Faecal bile acid loss and bile acid pool size during short-term treatment with ursodeoxycholic and chenodeoxycholic acid in patients with radiolucent gallstones

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SUMMARY Twelve non-obese patients with radiolucent gallstones were fed on a standard diet. After 10 days (period A), six patients received 15 mg/kg/day of ursodeoxycholic acid (UDCA) (group I) and the other six (group II) the same dose of chenodeoxycholic acid (CDCA) for 15 days (period B). An intravenous injection of 200 Ci of 14C-UDCA and of 14C-CDCA was given on the 11th day of period B to patients of group I and II respectively. Stools were collected at the end of period A and B and one bile sample was collected on the 12th day of period B. The faecal bile acid loss was higher during chenotherapy (36.12 μmol/kg/day) than during ursotherapy (23.94 μmol/kg/day), as was the proportion of lithocholic acid (73% vs 43%) in the faeces. Decay constant rate of faecal radioactivity was 0.365 day⁻¹ in group I and 0.642 in group II. The results indicate that faecal bile acid excretion and turnover rate are greater during CDCA than UDCA, while UDCA increases the bile acid pool size to an even greater extent than does CDCA (150.2 vs 94.9 μmol/kg). This is probably because the former is more slowly degraded to poorly reabsorbable compounds. In fact, the bile saturation index was 0.66 in group I and 1.05 in group II, even though biliary CDCA in the latter had risen to 69.6%.

Ursodeoxycholic acid (UDCA), the 7βepimer of chenodeoxycholic acid (CDCA), decreases the saturation of bile with cholesterol (Makino et al., 1975) and dissolves cholesterol gallstones using one-half to two-thirds of the doses normally used for chenotherapy (Maton et al., 1977; Salvioli et al., 1977; Mak no and Nakagawa, 1978). The fact that the intestinal metabolism of cheno and urso are different may be the cause of their different efficacies—for example, in vitro the 7βOH group is more slowly degraded by intestinal bacteria than is the 7αOH group, so that UDCA is less degraded to lithocholic acid (LCA) than is CDCA (Fedorowski et al., 1977a). The in vivo importance of such observations in the effectiveness of UDCA therapy is not known.

In the present study we have examined the effects of short-term feeding of UDCA and CDCA on (1) the bile acid pool size, and (2) faecal bile acid loss, on the assumption that the faecal excretion of bile acids indicates their fractional turnover rate.

Methods
Reference compounds (unconjugated and conjugated bile acids) were supplied from Supelco, Inc. 24-14C-CDCA and 24-14C-UDCA were purchased from Radio chemical Centre, Amersham; the radio-chemical purity was shown to be greater than 98% by thin-layer chromatography (TLC). The UDCA and CDCA administered to patients were supplied by Zambon spa (Bresso, Italy). The enzymatic reagent kit for fluorimetric determination of total bile acids was supplied by Nyegaard & Co, A/S, Oslo. Sulphated lithocholic acid and sulphated tauro- and glycolithocholic acid were synthesised according to the method of Eysen et al. (1976) and each was purified by TLC (solvent system chloroform:methanol:acetic acid:water 65:24:15:4 by volume) (Cass et al., 1975).

Protocol
Twelve non-obese inpatients (seven women and five men) with radiolucent gallstones were fed during the study on a standard diet (30 calories/kg; carbohydrates, 52%; lipids, 27%; and protein, 21%), containing 500 mg/day of Cr2O3 as an inert marker.
The daily cholesterol intake was about 500 mg. After 10 days (period A) six patients (group I) received UDCA (15 mg/kg/day) for 15 days (period B) and an intravenous infusion of 20 \( \mu \)Ci \( ^{14} \)C-UDCA on the 11th day of the period B. The other six patients (group II) received CDCA and an infusion of \( ^{14} \)C-CDCA at the same doses. Bile samples were collected one day after the injection with labelled bile acid in order to determine the bile acid pool, bile acid composition, and the saturation index. Stools were collected and weighed on the last three days of period A and on the last five days of period B; the samples were stored at \(-20^\circ\text{C}\) until analysed. No antibiotics were given during the course of the study.

**QUANTIFICATION OF BILE ACID POOL SIZE AND SATURATION INDEX**

The bile acid pool size was measured according to the one-day method of Duane et al. (1975). Fasting bile samples were obtained with a single-lumen polyvinyl tube positioned radiographically in the descending portion of the duodenum; gallbladder contraction was stimulated by intravenous cerulein 0·1 \( \mu \)g/kg (Farmitalia, Milan). Only a small aliquot (<5% of the total aspirate) of bile-rich duodenal aspirate was used and the remainder was returned via the tube. Bile acid mass and radioactivity were determined from a corresponding aliquot of methanolic extracts of bile. The biliary lipid composition (cholesterol, phospholipids, and bile salts) was expressed in molar percent and the saturation index (SI) was calculated from polynomial equation (Thomas and Hofmann, 1973) describing the cholesterol solubility line proposed by Hergardt and Dam (1971). The saturation index was not corrected according to the correction factor developed by Carey (1978) for UDCA-rich bile.

Biliary bile acid composition was determined by gas liquid chromatography (GLC); 0·05 ml of bile was hydrolysed with cholesteryl glycine hydrolase (Sigma Chemical Co.) for three hours at 37°C in buffer acetate pH 5·6. The internal standard (3 \( \times \) 12\% dihydroxy-7-one- 5 \( \beta \)cholan-24oic acid) was added after hydrolysis. The incubation mixture was acidified to pH 1 and the bile acids extracted with \( 3 \times 50 \) ml diethyl ether. The combined extracts, dried \textit{in vacuo}, were methylated with an excess of diazomethane for 15 minutes; the analysis was carried out on Packard-Becker 420 dual flame ionisation detector instrument, using 2 m \( \times \) 3 mm i.d. glass column (U shaped) packed with QF-1 3% on Gas-Chrom 100–200 mesh, kept at 220°C; the injection and detector temperature were 235°C and 245°C respectively.

**EXTRACTION OF BILE ACIDS FROM FAECAL SAMPLES**

Faeces were homogenised with a known amount of saline; 5 ml of faecal homogenates were refluxed for one hour at 70°C with NaOH 100 mmol in ethanol 70%. After cooling, 50 ml petroleum ether was added three times in order to extract the neutral sterols. The lower ethanolic phase was evaporated and the bile salt extracted with 5 g Amberlite XAD-2 by a batch procedure (Van Berge Henegouwen and Hofmann, 1976). The elution of bile acids and their derivatives was obtained with two 35 ml hot methanol washes. An aliquot of methanolic phase was used for measuring total radioactivity (Figure). Cr\(_2\)O\(_3\) was determined on faecal sample aliquots according to the method of Bolin et al. (1952).

**SEPARATION OF SULPHATED AND NON-SULPHATED BILE ACIDS**

The purified bile acid extracts were separated on a Sephadex LH-20 column (Figure) into two fractions of different polarities: the first fraction, eluted with 70 ml chloroform:methanol 1:1 containing 0·01 M NaCl, contained unconjugated bile acids (fraction I); the second fraction, eluted with 60 ml methanol, contained sulphated bile acids (fraction II). Because the sulphate fraction can be contaminated by trace amounts of non-sulphates, the efficacy of the separation was checked by rechromatography of the sulphate fraction on Sephadex LH-20. Total bile acids of fraction I and II (after solvolysis as described below) were determined with an enzymatic method and results were corrected daily for faecal flow by reference to the excretion of the inert marker chomatic oxide (Davignon et al., 1968).
ANALYSIS OF BILE ACID COMPOSITION OF FAEces

The faecal bile acid molar percentage of the second and third day stool samples of period A and B was determined by GLC as above. If fraction I is acidified and extracted with diethylether, it is possible to measure the percentage of unconjugated bile acids in faeces. According to this test, fraction I contained only a small amount of conjugated bile acids. This agrees with the analysis carried out on silica gel plates, developed in the system isooctane:isopropyl ether:acetic acid (50:25:25). Fraction II was subjected to solvolysis for 24 hours with acetone: methanol:HCl 10 N (9:1:0:1) at 37°C in order to cleave sulphate esters: the resulting mixture, neutralised with NH₄OH, was purified and desalted by Amberlite XAD-2 batch. Bile acids in the extracts thus obtained were quantified using the enzymatic method. Qualitative examination by TLC demonstrated that the extracts contained only non-sulphated bile acids. At the beginning of the procedure 5 μCi of 14C-CDCA were added to faecal samples of period A only, in order to check the efficiency of the extraction. The mean recovery of added CDCA was 92±6±3:8% after the purification on Amberlite and 79:7±7:6 after column chromatography and enzymatic hydrolysis.

MEASUREMENT OF RADIOACTIVITY

Total radioactivity was estimated on day 1, 2, 3, 4, and 5 after labelled injection using the method as below. Daily excretion rate of 14C, relatively to the Cr₂O₃ mass intake and excretion, was expressed according to the formula of Stanley and Nemchausky (1967). The decay constant (day⁻¹) of 14C-CDCA and 14C-UDCA was determined using a single-compartment kinetic model (Lindstedt, 1957).

The distribution of radioactivity in the different bile acids (cholic, CDCA, UDCA, deoxycholic, LCA, and 7-ketolithocholic acid) identified by reference to known standards run in parallel, was determined by TLC on the bands which were visualised by spraying with Bili Spra (8-hydroxyl pyrenetrusulfonic acid salt) (Supelco, Inc., Bellafonte, Pa.) and scraped off the plates, using a liquid scintillation counter; quenching was estimated by adding 14C-hexadecane as internal standard to the counting vials. This procedure was carried out only on the second day stool sample.

STATISTICAL ANALYSIS

The statistical significance of the differences between values obtained in groups I and II and during periods A and B was tested using the non-paired Student’s t test.

Results

BILE ACID COMPOSITION

The mean saturation index was lower in the group treated with UDCA (SI 0:66) than in the group taking CDCA (Table 1). The bile acid pool size was greater in UDCA-treated patients (150 μmol/kg) with respect to the group receiving CDCA (94 μmol/kg); the difference was statistically significant. The percentage composition of biliary bile acids after 10 days of treatment is shown in Table 2: the

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Weight (kg)</th>
<th>PL</th>
<th>CHO</th>
<th>BS</th>
<th>SI</th>
<th>Bile acid pool size (μmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>(molar %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>63</td>
<td>20:7</td>
<td>2:8</td>
<td>76:5</td>
<td>0:53</td>
<td>130:5</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>18:9</td>
<td>3:7</td>
<td>77:4</td>
<td>0:63</td>
<td>145:0</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>14:8</td>
<td>5:1</td>
<td>80:1</td>
<td>1:01</td>
<td>150:7</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>23:8</td>
<td>3:8</td>
<td>72:4</td>
<td>0:54</td>
<td>155:0</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>24:6</td>
<td>4:0</td>
<td>71:4</td>
<td>0:56</td>
<td>159:2</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>15:8</td>
<td>3:6</td>
<td>80:6</td>
<td>0:69</td>
<td>160:8</td>
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<tr>
<td>Mean ± SD</td>
<td></td>
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<td></td>
<td></td>
<td>0:66</td>
<td>150:2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0:18</td>
<td>11:2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td></td>
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<td></td>
<td></td>
<td>0:73</td>
<td>97:0</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>16:4</td>
<td>3:9</td>
<td>79:7</td>
<td>1:00</td>
<td>102:2</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>17:1</td>
<td>5:7</td>
<td>77:2</td>
<td>1:21</td>
<td>73:2</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>19:8</td>
<td>7:6</td>
<td>72:6</td>
<td>1:19</td>
<td>100:2</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>17:4</td>
<td>6:8</td>
<td>75:8</td>
<td>1:14</td>
<td>105:8</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>16:2</td>
<td>6:2</td>
<td>77:4</td>
<td>1:04</td>
<td>91:2</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>21:2</td>
<td>6:7</td>
<td>72:1</td>
<td>1:05</td>
<td>94:9*</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0:17</td>
<td>11:7</td>
</tr>
</tbody>
</table>

PL: phospholipids. CHO: cholesterol. BS: bile salts.

*<0:001 between group I and II; †<0:001 between group I and II.
Table 2 Effect of ursodeoxycholic acid and chenic acid treatment on biliary bile acid composition

<table>
<thead>
<tr>
<th></th>
<th>LCA (molar %)</th>
<th>DCA</th>
<th>CDCA</th>
<th>UDCA</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.8</td>
<td>10.8</td>
<td>15.9</td>
<td>53.7</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>±0.7</td>
<td>±1.7</td>
<td>±5.7</td>
<td>±8.9</td>
<td>±2.4</td>
</tr>
<tr>
<td>Group II</td>
<td>4.7</td>
<td>7.8</td>
<td>69.6</td>
<td>3.8</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>±0.9</td>
<td>±1.6</td>
<td>±8.5</td>
<td>±0.3</td>
<td>±2.3</td>
</tr>
</tbody>
</table>

The daily faecal bile acid mass of the ACID AND SULPHATED ACID EXCRETION group receiving UDCA in the first period was reported to the FAECAL BILE ACID PATTERN IN FECES group.

The percentage of lithocholic acid was higher in group II where CDCA became the major bile acid; in group I, although UDCA was the major bile acid fraction, CDCA and cholic acid were well represented.

FAECAL BILE ACID EXCRETION

The daily faecal bile acid loss, corrected by reference to the daily excretion of the inert marker Cr2O3, is reported in Table 3. During treatment the faecal bile acid loss grew to 23.94 µmol/kg/day in the group receiving UDCA and to 36.12 µmol/kg/day in the group treated with CDCA.

SULPHATED AND NON-SULPHATED BILE ACID IN FECES

The mass of sulphated faecal bile acid amounted to 13.5% and to 10.3% of total bile acid in period A and B respectively. The percentage rose to 17.4 during ursotherapy and to 22.3 during chenotherapy (Table 3).

Table 3 Sulphated and non-sulphated bile acids excreted with faeces before (period A) and during (period B) UDCA (group I) or CDCA (group II) administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Non-sulphated</th>
<th>Sulphated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(µmol/kg/day)</td>
<td>(µmol/kg/day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>8.12</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.02</td>
<td>±0.21</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>19.77*</td>
<td>4.17*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±3.27</td>
<td>±0.65</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>7.85</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.22</td>
<td>±0.17</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>28.07††</td>
<td>8.05††</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±5.07</td>
<td>±0.40</td>
</tr>
</tbody>
</table>

*p<0.01 between A and B of both groups I and II.
†p<0.01 between the two period Bs.
‡p<0.001 between A and B of group II.

for groups I and II respectively. The percentage rose to 17.4 during ursotherapy and to 22.3 during chenotherapy (Table 3).

BILE ACID PATTERN IN FECES

The composition of the non-sulphated fraction was very similar to that of the sulphated fraction (Table 4); LCA percentage was higher in patients taking UDCA, group II CDCA.

Table 4 Faecal bile acid composition before (period A) and during (period B) UDCA (group I) and CDCA (group II) administration

<table>
<thead>
<tr>
<th>Period</th>
<th>Fraction</th>
<th>LCA (molar %)</th>
<th>DCA</th>
<th>CDCA</th>
<th>UDCA</th>
<th>7-KLCA</th>
<th>CA</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>S</td>
<td>45.6</td>
<td>39.6</td>
<td>3.7</td>
<td>1.2</td>
<td>tr</td>
<td>1.2</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>±10.5</td>
<td>±7.6</td>
<td>±1.1</td>
<td>±0.3</td>
<td>±0.3</td>
<td>±3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>41.4</td>
<td>37.2</td>
<td>5.6</td>
<td>3.2</td>
<td>1.2</td>
<td>0.8</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±9.4</td>
<td>±9.2</td>
<td>±1.7</td>
<td>±1.5</td>
<td>±0.4</td>
<td>±0.1</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>44.6</td>
<td>36.5</td>
<td>3.4</td>
<td>5.6*</td>
<td>tr</td>
<td>tr</td>
<td>9.5</td>
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<tr>
<td></td>
<td></td>
<td>±12.2</td>
<td>±7.9</td>
<td>±6.6</td>
<td>±0.8</td>
<td>±3.2</td>
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</tr>
<tr>
<td></td>
<td>B</td>
<td>NS</td>
<td>37.7</td>
<td>29.1</td>
<td>10.5†</td>
<td>9.4†</td>
<td>4.7†</td>
<td>1.1</td>
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<tr>
<td></td>
<td></td>
<td>±8.7</td>
<td>±6.1</td>
<td>±2.2</td>
<td>±2.0</td>
<td>±1.4</td>
<td>±0.1</td>
<td>±4.6</td>
</tr>
<tr>
<td>Group II</td>
<td>S</td>
<td>45.4</td>
<td>37.4</td>
<td>4.6</td>
<td>1.0</td>
<td>tr</td>
<td>1.1</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>A</td>
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<td>±7.6</td>
<td>±0.8</td>
<td>±0.2</td>
<td>±0.3</td>
<td>±3.8</td>
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</tr>
<tr>
<td></td>
<td>NS</td>
<td>38.5</td>
<td>39.6</td>
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<td>1.4</td>
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<td></td>
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<td>±9.2</td>
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<td>S</td>
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<td>12.4†</td>
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<td>±3.4</td>
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<td></td>
<td>B</td>
<td>NS</td>
<td>70.5*</td>
<td>8.5*</td>
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<td>1.7</td>
<td>2.7</td>
<td>0.5</td>
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<tr>
<td></td>
<td></td>
<td>±13.8</td>
<td>±3.1</td>
<td>±1.9</td>
<td>±0.4</td>
<td>±0.8</td>
<td>±0.2</td>
<td>±2.3</td>
</tr>
</tbody>
</table>

*P<0.001 between period A and B.
†p<0.01 between period A and B.
‡p<0.001 between period B of group I and period B of group II.
S: sulphated. NS: non-sulphated.
Group I received UDCA, group II CDCA.
Table 5  Decay constant rate of faecal radioactivity and stool weight in patients taking UDCA (group I) or CDCA (group II)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Decay constant rate (day⁻¹)</th>
<th>Stool weight (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.358</td>
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</tr>
<tr>
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<td>0.403</td>
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</tr>
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<tr>
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<td>0.401</td>
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</tr>
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<td>6</td>
<td>0.345</td>
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</tr>
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<td>3</td>
<td>0.762</td>
<td>156</td>
</tr>
<tr>
<td>4</td>
<td>0.490</td>
<td>110</td>
</tr>
<tr>
<td>5</td>
<td>0.796</td>
<td>135</td>
</tr>
<tr>
<td>6</td>
<td>0.705</td>
<td>150</td>
</tr>
<tr>
<td>Mean</td>
<td>0.642*</td>
<td>138.8</td>
</tr>
<tr>
<td>±SD</td>
<td>0.135</td>
<td>26.2</td>
</tr>
</tbody>
</table>

*p < 0.001.
Stool weight is the mean value over a period of five days.

CDCA in both the sulphated and non-sulphated fractions.

FAECAL RADIOACTIVITY
The time course of faecal excretion of ¹⁴C after intravenous injection of ¹⁴C-UDCA and ¹⁴C-CDCA is shown in Table 5; the decrease of radioactivity remaining in the body was more rapid in patients taking CDCA. The decay constant was 0.365 day⁻¹ in group I and 0.642 in group II; the differences were statistically significant. Such changes occurred independently of any significant variation in stool weight. The stool weights before treatment (mean of three days) were 116.4 ± 15.5 in group I and 128.6 ± 19.7 (g/day) in group II.

Table 6 shows the distribution of radioactivity in the faecal bile acids of groups I and II the second day after the injection of labelled bile acids. The proportion of radioactivity found in the LCA fraction was greater in group II than in group I. During the following days the proportion of LCA radioactivity increased in group I also.

A portion of injected UDCA was excreted unchanged and 12.1% of total radioactivity was present in 7 keto LCA. The percentage of total radioactivity was higher in the non-sulphated fraction both in group I (87.6% ± 17.4) and in group II (81.5 ± 19.3).

Discussion
The results of this short-term study confirm in vivo the previous findings of Fedorowski et al. (1977a) that UDCA is more slowly degraded than is CDCA and that less LCA is formed by bacteria enzymes from UDCA. More faecal bile acid are excreted during short-term chenotherapy than during ursotherapy, the ¹⁴C retention being larger in the UDCA treated group given ¹⁴C-UDCA than in the CDCA group given ¹⁴C-CDCA. For these reasons the pool size may become greater after 10 days of ursotherapy than after the same period of chenotherapy. The study was a deliberately short-term one, so that patients could be followed in hospital during the experiment.

BILIARY LIPID COMPOSITION
Even though the cholesterol saturation index at the end of period A has not been determined, its value after treatment is lower in patients taking UDCA. From one to four weeks of treatment with CDCA are required for patients with cholesterol gallstones to produce an unsaturated bile (Iser et al., 1977). The bile desaturating capacity of UDCA appears to be greater than that of CDCA. In the experience of Maton et al. (1977a) a dose of 5 mg/kg/day of UDCA is sufficient to desaturate bile.

Bile acid pool size is greater in group I; in fact, during chenotherapy, the change in bile acid pool size is due to the increase in the CDCA pool and to the considerable decrease in the cholic and deoxycholic acid pools (Danzinger et al., 1973). During ursotherapy the pool size increases because CDCA, deoxycholic and cholic acids are slightly decreased and UDCA represent more than half the pool.

FAECAL BILE ACID LOSS
The mass of total bile acids in faeces is measured
Faecal bile acid loss and bile acid pool size during short-term treatment with UDCA and CDCA

using an enzymatic method which therefore does not estimate the bile acid conjugated at the C-3 position. However, if solvolysis is carried out, the detection of bile acids is complete, as the 3β-hydroxy bile acids do not have quantitative importance, as they represent only 4–5% of total faecal bile acids (Subbiah et al., 1973). The faecal bile acid loss is higher in patients given CDCA than UDCA. Although the results refer to a non-steady state equilibrium, they indicate that CDCA is eliminated more rapidly in the intestine. The mechanism for the more rapid elimination of CDCA than UDCA is not known. It is unlikely that rapid intestinal transit plays an important role, as faecal bile acid excretion increases only slightly when intestinal transit time is shortened by the oral administration of mannitol (Meihoff and Kern, 1968).

The increased percentage of LCA and sulphated products in the faeces of patients taking CDCA is consonant with the higher faecal bile acid excretion. With regard to the presence of bile acid sulphated in the human faeces, Podesta et al. (1978) reported that this fraction accounted for 27.4% of total bile acids in control subjects, and 25–60% and 17–22.5% in patients given UDCA and CDCA respectively. Intestinal flora such as Pseudomonas aeruginosa (Imperato et al., 1977) may desulphate bile acids (Cowen et al., 1975) and thus alter the proportion of bile acids excreted as sulphates. In our study the composition of sulphated and non-sulphated faecal bile acids is similar, but LCA accounts for about 40% of total bile acid in patients taking UDCA, whereas in patients treated with CDCA it accounts for 70%. Fedorowski et al. (1978) have found results similar to ours in patients fed with 1 g/day of ursodeoxycholic acid, whereas Makino and Nakagawa (1978) reported that LCA percentage in faeces increases to 60% of total bile acids after long-term treatment with low doses of UDCA. In the experience of Podesta et al. (1978) the percentage of total LCA was 33.7% of faecal bile acids in the controls, 24.0–45.7 after UDCA, and 48.5–54.5 after CDCA. The presence of unchanged UDCA in the faeces of group I indicates the possibility that this escapes intestinal degradation, as it would seem from its high faecal radioactivity (Table 6). Like Fedorowski et al. (1978) our results show that in patients fed with UDCA, 7-ketolithocholic acid represents about 10% of non-sulphated faecal bile acids. More unknown peaks are present on chromatograms; these act as dihydroxy bile acid on TLC.

EXCRETION OF RADIOACTIVITY

Total radioactivity
The patients taking CDCA had strikingly and significantly more rapid 14C excretion rates than those of the UDCA group. It seems that this result does not depend on a greater faecal weight or a greater bowel frequency. The higher decay constant rate of CDCA compared with UDCA indicates a different fractional turnover rate, equivalent to the daily fractional excretion rate. The validity of the faecal excretion method for determining bile acid turnover rates is well established (Beher et al., 1966). Our result, obtained over a period of 10 days, probably depends on the different metabolic pathway of the two epimers, as demonstrated from the greater percentage and radioactivity of LCA in CDCA-treated patients.

When large quantities of CDCA are given, a new steady state is attained in which the percentage of total pool as CDCA rises to 94.5%, the fractional turnover rate of CDCA, measured from specific decay curve in bile, becomes 0.59 day⁻¹ (0.27 before therapy), and the faecal excretion of bile acid increases (Danzinger et al., 1973). UDCA given at doses of 1 g/day increases its proportion to only about half of total biliary bile acids. In contrast with the equivocal effect of CDCA, UDCA clearly expands the bile acid pool (Fedorowski et al., 1978b). These authors show that during UDCA feeding, 91% of the UDCA pool turned over each day, whereas our results show a higher decay of CDCA with respect to UDCA, because CDCA is more extensively degraded to poorly absorbable compounds in the intestine. In this way, more bile acids are excreted daily and the pool size is only slightly increased because of the induced inhibition of other bile acid synthesis.

Distribution of radioactivity
In Rhesus monkey more than 95% of the faecal radioactivity after 14C-UDCA administration was present in the fraction of LCA, whereas the activity in the UDCA fraction was less than 5%; however, in this animal, the intestinal flora seems capable of carrying out the 7β-dehydroxylation of UDCA (Ota et al., 1977). In the man the intestinal metabolism of the 7βOH group of UDCA is different from that of the 7αOH group of CDCA (Fedorowski et al., 1977b). When 14C-UDCA is given intravenously to subjects fed 1 g/day of UDCA, the specific activity versus time decay of biliary UDCA and CDCA reveals a precursor-product relationship, whereas a relatively minute amount of CDCA is converted to UDCA. Our results demonstrate a higher LCA radioactivity in patients treated with CDCA both in the sulphated and non-sulphated fractions; in patients treated with UDCA, 7 keto LCA contains a high percentage of radioactivity (12.1%), while about 20% of total radioactivity is present in unknown fractions.
In conclusion, our results, although obtained during short-term therapy, show that UDCA is less degraded to poorly absorbed bile acid metabolites than is CDCA, so that small doses of UDCA may be effective in maintaining an expanded pool of bile acids.

References


