We examined the digestive and absorptive function of the small intestinal mucosa in three patients with pernicious anemia to determine the functional correlates of the morphologic changes previously described. Digestive brush border enzymes (disaccharidases and leucyl-naphthylamidase) in jejunal biopsy specimens from the patients followed a normal distribution compared with those in the control group. With the exception of one patient with mild steatorrhea, the rest of the absorption test results were within the normal range. Jejunal perfusion studies, however, with glucose, sodium and water showed intestinal secretion of sodium and water, i.e., net movement of sodium and water from plasma to lumen, in the presence of normal glucose absorption. Follow-up studies in two patients after treatment with vitamin B₁₂ did not reveal any significant improvement in the absorption rates from the pretreatment period. This abnormality of sodium and water transport in pernicious anemia represents another intestinal defect of a systemic disease which is not corrected by vitamin B₁₂ replacement therapy.

Morphologic alterations of the epithelial cells of the gastrointestinal tract, similar to macrocytosis in the peripheral blood and megaloblastosis in the bone marrow, have been described in patients with pernicious anemia [1–3]. Jejunal mucosal abnormalities include shortening of villi, decreased number of mitoses in the crypts and increased cellular infiltrate in the lamina propria [4,5]. Since the immature intestinal epithelium and the reduction of the absorptive surface may interfere with normal digestive and absorptive processes, it was of interest to further examine the functional significance of these morphologic abnormalities.

The purpose of this study was, therefore, to examine certain indices of intestinal digestion (brush border enzymes) and absorption (d-xylose, vitamin B₁₂). In addition, jejunal perfusion studies were performed, utilizing a test solution of glucose, sodium and water, to investigate the pattern of intestinal transport of these nutrients.

**PATIENTS AND METHODS**

The diagnosis of pernicious anemia was based on the following criteria: (1) abnormal Schilling test, i.e., urinary excretion of less than 10 per cent of an oral dose of ⁵⁷Co-labeled vitamin B₁₂; (2) gastric achlorhydria, as evidenced by the absence of gastric acid secretion, i.e., failure of pH in gastric aspirate to fall below 6 after maximal stimulation; (3) serum vitamin B₁₂ level of less than 200 pg/ml (normal range 200 to 1,000 pg/ml) and (4) megaloblastosis in the bone marrow and macrocytosis in the peripheral blood.
TABLE II  Results of Intestinal Absorption Tests

<table>
<thead>
<tr>
<th>Age</th>
<th>Serum Carotene (mg/dl)</th>
<th>D-Xylose Urinary Excretion (g/5 hr)</th>
<th>Serum Calcium (mg/dl)</th>
<th>Serum Albumin (g/dl)</th>
<th>Jejunal Biopsy (light microscopy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>44</td>
<td>58</td>
<td>4.8</td>
<td>9.5</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>45</td>
<td>4.6</td>
<td>9.0</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>74</td>
<td>5.5</td>
<td>9.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>
TABLE III Specific Activity of Brush Border Enzymes*

| Group                  | No. | Lactase | Sucrase | Maltase | Isomaltase | Trehalase | Alkaline | Phosphatase | LNA*
|------------------------|-----|---------|---------|---------|------------|-----------|----------|-------------|------
| Control                | 12  | 38 ± 18 | 85 ± 13 | 314 ± 240 | 198 ± 35 | 87 ± 15 | 290 ± 66 | N.S.        | 37 ± 5
| Pernicious anemia      | 3   | 35 ± 9  | 92 ± 16 | 345 ± 48 | 191 ± 35 | 82 ± 21 | 293 ± 110 | N.S.        | 39 ± 6
| P value                |     | N.S.    | N.S.    | N.S.    | N.S.      | N.S.     | N.S.     | N.S.        | N.S. |

* Values represent mean ± standard error of the mean. All values are given in μmol of substrate hydrolyzed per min per g protein.
N.S. = not significant.

+ LNA = leucyl-naphthylamidase.

Water absorption rates were calculated using standard formulas [20]. In two patients with pernicious anemia, jejunal perfusion studies were repeated after treatment with monthly vitamin B₁₂ injections (100 μg). The treatment period was approximately 18 months, and there was considerable hematologic and clinical improvement as compared to the pretreatment period.

RESULTS

The results of the hematologic studies are shown in Table I. All patients presented with megaloblastic, macrocytic type of anemia of varying degree, low serum vitamin B₁₂ levels and abnormal Schilling test results (excretion of 57Co-vitamin B₁₂ 10 per cent). It was of interest that in two patients 57Co-vitamin B₁₂ excretion was only partially corrected with intrinsic factor, indicating secondary malabsorption of vitamin B₁₂. Gastric achlorhydria, i.e., failure of maximal gastric pH to decrease below 6.0, was present in all patients.

The results of intestinal absorption tests are shown in Table II. The only abnormality was steatorrhea in one patient. The rest of the absorption test results were within the normal range. Light microscopy of the jejunal mucosal biopsy specimens did not reveal any significant abnormalities.

The results of intestinal absorption tests are shown in Table II. The only abnormality was steatorrhea in one patient. The rest of the absorption test results were within the normal range. Light microscopy of the jejunal mucosal biopsy specimens did not reveal any significant abnormalities.

The results of the specific activity of brush-border enzymes are summarized in Table III. Since mucosal disaccharidase activity follows a log distribution, statistical analyses of log₁₀ disaccharidase activity were performed using Student's t test (unpaired data). There was no significant difference in any of the brush-border enzyme activity in patients with pernicious anemia as compared to those in the control group. However, there was a marked net secretion of sodium and water into the intestinal lumen, a statistically significant difference compared with sodium and water net absorption rates in the control group (p <0.025).

Perfusion studies after treatment with vitamin B₁₂ in two patients demonstrated that in both patients glucose absorption was normal, but sodium and water absorption was inhibited, a pattern similar to that in the pretreatment period (Table IV).

COMMENTS

Several studies have previously shown that intestinal malabsorption may occur in patients with pernicious anemia [21–23]. However, considerable disagreement exists regarding the nutrients which are affected and the clinical significance of these abnormalities. Lindenbaum et al. [23], in a prospective study of 28 patients with pernicious anemia, found malabsorption of d-xylose in 29 per cent, fat in 9 per cent and vitamin B₁₂ with intrinsic factor in 75 per cent. Malabsorption of d-xylose and vitamin B₁₂ may be due to intestinal mucosal disease, bacterial overgrowth or both. Vitamin B₁₂ malabsorption is a well documented intestinal defect in pernicious anemia which occurs rather frequently [23,24]. Lack of vitamin B₁₂ may contribute to epithelial mucosal dysfunction and impairment of intestinal absorption.

Pena et al. [5] examined the morphologic and digestive enzyme abnormalities in jejunal biopsy specimens from untreated patients with pernicious anemia. They found shortening of villi and reduction of the absorptive surface, megalocytic changes of intestinal epithelial cells and a significant depression of mucosal

TABLE IV Glucose, Sodium and Water Absorption in Pernicious Anemia*

<table>
<thead>
<tr>
<th>Time of Study</th>
<th>No.</th>
<th>Glucose (mmol/hr/segment)</th>
<th>Sodium (meq/hr/segment)</th>
<th>Water (ml/hr/segment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>3</td>
<td>+14.5 ± 3.0</td>
<td>−42.0 ± 22</td>
<td>−428 ± 128</td>
</tr>
<tr>
<td>After treatment</td>
<td>2</td>
<td>+13.2 ± 2.6</td>
<td>−31.6 ± 26</td>
<td>−372 ± 165</td>
</tr>
</tbody>
</table>

NOTE: No statistically significant difference was detected between the pre- and post-treatment absorption rates of sodium and water. + indicates net absorption; − indicates net secretion, i.e., movement of solute and water from plasma to lumen.

* Values represent mean ± SEM.
Disaccharidases. These histologic and enzymatic defects diminished after treatment with vitamin B₁₂. We were unable, however, to demonstrate any significant reduction in any of the brush-border enzymes in jejunal biopsy specimens. It may be that alterations in the brush-border enzymes are related to the severity and duration of pernicious anemia, the nutritional status of the patients and their dietary intake. These factors probably did not play a significant role in our patients.

Since nutritional changes and morphologic abnormalities of the intestinal mucosa may affect the functional integrity of the intestinal epithelial cell, we examined glucose, sodium and water absorption utilizing the jejunal perfusion technic. We did not find any difference in jejunal glucose absorption rates between the patients with pernicious anemia and the control group. This is in contrast to the findings reported by Groen in 1938 [25], who demonstrated impaired intestinal glucose absorption in three patients with pernicious anemia. In his study, in which the Miller-Abbott technic of intestinal absorption was used, glucose absorption was restored to normal after vitamin B₁₂ replacement therapy.

There are several explanations to account for the difference in the results of our study. First, we utilized the segmental, triple-lumen intestinal perfusion technic which has been extensively evaluated in regard to its reproducibility and accuracy. The methodologic differences in the two studies are probably too important to allow any comparison of the results. Second, it was indicated that impaired glucose absorption in pernicious anemia was a nonspecific effect, possibly related to the low caloric intake or poor nutritional status of the patients [25], a factor which was probably not significant in our patients.

The most striking observation in our study, a finding not previously reported in pernicious anemia, was the demonstration of marked inhibition of sodium and water absorption in the jejunum, in the presence of a normal jejunal mucosal biopsy specimen. Not only sodium and water absorption was completely inhibited, but also a significant net secretion of sodium and water, i.e., net loss into the intestinal lumen occurred in all three patients. Luminal accumulation of sodium and water was apparent during the intestinal perfusion studies when our patients experienced abdominal pain and distress, evidently due to luminal distention. This phenomenon has been recognized in perfusion studies in which dihydroxy bile acids were used in the perfusate resulting in intestinal fluid secretion and distention of the intestinal wall [26]. Follow-up perfusion studies in two patients after a period of treatment with vitamin B₁₂ did not show any improvement in the absorption of sodium and water, although glucose absorption was normal as in the pretreatment period.
Sodium and water secretion into the lumen may occur in several clinical and experimental situations and in the absence of structural changes in the jejunal biopsy specimen. There are two conditions in pernicious anemia which may provide a reasonable explanation for our results. First, it is known that bacterial overgrowth in the jejunum commonly occurs in pernicious anemia [27]. Overgrowth in the lumen results in deconjugation of bile acids [28] which exert an inhibitory effect on intestinal electrolyte and water absorption [29]. Second, bacterial overgrowth may contribute to the formation of hydroxy-fatty acids [30], which are not ordinarily present in the diet, but they originate from bacterial hydroxylation of free fatty acids. Hydroxy-fatty acids exert a cathartic effect on intestinal mucosa as well as on the intestinal lumen [32]. The clinical significance of our observation is not clear, since none of the patients presented with secretory diarrhea or other electrolyte disorder. The fact, however, that this absorptive defect was not a reversible abnormality after treatment with vitamin B<sub>12</sub> suggests that it represents a primary intestinal manifestation of pernicious anemia, not necessarily related to the anemia per se. A recent study by Cook [33], showed that the presence of hypochromic or megaloblastic anemia does not affect glucose, glycine or folic acid absorption, whereas xylose absorption is impaired. This observation lends some support to the contention that luminal events (i.e., bacterial overgrowth, hydroxy-fatty acid formation or bile salt deconjugation) may account for the secretion of sodium and water in pernicious anemia.

**ACKNOWLEDGMENT**

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**REFERENCES**