Morphology, Dipeptidases and Disaccharidases of Small Intestinal Mucosa in Vitamin B₁₂ and Folic Acid Deficiency

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Mucosal morphology and intestinal enzyme activities (dipeptidases and disaccharidases) were studied in 33 biopsies from 24 patients with vitamin B₁₂ and/or folic acid deficiency. The villi were generally shorter in patients than in controls (34 subjects). The disaccharidases and L-alanyl-L-proline dipeptidase activity were depressed in about 30% of the patients, untreated and treated. Glycyl-L-leucine and L-alanyl-L-glutamic acid dipeptidase activity was normal in untreated patients but was depressed during treatment with vitamin B₁₂.

The present studies thus give further indication on intestinal defects in folate and vitamin B₁₂ deficiency.

When studying morphological and enzymatic data obtained from small intestinal biopsies from about 300 adults, we observed that some patients had low dipeptidase and disaccharidase activity in spite of essentially normal mucosa as seen in the microscope. Most of these patients turned out to be vitamin B₁₂ and/or folic acid deficient.

Intestinal absorptive defects have been described in patients with pernicious anaemia (Haurani et al. 1964). Carmel & Herbert (1967) observed intestinal malabsorption of vitamin B₁₂ in four out of ten patients with pernicious anaemia without evidence of intestinal disease. A jejunal biopsy was performed in one patient and was found to be normal.

The present study concerns enzymatic and morphological findings in 33 biopsies from 24 patients and changes observed following treatment.

MATERIALS AND METHODS

The 24 patients were arranged in four groups.

(1) Untreated vitamin B₁₂ deficiency
11 patients, 7 male and 4 female, 16-78 years. Two patients had had a gastric resection (Billroth II) and one patient suffered from Crohn's disease.
Four patients in this group were also studied after treatment and are, therefore, also included in group 2.

(2) Treated vitamin B₁₂ deficiency
11 patients, 4 male and 7 female, 38–70 years. The patients had been treated between one day and 20 years before the biopsy. Two had had a gastric resection (Billroth II) and one had had surgery for thyrotoxicosis.

(3) Combined vitamin B₁₂ and folic acid deficiency
Three patients, 2 male and 1 female, 47–58 years. In one male patient a gastric resection had been performed. All patients were untreated and one was also studied after treatment.

(4) Folic acid deficiency
Three females, 23–60 years. One of them had Crohn’s disease. All three were under treatment at the time biopsies were performed.

Reference group. This group consisted of 34 patients (20 female and 14 male), 17–75 years with normal serum B₁₂ and folate and with normal mucosa at the duodeno-jejunal flexure. Normality was based on the following criteria: normal villous structure under the dissecting microscope, histologically normal surface epithelium and absence of focal or diffuse inflammatory changes.

Laboratory tests. The laboratory investigation included determination of serum vitamin B₁₂ (Euglena gracilis), serum and whole blood folate (Lactobacillus casei), the Schilling test, urinary excretion of FIGLU, bone marrow examination, serum iron and total iron binding capacity, urinary xylose excretion, daily faecal fat excretion on 3-day specimens and determination of acidity of gastric secretion.

Biopsy technique. The biopsy was taken under fluoroscopic control with a Crosby & Kugler capsule (1957), at the duodeno-jejunal flexure in most cases. In the five patients with gastric resection the biopsy was taken from the upper jejunum and in another patient from the ileum.

Morphological procedure. Each biopsy was oriented on a fine mesh plastic net and fixed in Bouin’s solution or in a formaldehyde solution closely followed by fixation in Bouin. After examination in a dissecting microscope the biopsy was serially cut into 5–6 μm thick sections. Alternative slides were stained with haematoxylin and erythrosin, with van Gieson stain and periodic acid-Schiff according to McManus. The best oriented central cores of the specimens were used for assessment.

Assays. Dipeptidase activity* was determined according to Josefsson & Lindberg (1965) and Lindberg et al. (1968). Disaccharidase activity was assayed as described by Dahlqvist (1968). One unit of enzyme activity is defined as the activity hydrolysing 1 μmole of substrate per min. The dipeptidases were assayed at 40°C and the disaccharidases at 37°C.

Protein was determined according to the method of Lowry et al. (1951). In accordance with earlier publications, dipeptidase activity was expressed in units per mg of nitrogen (17% of the protein) and disaccharidase activity in units per g of protein.

RESULTS AND DISCUSSION
All untreated patients had megaloblastic anaemia with serum B₁₂ values less than 100 pg/ml, and/or serum folate less than 3.0 ng/ml and whole blood folate values less than 25 ng/ml. Direct assay of serum vitamin B₁₂ before treatment had been performed in only six of the eleven patients with treated vitamin B₁₂ deficiency, but all had typical haematological findings, achlorhydria and abnormal Schilling tests. The three patients with folic acid deficiency had subnormal serum and subnormal whole blood folate values before treatment. Haematological findings were normalized in all patients when the deficient vitamin was

* Abbreviations of dipeptides used: L-alanyl-L-glutamic acid (Ala-Glu), L-alanyl-L-proline (Ala-Pro), glycyl-L-leucine (Gly-Leu), glycyl-L-valine (Gly-Val), L-glutamyl-L-proline (Glu-Pro), L-glutamyl-L-valine (Glu-Val), L-valyl-L-glutamic acid (Val-Glu) and L-valyl-L-proline (Val-Pro).
either given parenterally or by mouth, constituting the sole form of therapy given.

A total of 33 biopsies were studied, 15 from untreated and 18 from treated patients.

All the biopsies had a villous appearance under the stereomicroscope with finger and leaf-like villi predominating. As a rule, the villi were histologically short, as seen in Figure 1. In one biopsy only moderate plasma cell infiltration and slight irregularities in the epithelial cells indicated definite but slight enteropathy. The ratio between the length of the villi from the bottom of the crypts to the top of the villi and the length of the crypts (the mucosal index) was lower in patients than in the reference group (Table I). The ratio was not higher in treated patients than in untreated patients. This may be due to the fact that some patients were only treated for a short period of time and regeneration of the mucosa is a rather slowly process. In many of the patients treated for a longer period of time, the biopsy was performed because of gastrointestinal symptoms (as in the reference group), and thus, they may be a ‘negative’ selection having short villi for reasons other than B$_{12}$ or folic acid deficiency. Our finding of short villi is in agreement with those of Veeger et al. (1965), Winawer et al. (1965), Foorozan & Trier (1967), and Bianchi et al. (1970). As did Winawer et al. (1965), we observed no megalocytosis and megaloblastosis, as has been reported by Veeger et al. (1965), Foorozan & Trier (1967) and Bianchi et al. (1970).

A definite decrease in at least two of the various enzyme activities was found in 20 of the biopsies. Disaccharidase activity (maltase, isomaltase, sucrase, trehalase and lactase) was low in 5 biopsies, 2 from untreated patients and 3 from treated. Isolated lactase deficiency was found in one patient with vitamin B$_{12}$ and folic acid deficiency and in one patient with vitamin B$_{12}$ deficiency. Figure 2 shows trehalase, lactase and maltase activity in untreated and treated subjects. No significant change was noted following treatment.

Dipeptidase activity behaved somewhat differently. As shown by Figure 3, it appears that activity with Gly-Leu and Ala-Glu as substrates is lower in treated patients than in untreated patients. The same was true for activity on Gly-Val, Val-Glu and Glu-Val. On the other hand, no such change was observed for activity on Ala-Pro, Val-Pro and Glu-Pro.

Five patients were studied before and after vitamin treatment. In four of them activity on Gly-Leu decreased considerably,
whereas an increase was observed in the fifth (Figure 4). No significant change in Ala-Pro dipeptidase activity occurred, whereas disaccharidase activity (represented by trehalase in Figure 4) was unchanged or increased.

Two of the findings appear to be of special interest:

1. Disaccharidase and Ala-Pro dipeptidase activity was depressed in several patients with vitamin B₁₂ or/and folic acid deficiency.

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![Figure 2. Intestinal disaccharidases in patients with vitamin B₁₂ and folic acid deficiency.](image1)

Open symbols denote biopsy from flexura duodenojejunalis. Filled symbols denote biopsies taken more distally. Horizontal lines denote ranges of reference group.

![Figure 3. Intestinal dipeptidases in patients with vitamin B₁₂ and folic acid deficiency.](image2)

For explanation of symbols see Figure 2.
These signs of dysfunction of the small intestinal mucosa are consistent with the findings of steatorrhoea (> 5 g faecal fat/day) in 8 of 13 patients and decreased xylose absorption (< 1.5 g xylose/5 hr; 5 g per oral dose) in 7 of 11 patients. No correlation was found between morphological findings and changes in enzyme activities in individual cases.

2. Certain dipeptidase activities, especially Gly-Leu dipeptidase, were depressed during treatment with vitamin B₁₂. The depression seemed to be more pronounced when the interval between vitamin B₁₂ administration and biopsy was brief. One patient with Crohn's disease in the distal ileum was biopsied one day before, one day after, and six days after parenteral administration of 1,000 µg of B₁₂. This patient was not included in the present material because he had no anaemia and no clear B₁₂ deficiency (B₁₂/serum: 115 pg/ml). Results of the dipeptidase analyses are given in Fig-

![Figure 4. Gly-Leu and Ala-Pro dipeptidase activity and trehalase in 5 patients before and after vitamin treatment.](image)

![Figure 5. Gly-Leu and Ala-Pro dipeptidase activity before and after administration of 1,000 µg of vitamin B₁₂.](image)
A definite decrease in Gly-Leu dipeptidase activity occurred the day after vitamin B_{12} administration. The different behaviour of Gly-Leu dipeptidase and of Ala-Pro dipeptidase activity is in accordance with earlier findings in various physiological and pathological situations (Lindberg 1966, Lindberg & Karlsson 1970, Berg et al. 1970). The explanation for depressed Gly-Leu and Ala-Glu dipeptidase activity during vitamin B_{12} treatment is unknown. In contrast to the disaccharidases, which are mainly brush border enzymes, the dipeptidases also take part in the intracellular metabolism and are, thus, also localized in the cytoplasm. In this context the findings of Rosensweig et al. (1969) are of interest. These authors showed that oral folic acid in obese and normal individuals increased jejunal glycolytic enzyme activity (cytoplasmic enzymes) whereas the disaccharidases (brush border enzymes) were unchanged. Oral vitamin B_{12} and intramuscular folic acid had, however, no effect.

Corcino et al. (1970) draw attention not only to the effect of vitamin B_{12} and folate deficiency on stomach and ileum, but also to protein and iron deficiency and exposure to chemical agents as ethanol.

Protein deficiency as a major factor with vitamin B_{12} and folate as contributory factors in the pathogenesis of jejunal villous atrophy was discussed by Saraya et al. (1971).

Factors other than vitamin B_{12} and folate may have to be considered for the understanding of the present findings.

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