Enterokinase was measured in peroral intestinal biopsies showing normal histology and in those from untreated coeliac patients which showed gross villous atrophy. There was no significant difference in the specific activity of enterokinase between these two groups. These results do not support the recent hypothesis that enterokinase is a brush border enzyme, but would be consistent with the idea that it is adsorbed to the cell membrane following secretion.

Enterokinase (enteropeptidase, E.C.3.4.4.8) is the key enzyme in the activation of the pancreatic proteolytic enzymes. It converts trypsinogen into trypsin, which then activates the other zymogens—chymotrypsinogen, procarboxypeptidase, and proelastase. The commonly held belief has been that enterokinase was secreted into the intestinal lumen from the epithelium, although the exact source remained unknown. Recently, however, this view has been challenged by reports that enterokinase is in fact a brush border enzyme, ie, located in or on the membrane of the microvilli of the epithelial cells, along with other brush border enzymes, such as sucrase, isomaltase, lactase, etc. In 1970, Holmes and Lobley reported that enterokinase was localized to the brush border membrane of guinea pigs, and subsequently Nordström and Dahlqvist (1970, 1971) have confirmed this observation with rats.

It is well established (Booth, 1970) that in the mucosa of untreated coeliac patients, which shows villous atrophy, there is a marked reduction of brush border enzymes. The levels of these enzymes return towards normal on the institution of a strict gluten-free diet. This is accompanied by a slow return to normal of the villous architecture. If enterokinase is a 'true' brush border enzyme then it would be expected to show a similar effect.

**Methods and Materials**

The coeliac patients were biopsied for diagnostic purposes. The controls were normal healthy volunteers, symptomatic patients, and non-symptomatic relatives of coeliac patients who were being investigated as part of a familial study. Biopsies were obtained using a modified Crosby capsule, and the position of the tube was checked fluoroscopically. The biopsies were examined macroscopically as soon as they were obtained, and a small piece was retained and fixed for histology. The remainder was frozen and stored at −20°C until the enzyme assays were performed.

For analysis, the biopsies were weighed and homogenized in distilled water at a concentration of approximately 20 mg/ml. Disaccharidases were measured by the method of Dahlqvist (1968) and enterokinase by the method of Nordstrom and Dahlqvist (1971). Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as standard. The results of the enzyme assays are expressed as mIU/mg protein.

**Results**

The biopsies were graded histologically according to the system of McNicholl and Egan (1968). In this system the biopsies were graded from 0 to III. Grade 0 represents normal histological appearance of the mucosa, and grade III total villous atrophy or the typical 'flat' mucosa as seen in untreated coeliac patients. Grades I and II are intermediate stages of mucosal damage, but in this study only biopsies which were clearly grade 0 (normal) or grade III (total villous atrophy) were included. The control group consisted of biopsies which appeared histologically normal (grade 0), regardless of whether they were obtained from normal volunteers, symptomatic controls, or relatives of coeliac patients.

The results are shown in Table I, duodenal samples,
Enterokinase in normal intestinal biopsies and those from patients with untreated coeliac disease

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Enterokinase</th>
<th>Sucrase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0 (normal)</td>
<td>2.77 ± 1.31</td>
<td>60.2 ± 25.1</td>
<td>26.8 ± 17.8</td>
</tr>
<tr>
<td>(1.08 - 5.4)</td>
<td>(9.7 - 75.0)</td>
<td>(21.8 - 100.0)</td>
<td></td>
</tr>
<tr>
<td>Grade III (flat)</td>
<td>2.61 ± 1.28</td>
<td>14.9 ± 12.6</td>
<td>4.8 ± 5.0</td>
</tr>
<tr>
<td>(1.0 - 4.24)</td>
<td>(4.5 - 30.0)</td>
<td>(0.9 - 18.9)</td>
<td></td>
</tr>
</tbody>
</table>

Table I  Activities of enterokinase, sucrase, and lactase in duodenal biopsies

Activities are specific activities expressed as mIU/mg protein. Ranges are shown in parentheses.

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Enterokinase</th>
<th>Sucrase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0 (normal)</td>
<td>1.49 ± 0.82</td>
<td>76.5 ± 32.0</td>
<td>50.5 ± 23.1</td>
</tr>
<tr>
<td>(0.28 - 4.1)</td>
<td>(17.2 - 146.6)</td>
<td>(13.4 - 99.0)</td>
<td></td>
</tr>
<tr>
<td>Grade III (flat)</td>
<td>1.28 ± 0.88</td>
<td>11.9 ± 8.0</td>
<td>3.4 ± 2.5</td>
</tr>
<tr>
<td>(0.46 - 3.18)</td>
<td>(4.3 - 30.9)</td>
<td>(1.4 - 10.6)</td>
<td></td>
</tr>
</tbody>
</table>

Table II  Activities of enterokinase, sucrase, and lactase in jejunal biopsies

Activities are specific activities expressed as mIU/mg protein. Ranges are shown in parentheses.

and Table II, jejunal samples. The distinction was made between the two as it is known that enterokinase levels are higher in the duodenum than in the jejunum (Nordström and Dahlgqvist, 1971). Table I shows that in the duodenal samples there is a considerable reduction of the levels of sucrase and lactase in the grade III biopsies with no concomitant significant reduction in enterokinase levels. Results from the jejunal samples (Table II) show a similar trend, again a dramatic reduction of the disaccharidases in the grade III biopsies, and although there is some reduction of enterokinase, it is not statistically significant. Table III shows the enzyme levels of the grade III biopsies as a percentage of the normals. The normal values agree well with those of Dahlgqvist (1970).

Discussion

Reports suggesting that enterokinase is a brush border enzyme such as those by Lobley and Holmes (1970) and Nordström and Dahlgqvist (1971) are based on the technique of preparing brush border fractions from mucosal homogenates, and showing that there is an enrichment of both enterokinase and other well established brush border enzymes. In some cases (Holmes and Lobley, 1970; Hadorn, Steiner, Sumida, and Peters, 1971) the enrichment of enterokinase activity is considerably greater than of sucrase. These methods do not preclude the possibility that enterokinase is binding to the membranes of the microvilli, following secretion from some other locus in the intestine. Certainly the association of enterokinase with the brush border membrane is much more superficial than the other enzymes. Enterokinase is much more easily released from the membranes by the action of proteolytic enzymes (Nordström, 1972). Bile salts release enterokinase from the membranes (Hadorn et al, 1971) but also release other enzymes such as the disaccharidases (Nordström, 1972). Our results would not support the idea that enterokinase is a true brush border enzyme. If it were a true brush border enzyme, the level would be expected to be drastically reduced in biopsies of coeliac patients as is demonstrable with the disaccharidases. Our results, however, would be consistent with the binding of enterokinase to the microvillous membrane, following secretion from cells or tissues which are unaffected by coeliac disease.

By using an immunohistochemical technique, Takano, Suzuki, and Yasuda (1971) demonstrated that in the porcine intestine, there was a concentration of enterokinase in the goblet cells, as well as a diffuse distribution on the epithelial cell surface. These authors suggest that enterokinase is secreted by the goblet cells, and our results would not contradict this view. While the mucosa of coeliac patients shows severe villous atrophy, there is no evidence that there is a reduction in the concentration of goblet cells.

While undoubtedly enterokinase is found in brush border preparations, it may be premature to describe it as a 'brush border' enzyme comparable with the disaccharidases. The results also indicate that enterokinase cannot be implicated in the aetiology of coeliac disease.

We wish to thank the following from Galway...
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References


The October 1972 Issue

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