Improved Human Faecal Bile Acid Extraction

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There is good experimental and epidemiological evidence linking increased faecal bile acid excretion with increased risk of developing colorectal cancer (1,2,3,4). Case control studies give far less clear cut differences and methodological problems have been suggested as a major source of discrepancies (5).

The extraction of faecal bile acids for GLC-FID analysis is a complex and labour intensive task. A number of different extraction methods have been developed, but the most widely accepted, and that leading to the fewest methodological distortions of individual or group concentrations is Setchell's 1983 method (6). Setchell described a modification of Alme's (7) urinary bile acid group separation using Lipex-DEAP. We describe a minor modification to Setchell's liquid-gel and liquid-solid extraction/separation techniques prior to GLC analysis.

Using established methodology (6) with 3H-cholic acid and nordeoxycholic acid as dual internal standards, recoveries for faecal bile acids from Golden Syrian Hamsters were >85% in 36 of 50 (72%) samples. However, using identical equipment and techniques from humans (colorectal cancer patients, polyps, and controls) was unsatisfactory with >85% recovery in only 12/56 (21.4%) samples. The apparent difference in recovery rates appeared to be due to much higher faecal fat excretion in the human faeces.

In an attempt to improve human faecal bile acid recoveries a preliminary hexane wash was incorporated prior to Setchell extraction method (6).

Approximately 0.2 gms of homogenized lyophilized faeces equivalent to 1/100 daily output was resuspended in either 30 ml of hexane/water (2:1 vv) or 30 ml of hexane:ethanol:water (10:4:1 w) and sonicated for 15 minutes. The solutions were allowed to stand for 30 minutes to allow the biphasic mixture to partition. The hexane supernatant containing fats and neutral sterols was removed and retained for analysis with the final neutral sterol extract. The residual water or ethanol water extract contained free and conjugated bile acids, which were then extracted in the standard fashion (6). Seven radioactive sterols were used to assess relative partitions.

The results (Table 1) indicate that the hexane:ethanol:water (10:4:1 vv) wash is a highly selective and reproducible neutral sterol wash. Using this wash prior to standard extraction techniques improved the subsequent human faecal bile acid extractions significantly. Acceptable recoveries (defined as >85% recovery) improved from 21.4% of sample analysed to 77.3% (n=44). It has the added theoretical advantage of reducing fatty acid-bile transesterifation that is known to take place during the refluxing phases. Since the hexane wash is returned to the neutral sterol fraction prior to GLC-FID analysis there is no loss of this fraction.

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**REFERENCES**


**TABLE 1**

<table>
<thead>
<tr>
<th>Radioactive Sterol</th>
<th>Hexane:Water</th>
<th>Hexane:Ethanol:Water</th>
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</thead>
<tbody>
<tr>
<td>14C C-oleate</td>
<td>17.32% (3.1)</td>
<td>85.68% (4.3)</td>
</tr>
<tr>
<td>3H CA</td>
<td>17.32% (0.02)</td>
<td>0.028% (0.01)</td>
</tr>
<tr>
<td>14C DCA</td>
<td>10.6% (0.12)</td>
<td>1.9% (0.21)</td>
</tr>
<tr>
<td>14C CDCA</td>
<td>0.75% (0.19)</td>
<td>1.1% (0.23)</td>
</tr>
<tr>
<td>14C LCA</td>
<td>26.86% (5.13)</td>
<td>4.76% (1.13)</td>
</tr>
<tr>
<td>14C GCA</td>
<td>0.288% (0.08)</td>
<td>0.43% (0.02)</td>
</tr>
<tr>
<td>14C TCA</td>
<td>0.0762% (0.03)</td>
<td>0.12% (0.015)</td>
</tr>
</tbody>
</table>

The use of a hexane:ethanol:water wash appears to be a quick, simple and useful methodological improvement for human faecal bile acid extraction.