The faecal ratio of lithocholic acid to deoxycholic acid may be an important aetiological factor in colorectal cancer

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There is strong evidence from metabolic epidemiology correlating the incidence of CRC in populations with high FBA concentrations and the carriage of bacteria able to degrade bile acids (Aries et al., 1969; Reddy & Wynder, 1977). Case control studies have proved equivocal and the reasons for this have been discussed by Hill (1981). Several studies (Narisawa et al., 1974; Silverman & Andrews, 1977; Wilpart et al., 1983) have yielded strong evidence implicating the major FBA, DCA and LA, as promoters of colon carcinogenesis.

We have adapted (Owen, 1981) the methods of Alme et al. (1977) and applied them to the fractionation of faecal extracts in a CRC versus control study. Faecal extracts were obtained by two-stage Soxhlet extraction using petroleum ether (40-60°) and chloriform/methanol (1:1) to remove sequentially neutral and acidic steroids respectively. The extracts were fractionated by anion-exchange column-chromatography on diethylaminohexypropyl-Sephadex into neutral steroids, free bile acids, glycine-conjugated, tauro-conjugated and sulphated steroids. The results of the bile acid analyses are reported here.

After fractionation the bile acids were quantified by g.l.c. on a Pye 304 gas chromatograph fitted with a 3% OV-1 column, using a temperature gradient from 160 to 260°C at 4°C/min. The bile acids were methylated with ethereal diazomethane (De Boer & Backer, 1954) and methyl 7α,12α-dihydroxy-3-oxo-5β-cholanoic acid was incorporated as an internal standard before g.l.c.

The results show (Table 1) that the primary bile acids CDCA and CA were not detected in faecal extracts, the major bile acids present being DCA and LA. There was no statistical difference between the CRC and control group in the faecal excretion of total bile acids. However, the faecal concentration of LA was significantly higher \( (P < 0.05) \) in the CRC group compared with the control group.

The most striking difference in the excretion patterns between the study groups was evident in the ratio of LA to DCA. The ratio was significantly higher \( (P < 0.01) \) in the CRC group \( (1.43 \pm 0.72) \) compared with the control group \( (0.72 \pm 1.10) \). On a percentage basis, 75% of the CRC group excreted more DCA than LA, whilst 85% of the control group excreted more LA than DCA, although the difference was not statistically significant.

In conclusion this study shows that the LA : DCA ratio in faeces may be an important discriminant marker for CRC susceptibility. What factors influence the LA : DCA ratio have yet to be elucidated. Because faecal bile acid profiles reflect hepatic bile acid synthesis it indicates that individuals who are susceptible to CRC synthesise more DCA than CA in the liver, the reverse being true in non-susceptibility. A low LA : DCA ratio occurs consistently in healthy individuals (R. W. Owen, unpublished work) and thus it is imperative to elucidate which factors influence the synthesis of CDCA and CA in the liver.

The detection of this new marker for CRC susceptibility may now help to elucidate more clearly which factors influence the onset of CRC. Evidently FBA analyses must be related directly to dietary cholesterol intake and hepatic and biliary bile acid profiles. This is especially true since vegetarians who have a low intake of dietary cholesterol have a very low incidence of CRC (Turjman et al., 1981).

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Abbreviations used: CA, cholic acid; CDCA, chenodeoxycholic acid; CRC, colorectal cancer; DCA, deoxycholic acid; FBA, faecal bile acids; g.l.c., gas-liquid chromatography; LA, lithocholic acid.

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Table 1. Composition of major faecal bile acids in the study groups

<table>
<thead>
<tr>
<th>Bile acid</th>
<th>CRC group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Cholic acid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>3.6 ± 0.8</td>
<td>3.5 ± 0.5</td>
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<tr>
<td>Lithocholic acid</td>
<td>4.7 ± 1.1*</td>
<td>2.3 ± 0.3</td>
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<tr>
<td>Total bile acids</td>
<td>9.3 ± 1.8</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>LA</td>
<td>1.43 ± 1.22†</td>
<td>0.72 ± 1.10</td>
</tr>
</tbody>
</table>

ND, Not detected. Values are means ± S.E.M. and are expressed in mg/g of faecal dry weight except for the ratio. Statistical analysis was conducted by Students t-test; *significantly different from control group \( (P < 0.05) \); †significantly different from control group \( (P < 0.01) \).