DISACCHARIDASE LEVELS IN JEJUNAL BIOPSY SPECIMENS FROM AMERICAN AND SOUTH INDIAN CONTROL SUBJECTS AND PATIENTS WITH TROPICAL SPRUE*

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SUMMARY

Disaccharidase activities have been studied in biopsy specimens obtained from control Indian and American subjects and from Indian patients with tropical sprue. The values in the Americans were similar to those of Caucasian subjects reported in the literature. Lactase, maltase and trehalase values in the Indian controls were significantly lower than the values in the Americans, but there was no difference in the sucrase. Lactase levels were uniformly low in the Indian controls, indicating a very high prevalence of lactase "deficiency". In patients with sprue the maltase, sucrase and trehalase levels were significantly lower than in the control groups. Enzyme levels along the length of the intestine of a sprue patient, obtained at post mortem, were decreased to a greater extent in the proximal region.

The enzymes responsible for splitting disaccharides are present in the brush border region of the intestinal cells. The enzymes have been shown to be diminished or absent in various disaccharide intolerance states both congenital and acquired. Racial differences in enzyme levels have also been described. The present study was undertaken to compare and contrast the disaccharidase levels in jejunal biopsy specimens from American subjects, Indian control subjects and Indian patients with tropical sprue.

MATERIALS AND METHODS

Three groups of subjects were studied. The first comprised 30 American control subjects studied within 10 days of their arrival in India, and the second consisted of 38 Indian control subjects matched with the patient group with respect to dietary habits and socio-economic status. None of the control subjects had a history of

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chronic diarrhoea and in all, fat and vitamin \( B_{12} \) absorption were normal. Xylose excretion was normal (25% or more) in the majority, but in 2 American and 10 Indian subjects xylose excretion was only 15–25% of the administered dose.

The third group comprised 22 Indian patients with tropical sprue—either endemic or epidemic. All these patients had a history of chronic diarrhoea with malabsorption of fat and xylose, together with histological and radiological changes typical of this condition. Sixteen also had vitamin \( B_{12} \) malabsorption.

All subjects were hospitalized and studied in a metabolic ward. Jejunal biopsies were obtained from just beyond the ligament of Treitz, with a Crosby capsule, passed under fluoroscopic control. The biopsy fragment was divided into two, one was used for pathological examination and the other was placed immediately in ice-cold 1.15% KCl for enzyme studies.

The portion of the specimen for enzyme studies was gently blotted, weighed and homogenized in 1 ml of 1.15% KCl for 1 min at 0–5° using a glass microhomogenizer (Kontes) placed in ice. Sucrase, maltase, trehalase and lactase activities were determined by the Tris–glucose oxidase procedure of Dahlqvist as described previously, with the corresponding disaccharide as substrate (final concentration, 0.028 M) in 0.1 M sodium phosphate buffer, pH 6.0. Protein was estimated by the procedure of Lowry et al. using crystalline bovine serum albumin as the standard. One enzyme unit is defined as the amount of enzyme required to hydrolyze one \( \mu \)mole of disaccharide per min, at 37° under the assay conditions. Disaccharidase levels are expressed as enzyme units per g wet weight of tissue or as units per g of protein.

The portion of the biopsy for pathological examination was fixed in Bouin’s fixative. Each specimen was examined under the dissecting microscope, and then processed by standard histological techniques. In the sprue patients the biopsies were placed into one of two groups—“group A”, those with more severe changes where the glands occupied half or more than half the distance from the base of the glands to the villus tip, and “group B”, those with less severe changes, where the glands occupied less than half this distance.

In one patient, who died during an acute exacerbation of tropical sprue, the intestine was obtained at post-mortem examination within 2 h of death. The intestine was divided into 10 equal segments and processed as described earlier.

RESULTS

In the Americans, dissecting microscopic examination of the jejunal biopsy showed a mixture of finger- and leaf-shaped villi in all except four subjects who had only leaves. Histologically the majority appeared normal, but in 5 there was some increase in the thickness of the glandular layer and a slight increase in cellular infiltration of the lamina propria. The Indian control subjects showed minor abnormalities similar to those in the normal population. Nine showed a convoluted mucosal pattern and the remainder had leaf-like villi. Histologically two were similar to the American biopsies and the remainder had varying degrees of mild glandular hypertrophy and reduction in villus height. The patients with sprue showed changes similar to those described previously. Seventeen had a convoluted mucosa and the remainder

had leaf-like villi. Ten had severe histological changes (group A) and the other 12 had less severe changes (group B).

The individual enzyme levels (units/g of tissue) in the biopsy specimens are shown in Fig. 1 (a–d). The means of the values are given in Tables I and II. In the Indian controls the values for maltase, trehalase and lactase, expressed either way, were significantly lower than the corresponding values in the American subjects. In the patients with sprue the sucrase and maltase values, expressed either way, were lower than in the Indian controls. The trehalase values expressed per gram of tissue were also significantly lower in the sprue patients, but this was not evident when expressed on a protein basis.

![Fig. 1 (a–d). Scattergrams of the disaccharidase activities in the jejunal biopsy specimens from American and Indian controls and in patients with tropical sprue. (a) Maltase, (b) sucrase, (c) trehalase and (d) lactase.](image)

When the sprue patients were divided into the two groups A and B, according to the severity of the histological changes, the following mean values (units/g tissue) were obtained: sucrase, A, 1.7; B, 2.7 ($P > 0.05$); maltase, A, 6.5; B, 6.6 (N.S.); trehalase, A, 0.39; B, 0.52 (N.S.) and lactase, A, 0.1; B, 0.3 (N.S.). In the sprue group there was no detectable correlation between enzyme levels and the dissecting micro-

TABLE I
DlSACCHARIDASE ACTIVITIES IN BIOPSY SPECIMENS (enzyme units/g tissue)
The mean values ± SD are given. The P values are for the difference in the means between the
groups on either side of the column; NS = non-significant.

<table>
<thead>
<tr>
<th>Maltase</th>
<th>Sucrase</th>
<th>Trehalase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Americans</td>
<td>23.2 ± 12.4</td>
<td>5.9 ± 3.7</td>
<td>2.9 ± 2.3</td>
</tr>
<tr>
<td>Indian controls</td>
<td>13.2 ± 7.6</td>
<td>4.4 ± 2.3</td>
<td>1.2 ± 1.2</td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
<td>NS</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NS  = non-significant.

TABLE II
DlSACCHARIDASE ACTIVITIES IN BIOPSY SPECIMENS (enzyme Units/g protein)
The mean values ± SD are given. The P values are for the difference in the means between the
groups on either side of the column; NS = non-significant.

<table>
<thead>
<tr>
<th>Maltase</th>
<th>Sucrase</th>
<th>Trehalase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Americans</td>
<td>363 ± 244</td>
<td>88.4 ± 58.7</td>
<td>43.0 ± 34.7</td>
</tr>
<tr>
<td>Indian controls</td>
<td>224 ± 126</td>
<td>80.5 ± 45.6</td>
<td>19.3 ± 22.7</td>
</tr>
<tr>
<td>P value</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
</tbody>
</table>

sloscopic appearance of the biopsy, the duration of illness, the degree of steatorrhoea,
the degree of xylose or glucose malabsorption or the presence or absence of vitamin
B₁₂ malabsorption. In the Indian controls there was no correlation between enzyme
levels and the biopsy findings or the xylose absorption.

The activities of maltase and sucrase along the length of the intestine of the
patient who died in an acute exacerbation of tropical sprue are shown in Fig. 2 com-
pared with the levels from 3 intestines of subjects dying from non-gastrointestinal
diseases. In each case the reduction in enzyme activity was relatively greater in the
proximal part of the small intestine. A similar pattern was also found with trehalase
activity.

![Fig. 2](image)

Fig. 2. The distribution of disaccharidase activities along the length of human small intestines
obtained at post mortem. Maltase (●—●), and sucrase (■—■), average values from 3 normal
controls (data from ref. 5); maltase (○—○) and sucrase (□—□) in the small intestine of a
patient with tropical sprue.

DISCUSSION

The sucrase, maltase and lactase levels in the biopsies from the Americans are similar to those found by others in Caucasian subjects\textsuperscript{3-8}. Trehalase activity has not often been studied, but the values obtained in the Americans are similar to those reported by Kerry and Townley\textsuperscript{10}.

The outstanding difference between the Indian control subjects and the Americans is in the lactase levels. If lactase deficiency is defined as less than 1 unit per g wet weight of tissue\textsuperscript{11} then 7 out of 26 American subjects tested (27%) and all of 38 Indian control subjects (100%) had lactase deficiency. Racial differences in the prevalence of low lactase levels is well documented. Negroes have a prevalence of 70–75% against a 5–20% prevalence in Caucasians studied in the same laboratory\textsuperscript{3,11}. Ugandan Bantus but not the Hamitic peoples\textsuperscript{12,13} have low lactase levels. Oriental subjects\textsuperscript{14,15} and subjects in Western India\textsuperscript{16} also have a high incidence of lactase deficiency. It is not clear whether these racial differences are hereditary in nature, or acquired, due to prolonged substrate (lactose) deprivation. In Ugandan subjects\textsuperscript{19} there was no correlation between milk intake and the lactase levels. Attempts to increase lactase activity in man by feeding lactose\textsuperscript{17}, or to decrease it by withholding lactose\textsuperscript{18} for periods of up to 6 weeks have failed, although in rats increases in lactase have been found following lactose feeding\textsuperscript{19}. Cook\textsuperscript{20} found normal lactase levels in four Baganda neonates and suggested that the high prevalence of lactase deficiency in Baganda adults was a genetically determined change which becomes overt in early life. A study of lactase levels in Caucasians deprived of lactose over a long period of time (e.g. "Vegans") might throw further light on this question.

The mean values for maltase and trehalase in the Indian controls were also significantly lower than those found in the American subjects, but there was no such difference in the sucrase values. It is probable that the reduction of maltase and trehalase activity in the Indian controls is the result of the mild intestinal damage prevalent in the community rather than being genetically determined. It also seems unlikely that the differences can be explained on differences in substrate intake.

In the Indian controls the finding of a reduction in maltase levels without concomitant reduction in sucrase levels, is of interest. Sucrase activity is known to be associated with maltase I, one of the three maltase fractions. This fraction, exhibiting sucrase, maltase and isomaltase activities has been obtained in a homogeneous state\textsuperscript{21} and comprises about 60% of the total intestinal maltase activity. The sucrase activity of this fraction corresponds to about 70% of the total maltase I, while the remainder corresponds to isomaltase activity\textsuperscript{22,23}. It is, therefore, possible that a reduction of maltase activity could occur without a similar change in sucrase levels.

In the sprue patients, lactase levels were very low but not significantly different from the Indian controls. Sucrase, maltase and trehalase (units/g tissue) were significantly lower in the sprue patients than in the Indian controls. Lowered levels of disaccharidases have also been found in patients with tropical sprue studied in Puerto Rico and America\textsuperscript{24-26}. On the other hand, in Western India, Desai et al.\textsuperscript{16} found significant depression only in lactase levels.

Depression of disaccharidase levels in the intestinal mucosa has been found in many diseases\textsuperscript{5,8} and particularly in coeliac disease, where depression of all enzymes has been found to occur\textsuperscript{8,17,28}. In both sprue and coeliac disease this reduction in

enzymes is presumably the biochemical counterpart of the morphologic changes in the mucosa. In the present study, in the sprue patients, the depression of sucrase was more marked in those with more severe histological change. Welsh et al.\(^{28}\) in patients with coeliac disease also found a greater reduction in enzyme levels in histologically more abnormal biopsies. Whether the reduction in enzyme levels is simply due to the fact that with severe histological damage there is a gross reduction in the number of mature columnar epithelial cells\(^{29,30}\), or whether there is also a reduction in the amount of enzyme per mature epithelial cell is unknown.

The one post mortem intestine studied showed a proportionately greater reduction in enzyme activity in the upper small intestine as compared with the levels lower down. This is also in keeping with the histological changes which in this specimen, as in tropical sprue in general, were less severe distally than proximally.

ACKNOWLEDGEMENTS

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REFERENCES