symmetrical dimethylhydrazine on jejuno-colic transposition in Sprague-Dawley rats. *Cancer Res* 1981; **41**:2908–11.
- 34 Blake JRS, Reeve RS, Hardcastle JD, Dawson IMP, Metcalf MJ, Thompson MJ. A study of the effect of colectomy and mucosal field changes in experimental colon cancer. *Clin Oncol* 1980; **6**:113–23.
- 35 Harte PJ, Rayner AA, Munroe A, Wilson RE, Steele GD. Potentiation of dimethylhydrazine-induced adenocarcinoma at the suture line by major bowel resection. *Surg Forum* 1980; **31**:411–3.
- 36 Winkler R, Heitmann C, Otto HF. Division of fecal stream and colorectal carcinogenesis. *Chir Gastroenterol* 1977; **11**:105–8.
- 37 Curtis MR, Bullock FD, Dunning WF. A statistical study of the occurrence of spontaneous tumors in a large colony of rats. *Am J Cancer* 1931; **15**:67–121.
- 38 Willis RA. Carcinoma of the intestine in rats. *J Pathol Bact* 1935; **40**:187–8.
- 39 Crain RC. Spontaneous tumors in the Rochester strain of Wistar rat. *Am J Pathol* 1958; **34**:311–35.
- 40 Gilbert C, Gillman J. Spontaneous neoplasms in the albino rat. *S Afr J Med Sci* 1958; **23**:257–72.
- 41 Snell KC. Spontaneous lesions of the rat. In: Ribelin WE, McCoy JR, eds. *The pathology of laboratory animals*. Springfield, Ill., Thomas, 1965:241–300.
- 42 Burn JI, Sellwood RA, Bishop M. Spontaneous carcinoma of the colon of the rat. *J Pathol Bact* 1966; **91**:253–4.
- 43 Mitra SK. Carcinoma of the colon in a rat. *Br J Cancer* 1966; **20**:399–401.
- 44 Rowlatt UF. Neoplasms of the alimentary canal of rats and mice. In: Cotchin E, Roe FJC, eds. *Pathology of Laboratory Rats and Mice*. Oxford: Blackwell Scientific Publications, 1967: 57–84.
- 45 Grasso P, Creasey M. Carcinoma of the colon in a rat. *Eur J Cancer* 1969; **5**:415–9.
- 46 Heslop BF. Cystic adenocarcinoma of the ascending colon in rats occurring as a self-limiting outbreak. *Lab Anim* 1969; **3**:185–95.
- 47 Miwa M, Takenaka S, Ito K, Fujiwara K, Kogure K, Tokunaga A, Hozumi M, Fujimura S, Sugimura T. Spontaneous colon tumors in rats. *J Natl Cancer Inst* 1976; **56**:615–21.
- 48 Barthold SW, Beck D. Modification of early dimethylhydrazine carcinogenesis by colonic mucosal hyperplasia. *Cancer Res* 1980; **40**:4451–5.