The Increased Rate of Loss, and Non-Exponential Excretion of Bile Salts in Patients with Tropical Sprue and Ileectomy

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Summary: Cholic acid-carboxyl14C was administered to five control subjects, seven patients with tropical sprue and two patients with ileal resection and the daily excretion of the label in the faeces measured for seven to 13 days. Contrary to previous assumptions, the excretion was not always exponential. The practice of calculating decay constants from faecal excretion studies is therefore not valid.

Four out of seven patients with sprue had an increased rate of excretion of the label, which in two subjects was similar to that of the patients with ileal resection. This indicates defective reabsorption of bile salts and may contribute to the pathogenesis of the steatorrhoea of tropical sprue.

Tropical sprue has been defined as a syndrome of intestinal malabsorption of unknown aetiology, occurring in residents of, or visitors to, certain areas of the tropics. Although steatorrhoea of different degrees occurs in the majority of subjects with tropical sprue, its pathogenesis has not as yet been fully elucidated.

A previous study of intraluminal bile salts in control subjects and in patients with tropical sprue has shown that an increase in the ratio of glycine to taurine conjugates occurs in the jejunum, but in the ileum the ratios are similar to those in control subjects. It was postulated that this altered ratio in the jejunum could be the sequela of bile salt malabsorption in the ileum which is the site of active bile salt transport in man. When the ileum is diseased, passive reabsorption from the jejunum is increased. The physiological pH of the gut, favours the reabsorption of glycine conjugates, which are thus conserved to a greater extent than taurine conjugates. This results in a higher ratio of glycine conjugated bile acids to taurine conjugated ones in the upper small intestine.

The present study was undertaken to test this hypothesis of ileal bile salt malabsorption in tropical sprue.

Methods
Five control subjects, seven patients with tropical sprue and two patients with ileal resection were studied. The control subjects were apparently healthy individuals. The patients with tropical sprue had features typical of that disease and all other causes of malabsorption were excluded. The ileal resections were done, in one case for mesenteric thrombosis and in the other several years previously for a non specific granulomatous lesion of the ileum. Prior informed consent for the investigations was obtained from all subjects. They were hospitalised in a metabolic ward and given a diet containing 50-60g of fat per day. All stools were collected and the daily fat...
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<tr>
<th></th>
<th>Fat Excretion g/day</th>
<th>Xylose Excretion % 5 hrs</th>
<th>Vitamin B₁₂ Absorption % dose/hour plasma</th>
<th>Barium meal (grade)</th>
<th>Jejunal biopsy</th>
<th>Rate of loss of label</th>
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L = Leaves; C = Convolutions; MGH = Mild glandular hypertrophy; GH = Glandular hypertrophy; PVA = Partial villous atrophy; TVA = Total villous atrophy.

Excretion measured by the method of van der Kaaer et al. Xylose absorption was tested using a 5g dose of D-xylose and measuring the five hour urinary excretion. Vitamin B₁₂ absorption was tested by giving a 1 μg dose of 14C vitamin B₁₂ (0.5 μCi) and measuring the rise in plasma radioactivity after eight hours. Jejunal biopsies were done using a Crosby capsule. Barium meals were graded using the criteria of Paterson et al.

After an overnight fast the subjects were given 5 or 10 μCi of cholico acid-carboxyl14C. The cholico acid was dissolved in alcohol and injected into the tubing of a freely running intravenous saline infusion. Twenty-four hour stool collections were made for seven to 14 days. The faecal radioactivity was measured by the method of Radhakrishnan and Bicker. Each twenty-four hour collection was thoroughly homogenised. A tared aliquot of approximately 20 g of the homogenate was treated with 0.5 ml of 2N sodium hydroxide and 4 ml of 30 per cent hydrogen peroxide in a flask and allowed to stand, with frequent agitation, until it was decolourised. A tared aliquot of this was then added to a dioxane based scintillation mixture containing a thiazotrope gel and counted at 4°C in a Packard Tricarb liquid scintillation counter. Allowance was made for quenching by the channel ratio method, checked from time to time by the addition of an internal standard. Using this technique recovery experiments were carried out by addition of a known amount of radioactivity to a variety of stool specimens. Nine different stools tested in duplicate gave recoveries ranging from 87.0 to 99.8% (mean 94.9%, SD 3.2). The amount of radioactivity in each day's stool was calculated. The percentage of the label retained in the body at the end of each twenty-four hour period was calculated by subtracting the cumulative excretion from the amount given. For each subject decay curves were made by plotting the percentage remaining in the body on a logarithmic scale, against time on a linear scale.

Results

Intraintestinal structure and function

The control subjects had no evidence of malabsorption and their barium meals and jejunal biopsies were within the normal range for this population. The patients with tropical sprue all had steatorrhoea, xylose malabsorption,
barium meal and jejunal biopsy changes. Five also had vitamin $B_{12}$ malabsorption. The patients with ileal resection had steatorrhoea and vitamin $B_{12}$ malabsorption. The full details of all subjects are given in Table 1.

Rate of loss of label

The decay curves in the five control subjects fell between those of P.T. and A.N. shown in Figure 1. The two patients with ileal resection showed a rapid decline in radioactivity (Fig. 1). In three patients with tropical sprue (K.A., M.S. and I.N.) the decay curves are similar to those of the control subjects. In two (D.V. and S.W.) the excretion of the label appears moderately increased and in two others (K.N. and G.V.) the decay curves are in the range of the two patients with ileal resection (Fig. 2).

In one control subject with a slightly more rapid decline in radioactivity (A.N.), in four patients with tropical sprue (I.N., D.V., K.N. and G.V.) and in both patients with ileal resection, the decay curves are not linear.

Discussion

It had initially been assumed that the excretion of the $^{14}C$ label would follow an exponential pattern as has been described by other workers. However in this study the two ileectomised subjects, four patients with tropical sprue and one control had decay curves which were not exponential. This is most clearly seen in the subjects with the greatest initial losses. It appears that in the above cited papers the patients with rapid excretion were not followed long enough to allow for the clear demonstration of non-linearity. The $^{14}C$ labelled cholate initially is converted into both $^{14}C$ taurocholate and $^{14}C$ glycocholate. Subsequently those primary bile acids that escape reabsorption in the ileum are subject to degradation by colonic microorganisms. A proportion of these metabolites then undergo enterohepatic recirculation. In studies which measure the faecal excretion of the radioactivity, the manner of distribution of the label between the primary bile acids and the metabolites is unknown. The metabolites may well have differing rates of recirculation. In such a complex situation an exponential decay of the label is scarcely to be expected and it is not justifiable to calculate rate constants. In this study the rate of loss of the label has therefore been designated as normal (N), moderately increased (+) and markedly increased (++) (Table 1).

In the patients with tropical sprue the decay curves of $^{14}C$ labelled cholic acid range from normal to being similar to that seen in subjects with ileal resection (Figure 2). The increased rate of excretion of the label could theoretically result from very rapid passage through the ileum or from ileal disease. In mannitol-induced diarrhoea, in which there is rapid passage through the ileum, studies have shown only a
moderately increased rate of excretion of bile salts. However the decay curves in patients G.V. and K.N. (Fig. 2) indicate a considerably more rapid excretion of the label than could be accounted for on this basis. Furthermore, passage through the intestine, as seen on the barium meal, was either normal or delayed in all seven cases. The rapid excretion must therefore be the result of ileal disease.

The only other study of bile salt metabolism in tropical sprue using isotopically labelled material appears to be that of Turner et al. This was a preliminary report in six Puerto Rican subjects, four of whom had a slightly shortened half-time of injected $^{14}$C cholate in duodenal bile. The authors attributed this finding to an ileal lesion.

Low-Beer et al. studying patients with coeliac disease showed that labelled bile acid remained in the enterohepatic circulation for longer than normal. They attribute this to failure of adequate contraction of the gall bladder due to cholecystokinin deficiency resulting from upper small intestinal disease. The fact that none of their patients showed a decrease in the time the label was in the enterohepatic circulation suggests that their patients did not have a significant ideal lesion. It is possible that subjects with sprue may also have cholecystokinin deficiency, though this has not been studied. If so, this would tend to decrease the rate of excretion of the label, and mask, to some extent the effect of the ileal lesion.

The four sprue patients in the present study with rapid excretion of the label had other evidence of ileal disease in that they also had defective absorption of vitamin B$_{12}$ which was not corrected by intrinsic factor. Of the three patients with tropical sprue who had decay curves that fell within the normal range, two had a normal vitamin B$_{12}$ absorption and one an abnormal absorption. This would indicate that defective vitamin B$_{12}$ absorption and defective bile salt reabsorption may occur independently of each other.

There was no correlation between the degree of steatorrhoea and the normality or otherwise of the decay curves. Nevertheless the two patients (G.V. and K.N.) who had the most rapid excretion had marked steatorrhoea and it is probable that excessive bile salt loss is important in the pathogenesis of steatorrhoea in some cases of tropical sprue. A direct correlation is not to be expected because other factors, such as the damage to the jejunal mucosal cells which interferes with fatty acid absorption, must also be significant in its pathogenesis.

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References