

## Alimentary tract and pancreas

# Intracolonic environment and the presence of colonic adenomas in man

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**SUMMARY** A promoting effect of large bowel contents on colonic carcinogenesis as seen in the animal model is still incompletely explored in man. We investigated simultaneously deoxycholate absorption (as marker of colonic mucosal exposure to tumour promoting bile salt metabolites), mouth-anus transit time, and the ratio of anaerobic to aerobic bacteria in stool in 10 persons with colonic adenomas and in 10 age matched control subjects. We found that anaerobic/aerobic ratios and colonic deoxycholate absorption were higher in patients with colonic adenomas ( $p < 0.002$  and  $p < 0.001$ ) and that these parameters were clearly interrelated, which also applied to intestinal transit times and the anaerobic/aerobic ratios. These data are consistent with a promoting effect of the intracolonic environment on development of adenomas in man. Long term induction of a more aerobic colon flora and shortening of intestinal transit time may diminish bile-salt induced tumour promotion in adenoma patients.

High faecal bile acid concentrations in the presence of high numbers of some species of anaerobic bacteria, able to dehydrogenate the bile acid nucleus, have been reported in patients with colon cancer.<sup>1,2</sup> This led to renewed interest in the theory that formation of (co)-carcinogenic bile acid metabolites in the large bowel and subsequent mucosal exposure to these products may be involved in colonic carcinogenesis in man.<sup>3</sup> Later studies could not confirm that patient groups with colonic carcinoma or with colonic adenoma (having a high risk of colon cancer) could be identified by counts of bile acid degrading bacteria.<sup>4,5</sup> Both  $7\alpha$ -dehydroxylation, the initial step in bile acid nucleus conversion, however, and the ratio of anaerobic to aerobic – that is, facultative anaerobic – bacteria proved to be increased in their stool<sup>4,6</sup> reflecting better growth conditions and higher enzymatic activity (*in vitro*) of their anaerobic faecal flora.

The view that colonic stasis evidenced by a slow gut transit may have an additional influence is still controversial.<sup>7,8</sup> Other data supporting the bile acid hypothesis have been confined almost exclusively to animal and *in vitro* experiments.<sup>9,10</sup>

In our studies of colonic carcinogenesis in man we

used endogenous deoxycholate (DCA) absorption as a marker of mucosal exposure to secondary bile acids. Deoxycholate absorption can be assessed from the daily DCA input into the circulating bile acid pool using isotopic bile salt studies in bile.<sup>11,12</sup> An enhanced colonic DCA absorption could indeed be shown in adenoma patients.<sup>13</sup> Another study objective was investigation of possible relationships of colonic DCA absorption with quantitative data of the anaerobic and aerobic faecal flora as indicators of the colonic microenvironment.<sup>14</sup>

This report describes these additional findings in adenoma patients at high risk of colonic carcinoma as compared with control subjects matched for age and (as closely as possible) also for intestinal transit time because of their influence on DCA absorption shown previously.<sup>15</sup>

## Methods

### SUBJECTS

Ten patients with histologically proven colonic adenomas participated in the study. All were at high risk for colon cancer on account of severe epithelial dysplasia (five patients) and/or size (mean diameter  $\pm$  SD:  $1.5 \pm 0.7$  cm) and number of their adenomas (six patients with two or more recurrent adenomas),<sup>16</sup> but none suffered from adenomatous polyposis or had any other illness. No laxatives,

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antibiotics, sedatives, hypnotics, or oestrogens were used. Ten healthy volunteers without medication, matched for age and with comparable gut transit times, served as control subjects. Eligibility required guiac negative stools, a non-compromised intestinal, hepatic, renal, and gall bladder function and normal fasting serum lipids. Basal data are given in Table 1.

#### STUDY DESIGN

All subjects were studied as outpatients. Food intake was, starting one week before the isotopic bile salt study, individually and carefully standardised.<sup>15</sup> Each participant passed in this period one stool, using the toilet of the laboratory, which allowed immediate microbiological processing. Deoxycholate metabolism was investigated subsequently using a previously reported isotope dilution method.<sup>11, 12</sup> After intravenous administration of 10  $\mu$ Ci sodium [24-<sup>14</sup>C] deoxycholate fasting duodenal bile (less than 2 ml) was aspirated after cholecystokinin induced gall-bladder contraction on the next five mornings.

Mouth-anus transit time was measured during this period with radio-opaque pellets.<sup>15, 17</sup>

#### ANALYSES

##### Bile

Pool sizes and fractional turnover rates of DCA, allowing calculation of DCA input rate, were derived from its semilogarithmic specific activity decay curves in bile.<sup>18</sup> The required measurements of DCA in each bile sample (0.5 ml) were performed by gas-liquid chromatography after purification by thin layer adsorption chromatography and conversion into trifluoroacetate derivatives of its methyl esters.<sup>19, 20</sup> <sup>14</sup>C-radioactivity was determined by scintillation counting.<sup>12</sup>

Previously reported methods for examination of fasting serum lipids were used.<sup>21, 22</sup>

##### Faecal microbiology

A weighted stool sample of approximately one gram was introduced without delay into an anaerobic

Table 1 Basal data on investigated subjects (mean  $\pm$  SD)

	Patients	Controls	p
Number of subjects	10	10	
Age (yr)	50 $\pm$ 9	50 $\pm$ 9	
Sex ratio (male/female)	6/4	5/5	
Relative weight (%) <sup>*</sup>	103 $\pm$ 11	105 $\pm$ 18	NS
Serum triglyceride (mmol/l)	1.43 $\pm$ 0.54	1.19 $\pm$ 0.76	NS
Serum cholesterol (mmol/l)	5.8 $\pm$ 1.0	4.7 $\pm$ 1.1	<0.05
Gut transit time (h)	72 $\pm$ 31	67 $\pm$ 40	NS

NS: not significant.

<sup>\*</sup> As compared with ideal weight:  $\frac{\text{actual weight (kg)}}{\text{length (cm)} - 100} \times 100$ .

glove box.<sup>23</sup> After rapid preparation of a series of faeces dilutions in saline, amounts of 100 microlitre were pipetted from the appropriate homogenised suspensions (10<sup>-5</sup>, -6, -7 and 10<sup>-8</sup>, -9, -10 for aerobic and anaerobic cultures respectively) and spread onto an agar medium, enriched with sheep blood (7%).<sup>24</sup> One of these (duplicate) sets was incubated aerobically (one to two days), the other anaerobically (one week).<sup>25</sup> The total counts of colony forming units are given as their logarithms.<sup>26</sup>

Direct microscopic counts were obtained according to Holdeman and Moore<sup>27</sup> and compared with the total of anaerobic colony forming units to define the recovery of vital anaerobes. Culture yields of less than 15% were not reproducible and were not accepted for evaluation (Table 2).

Faecal pH was measured in another part of the stool after saline dilution (v/v: 1/1) using a pH electrode (Radiometer Copenhagen Pm22r).

#### STATISTICS

Differences between both groups were analysed with the Wilcoxon's rank sum test, significance of associations was derived from Spearman correlation coefficients.<sup>28</sup>

#### Results

Input rates of DCA into the bile acid pool were

Table 2 Data on colonic deoxycholate absorption, faecal microbiology, faecal pH, and faecal water content in patients with adenomatous polyps and control subjects (medians and ranges)

	Patients (n=10)	Controls (n=10)	p
Deoxycholate absorption ( $\mu$ mol/kg/d)	3.6 (2.5-7.5)	1.9 (1.5-3.8)	<0.001
Total anaerobic bacteria (g/dry weight) <sup>*</sup>	11.31 (10.49-11.49)	11.07 (10.23-11.87)	NS
Total aerobic bacteria (g/dry weight) <sup>*</sup>	8.16 (5.62-8.64)	8.20 (7.56-9.92)	NS
Anaerobic/aerobic ratio <sup>*</sup>	3.22 (2.23-5.00)	2.66 (1.95-3.68)	<0.002
Recovery of vital anaerobes (%)	30 (15-81)	38 (15-97)	NS
Faecal pH	6.9 (6.2-8.0)	7.1 (6.7-7.5)	NS
Faecal water (%)	72 (66-78)	76 (66-83)	NS

NS: not significant.

<sup>\*</sup> Data transformed into Log<sub>10</sub>.

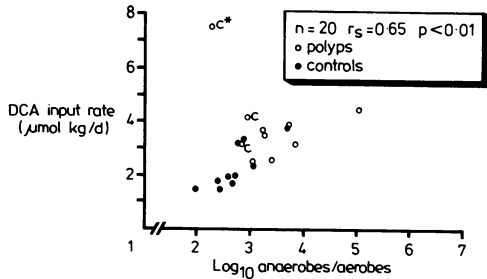


Fig. 1 Scatter diagram of anaerobic/aerobic ratio and deoxycholate absorption in patients with adenomatous polyps (○) and control subjects (●). C: previous cholecystectomy, \*: see text (discussion), DCA: deoxycholate.

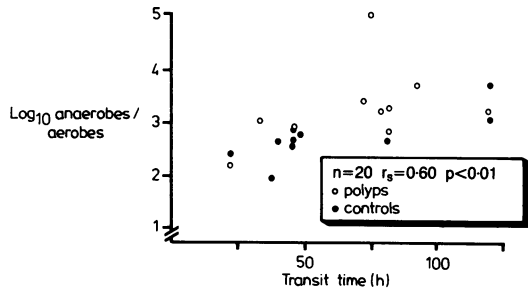


Fig. 2 Scatter diagram of gut transit time and anaerobic/aerobic ratio in patients with adenomatous polyps (○) and control subjects (●).

higher in adenoma patients than in control subjects (medians 3.6 and 1.9 μmol/kg/d,  $p < 0.001$ , Table 2 and Fig. 1), whereas total anaerobic and aerobic counts showed no significant distinction (Table 2). The ratio of anaerobes to aerobes in adenoma patients, however, proved to exceed that in control subjects (medians 3.22 and 2.66, a 3.6 times greater predominance of anaerobes,  $p < 0.002$ , Table 2).

In contrast with a lack of correlation of both total anaerobic and total aerobic counts with colonic DCA absorption (both  $r_s < 0.37$ , NS) was the clear relationship between the anaerobic/aerobic ratio and DCA absorption ( $r_s = 0.65$ ,  $p < 0.01$ , Fig. 1). No difference in faecal pH was observed (Table 2); however, slower intestinal transit times were associated with higher anaerobic/aerobic ratios (Fig. 2).

## Discussion

This study shows an enhanced DCA absorption and a greater predominance of the anaerobic faecal flora (as judged from the anaerobic/aerobic ratio) in patients with colonic adenomas. In addition, it shows that these parameters are interrelated, which

applied to patients as well as to control subjects (Fig. 1, Table 2). This favours a link between a colonic environment well suited to the growth of anaerobic bacteria and exposure of colonic mucosa to tumour promoting bile acid metabolites. The results of this study are also consistent with epidemiological reports of relatively low ratios of anaerobic to aerobic faecal bacteria in populations from Africa and Asia with a low risk of colon cancer.<sup>29</sup> The marked predominance of faecal anaerobes in adenoma patients is in agreement with other studies.<sup>4, 8</sup>

Both low and high anaerobic/aerobic ratios have been described in faecal cultures of colon cancer patients.<sup>8, 30</sup> These apparently conflicting results can be reconciled by assuming that large bowel tumours may accelerate gut transit in some cases, which appears to be accompanied by a lower anaerobic/aerobic ratio of the gut flora according to our data. This may apply to those colon cancer patients, who present with a history of more frequent bowel motions.

Three of the adenoma patients had a previous cholecystectomy, another possible risk factor of colon cancer justifying their inclusion into the patient group. Exclusion of the data from these subjects did not affect essentially the statistical analysis of the results reported.

The rapid intestinal transit in one of our cholecystectomised adenoma patients (\* Fig. 1) seems to be secondary to an increased turnover of her cholic acid pool,<sup>13</sup> a possible effect of cholecystectomy.<sup>31</sup> The ensuing increase in DCA formation enhances colonic water secretion<sup>32</sup> and intestinal motility<sup>33</sup> and also suppresses growth of anaerobic bacteria,<sup>34</sup> which may account for the rapid transit and low anaerobic/aerobic ratio in this patient.

Our finding that a rapid mouth-anus intestinal transit was associated with a more aerobic flora in all subjects (Fig. 2) might be ascribed to a lack of extensive intracolonic stasis. Experimental data on anaerobic colonisation of self-filling, but not of self-emptying blind loops leave no doubt on such effect of intestinal stasis.

The impact of slow intestinal transit on colonic carcinogenesis as emphasised by Burkitt<sup>6</sup> may be attributed to a more anaerobic intracolonic environment, which appears to enhance mucosal exposure to secondary bile acids (Fig. 1). Although the mean intestinal transit of our adenoma patients was slower than in our control subjects (Table 1) the difference was small and not statistically significant. In our opinion it may be a contributory but not the sole underlying mechanism of the higher DCA absorption in our adenoma patients. This accords with our earlier observation that DCA absorption

and gut transit time are only clearly correlated in young adults.<sup>15</sup>

Rapid formation of DCA in the caecum is also dependent on an alkaline pH and may facilitate DCA absorption from the colon. As it is unlikely that faecal pH will reflect always the pH in the caecum, we were not surprised that no correlation could be detected between DCA absorption from the colon and the pH in a fresh stool sample of our subjects (Table 2).

In conclusion, this study reveals a relationship of DCA absorption, anaerobic growth conditions in the large bowel and gut transit. Mucosal exposure to bile acid metabolites appeared to be higher in patients with adenomas and was associated to their more anaerobic gut flora as compared with control subjects. These data suggest that induction of a more aerobic bacterial flora may lead to a less tumour promoting environment in the large bowel, a possibility which should be further explored. Previous observations on the influence of dietary composition on faecal flora have shown that this may require rather long term studies.<sup>35 36</sup>

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