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Comparison of faecal bile acid profiles between patients with adenomatous polyps of the large bowel and healthy subjects in Japan

N TANIDA, Y HIKASA, T SHIMOYAMA, AND K D R SETCHELL

From the Department of Internal Medicine 4, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan, and Division of Clinical Chemistry, Clinical Research Centre, Harrow, Middlesex

SUMMARY Faecal bile acid excretion was examined in 13 patients with adenomatous polyps of the large bowel and compared with a series of matched healthy subjects. Bile acids were analysed in detail with respect to the composition of individual bile acids and their mode of conjugation. The total excretion of bile acids by the patient group and the healthy subjects ranged from 55·0–837·6 μmol/day (median 233·8, mean 346·9) and 93·8–712·3 μmol/day (median 489·2, mean 386·7) respectively. Expressed as μmol/g faecal weight these values were 0·6–4·8 (median 2·2, mean 2·4) and 0·4–5·8 (median 2·2, mean 2·8) and in terms of μmol/g faecal dry weight, 1·9–50·7 (median 10·1, mean 16·5) and 3·9–32·4 (median 16·3, mean 16·7) respectively for the two groups. The composition of the individual bile acids and their distribution within the various conjugate fractions was essentially the same for both groups. Cholenoic acid (5β-chol-3-enoic acid), an unusual bile acid, was detected in one patient and three healthy subjects. These results revealed no significant quantitative differences in bile acid excretion between the group of patients with adenomatous polyps and those of healthy subjects.

Epidemiological studies have suggested a link between patients with adenomatous polyps and cancer of the large bowel.[1–4] Although an adenoma–carcinoma sequence has been the subject of controversy,[5 6] it is generally accepted that patients with adenomatous polyps are at greater risk of developing colorectal cancer[7] than the normal population.

Bile acids have been shown to promote the formation of tumours in an experimental animal model[8 9] and have been implicated in carcinogenesis of the large bowel in man, because their faecal excretion has been shown to be greater in patients with colon cancer compared with controls.[10 11]

Studies on the faecal excretion of bile acids in patients with adenomatous polyps, although limited and not well defined, has suggested that this group of patients excrete higher quantities of bile acids in the faeces compared with healthy subjects.[10 11] All of the studies to date on faecal bile acid excretion in relation to colorectal cancer have been restricted to the measurement of the 'total' or the principal primary and secondary bile acids in faeces using techniques or modifications of techniques developed in the mid 1960s.[12–15] We have recently developed more sophisticated methods for the detailed determination of metabolic profiles of bile acids in faeces which permit the detection of a large number of individual bile acids and provide information on their mode of conjugation in the original faecal sample.[16–18]

We report here the first 'in depth' study of the qualitative and quantitative faecal bile acid excretion by healthy Japanese subjects, in a population at low risk of developing colon cancer and in a group of Japanese patients with adenomatous polyps of the large bowel who are therefore at greater risk of developing colon cancer within this population.

Methods

PATIENTS AND HEALTHY SUBJECTS

The age, sex, body weight, and faecal weights of the 13 patients studied are shown in Table 1 together with those of healthy subjects. Patients attended

Address for correspondence: Dr K D R Setchell, Clinical Research Centre, Clinical Mass Spectrometry, Watford Road, Harrow, Middlesex HA1 3UJ.

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Table 1 *Age, sex, body weight, and faecal weights of patients with adenomatous polyps of the large bowel and healthy subjects*

	Patient/healthy subject (no)													
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*Mean ± SD*
Patient														
Age (yr)	34	38	44	46	51	61	61	63	66	70	72	72	78	58±14
Sex	M	F	M	M	M	M	M	M	M	F	M	M	F	
Body weight (kg)	57	55	67	77	61	62	67	47	55	44	50	51	58	58±9
Faecal wet weight (g/day)*	60	173	132	104	175	190	230	165	108	140	103	137	95	139±46
Faecal dry weight (g/day)*	16	29	28	27	45	15	33	41	8	27	36	23	22	27±10
Healthy subject														
Age (yr)	32	39	49	49	50	50	50	54	57	64	66	67	74	54±12
Sex	M	M	M	M	M	M	M	F	F	M	M	M	M	
Body weight (kg)	65	58	60	76	70	57	53	40	54	50	56	48	53	57±10
Faecal wet weight (g/day)*	122	133	57	133	152	37	113	245	270	220	140	140	220	153±69
Faecal dry weight (g/day)*	20	27	10	18	25	10	23	42	50	30	22	17	25	25±11

* Calculated from a 72 hour faecal collection.

hospital with abdominal symptoms such as dull pain, discomfort, transient constipation, and soft stools, but no evidence of organic disorder was found in the upper gastrointestinal tract, large intestine, or other digestive organs using radiology, endoscopy, and ultrasonography except for the presence of colorectal polyps. The diagnosis of adenomatous polyps was based on the radiological, colonoscopic, and histopathological findings. The large intestine, from the rectum to caecum of the patients was fully examined by colonoscopy, which allowed the detection of polyps as small as 2 mm in diameter. After a period of at least four weeks from the final examination, all patients were admitted to the Hospital of Hyogo College of Medicine for endoscopic polypectomy. No carcinoma was found in the resected polyps, and their distribution along the gastrointestinal tract is shown in Table 2.

The healthy subjects consisted of 11 hospital staff and two patients with old cerebral infarctions who were otherwise healthy. None of the subjects including the patients had any previous history of gastrointestinal problems. Only the eldest two subjects from the healthy controls had radiological examination, with no evidence of polyps. No drugs had been administered for at least four weeks before the faecal collections. Liver function tests and serum cholesterol concentrations were within the normal range. An oral and written explanation of the protocol was given and full consent obtained from all of the subjects in the study.

ANALYTICAL PROCEDURE

Faeces were collected over a 72 hour period, and in the case of the patients this was during the first three days of admission. To minimise the effect of daily

Table 2 *Distribution of adenomatous polyps of the large bowel in 13 patients studied*

Patient (no)	*Caecum*	*Ascending colon*	*Transverse colon*	*Descending colon*	*Sigmoid colon*	*Rectum*	*Total number*
1	–	–	–	–	1	–	1
2	–	–	–	–	1	3	4
3	–	–	–	–	1	–	1
4	–	–	–	1	–	–	1
5	–	–	1	–	–	–	1
6	1	–	2	3	1	–	7
7	–	–	–	–	–	2	2
8	–	–	–	–	2	–	2
9	1	–	1	1	3	–	6
10	–	–	–	–	1	–	1
11	–	–	–	–	1	–	1
12	–	–	1	2	1	–	4
13	–	–	–	–	1	–	1
Total	2	–	5	7	13	5	32

variations in faecal output only the subjects who had regular bowel movements were included in the study. The faeces were stored immediately at −20°C after voiding. The analytical procedure is described in detail elsewhere.[16–18] Bile acids were extracted from faecal homogenates by sequential refluxing in organic solvents. The extracts were then purified and the bile acids were separated according to their mode of conjugation using the lipophilic anion exchange gel, Lipidex-DEAP (Packard Instrument Co Inc, Downers Grove, Ill, USA). Fractions comprising (i) neutral, (ii) unconjugated bile acids, (iii) glycine conjugates, (iv) taurine conjugates, and (v) bile acid sulphates were obtained. After deconjugation and/or preparation of the methyl ester-trimethylsilyl ether derivatives, bile acids were quantified in each fraction by capillary column gas chromatography and identified using mass spectrometry. The conditions for capillary column gas chromatography and mass spectrometry have been described previously.[16] Samples were also analysed as methyl esters using a 2 m 1·5% SE-30 packed column to enable the separate determination of 5β-chol-3-enoic acid.

All analyses were performed in duplicate, and the recovery of added radiolabelled bile acids using this analytical procedure was quantitative. The variation in the duplicates ranged from 5·2±2·2% to 21·3±10·0% (mean ± SD) for the major and minor components respectively and for the total excretion this value was 7·5±3·1%. The results are expressed as the mean value of duplicate determinations and no correction for recovery was applied. The data were analysed with the paired Wilcoxon's rank test, median test, and χ^2 test.

Results and Discussion

For over a decade now bile acids have been implicated in the aetiology of colorectal cancer.[19 20] This hypothesis is based upon a considerable amount of circumstantial data, involving epidemiological studies and biochemical and microbiological studies both *in vivo* and *in vitro*. Bile acids have been shown to have a promoting effect on carcinogenesis of the large bowel in an experimental animal model[8 9] and at the same time the faecal excretion of bile acids, notably secondary bile acids, has been reported to be higher in patients with colon cancer compared with healthy subjects.[10 11] Correlations have been observed between the dietary intake of animal fat,[21–23] which increases faecal bile acid excretion,[24 25] and the incidence of colon cancer. *In vitro* experiments have also shown the capability of faecal bacteria to transform the bile acid molecule into compounds with potential carcinogenic or cocarcinogenic activity.[26–29] The numbers of nuclear dehydrogenating Clostridia, which are capable of these chemical degradations, in the faeces of patients with large bowel cancer have also been shown to be significantly greater than in controls.[10]

Despite all of the above observations the actual carcinogens responsible for tumours of the large bowel have yet to be recognised and the aetiology of this disease remains unclear. To some extent the techniques used for faecal bile acid determination, which represent little advance upon those developed in the mid 1960s, can be criticised for lacking in specificity and being incapable of detecting trace quantities of the types of compounds proposed as carcinogens or cocarcinogens. Furthermore, all knowledge of the state of conjugation of the bile acids excreted is lost in the initial saponification procedures. To overcome these problems and criticism, we have applied recently developed techniques for the metabolic profiling of bile acids in faeces[16–18] which provides a two dimensional analysis in terms of the types of bile acid structures which are present and gives information about their mode of conjugation in the original faecal sample. Quantification of the bile acids is attained by high resolution glass capillary column gas chromatography and identification by mass spectrometry.

Patients with adenomatous polyps of the large bowel are at greater risk of developing colon cancer than healthy adults[1–4 7] and it has been suggested that carcinogenesis proceeds through an adenoma–carcinoma sequence.[5 6] It was therefore of interest to apply these recently developed techniques to study the pattern of faecal bile acid excretion in this type of patient, particularly as it has been shown in previous studies that the quantitative excretion of faecal bile acids in patients with adenomatous polyps is similar to that of colon cancer patients and greater than in normal controls.[10 11] Both of these studies[10 11] were carried out using white subjects from a western population. Our data reported here, however, differ in that the patients with adenomatous polyps and the controls are from Japan, a country with a low incidence of colorectal cancer.[22 30]

It was difficult to strictly match the patients and controls for age and sex but a reasonable matching was attained (Table 1) and the differences are unlikely to affect the faecal bile acid excretion.[31] With the exception of two subjects the healthy controls were not colonoscoped, the possibility that some may have polyps cannot be excluded. Prevalance rates for adenomatous polyps in the Japanese population have been reported from necropsy studies to be 18·3–30%.[32] Our clinical observations reveal a prevalence rate of 6·5%.

As one of the objectives of the study was to determine the 'natural status' of faecal bile acid excretion in patients with colon cancer and in matched controls, to avoid manipulating bile acid excretion by placing these subjects on strict diets which would require an equilibrium period, samples were collected from the control group as outpatients while to minimise the effect of hospital diets the stools from the patients were collected during the first three days of hospital admission. The difference in the collection procedure between the patients and the healthy subjects and the fact that all of the patients and two of the controls had received large bowel radiological examination before collection of their faeces should be taken into account when interpreting these data. While differences in faecal bile acid excretion have been noted immediately after barium enemas (Setchell, unpublished observations) all of the individuals examined returned to their previous bowel habits within one week after examination and as a precaution a further three weeks were allowed before commencement of collection of stools. Furthermore it should be stressed that all of the subjects studied had regular bowel movements with no indication of constipation during the collection period.

Despite collecting faeces over a 72 hour period a wide variation in the excretion of bile acids was observed and this was apparent irrespective of whether these data were expressed in terms of the total daily excretion, or as a concentration – that is, excretion/g wet weight or excretion/g dry weight of faeces (Fig. 1).

Wide variations in the excretion of bile acids in faeces have been reported between individuals[10 11] and from day to day within the same individual[31] and despite prolonging the collection time of the stools these variations are still evident. The mean total excretion of bile acids in the control group (386·7 μmol/day) was not found to be significantly different from the patients with adenomatous polyps (346·9 μmol/day) and this was also the case when these data were expressed as μmol/g wet faeces and μmol/g dry faeces. The quantitative excretion by the healthy subjects studied here was 16·6 μmol/g faecal dry weight (approximately 6·6 mg/g faecal dry weight) which is similar to previous reports for the Japanese population[11 31 33] (mean 6·0–8·6 mg/g dry weight) using different methodology, and considerably lower than in healthy subjects in western populations (cf 10·8 mg/g dry weight[11]).

Several previous reports have indicated that patients with adenomatous polyps excrete greater quantities of bile acids in the faeces than healthy subjects.[10 11] This was not the case in this study but it is difficult to make comparisons as the types of

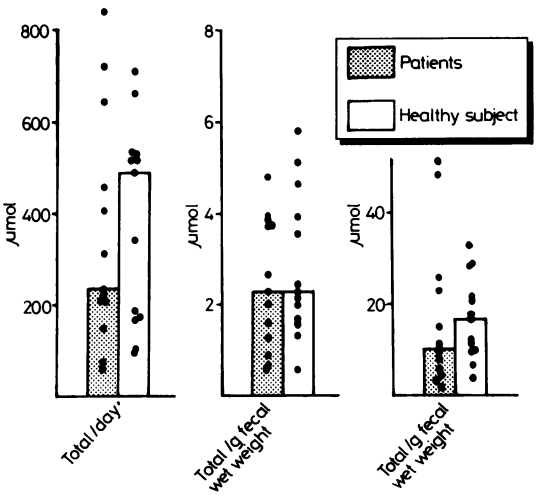

Fig. 1 *Total faecal bile acid excretion of patients with adenomatous polyps of large bowel and healthy subjects. Each symbol represents one individual and columns indicate the median values for each group.*

subjects – that is, western population – and the methods used for analysis of bile acids in these previous investigations differ markedly from those described here.

The complexity of faecal bile acids has been indicated in previous studies of both western[18] and Japanese healthy adults and the diversity of bile acid structures which have been identified by gas chromatography–mass spectrometry in these samples has been described previously.[16]

In qualitative terms these data are similar to those reported by Eneroth *et al*[14 34 35] using gc-ms, however since the primary objectives of these early elegant studies of Eneroth *et al* were to develop suitable techniques for faecal bile acids[14] and to characterise bile acids in faeces,[34 35] accurate faecal collections were not made and only a few subjects were studied. While it is difficult therefore to compare critically the quantitative excretion of faecal bile acids in our studies with those of Eneroth *et al,* in general terms the range and variations in total and individual bile acid excretion were similar despite the obvious differences that probably exist between the diets of the individuals from these two populations. Quantitatively greater than 90% of the total bile acids excreted were present in the unconjugated form, the remainder being distributed between the glycine, taurine, and sulphate conjugated fractions (Fig. 2). Faecal bile acid sulphates accounted for less than 5% of the total bile acids excreted in both patients and controls.

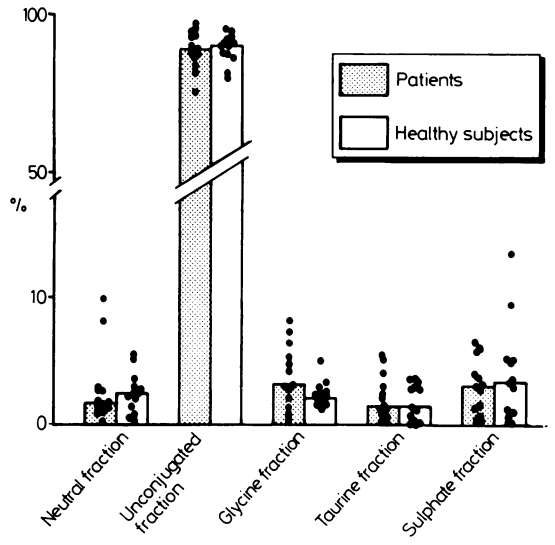

Fig. 2 *Composition of faecal bile acids by their mode of conjugation in patients with adenomatous polyps of large bowel and healthy subjects. Each symbol represents one individual, and median values for each groups are indicated. NF = neutral fraction, UF = unconjugated fraction, GF = glycine fraction, TF = taurine fraction, SF = sulphate fraction.*

A list of the bile acids which were quantified and identified in each of the conjugate fractions is summarised in Tables 3 and 4 for the patient group and the controls respectively. The quantitative excretion in μmol/day for each bile acid and the frequency with which they were detected in each of the groups is shown. In some instances because of the lack of authentic standards it is only possible to assign a general chemical structure to the compound based upon the mass spectrometric fragmentation pattern. In this case the quantitative excretion of the unidentified bile acids are expressed collectively according to their general chemical structure.

The detection of 5β-chol-3-enoic acid in the faeces of four subjects (one patient and three controls) is particularly interesting. This unusual bile acid has been previously identified in the faeces of a patient with gall stones[36] and is most probably formed from lithocholic acid sulphate by intestinal microflora.[37 38] It is possible that it could be an intermediate in the pathway to the formation of a fully aromatised carcinogenic structure as suggested by Hill *et al*[10 19 28] but such compounds were not identified in this present study.

On examination of the quantitative excretion (Tables 3 and 4) of the principal primary and secondary bile acids, no significant differences were found between patients with adenomatous polyps and healthy subjects (Fig. 3).

Microbiological studies of the stools were carried out and are reported elsewhere,[39] but in summary revealed no significant differences in the obligate anaerobes between the patients with adenomatous polyps and the control group. Although the numbers of nuclear dehydrogenating Clostridia were small, a higher incidence of nuclear dehydrogenating Clostridia was not found in the patient group compared with the controls (unpublished data).

These data are in agreement with studies in western populations where, despite their high risk for carcinoma, patients with adenomatous polyps surprisingly had a similar[40] or slightly lower[41] carriage rate for nuclear dehydrogenating Clostridia compared with healthy subjects. Boriello found that 41% of adenomatous polyp patients carried nuclear dehydrogenating Clostridia compared with 32% of the controls; however, of this 41% the majority tended to carry relatively high levels of nuclear dehydrogenating Clostridia which bore no relationship to the polyp size.

It is difficult to make direct comparisons between subjects from western and Japanese populations; however, an indirect assessment of the degree of microbial degradation can be determined by comparing the ratios of individual bile acids. In this way, the extent to which dehydroxylation and/or oxidoreduction occur can be seen (Table 5). The

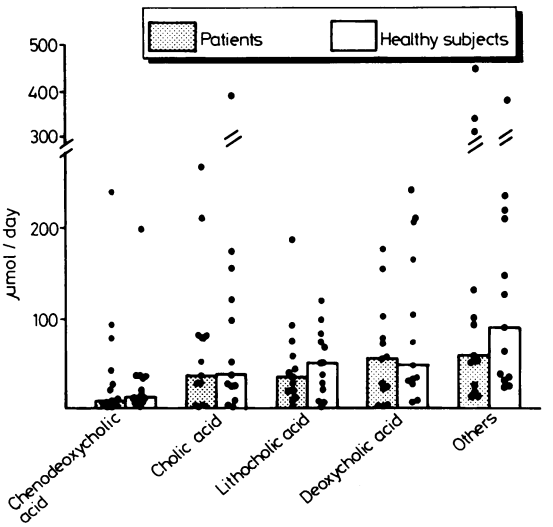

Fig. 3 *Composition of individual bile acids in faeces of patients with adenomatous polyps of large bowel and healthy subjects. Each symbol represents one individual. Columns indicate median values for each group.*

Table 3 *Faecal bile acid profiles of 13 healthy subjects. The figures indicate the ranges (μmol/day) in the upper row, the frequencies of the detection (individuals out of 13 subjects) and the median values of the ranges in the lower row of each compound**

	NF	*UF*	*GF*	*TF*	*SF*	*Total*
Lithocholic	0·1–3·2	2·1–101·9	0·2–5·7	0·2–13·7	0·2–11·9	6·3–121·4
	11, 0·8	12, 42·7	11, 1·6	10, 2·1	10, 3·5	12, 52·0
Deoxycholic	0·1–6·9	7·4–229·4	0·1–4·5	tr–3·8	0·1–7·6	8·2–242·1
	10, 1·3	13, 64·9	11, 1·0	8, 0·2	9, 2·5	13, 71·1
Chenodeoxycholic	0·2–2·5	tr–190·8	0·2–4·3	tr–0·5	0·1–33·8	0·3–197·0
	10, 0·4	11, 9·9	12, 1·3	12, 0·3	12, 0·3	13, 12·5
Cholic	0·3–4·0	3·6–383·0	0·4–4·0	0·1–1·1	tr–3·9	tr–391·1
	9, 0·8	12, 40·4	9, 1·3	7, 0·5	7, 0·2	13, 35·7
Monohydroxy	0·1–1·7	0·1–19·8	0·1–9·7	0·1–4·6	0·1–0·9	0·1–31·3
	6, 0·5	12, 6·3	10, 0·2	7, 1·1	7, 0·4	13, 10·3
Dihydroxy	0·2–4·0	3·4–120·1	tr–4·4	tr–4·2	0·1–53·9	3·9–127·2
	12, 1·3	12, 33·12	11, 2·7	12, 1·4	12, 2·0	13, 61·3
Trihydroxy	tr–8·9	0·4–63·2	0·1–1·0	tr	0·1–0·4	0·5–65·6
	5, 0·5	6, 9·8	3, 0·3	1	3, 0·2	8, 7·8
Mono-oxo	0·5	2·7				3·2
	1	1				1
Monohydroxy-mono-oxo	0·6–1·2	4·9–179·9	0·2–1·7	0·1–1·9	0·1–3·9	0·1–182·1
	6, 0·5	10, 18·56	6, 0·3	8, 0·1	7, 0·3	12, 11·9
Dihydroxy-mono-oxo	0·1–0·8	2·2–56·4	0·1–0·9	0·4–1·20	0·2–0·3	2·1–57·5
	6, 0·3	9, 8·8	8, 0·5	7, 0·6	2, 0·3	10, 9·2
Monohydroxy-di-oxo		3·1	0·1			0·1–3·1
		1	1			2, 1·6
5β-Chol-3-enoic acid		tr–25·8				tr–25·8
		3, tr				3, tr
Total	0·2–13·8	86·4–622·4	1·3–29·6	0·5–22·8	0·9–96·0	93·8–712·3
	13, 8·0	13, 435·9	13, 8·2	12, 6·2	13, 9·0	13, 489·2

* NF, UF, GF, TF, and SF indicate fractions of neutral (side chain esterified), unconjugated, glycine conjugated, taurine conjugated, and sulphate bile acids; tr = trace for less than 0·1. Identification of bile acids in these samples has been reported previously.[16] For the purpose of quantification compounds were grouped according to their general structure. Thus, monohydroxy included 3β, 7α, 12β, and one other unidentified bile acid; dihydroxy included epimers of 3,7- and 3,12- and as a minor component 3,6-; trihydroxy included mainly 7β-epimers of cholic acid and as a minor component hyocholic acid; mono-oxo included 3-oxo and 7-oxo compounds; monohydroxy-mono-oxo included 3α-hydroxy-7-oxo and 3α-hydroxy-12-oxo with small amounts of 7α-hydroxy-3-oxo bile acids; dihydroxy-mono-oxo and mono-hydroxy-di-oxo included epimers of 3,7,12 hydroxy positions with oxo groups being in the 7 and/or 12 positions. No other compound was found in appreciable amounts.

ratio of lithocholic/chenodeoxycholic in the patient group (11·1±14·7) was higher than in the healthy control group (2·9±2·5), suggesting increased 7α-dehydroxylation of bile acids although this was not significant when analysed statistically. This was also the case for the deoxycholic/cholic acid ratio.

Whether faecal bile acids reflect the state of bile acids in the intestinal lumen is difficult to determine. For example, 3-oxo-nuclear dehydrogenated bile acids, which were not detected, may be formed within the intestine and later rehydrogenated after passage of the stools. To minimise the possibility of such changes occurring, freshly voided faeces were collected and frozen immediately and we can only assume, as with all previous studies on faecal bile acid excretion, that the metabolic profiles which have been determined reflect events which have occurred in the gastrointestinal tract.

In this instance then, we could find no significant quantitative differences in the total faecal bile acid excretion or concentration, the extent of conjugation and deconjugation and the amounts of the individual bile acids, save some alterations in dehydroxylation and/or oxidoreduction, between patients with adenomatous polyps of the large bowel and healthy controls. Our findings, using more sophisticated analytical techniques, are at variance with previous studies in patients with adenomatous polyps.[10 11 42] This may be explained, however, by the differences in geographical locations of the subjects and corresponding prevalence rates of the disease.

A recent study has shown that an increased colonic absorption of deoxycholic acid, associated with a more rapid turnover of cholic acid, occurs in adenoma patients.[43] Kinetic studies were not carried out here, however the relative rates of synthesis of cholic and chenodeoxycholic acids calculated from the quantitative faecal excretion of the metabolites of these two primary bile acids are shown in Table 6.

Table 4 *Faecal bile acid profiles of 13 patients with adenomatous polyps of large bowel. Figures indicate the ranges (μmol/day) in the upper row, the frequencies of the detection (individuals out of 13 patients) and the median values of the ranges in the lower row for each compound**

	NF	*UF*	*GF*	*TF*	*SF*	*Total*
Lithocholic	0·1–1·5 11, 0·7	4·8–175·0 12, 34·8	0·2–9·9 9, 0·6	0·1–10·5 13, 0·6	0·1–7·0 12, 1·4	0·6–186·0 13, 34·0
Deoxycholic	0·1–3·1 12, 1·8	tr–172·1 12, 50·6	0·5–8·4 10, 1·1	0·1–1·3 9, 0·2	0·1–9·2 11, 2·4	0·6–176·8 13, 54·5
Chenodeoxycholic	tr–5·3 9, 0·6	3·9–232·2 9, 22·0	0·1–8·6 11, 1·3	0·1–3·0 9, 0·4	0·1–4·7 12, 0·4	0·2–242·6 13, 11·2
Cholic	0·1–4·5 7, 1·5	tr–239·2 13, 26·8	0·1–21·5 9, 1·4	0·1–4·2 7, 0·6	0·1–5·1 7, 0·2	1·0–269·5 13, 35·2
Monohydroxy	0·3–25·0 4, 0·5	1·3–59·2 10, 10·5	tr–1·2 8, 0·3	0·1–0·7 6, 0·4	0·1–1·1 9, 0·5	0·6–85·6 11, 5·8
Dihydroxy	0·3–3·9 12, 1·0	1·1–144·9 12, 31·5	0·3–5·2 10, 2·5	0·2–5·5 10, 0·7	0·3–3·8 10, 1·3	3·0–152·9 13, 31·4
Trihydroxy	0·4 1	tr–99·4 3, 6·5	0·8–1·3 2, 1·0	0·1–0·2 2, 0·2	1·2–2·4 2, 1·8	tr–101·3 5, 1·5
Tetrahydroxy	0·2 2, 0·2	tr 1		0·1–0·4 3, 0·1		tr–0·4 4, 0·2
Mono-oxo	1·1 1	9·2 1				0·1–9·2 2, 5·1
Monohydroxy-mono-oxo	0·1–1·1 6, 0·6	1·6–222·9 11, 21·4	0·1–7·1 7, 0·9	0·1–0·7 6, 0·3	0·1–3·8 5, 0·3	0·5–225·8 13, 9·2
Dihydroxy-mono-oxo	4·1 1	1·8–12·3 3, 9·0	0·5 1	0·1–1·3 2, 0·7		0·1–12·3 5, 4·7
Monohydroxy-di-oxo	0·2 1	10·5 1	0·7–1·5 2, 1·1	0·3–2·0 5, 0·5	0·4–0·5 2, 0·5	0·4–13·3 5, 1·5
5β-Chol-3-enoic acid		3·9 1				3·9 1
5β-Cholanoic acid			1·1 1	5·2 1		6·3 1
Total	0·1–31·9 13, 5·7	47·0–811·3 13, 218·6	0·5–37·3 13, 6·9	0·6–16·5 13, 3·5	0·5–26·9 13, 7·1	55·0–837·6 13, 233·8

* See footnote to Table 3.

Table 5 *Ratios of various faecal bile acids indicating the extent of deconjugation, dehydroxylasion, and oxidoreduction of the faecal bile acids in the patients with adenomatous polyps of the large bowel and healthy subjects**

	Patients	*Healthy subjects*
Mode of conjugation (% in total excretion)		
NF	2·7±2·8	2·4±1·6
UF	88·5±6·0	88·9±4·4
GF	3·7±2·5	2·3±1·0
TF	1·9±1·9	1·8±1·4
SF	3·0±2·2	4·0±3·9
CDC+C/total	0·3±0·2	0·3±0·2
LC/CDC	11·1±14·7	2·9±2·5
DC/C	2·3±2·4	1·6±1·3
Epimers and oxo metabolites of LC/LC	0·3±0·2	0·2±0·1
Epimers and oxo metabolites of DC/DC	1·4±2·3	0·4±0·5
Epimers and oxo metabolites of CDC/CDC	1·9±2·0	2·3±2·4
Epimers and oxo metabolites of C/C	0·3±0·3	0·4±0·2

* The figures are expressed as mean ± SD of the ratios calculated from the total amounts of individual bile acids.
NF = neutral fraction; UF = unconjugated fraction; GF = glycine fraction; TF = taurine fraction; SF = sulphate fraction; LC = lithocholic acid; DC = deoxycholic acid; CDC = chenodeoxycholic acid; C = cholic acid.

While the proportion of cholic acid derived compounds excreted in the patient group was less than that of the control group, this difference was not statistically significant. The differences in the qualitative patterns, however, particularly with respect to the extent of dehydroxylation and oxido-

Table 6 *Faecal excretion of cholic acid and chenodeoxycholic acid related compounds in patients with adenomatous polyps of the large bowel and healthy subjects.**

	Cholic acid derived†		*Chenodeoxycholic acid derived†*	
	μmol/day	*% of total*	*μmol/day*	*% of total*
Patients	171·7±127·7	49±17	146·0±117·0	46±18
Healthy subjects	218·3±152·0	57±16	153·3±103·0	40±16

* The figures are expressed as mean ± standard deviation.
† Cholic acid derived includes, 12-mono-, 3,12-di- and 3,7,12-trisubstituted compounds. Chenodeoxycholic acid derived includes 3-, 7-mono- and 3,7-disubstituted compounds together with 5β-chol-3-enoic acid. C-6 substituted and unidentified compounds were minor components and were excluded from the calculation.

reduction, would not exclude a role for bile acids in colonic carcinogenesis, despite several reports which cast doubt upon this theory for the aetiology of the disease.[42 44–46]

What is clear from this study, however, is the need to apply a more critical approach to the accurate qualitative analysis of bile acids in faeces,[47] and these studies are being extended to investigate colon cancer patients in the western and Japanese populations.

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References

1 Corre P, Strong JP, Reif A, Johnson WD. The epidemiology of colorectal polyps. Prevalence in New Orleans and international comparisons. *Cancer* 1977; **39:** 2258–64.
2 Bremner CG, Ackerman LV. Polyps and carcinoma of the large bowel in South African Bantu. *Cancer* 1970; **26:** 991–9.
3 Correa P, Duque E, Cuello C, Haenszel W. Polyps of the colon and rectum in Cali, Columbia. *Int J Cancer* 1972; **9:** 86–96.
4 Stemmermann GN, Yatani R. Diverticulosis and polyps of the large intestine. A necropsy study of Hawaii Japanese. *Cancer* 1973; **31:** 1260–70.
5 Morson BC. The polyp-cancer sequence in the large bowel. *Proc R Soc Med* 1974; **67:** 451–7.
6 Hill MJ, Morson BC, Bussey HJR. Aetiology of adenoma-carcinoma sequence in large bowel. *Lancet* 1978; **1:** 245–7.
7 Buntain WL, ReMine WH, Farrow GM. Premalignancy of polyps of the colon. *Surg Gynecol Obstet* 1972; **134:** 499–508.
8 Narisawa T, Magadia NE, Weisburger JH, Wynder EL. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. *J Natl Cancer Inst* 1974; **53:** 1093–7.
9 Reddy BS, Narisawa T, Weisburger JH, Wynder EL. Promoting effect of sodium deoxycholate on colon adenocarcinomas in germfree rats. *J Natl Cancer Inst* 1976; **56:** 441–2.
10 Hill MJ, Drasar BS, Williams REO, Meade TW, Cox AG, Simpson JEP, Morson BC. Faecal bile-acids and Clostridia in patients with cancer of the large bowel. *Lancet* 1975; **1:** 535–9.
11 Reddy BS, Wynder EL. Metabolic epidemiology of colon cancer. Fecal bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps. *Cancer* 1977; **39:** 2533–9.
12 Grundy SM, Ahrens EH Jr, Miettinen TA. Quantitative isolation and gas liquid chromatographic analysis of total fecal bile acids. *J Lipid Res* 1965; **6:** 397–419.
13 Ali SS, Kuksis A, Beveridge JMR. Excretion of bile acids by three men on a fat-free diet. *Can J Biochem* 1965; **44:** 957–69.
14 Eneroth P, Hellström K, Sjövall J. A method for quantitative determination of bile acids in human feces. *Acta Chem Scand* 1968; **22:** 1729–44.
15 Evrard E, Janssen G. Gas-liquid chromatographic determination of human fecal bile acids. *J Lipid Res* 1968; **9:** 226–36.
16 Tanida N, Hikasa Y, Hosomi M, Satomi M, Oohama I, Shimoyama T. Fecal bile acid analysis in healthy Japanese subjects using a lipophilic anion exchanger, capillary column gas chromatography and mass spectrometry. *Gastroenterol Jpn* 1981; **16:** 363–71.
17 Gilbert JM, Lawson AM, Worthington J, Setchell KDR. Improved methods for the metabolic profiling of bile acids in faeces. [Abstract] *Gut* 1981; **23:** A912.
18 Setchell KDR, Lawson AM, Tanida N, Sjövall J. General methods for the analysis of metabolic profiles of bile acids and related compounds in feces. *J Lipid Res* 1983; **24:** 1085–100.
19 Aries VC, Crowther JS, Drasar BS, Hill MJ, Williams REO. Bacteria and the aetiology of cancer of the large bowel. *Gut* 1969; **10:** 334–5.
20 Hill MJ, Crowther JS, Drasar BS, Hawksworth G, Aries VC, Williams REO. Bacteria and aetiology of cancer of the large bowel. *Lancet* 1971; **1:** 95–100.
21 Wynder EL. Dietary habits and cancer epidemiology. *Cancer* 1979; **43:** 1955–61.
22 Wynder EL, Shigematsu T. Environmental factors of cancer of the colon and rectum. *Cancer* 1967; **20:** 1520–61.
23 Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices. *Int J Cancer* 1975; **15:** 617–31.
24 Hill MJ. The effect of some factors on the faecal excretion of acid steroids, neutral steroids and urobilins. *J Pathol* 1970; **104:** 239–45.
25 Cummings JH, Wiggins HS, Jenkins DJA, Hill MJ, Pearson JR. Influence of diets high and low in animal fat on bowel habit, gastro-intestinal transit, faecal microflora, bile acid and fat excretion. *J Clin Invest* 1978; **61:** 953–63.
26 Aries VC, Goddard P, Hill MJ. Degradation of steroids by intestinal bacteria III 3-oxo-5β-steroid-Δ^1-dehydrogenase and 3-oxo-5β-steroid-Δ^4-dehydrogenase. *Biochim Biophys Acta* 1971; **248:** 482–8.

27 Goddard P, Hill MJ. Degradation of steroids by intestinal bacteria. IV. The aromatisation of ring A. *Biochim Biophys Acta* 1972; **280:** 336–42.
28 Drasar BS, Hill MJ. *Human intestinal flora.* London: Academic Press, 1974.
29 Silverman SJ, Andrews AW. Bile acids: co-mutagenic activity in the salmonella-mammalian-microsome mutagenicity test: brief communication. *J Natl Cancer Inst* 1977; **59:** 1557–9.
30 Thompson MH. The role of diet in relation to faecal bile acid concentration and large bowel cancer. In: Malt RA, Williams RCN, eds. *Colonic carcinogenesis.* Lancaster: MTP Press, 1982: 49–57.
31 Mower HF, Ray RM, Stemmermann GN, Nomura A, Glober GA. Analysis of fecal bile acids and diet among the Japanese in Hawaii. *J Nutr* 1978; **108:** 1289–96.
32 Sato E, Ouchi A, Sasano N, Ishidate T. Polyps and diverticulosis of large bowel in autopsy population of Akita prefecture, compared with Miyagi. High risk for colorectal cancer in Japan. *Cancer* 1976; **37:** 1316–21.
33 Mower HF, Ray RM Shoff R *et al.* Fecal bile acids in two Japanese populations with different colon cancer risks. *Cancer Res* 1979; **39:** 328–31.
34 Eneroth P, Gordon B, Sjövall J. Identification of mono- and dihydroxy bile acids in human feces by gas liquid chromatography and mass spectrometry. *J Lipid Res* 1966; **7:** 511–23.
35 Eneroth P, Gordon B, Sjöall J. Characterization of trisubstituted cholanoic acids in human feces. *J Lipid Res* 1966; **7:** 524–30.
36 Norman A, Palmer RH. Metabolites of lithocholic acid-24-^{14}C in human bile and feces. *J Lab Clin Med* 1964; **63:** 986–1001.
37 Kelsey MI, Molina JE, Huange S-KS, Huange K-K. The identification of microbial metabolites of sulfolithocholic acid. *J Lipid Res* 1980; **21:** 751–9.
38 Borriello SP, Owen RW. The metabolism of lithocholic acid-3α-sulphate by human fecal bacteria. *Lipids* 1982; **17:** 477–82.
39 Hikasa Y, Tanida N, Ohno T, Shimoyama T. Fecal bile acids and microflora in the patients with adenomatous polyps of the large bowel. *Scand J Gastroenterol* 1982; **17** suppl 78: A299.
40 Borriello SP. *Clostridial flora of the gastrointestinal tract in health and disease.* University of London: PhD Thesis, 1981: 188–9.
41 Finegold SM, Flora DJ, Attebery HR, Sutter VL. Fecal bacteriology of colonic polyp patients and control patients. *Cancer Res* 1975; **35:** 3407–17.
42 Mudd DG, McKelvey STD, Norwood W, Elmore DT, Roy AD. Faecal bile acid concentrations of patients with carcinoma or increased risk of carcinoma of the large bowel. *Gut* 1980; **21:** 587–90.
43 Van der Werf SDJ, Nagengast FM, Van Berge Henegouwen GP, Huybregts AWM, Van Tongeren JHM. Colonic absorption of secondary bile acids in patients with adenomatous polyps and in matched controls. *Lancet* 1982; **1:** 759–62.
44 Murray WR, Blackwood A, Trotter JM, Calman KC, MacKay C. Fecal bile acids and Clostridia in the aetiology of colorectal cancer. *Br J Cancer* 1980; **41:** 923–8.
45 Cruse JP, Lewin MR, Ferulano GP, Clark CG. Experimental evidence against the bile salt theory of colon carcinogenesis. *Eur Surg Res* 1981; **13:** 117–24.
46 Moskovitz M, White C, Barnett RN *et al.* Diet, fecal bile acids, and neutral sterols in carcinoma of the colon. *Dig Dis Sci* 1979; **24:** 746–51.
47 Setchell KDR, Gilbert JM, Lawson AM. Fat and cancer. *Br Med J* 1983; **286:** 1750.