Colonic fermentation of dietary fibre to short chain fatty acids in patients with adenomatous polyps and colonic cancer

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Abstract

Short chain (C_2 - C_6) fatty acids are produced in the colon through bacterial fermentation of mainly dietary fibre. Butyrate (C_4) possesses antineoplastic effects on human colon carcinoma cells, and epidemiological studies indicate that high fibre diets may reduce the incidence of colorectal cancer. The role of dietary fibre during colorectal carcinogenesis might therefore be related to its fermentation to butyrate. Faecal concentrations of total short chain fatty acids and concentrations and ratios of the individual C_2 - C_6 fatty acids did not differ between 16 healthy controls, 17 patients with colonic adenomas, and 17 patients with colonic cancer. Comparison of the molar production velocities (mmol/l.hour) of total and individual short chain fatty acids from glucose, ispagula, wheat bran, and albumin in six and 24 hour faecal incubations showed no differences. The ratio of butyrate production to total short chain fatty acid production from fibre, however, was reduced in patients with colonic cancer and adenomas compared with healthy controls (ispagula, six hours: 6.4, 7.6, and 11.5% respectively, $p=0.005$ and 24 hour: 9.1, 9.9, and 15.4%, $p=0.002$; wheat bran, six hours: 9.9, 10.2, and 14.7% respectively, $p=0.06$ and 24 hours: 15.1, 16.8, and 21.0%, $p=0.01$). It may be that the low ratios of colonic butyrate formation combined with low fibre diets increase the risk of colonic neoplasia.

Epidemiological studies have indicated that environmental factors influence the incidence of colorectal cancer, and diet seems to be the most important of these.¹ Among North Americans and Western Europeans, the disease is more frequent than in residents of Asia, South America, and rural Africa,²⁻⁸ and migrants from areas of high incidence to those of low incidence generally attain rates of colonic cancer similar to those of their new environment.⁹ The critical difference between these groups seems to be the characteristic western diet, which is high in beef, fat, and protein and low in dietary fibre. The relative importance of dietary fibre in the prevention of colorectal cancer, however, is still not clear and the way in which fibre exerts a possible protective role cannot be shown with certainty until the carcinogenic process by which large bowel cancer arises is known. Hypothetically, dietary fibre may protect against intestinal cancer in several ways – for example, through its fermentation to short chain fatty acids (SCFAs) and its effect on colonic pH.¹⁰

SCFAs C_2 - C_6 are the predominant anions in human faeces and originate in the colon through bacterial fermentation of mainly non-absorbed dietary carbohydrate.¹¹ Despite their presence in the colon in considerable concentrations (approximately 100 mmol/l), relatively little information regarding the role of SCFAs in health and disease exists. In colorectal cancer, butyrate (C_4) is of special interest because of its effects on nucleic acid metabolism.¹² Butyrate induces cell differentiation in various mammalian cells, including several human colorectal cancer cell lines,¹³⁻²¹ reduces the growth rate of these cell lines in vitro and increases their doubling time,¹³⁻²¹ and represents an important fuel for energy metabolism in colonocytes.²²⁻²³ Thus, butyrate is seen as promoting growth and proliferation of normal colonic mucosa, while suppressing cancer cells. These observations are interesting because when fibre is fermented by the colonic flora, butyrate is a major metabolite.¹¹ The protective effect of dietary fibre on colonic neoplasia might therefore be related to the colonic production of SCFAs, and especially the production of butyrate.

Since the adenoma-carcinoma sequence is accepted as the natural developmental pathway for most cases of colorectal cancer,²⁴ we found it of interest to characterise possible differences in concentration, proportion, and fermentative production of SCFAs in faeces from patients with former colonic adenomatous polyps or cancer *versus* those from healthy controls. The results presented in this report show that the fermentation of dietary fibre to butyrate in proportion to total SCFA production is reduced in faecal incubations from patients with colonic cancer or adenomas.

Methods

SUBJECTS

Sixteen healthy control subjects with no history of gastrointestinal disease (seven men and nine women) aged 30-82 years (mean age 60 years), 17 patients with former colonic adenomas (11 men and six women) aged 49-87 years (mean age 66 years), and 17 patients with former colonic cancer (eight men and nine women) aged 44-77 years (mean age 63 years) participated in the study. The patients with former adenomas had undergone colonoscopic polypectomy at least three months before faecal sampling. The adenomas were neoplastic adenomas – that is either tubular (9), tubulovillous (6), or villous (2) adenomas. All adenomas found during colonoscopy were removed. The patients with former

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TABLE 1 Concentrations (mmol/l) and ratios (% of total) of short chain fatty acids in faeces (0 hours) from normal individuals (controls) and patients with colonic adenomas or colonic cancer

	Concentration (mmol/l)										Ratio (% of total)							
	Total	C ₂	C ₃	C ₄	LSD, p	iC ₄	C ₅	iC ₅	C ₆	pH	C ₂	C ₃	C ₄	LSD, p	iC ₄	C ₅	iC ₅	C ₆
Controls: Mean (SEM)	91.3 (6.8)	59.3 (5.2)	14.4 (1.3)	10.9 (1.0)		1.6 (0.2)	1.9 (0.2)	2.5 (0.3)	0.7 (0.2)	7.1 (0.1)	64.6 (1.7)	15.9 (1.0)	12.0 (0.8)		1.8 (0.2)	2.1 (0.1)	2.8 (0.3)	0.8 (0.2)
Adenomas: Mean (SEM)	85.8 (7.1)	58.3 (5.1)	13.2 (1.4)	8.0 (0.9)	NS	1.5 (0.1)	1.9 (0.2)	2.3 (0.2)	0.6 (0.1)	7.2 (0.1)	67.9 (1.4)	15.2 (0.9)	9.1 (0.6)	NS	1.9 (0.2)	2.2 (0.2)	3.0 (0.3)	0.7 (0.1)
Cancer: Mean (SEM)	114.9 (12.9)	73.4 (8.6)	18.3 (2.1)	14.6 (3.0)	NS	2.1 (0.3)	2.3 (0.3)	3.1 (0.4)	1.2 (0.3)	7.2 (0.1)	64.2 (1.7)	16.4 (0.9)	11.6 (1.5)	NS	1.9 (0.2)	2.1 (0.2)	2.9 (0.3)	0.9 (0.2)
ANOVA, p	0.08	0.21	0.09	0.07		0.12	0.51	0.19	0.25	0.85	0.22	0.66	0.11		0.90	0.88	0.94	0.75

C₂, acetate; C₃, propionate; C₄, butyrate; iC₄, isobutyrate; C₅, valerate; iC₅, isovalerate; C₆, hexanoate. ANOVA: one way analysis of variance. LSD: least significant difference compared with controls. NS: p > 0.05.

colonic cancer had undergone right-sided (7) or left-sided (3) hemicolectomy, or segmental sigmoid resection (7) because of colonic adenocarcinoma (Dukes A (2), Dukes B (11), and Dukes C (4)). No patient showed signs of recurrence at the time of faecal sampling, which was at least three months after surgery. All the patients were chosen consecutively, but were excluded from the group with former adenomas if they had previously had abdominal surgery, and from the group with former colonic cancer if the cancer operation had resulted in colostomy. Furthermore, intake of antibiotics within the previous two weeks caused exclusion. Both normal subjects and patients were on a ordinary Danish diet, and no dietary restrictions or recommendations were given. All subjects gave informed consent to the study, which was approved by the local ethical committee.

PREPARATION AND ANALYSIS OF FAECAL SAMPLES

Freshly passed faeces was homogenised with five times its weight of isotonic NaCl (100 mmol/l NaCl; 50 mmol/l KCl) for study in an anaerobic faecal incubation system using the method of Vince *et al.*²⁵ Several aliquots of 10 ml from each faecal sample were incubated in duplicate simultaneously to allow comparison of different substrates. Glucose, ispagula husk, wheat bran, or albumin were added at 0 hours in concentrations of 10 mg/ml. Control incubations were faecal suspensions prepared with no addition of substrate. Incubation times were 0, 6, and 24 hours at 37°C, and termination was performed by freezing. Specimens were stored at -18°C until analysis by steam distillation as described by Zijlstra *et al.*²⁶ before gas liquid chromatographic (GLC) analysis of the SCFAs. Some 0.5 µl of the distilled sample was automatically injected splitless into a Hewlett-Packard 5890A gas chromatograph equipped with a wide bore, 530 µm (internal diameter), 30 m HP-FFAP cross linked fused silica capillary column with a film thickness of 1 µm (Hewlett-Packard, Palo Alto, CA, USA). Carrier and make up gas was helium with a flow rate of 8 and 20 ml/minute respectively. Injection and flame ionisation detector temperatures were 200°C. The oven temperature was 115°C for two minutes before the temperature was raised 5°C/minute to 150°C. SCFA concentrations were calculated from areas of GLC peaks (internal standard was 2-ethylbutyrate) automatically cal-

culated on a Hewlett-Packard 3396A integrator connected on-line to the gaschromatograph. The pH of faecal suspensions was measured by a pH meter (Radiometer, Copenhagen).

MATERIALS

SCFAs, 2-ethylbutyric acid (internal standard), D-glucose, and albumin were from Merck Chemical Co, Darmstadt, Germany. Ispagula was a gift from Park-Davis (stock no 105-8680).

STATISTICS

Statistical analysis was by one way analysis of variance (one way ANOVA) with the difference between individual groups determined using the least significant difference (LSD).

Results

FAECAL CONCENTRATIONS AND RATIOS OF SHORT CHAIN FATTY ACIDS

The total concentrations of SCFAs and the concentrations of the individual acids, including butyrate, in faecal suspensions from 16 normal subjects, 17 patients with former colonic adenomas, and 17 patients with former colonic cancer were not significantly different (Table I). Moreover, the ratios (% of total concentrations) of the individual SCFAs in faeces from patients with colonic adenomas or cancer were identical to proportions seen in faeces from normal subjects (Table I). No differences in faecal pH were found between the three groups investigated (Table I).

PRODUCTION RATES OF SHORT CHAIN FATTY ACIDS FROM GLUCOSE, ISPAGULA HUSK, WHEAT BRAN, AND ALBUMIN

The production of SCFAs in samples from normal subjects and patients with colonic adenomas or cancer was evaluated in relation to time of incubation (6 and 24 hours) with no addition of external substrate (control) and with addition of glucose, ispagula husk, wheat bran, and albumin (Table II). The production rates of total SCFAs in control incubations after 6 hours (V₆) or 24 hours (V₂₄) were not significantly different between healthy controls, patients with adenomas, and patients with cancer (V₆: p=1.00 and V₂₄: p=0.46). The production rate of buty-

TABLE II Production rates (0–6 hours and 0–24 hours) of short chain fatty acids (V_6 , mmol/l.hour and V_{24} , mmol/l.hour) in faecal incubations with and without addition of external substrates from normal individuals (controls) and patients with colonic adenomas or colonic cancer

	V_6 (mmol/l.hour)						V_{24} (mmol/l.hour)							
	Total	C_2	C_3	$\overline{C_4}$	$\overline{LSD}, p$	iC_4-C_6	pH	Total	C_2	C_3	$\overline{C_4}$	$\overline{LSD}, p$	iC_4-C_6	pH
No substrate added:														
Controls														
Mean (SEM)	11.9 (1.6)	6.9 (1.1)	2.6 (0.3)	1.6 (0.2)		0.9 (0.1)	6.7 (0.2)	5.0 (0.6)	2.5 (0.3)	1.3 (0.1)	1.1 (0.1)		0.5 (0.1)	6.5 (0.1)
Adenomas														
Mean (SEM)	12.0 (7.1)	7.2 (5.1)	2.6 (1.4)	1.2 (0.9)	NS	1.0 (0.7)	6.6 (0.1)	4.9 (0.5)	2.3 (0.3)	1.2 (0.2)	0.7 (0.1)	<0.05	0.6 (0.1)	6.5 (0.1)
Cancer														
Mean (SEM)	12.0 (1.6)	7.0 (1.0)	2.9 (0.4)	1.3 (0.2)	NS	0.9 (0.2)	6.5 (0.2)	5.8 (0.5)	2.9 (0.3)	1.5 (0.1)	0.9 (0.1)	NS	0.6 (0.1)	6.6 (0.1)
ANOVA, p	1.0	0.97	0.87	0.40		0.87	0.79	0.46	0.36	0.43	0.05		0.96	0.84
D-glucose (10 mg/ml) added:														
Controls														
Mean (SEM)	43.6 (4.1)	30.6 (3.4)	8.9 (1.6)	3.7 (0.5)		0.4 (0.2)	4.8 (0.1)	14.3 (1.0)	9.7 (1.0)	2.6 (0.4)	1.7 (0.3)		0.2 (0.1)	4.6 (0.1)
Adenomas														
Mean (SEM)	47.4 (4.9)	34.4 (4.3)	9.5 (1.5)	3.0 (0.6)	NS	0.6 (0.3)	4.9 (0.1)	15.8 (1.3)	10.4 (1.0)	3.3 (0.5)	1.7 (0.3)	NS	0.4 (0.2)	4.7 (0.2)
Cancer														
Mean (SEM)	59.2 (5.8)	43.2 (5.0)	11.7 (1.8)	3.9 (0.9)	NS	0.4 (0.2)	4.7 (0.2)	18.7 (1.3)	12.7 (1.0)	3.6 (0.5)	2.2 (0.4)	NS	0.2 (0.1)	4.8 (0.1)
ANOVA, p	0.09	0.12	0.46	0.66		0.77	0.50	0.05	0.11	0.41	0.55		0.59	0.52
Ispagula husk (10 mg/ml) added:														
Controls														
Mean (SEM)	22.4 (2.2)	14.4 (1.9)	4.9 (0.4)	2.4 (0.3)		0.8 (0.1)	6.2 (0.1)	10.1 (0.9)	5.7 (0.6)	2.7 (0.3)	1.5 (0.2)		0.5 (0.1)	5.6 (0.1)
Adenomas														
Mean (SEM)	24.2 (2.6)	15.7 (2.0)	5.8 (0.7)	1.8 (0.2)	NS	0.9 (0.2)	6.2 (0.2)	10.5 (1.1)	5.5 (0.7)	3.0 (0.3)	1.0 (0.1)	<0.05	0.6 (0.1)	5.7 (0.1)
Cancer														
Mean (SEM)	28.8 (3.6)	19.6 (2.8)	6.5 (0.8)	1.9 (0.3)	NS	0.8 (0.2)	6.1 (0.2)	12.6 (1.3)	7.1 (0.8)	3.4 (0.3)	1.2 (0.2)	NS	0.5 (0.1)	5.6 (0.1)
ANOVA, p	0.31	0.27	0.24	0.28		0.77	0.83	0.26	0.28	0.37	0.08		0.80	0.74
Wheat bran (10 mg/ml) added:														
Controls														
Mean (SEM)	21.9 (1.7)	13.8 (1.4)	4.5 (0.3)	3.1 (0.3)		1.0 (0.2)	6.0 (0.2)	10.4 (0.7)	5.7 (0.4)	2.2 (0.2)	2.2 (0.2)		0.7 (0.1)	5.8 (0.1)
Adenomas														
Mean (SEM)	21.8 (2.0)	14.1 (1.5)	4.4 (0.4)	2.2 (0.3)	NS	1.1 (0.3)	6.0 (0.1)	10.5 (0.8)	5.8 (0.5)	2.2 (0.2)	1.8 (0.2)	NS	0.7 (0.1)	5.8 (0.1)
Cancer														
Mean (SEM)	27.6 (2.4)	17.9 (1.8)	5.8 (0.6)	2.7 (0.4)	NS	1.1 (0.2)	5.8 (0.2)	12.7 (0.8)	7.3 (0.5)	2.7 (0.2)	2.0 (0.2)	NS	0.8 (0.1)	5.8 (0.1)
ANOVA, p	0.10	0.13	0.09	0.18		0.87	0.79	0.08	0.06	0.15	0.41		0.81	0.99
Albumin (10 mg/ml) added:														
Controls														
Mean (SEM)	22.5 (2.5)	11.2 (1.6)	5.1 (0.6)	3.8 (0.5)		2.4 (0.4)	6.7 (0.1)	14.2 (1.1)	5.3 (0.5)	3.4 (0.3)	3.1 (0.3)		2.4 (0.3)	6.7 (0.1)
Adenomas														
Mean (SEM)	24.6 (2.3)	13.2 (1.3)	5.7 (0.6)	3.3 (0.4)	NS	2.4 (0.4)	6.5 (0.1)	15.3 (1.1)	6.7 (0.6)	3.3 (0.2)	2.8 (0.2)	NS	2.4 (0.3)	6.6 (0.1)
Cancer														
Mean (SEM)	27.1 (3.9)	14.3 (2.4)	6.5 (0.9)	3.6 (0.6)	NS	2.7 (0.5)	6.4 (0.2)	16.0 (1.6)	6.7 (0.8)	3.8 (0.4)	3.0 (0.3)	NS	2.5 (0.3)	6.7 (0.1)
ANOVA, p	0.57	0.47	0.35	0.74		0.84	0.52	0.60	0.22	0.50	0.83		0.98	0.73

C_2 , acetate; C_3 , propionate; C_4 , butyrate; iC_4-C_6 , isobutyrate, valerate, isovalerate and hexanoate. ANOVA: one way analysis of variance. LSD: least significant difference compared with controls. NS: $p>0.05$.

rate after 24 hours was reduced in patients with adenomas compared with controls ($p<0.05$), but not in patients with cancer ($p>0.05$). Addition of glucose, ispagula husk, wheat bran, and albumin (10 mg/ml) increased the production of SCFAs, glucose increasing the production almost four times and the other substrates increasing the production approximately twice (Table II). The production of butyrate from ispagula husk after 24 hours was reduced in patients with adenomas compared with controls ($p<0.05$), but not in patients with cancer ($p>0.05$). Applied to the other substrates, no significant differences in the molar production of any SCFAs were found between the three groups.

A small and equal decrease in pH was seen after 6 or 24 hours in control incubations from healthy subjects and patients with colonic adenomas or cancer (Tables I and II). Addition of glucose to incubations caused a noticeable decrease in the pH of the incubate while the addition of ispagula husk and wheat bran resulted in only a small reduction in pH. The addition of albumin did not influence pH. The change in assay pH in relation to incubation time did not differ between the three clinical groups.

RATIOS OF INDIVIDUAL SHORT CHAIN FATTY ACIDS PRODUCED FROM GLUCOSE, ISPAGULA HUSK, WHEAT BRAN, AND ALBUMIN
The production ratios of specific SCFAs,

expressed as percentages of total SCFA formation, showed a reduced ratio of butyrate production after 6 hours in faecal control incubations from patients with colonic adenomas compared with healthy controls ($p<0.05$). Furthermore, when the incubation time was extended to 24 hours, butyrate ratios were reduced in faecal incubations from both patients with adenomas ($p<0.01$) and cancer ($p<0.01$) compared with control subjects (Table III). Addition of glucose to incubations primarily increased the ratios of acetate at the expense of the C_4-C_6 SCFAs, but significant differences in the relative production rates of individual SCFAs between the three groups were not registered (Table III). The in vitro fermentation of the fibre substrates ispagula husk and wheat bran showed a significantly reduced relative production of butyrate in patients with both colonic adenomas and colonic cancer compared with healthy control subjects (Table III). Also, the relative production of butyrate was diminished in faecal suspensions incubated with albumin in patients with colonic cancer compared with control subjects after 6 and 24 hours ($p<0.05$), but did not reach the level of significance ($p>0.05$) in patients with colonic adenomas. Regarding the relative production of the other short chain fatty acids, acetate, propionate, isobutyrate, valerate, isovalerate, and hexanoate, no significant differences were found between the three groups investigated.

TABLE III Production rates of short chain fatty acids (SCFAs) (V_6 : 0–6 hours and V_{24} : 0–24 hours) expressed as % of total SCFAs production rates in faecal incubations with and without addition of external substrates from normal individuals (controls) and patients with colonic adenomas or colonic cancer

V_6 (mmol/l.h)/total V_6 (mmol/l.h)×100%:					V_{24} (mmol/l.h)/total V_{24} (mmol/l.h)×100%:					
	C_2	C_3	$\overline{C_4}$	$\overline{LSD, p}$	iC_5-C_6	C_2	C_3	$\overline{C_4}$	$\overline{LSD, p}$	iC_5-C_6
No substrate added:										
Controls										
Mean (SEM)	54.8 (2.3)	23.6 (1.3)	14.1 (1.6)		7.5 (0.8)	44.7 (2.2)	25.4 (1.5)	19.5 (1.4)		10.4 (1.0)
Adenomas										
Mean (SEM)	59.5 (2.1)	23.2 (1.9)	9.6 (0.8)	<0.05	7.7 (1.2)	46.7 (2.4)	26.4 (1.6)	14.5 (1.1)	<0.01	12.4 (1.3)
Cancer										
Mean (SEM)	57.6 (2.1)	24.2 (1.6)	10.9 (1.2)	NS	7.3 (1.0)	48.5 (1.9)	26.3 (1.4)	14.7 (1.0)	<0.01	10.5 (1.0)
ANOVA, p	0.34	0.90	0.04		0.96	0.56	0.87	0.02		0.33
D-glucose (10 mg/ml) added:										
Controls										
Mean (SEM)	68.4 (3.3)	20.2 (2.7)	10.0 (1.9)		1.4 (1.0)	65.4 (3.6)	19.3 (2.5)	13.2 (2.6)		2.1 (1.5)
Adenomas										
Mean (SEM)	69.6 (3.4)	22.5 (3.1)	6.4 (1.0)	NS	1.5 (0.9)	66.7 (3.3)	21.3 (2.8)	9.4 (1.6)	NS	2.6 (1.3)
Cancer										
Mean (SEM)	70.7 (3.9)	22.5 (3.7)	5.9 (1.1)	NS	0.9 (0.5)	67.9 (3.4)	20.5 (3.2)	10.4 (2.0)	NS	1.2 (0.5)
ANOVA, p	0.91	0.84	0.10		0.84	0.88	0.89	0.43		0.69
Ispagula husk (10 mg/ml) added:										
Controls										
Mean (SEM)	61.6 (2.4)	23.2 (1.6)	11.5 (1.5)		3.7 (0.5)	52.5 (1.9)	27.2 (1.9)	15.4 (1.7)		4.9 (0.5)
Adenomas										
Mean (SEM)	62.9 (2.6)	25.3 (2.2)	7.6 (0.8)	<0.05	4.2 (1.3)	52.8 (2.5)	31.5 (1.5)	9.9 (0.9)	<0.01	5.8 (1.2)
Cancer										
Mean (SEM)	65.4 (3.1)	25.6 (2.9)	6.4 (0.8)	<0.01	2.6 (0.4)	56.2 (2.6)	30.8 (2.6)	9.1 (0.6)	<0.01	3.9 (0.4)
ANOVA, p	0.64	0.74	0.005		0.44	0.60	0.39	0.002		0.28
Wheat bran (10 mg/ml) added:										
Controls										
Mean (SEM)	60.1 (2.7)	21.0 (1.3)	14.7 (1.9)		4.2 (1.2)	52.3 (1.6)	21.1 (1.1)	21.0 (1.4)		6.5 (1.0)
Adenomas										
Mean (SEM)	62.2 (3.6)	22.7 (2.5)	10.2 (1.2)	<0.05	4.9 (1.1)	55.4 (2.0)	21.4 (1.5)	16.8 (0.9)	NS	6.4 (1.2)
Cancer										
Mean (SEM)	63.7 (2.9)	22.5 (2.4)	9.9 (1.3)	<0.05	3.9 (0.7)	57.9 (1.8)	21.1 (1.2)	15.1 (1.1)	<0.01	5.9 (0.7)
ANOVA, p	0.71	0.84	0.06		0.58	0.13	0.95	0.01		0.80
Albumin (10 mg/ml) added:										
Controls										
Mean (SEM)	49.2 (2.8)	22.8 (1.2)	16.9 (1.3)		11.1 (2.0)	37.0 (1.9)	24.5 (1.1)	21.9 (1.4)		16.6 (1.3)
Adenomas										
Mean (SEM)	52.8 (2.3)	23.8 (1.7)	13.7 (1.0)	NS	9.7 (1.2)	43.2 (1.6)	22.6 (1.4)	18.7 (1.0)	NS	15.5 (1.2)
Cancer										
Mean (SEM)	51.6 (2.1)	25.1 (1.4)	13.2 (1.0)	<0.05	10.1 (1.1)	41.0 (1.8)	25.1 (1.4)	18.5 (1.0)	<0.05	15.4 (1.1)
ANOVA, p	0.59	0.59	0.07		0.82	0.06	0.41	0.08		0.78

C_2 , acetate; C_3 , propionate; C_4 , butyrate; iC_4-C_6 , isobutyrate, valerate, isovalerate, and hexanoate. ANOVA: one way analysis of variance; LSD: least significant difference compared with controls. NS: $p > 0.05$.

EFFECT OF COLONIC SURGERY ON CONCENTRATIONS AND RATIOS OF FAECAL SHORT CHAIN FATTY ACIDS

The effects of colonic surgery on concentrations and ratios of faecal SCFAs is evaluated in Table IV by comparing healthy unoperated control subjects (16) and patients with cancer treated by segmental sigmoid resection (7) and right-sided (7) or left-sided (3) hemicolectomy. There was a tendency to higher faecal concentrations of SCFAs in the patients who had undergone colonic surgery, but only the left-sided hemicolectomised patients differed significantly from control subjects ($p < 0.05$). Faecal pH was significantly lower in patients who had undergone left-sided hemicolectomy compared with control subjects or patients who had undergone other types of surgery ($p < 0.05$; Table IV).

Discussion

Since Burkitt in 1971 proposed the hypothesis of a protective role of dietary fibre in the colonic carcinogenesis,⁴ the mechanism behind such a role has been the subject of much speculation. There is still no conclusive evidence, however, as to how this possible protection occurs. Burkitt suggested that a high fibre diet causes a more rapid gastrointestinal transit time and bulkier stools, resulting in dilution of carcinogens which may be in the large intestine.⁴ While these

properties of dietary fibre are clearly important, its major role may be mediated through fermentation. The production of SCFAs, including butyrate, may affect colonic cell function and decrease colonic pH, preventing or reducing carcinogen formation.²⁷

This study did not show any significant differences in the pH of faecal suspensions from healthy control subjects and patients with former colonic adenomas or colonic cancer. This does not agree with the results of Pietroiusti *et al.*,²⁸ who found a higher pH in faeces from patients with colorectal cancer than in healthy subjects, and with those of Macdonald *et al.*,²⁹ who found that subjects with colorectal cancer had a significantly higher pH than Seventh Day Adventists, a population at low risk for the development of large bowel cancer. The patients with colonic cancer in this study, however, unlike those in the above mentioned studies, were examined after colonic resection had been performed, which may reduce faecal pH (Table IV). Hence, patients who had undergone left-sided hemicolectomy had significantly lower faecal pH, suggesting an approximation to the more acid conditions normally found in the caecum.³⁰ When fermentable substrate was added to the faecal incubations all three groups responded in the same way concerning pH – that is the biochemical capability to lower pH in colonic contents is probably normal in patients with

TABLE IV Effect of colonic surgery on concentrations (mmol/l) and ratios (%) of faecal short chain fatty acids

	Concentrations (mmol/l)						Ratio (%)			
	Total	C ₂	C ₃	C ₄	iC ₄ -C ₆	pH	C ₂	C ₃	C ₄	iC ₄ -C ₆
Controls (A)										
Mean (SEM)	91.3 (6.8)	59.3 (5.2)	14.4 (1.3)	10.9 (1.0)	6.7 (0.6)	7.1 (0.2)	64.6 (1.7)	15.9 (1.0)	12.0 (0.8)	7.5 (0.6)
Sigmoid resection (B)										
Mean (SEM)	94.9 (11.1)	64.9 (7.8)	14.0 (1.6)	9.4 (2.0)	6.6 (0.5)	7.2 (0.1)	68.5 (1.8)	14.8 (0.9)	9.2 (1.2)	7.5 (0.7)
Right hemicolectomy (C)										
Mean (SEM)	118.8 (15.0)	75.7 (9.0)	20.8 (2.4)	13.9 (4.1)	8.4 (2.9)	7.3 (0.2)	64.3 (3.0)	18.4 (1.8)	10.7 (1.7)	6.6 (1.4)
Left hemicolectomy (D)										
Mean (SEM)	146.9 (9.8)	82.2 (1.3)	21.1 (2.0)	29.6 (7.0)	14.0 (0.1)	6.5 (0.3)	56.8 (4.2)	14.5 (2.3)	19.0 (6.1)	9.7 (0.6)
ANOVA, p	0.003	0.02	0.003	0.001	0.05	0.06	0.14	0.37	0.05	0.51
LSD, 95%	A<D	NS	A<C	A=B<C<D	NS	D<A=B=C	NS	NS	A=B=C<D	NS

C₂, acetate; C₃, propionate; C₄, butyrate; iC₄-C₆, isobutyrate, valerate, isovalerate, and hexanoate. ANOVA: one way analysis of variance; LSD (least significant difference) test was used to evaluate differences between individual groups at 95% level, eg, A<D, A less than D, but neither B nor C different from either A or D; A=B<C<D, A, B and C significantly lower than D, NS=all groups homogeneous.

colonic neoplasia. Results in Table II indicate that it is to be expected that colonic acidity is increased by bacterial degradation of carbohydrates (glucose, ispagula, and wheat bran) only, and not by peptides (albumin). That is antineoplastic effects secondary to pH reduction are probably restricted to fermentation of carbohydrates. Therefore, the preoperative higher faecal pH found in former studies may be a consequence of a diminished supply of carbohydrates for colonic fermentation, either because of the ingestion of a low fibre diet or a more efficient absorption of carbohydrates (including 'resistant starch') in the small intestine in patients with colonic cancer. Intestinal malabsorption of carbohydrates to the colon has not been investigated in patients with colonic cancer, but it has been shown that small bowel absorption of starch is very efficient in patients with colonic adenomas, probably reducing colonic fermentation and, hypothetically, increasing the risk of colonic neoplasia.³¹

No differences in faecal concentrations or molar ratios of SCFAs were found among healthy subjects and patients with former colonic adenomas or colonic cancer in the present study. A former study³² investigated SCFA distributions in enema samples from a sigmoidoscopy population and found a significantly higher ratio of acetate and a lower ratio of butyrate in patients with adenomatous polyps. We also found the faecal ratio of butyrate reduced (9.1%) in the patients with adenomas compared with normal subjects (12.0%). Although this was significant ($p<0.05$) when an ordinary *t* test was used, it was not significant when the least significant difference test or one way analysis of variance were applied to the data. Moreover, the butyrate ratio in the cancer group (11.6%) was close to the control value.

The present experiments used an incubation system as an *in vitro* model of colonic bacterial metabolism. This system has been applied to a number of different biochemical events in the intestine and it has earlier been shown that the numbers of the main groups of colonic organisms remain unchanged during an incubation time of up to 48 hours.²⁵ In this faecal incubation assay, the 'endogenous' (assay with no additions) production ratio of butyrate was significantly reduced in patients with colonic adenomas and cancer in contrast to all other specific SCFA production ratios. Furthermore, the relative production of butyrate from the fibre ispagula and

wheat bran remained reduced in the same groups of patients when fermentation was increased by the external addition of these polysaccharides to incubations. This effect on butyrate formation was not restricted to fibre fermentation but was also seen in 24 hour incubations with albumin (Table III).

Previous work¹³⁻²¹ has shown that exposure to butyrate induces alteration of several growth properties and morphological and biochemical changes consistent with a more differentiated state on a number of cultured human colon cancer cell lines. The changes include increased doubling times,^{13-17,21} altered colony forming abilities in soft agar,^{13,17,19,21} changes in morphology,^{13,16,18-21} increased activities of certain membrane bound enzymes,^{13,16,18-20} increased expression of carcinoembryonic antigen,^{13-15,17} and modification of other oncogene markers.¹⁸ It might therefore be postulated that a major role of dietary fibre is to raise colonic butyrate concentrations, thus increasing the differentiation pressure in colonic enterocytes. This theory has some indirect support from recent findings that butyrate concentrations are lower in the sigmoid and descending colon,^{30,33} the region where the incidence of colonic polyps and cancers is highest.

In this study, the significantly reduced capacity of stool bacteria from patients with colonic adenomas and cancers to produce butyrate suggests that butyrate may be important in the genesis of colonic neoplasia *in vivo*, and, hypothetically, subjects characterised by a colonic flora with a relatively low butyrate formation may have an increased risk of developing colonic adenomas and cancer. If so, this risk might be overcome by a diet rich in carbohydrates, which are malabsorbed by the colon and readily fermented to butyrate.

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