Faecal bile acid concentrations of patients with carcinoma or increased risk of carcinoma in the large bowel

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SUMMARY Faecal 3-hydroxy bile acids were assayed enzymatically in patients with carcinoma, or at increased risk of developing carcinoma of the large bowel. No rise in bile acid concentration was demonstrated in patients with ulcerative colitis, previously resected adenoma, or resected carcinoma. Patients with carcinoma, before treatment, had faecal bile acid concentrations similar to control values, and surgery did not affect the mean level. These findings cast doubt on the importance of the 3-hydroxy bile acid concentration in the faeces in the pathogenesis of large bowel cancer.

Carcinoma of the large bowel is common in developed countries with a North-West European culture.¹ Diets rich in fat² or meat³ and low in fibre⁴ may account for the higher incidence in developed nations when compared with underdeveloped societies.

Faecal bile acid concentrations are quantitatively related to the amount of fat eaten⁵ and were reported to be raised in populations with a high incidence of large bowel carcinoma.⁶ Subsequent studies on healthy subjects in Scandinavia only partially confirmed the association between faecal bile acid concentration and the risk of cancer.⁷

In previous studies, patients with established carcinoma of the large bowel had raised levels of bile acids in the faeces⁸⁻⁹ suggesting either a causal relationship or, alternatively, the effect of an obstructing lesion. In other studies patients with adenomatous polyps⁹ had raised faecal bile acid concentrations, while those with familial polyposis¹⁰ and ulcerative colitis¹¹ did not.

We report here the faecal bile acid concentrations of normal subjects and compare them with patients at increased risk of large bowel carcinoma and with cancer patients before and after excision of the tumour.

Methods

PATIENTS

Patients at increased risk of large bowel cancer were

studied initially. They included 20 patients who had undergone local resection of an adenomatous polyp (age 49-75 years; 10 male and 10 female), 11 patients with ulcerative colitis (age 23-72 years, seven male and four female), 15 patients who had undergone prior excision of the rectum for carcinoma (age 46-79 years, 11 male, four female) and 10 patients who had sigmoid colectomy for carcinoma (age 48-78 years, seven male, four female). These outpatients had undergone surgery more than four months before the study and none had had a barium enema examination or antibiotic in the month before study. Subsequently, 19 patients with large bowel carcinoma (right colon, five; transverse colon, one; left colon, four; rectum, nine) were studied as outpatients before operation, and in 10 of these the measurements were repeated two to nine months after surgery. Again stools were obtained before administering purgatives or antibiotics and before performing barium studies. The healthy age- and sex-matched controls were outpatients attending for physiotherapy. Both patients and controls were eating a mixed unrestricted diet and those who had recently modified their diet (with wheat bran, for example) were excluded.

TECHNIQUES

A whole stool was collected by the patient in a polythene bag and deep-frozen within 12 hours of voiding. Many of the cancer patients submitted only small faecal samples (10-20 g) because of their bowel disturbance. After thawing and homogenising the stool with methanol, a small sample (6-10 g) was freeze-dried and about 1 g extracted for bile

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acids by the method of Grundy *et al.*¹² This entailed mild saponification of faeces with M sodium hydroxide, extraction of neutral steroids with petroleum ether, strong saponification with 10 M sodium hydroxide, and extraction of the bile acids with chloroform. The 3-hydroxy bile acids were estimated using hydroxysteroid dehydrogenase (Sigma Chemicals)¹³ after purifying the crude extract by heptane washing but omitting reduction by sodium borohydride. Each batch of four faecal extracts (from patients and controls) was assayed along with four standard solutions of deoxycholic acid (100, 200, 300, 400 μ M) for calibration. The faecal bile acid concentration of each subject was expressed as the mean of two determinations in μ mol per gram of dried faeces.

Preliminary studies were made on normal subjects to test the reproducibility of the method and to determine whether random sampling from one point in a stool was adequate. The value of borohydride reduction, which is necessary to detect 3-keto bile acids, was assessed.

Results

METHODOLOGY

When 10 replicate measurements were made on each of five faecal extracts the coefficients of variation of the results were in the range 4.3 to 16.9%

Table 1 Reproducibility of measurements of bile acid concentration of five different faecal extracts (mean of 10 replicate observations)

	Concentration of bile acid (μ M)				
Mean	115	134	230	331	344
SD	13.4	22.6	23.9	19.4	14.9
Coefficient of variation (%)	11.7	16.9	10.4	5.9	4.3

Table 2 Faecal bile acid concentration in samples taken at three sites (A, B, C) in 10 different stools, expressed as the mean of duplicate estimations

Stool number	Faecal bile acid concentration (μ mol/g faecal solids)		
	A	B	C
1	23.0	25.4	40.2
2	7.9	8.6	10.2
3	25.3	20.1	18.8
4	7.2	8.6	12.3
5	19.1	21.8	23.1
6	8.1	11.4	11.2
7	57.0	35.3	43.1
8	53.5	14.1	18.8
9	23.5	45.6	35.3
10	9.4	14.8	15.3

(Table 1). In practice, faecal extracts of concentration 200–400 μ M were used, as the coefficients of variation were smaller in this range.

When samples were taken from three diverse points in a stool from each of 10 subjects, there was wide variation in the bile acid concentration (Table 2). Homogenisation of the stool before sampling reduced this variation (Table 3), and this procedure was therefore adopted.

In six samples, borohydride reduction decreased the detected amount of bile acid by 24% (11–40%) (mean and range). This step was therefore omitted from the procedure, accepting that the small proportion of 3-keto acids would not be detected.

PATIENTS AT INCREASED RISK OF LARGE BOWEL CANCER

The mean bile acid concentrations in the stools of patients with large bowel adenoma, ulcerative colitis, and previous resection for carcinoma are shown in Table 4. The values did not differ statistically from those of controls (paired *t* test).

Table 3 Faecal bile acid concentration in three samples taken from homogenates of five stools, expressed as the mean of duplicate estimations

Subject number	Faecal bile acid concentration (μ mol/g faecal solids)		
	Sample		
1	35.4	33.4	31.7
2	29.7	26.2	24.0
3	24.9	23.2	21.9
4	30.0	34.2	37.4
5	49.7	46.9	44.8

Table 4 Faecal bile acid concentrations (mean $\pm$ SEM) of patients at increased risk of large bowel carcinoma

Diagnosis	No.	Faecal bile acid concentration (μ mol/g)		<i>t</i> test
		Patients	Controls	
Adenoma	20	25.4 $\pm$ 2.3	27.8 $\pm$ 2.6	0.5 > <i>P</i> > 0.1
Ulcerative colitis	11	26.9 $\pm$ 3.3	32.3 $\pm$ 3.4	0.5 > <i>P</i> > 0.1
Rectal resection	15	27.0 $\pm$ 2.9	27.6 $\pm$ 2.7	<i>P</i> > 0.5
Sigmoid resection	10	25.7 $\pm$ 3.4	25.6 $\pm$ 4.0	<i>P</i> > 0.5

PATIENTS WITH CARCINOMA OF COLON OR RECTUM

Nineteen patients with untreated carcinoma of the large bowel had bile acid concentrations similar to those of healthy controls (Table 5). No significant change was detected when 10 patients were tested again two to nine months after resection of the tumour.

Table 5 Faecal bile acid concentrations (mean $\pm$ SEM) of patients with large bowel carcinoma

Diagnosis	No.	Faecal bile acid concentration (μ mol/g)		t test
		Patient	Control	
Carcinoma	19	26.3 $\pm$ 2.5	28.1 $\pm$ 2.7	0.5 > P > 0.1
		Preoperative	Postoperative	
Carcinoma	10	26.8 $\pm$ 3.3	25.2 $\pm$ 4.3	P > 0.5

Discussion

The first part of the study concerned patients at increased risk of developing carcinoma. The normal faecal bile acid concentration found in patients with ulcerative colitis agrees with the finding of other workers.¹¹ Miettinen¹⁴ also showed that the total mass of faecal bile acids excreted daily is not raised in patients with ulcerative colitis. A recent report of raised concentrations in adenoma patients⁹ could not be confirmed. These authors used gas-liquid chromatography to quantify the bile acids and tested patients while the adenoma was present. We have used an enzymatic method after local removal of the adenoma. The latter fact in particular may explain the discrepancy in results, as the adenoma might have an effect on bile acid levels. Similarly, the normal bile acid levels observed after resection of the rectum or sigmoid colon for carcinoma raised the possibility that high levels previously observed in untreated patients^{8,9} were an effect of the tumour rather than a precursor of the lesion.

A study was therefore made of patients with symptoms suggestive of large bowel carcinoma. This required faecal collections from many patients so that the minority who were confirmed to have a carcinoma could be studied without the disturbance of the colonic environment associated with a barium enema examination. Despite these precautions, we failed to show a difference in faecal bile acid concentrations in patients with large bowel cancer and the levels were not significantly altered by subsequent resection.

Our findings in preoperative cancer patients conflict with those of two previous studies^{8,9}, possibly because of differences in the bile acid assay, the timing of samples, or the selection of patients.

Enzymatic assay of faecal bile acids has been used by other investigators in this field.⁸ These authors used sodium borohydride to reduce 3-keto bile acids, but we have shown that this procedure causes an overall loss of bile acids. We have omitted this step, as 3-keto steroids make up a small proportion (about 2–3%) of the total faecal bile acid

mass in normal subjects,^{12,15} although their proportion in patients with tumours is unknown. The absolute concentrations found in this study cannot, therefore, be compared with those of Hill *et al.*,⁸ nor with those of Reddy and Wynder.⁹ The latter corrected their values for losses by incorporating radioactive cholic acid in the stools.

When stools are obtained after the development of carcinoma or adenoma of the large bowel, their bile acid concentrations cannot be assumed to have significance in the aetiology of the tumour. A prospective study of a large healthy population would be necessary to show whether high levels predispose to carcinoma. Equally, the study of faecal constituents in cancer patients is misleading after procedures which interfere with the colonic contents, such as barium enema examination. We have excluded this source of error by studying outpatients before investigation or preparation for surgery. We could not exclude, however, the effect of a decrease in appetite or disturbance of bowel habit experienced by most of the cancer patients. All of the other groups of patients and all controls were eating a normal mixed diet, and had a good appetite with normal bowel habit when studied.

As right- and left-sided carcinomas might be expected to affect faecal bile acids in different ways, we ensured that the distribution of carcinomas in our series followed the usual frequency distribution along the large bowel. Previous series included a preponderance of rectal carcinomas⁸ or colonic tumours of unspecified site.⁹ The selection of healthy controls from the same high-risk population as the patients follows the procedure in previous studies. We have failed to show that patients with bowel cancer, or increased risk of cancer, have higher faecal bile acid concentrations than those found in the general population.

We conclude that the concentration of total faecal bile acids is not simply correlated with the presence of carcinoma or the risk of carcinoma in the large bowel. This does not exclude a carcinogenic role for individual bile acids or products of their metabolism by the colonic bacteria.¹⁶

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