A Study of the Ratios of Bile Salt Conjugates of Glycine to Taurine in the Jejunum and Ileum in Patients with Tropical Sprue

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Ratios of glycine to taurine conjugates of dihydroxy and trihydroxy bile acids were estimated in intestinal juice in 6 control subjects and 8 patients with tropical sprue. Elevated ratios were found in the patients with tropical sprue as compared to the control subjects, at jejunal levels. In the patients, ratios were reduced in the ileum. Follow-up in 2 patients in remission showed a normal ratio in the jejunum in each case. These findings are compatible with an interference of bile salt transport in patients with sprue.

Key-words: Bile salts; glycine/taurine ratio; tropical sprue
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The pathogenesis of steatorrhoea in tropical sprue has not been fully elucidated. Alterations in bile salt metabolism have been shown to contribute to the steatorrhoea in various forms of secondary malabsorption (6, 16, 18, 19), and it is possible that such may occur in tropical sprue. In a previous study, it was demonstrated that bile salt deconjugation and dehydroxylation does not occur to any significant extent in patients with tropical sprue (11). The aim of the present investigation was to study the relative concentrations of glycine and taurine conjugates of dihydroxy and tribhydroxy bile acids at different levels of the small intestine in patients with tropical sprue and compare these with the findings in normal subjects.

SUBJECTS AND METHODS
Six control subjects and 8 patients with tropical sprue were studied in a metabolic ward. All subjects were on a diet containing 50 g of fat per day. Repeat studies during periods of remission were performed on 2 subjects.

CLINICAL INVESTIGATION
The following investigations were carried out. The daily excretion of stool fat was estimated by the method of van der Kamer, Huinink & Weyers (10) in all subjects and the results expressed as a 3-day running mean. d-Xylose absorption was tested by measuring urinary excretion in the first five hours following a 5 g oral dose. Vitamin B12 absorption was measured in every subject either by the urinary excretion method of Schilling (17) (normal 7 per cent excretion or more of administered dose), or by

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measuring the rise in serum radioactivity 8 hours after an oral dose of radioactive vitamin B₁₂ (13) (normal 0.2 per cent of administered dose/litre plasma). In each case a 1 µg dose of Co₄₀ labelled vitamin was employed. Jejunal biopsies using a Crosby capsule and barium meal examinations using non-flocculating barium were done in all subjects. The barium meals were graded using the criteria of Paterson, David & Baker (15).

**Sampling technique**

After an overnight fast a double lumen, radiopaque, polyvinyl tube 400 cm long, weighted with a mercury bag and with an attached balloon which could be inflated via one lumen, was passed per mouth under fluoroscopic control. The balloon was inflated after the tip of the tube had reached the upper jejunum. Samples were collected from the upper jejunum (just beyond the ligament of Treitz, 75 to 110 cm from the incisor teeth), lower jejunum (140 to 200 cm), upper ileum (220 to 250 cm), and lower ileum (280 to 315 cm). Samples were collected on ice and stored at -20 °C till further use. A liquid diet was given only after lower jejunal collections were completed.

**Quantitative analysis**

After thawing, the samples of intestinal juice were processed by precipitation of proteins by repeated ethanolic extraction and the removal of extraneous lipids by partitioning with n-heptane.

The individual bile salt conjugates in each sample were quantitated by the method of Gänshirid, Kous & Merianez (7) using thin-layer chromatography on 20 × 20 cm plates coated with 250 µ thickness silica gel G (E. Merck). The individual bile salt conjugates were identified by comparison with a reference chromatogram run on the same plate and sprayed with 5 per cent phosphomolybdic acid in ethanol and developed by heating at 100° C for 15 minutes. In many of the controls and patients the concentration of bile salts in the lower

<p>| Tab I. Details of control subjects (1 to 6) and patients with tropical sprue (7 to 14) |
|---|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of symptoms (weeks)</th>
<th>Fat excretion g/day</th>
<th>Xylose excretion % in 5 hrs.</th>
<th>Vitamin B₁₂ absorption (%)</th>
<th>Barium meal (grade)</th>
<th>Jejunal biopsy microcopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1 24</td>
<td>M</td>
<td>0</td>
<td>2.7</td>
<td>26</td>
<td>N</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>F</td>
<td>0</td>
<td>4.5</td>
<td>34</td>
<td>N</td>
<td>0</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>F</td>
<td>0</td>
<td>4.2</td>
<td>20</td>
<td>N</td>
<td>0</td>
<td>L</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>M</td>
<td>0</td>
<td>4.0</td>
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<td>0</td>
<td>L</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>M</td>
<td>0</td>
<td>4.7</td>
<td>42</td>
<td>N</td>
<td>0</td>
<td>L</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>M</td>
<td>0</td>
<td>3.7</td>
<td>27</td>
<td>N</td>
<td>0</td>
<td>L</td>
</tr>
<tr>
<td>Patients</td>
<td>7 45</td>
<td>M</td>
<td>81</td>
<td>21.0</td>
<td>0</td>
<td>N</td>
<td>II</td>
<td>C</td>
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<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>162</td>
<td>32.0</td>
<td>6</td>
<td>N</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>M</td>
<td>162</td>
<td>28.0</td>
<td>1</td>
<td>N</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>M</td>
<td>7</td>
<td>10.0</td>
<td>6</td>
<td>A</td>
<td>I</td>
<td>L</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>F</td>
<td>8</td>
<td>15.0</td>
<td>9</td>
<td>A</td>
<td>I</td>
<td>L</td>
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<tr>
<td>12</td>
<td>23</td>
<td>M</td>
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<td>26.0</td>
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<td>A</td>
<td>I</td>
<td>R</td>
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<td>M</td>
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<td>A</td>
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<td>C</td>
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<tr>
<td>14</td>
<td>19</td>
<td>M</td>
<td>100</td>
<td>18.0</td>
<td>9</td>
<td>A</td>
<td>I</td>
<td>C</td>
</tr>
</tbody>
</table>

N = Normal; A = Abnormal; L = Leaver; R = Ridges; C = Convolutions; MGH = Mild glandular hypertrophy; GH = Glandular hypertrophy; PVA = Partial villous atrophy.
DISCUSSION

In man, the ileum is the site of active transport of bile acids (4), consequently ileal resection and ileal disease may cause interference with bile salt absorption resulting in a depletion of the body bile acid pool. The symptomatic and biochemical alterations resulting from this dysfunction in bile salt metabolism have been termed the 'steroid wasting syndrome'. In this condition steatorrhoea is a common manifestation (9, 18), and there is an increase in the ratio of glycine to taurine conjugates of dihydroxy and trihydroxy bile salts in the upper small intestine (8, 12).

The findings in this study have demonstrated an elevation in the ratio of glycine to taurine conjugates in intestinal juice collected from upper and lower jejunal levels in patients with tropical sprue, as compared with control subjects. In each case in the ileum the ratios were lower (Fig. 1). In two cases studied in remission the ratios even in the jejunum were normal (Table II). The precise mechanism responsible for the elevated ratio of glycine to taurine conjugates in jejunal bile must at the present juncture remain speculative. It is probable that this is a result of ileal disease. It is well known that patients with tropical sprue often have other evidence of ileal disease, such as histological abnormalities (3) and malabsorption of vitamin B₁₂ which is not corrected by the simultaneous administration of intrinsic factor (2). This ileal dysfunction may result in a reduction of the active transport of bile salts in the ileum. Passive non-ionic diffusion would then contribute a relatively greater amount to bile salt reabsorption. The pKₐ values of the glycine and taurine conjugates (9) may contribute to this observed ratio.

RESULTS

All control subjects had stool fat excretion less than 5 g per 24 hours, normal xylose and vitamin B₁₂ absorption and normal barium meals (Table I). Jejunal biopsies in control subjects were consistent with those seen in the general population (5).

All patients with tropical sprue had steatorrhoea ranging from 10 g to 32 g per day. All had d-xylose malabsorption and abnormal jejunal biopsies, and 5 had vitamin B₁₂ malabsorption. Investigations did not reveal any other cause for the malabsorption.

The mean ratio of glycine to taurine conjugates of dihydroxy and trihydroxy bile salts in the upper jejunum was 2.1 in the controls and 6.2 in the patients (Fig. 1). The difference between these means is significant at the 0.5 per cent level. In the lower jejunum, in controls the mean was 2.0 and in the patients 6.9. In the upper ileum the mean was 1.7 in the controls and 3.6 in the patients.

Table II. The ratios of glycine to taurine conjugates in the upper jejunum in 2 patients during the initial admission and during remission

<table>
<thead>
<tr>
<th>No.</th>
<th>Initial G/T ratio</th>
<th>Follow-up G/T ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(months)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>7.4</td>
<td>3.1</td>
</tr>
<tr>
<td>12</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6.3</td>
<td>1.5</td>
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</tbody>
</table>
taurine conjugates would result in preferential absorption of the former at the existing physiological pH in the gut, leading to an increase in the ratio of glycine to taurine conjugates in the jejunum, lowering of the ratio in the ileum as compared with the jejunum supports the concept of preferential absorption of glycine conjugates.

Garbutt et al. (8), in their work on ileal resection and disease, have clearly implicated the role of altered hepatic synthesis to explain the increase in the ratio of glycine to taurine conjugates. They have also suggested the possible role of a selective deficit of available taurine. Work is currently in progress to investigate the role of these two factors in tropical sprue.

In the present study there was no correlation between the ratio of glycine to taurine conjugates and the degree of steatorrhoea. It is therefore not clear what role, if any, the observed abnormalities play in the pathogenesis of the steatorrhoea. There was also no correlation between vitamin B12 absorption and the ratios. This is not surprising, however, in view of the different mechanisms of absorption of vitamin B12 and bile salts from the intestinal lumen. Since this work was undertaken a brief report (20) on six Puerto Rican subjects with tropical sprue, has shown reductions in bile salt pool size in four, but the ratios of glycine to taurine conjugates are not given. It is probable that this reduction in pool size is also a manifestation of the ileal lesion of tropical sprue.

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