ILEAL MUCOSA IN FAMILIAL SELECTIVE VITAMIN B₁₂ MALABSORPTION
I. L. Mackenzie, M.D., R. M. Donaldson, Jr., M.D., J. S. Trier, M.D., and V. I. Mathan, M.D.
ILEAL MUCOSA IN FAMILIAL SELECTIVE VITAMIN B₁₂ MALABSORPTION

I. L. Mackenzie, M.D., R. M. Donaldson, Jr., M.D., J. S. Thier, M.D., and V. I. Mathan, M.D.

Abstract

Three brothers with selective vitamin B₁₂ malabsorption and proteinuria were studied to characterize the absorptive defect in this disorder. Gastric juice from all three brothers contained adequate quantities of biologically active intrinsic factor. Serum concentrations of transcobalamin II were normal, and the patients had no demonstrable serum antibodies against intrinsic factor or against ileal receptors for intrinsic-factor-vitamin B₁₂ complex (IF-B₁₂). Mucosal biopsies from the terminal ileum of one brother were normal on light and electron microscopy. Intrinsic factor markedly stimulated uptake of cyanocobalamin by homogenates of the biopsies. Thus, selective vitamin B₁₂ malabsorption is not associated with a morphologically identifiable lesion, and the absorptive defect does not seem to result from lack of ileal receptors for intrinsic factor. However, peroral administration of vitamin B₁₂ absorption that occurs after IF-B₁₂ attaches to the surface of the ileal cell and before the absorbed vitamin binds to transcobalamin II.

IN 1960 Intersland and Gräsbek described a familial disorder in which megaloblastic anemia develops because of vitamin B₁₂ deficiency. These patients appear to be vitamin B₁₂ malabsorbers, but serum gastric juice contains intrinsic factor (IF). Intestinal absorption of fat and xylose is not impaired, and the patients have no other clinical or radiologic evidence of small-bowel disease. Persistent idiopathic proteinuria occurs regularly. Familial selective vitamin B₁₂ malabsorption associated with proteinuria has now been described in 94 children, but the pathogenesis of vitamin B₁₂ malabsorption in this syndrome remains unknown.

This report summarizes studies performed in three siblings with selective vitamin B₁₂ malabsorption and proteinuria. Mucosal biopsies from the terminal ileum of one of these patients were examined by light and electron microscopy. In addition, the in vitro uptake of free and IF-bound vitamin B₁₂ by homogenized ileal-mucosa biopsies was measured in view of earlier speculations that patients with this disorder might lack ileal surface receptors specific for IF-vitamin B₁₂ complex (IF-B₁₂).

CASE REPORTS

Case 1. Weakness, easy bruising, recurrent oral ulcers and difficulty walking developed in the oldest of 6 children at the age of 14 years. He was pale and had decreased vibratory sensation over both lower legs. Laboratory studies demonstrated a macrocytic anemia, with a hemoglobin of 8.0 g per 100 ml. The Schilling test was abnormal with and without added IF. Five days after the parental administration of 1000 μg of cyanocobalamin, the reticulocyte count rose to 25 per cent. Urinalysis was unremarkable except for mild proteinuria. After treatment with monthly injections of cyanocobalamin, the anemia was corrected; all symptoms disappeared, and physical examination was unremarkable. The patient has remained in excellent health for 7 years.

Case 2. In a younger brother weakness, nausea, vomiting, diarrhea and a sore tongue developed at 11 years of age. Physical examination showed only pallor and redness of the tip of the tongue. The patient had a macrocytic megaloblastic anemia with a hemoglobin of 7.5 g per 100 ml. Urinalysis disclosed persistent proteinuria, a finding that had also been noted on several occasions when he was approximately 1 year old. Serum iron and total iron binding capacity were normal. A Schilling test showed impaired vitamin B₁₂ absorption. Gastric analysis documented the presence of free acid. With monthly injections of 1000 μg of cyanocobalamin the patient became asymptomatic, his anemia was corrected, and he has remained in excellent health for 5 years.

Case 3. A third brother had fatigue and pallor at the age of 11 years. On the basis of the previous experience with his brothers, the father merely instituted monthly injections of 1000 μg of cyanocobalamin without consulting a physician. The patient began to feel well after the 1st injection and has remained asymptomatic for 4 years.

In 1971 when Case 1 was 21, Case 2 16 and Case 3 15 years old, the brothers were re-examined and found to be in excellent health. The following studies were all within normal limits: complete blood counts; serum electrolytes, including calcium and phosphorus; serum protein electrophoresis, and serum levels of IgG, IgA and IgM. All 3 patients secreted acid gastric juice under basal conditions and had peak acid outputs in response to betazole hydrochloride (Histalog) of 46 to 51 mEq per hour. Xylose tolerance tests and 72-hour fecal fat excretion tests were normal. Nevertheless, all 3 brothers had vitamin B₁₂ malabsorption as determined by Schilling tests (1.3 to 3.6 per cent urinary excretion), and this malabsorption was not corrected by IF (0.8 to 3.1 per cent urinary excretion from vitamin B₁₂ alone). X-ray films of the upper gastrointestinal tract showed normal small bowel. Urinalyses were unremarkable except for persistent mild proteinuria, all of which was thought to be due to low-grade glomerular disease; leukocytes, cast cells, erythrocytes and pigment casts were found in urine and the polycystic kidney disease was confirmed by ultrasonographic examination. Creatinine clearances were within normal limits, and urinary amino acid analyses showed no abnormalities. Intravenous pyelograms demonstrated blunting of the superior pole of the right kidney in Cases 1 and 2 but was normal in Case 3.

The other brothers, 1 sister, the mother and the father were also examined and found to be in good health. None were anemic, and all had normal results on Schilling tests. The mother and father were not related, and no other relatives were known to have had anemia.

METHODS

Vitamin B₁₂ absorption was measured by the urinary excretion test described by Schilling. Tests were performed with 0.5 μg of 99Cu-labeled cyanocobalamin (specific activity, 1.0 μCi per microgram), which was administered either as free vitamin or...
bound to IF. Pooled gastric juice obtained from healthy volunteers and gastric juice specimens from each of the three patients served as sources of IF. Gastric juice was collected, and IF-bound *Co-la- beled cyanocobalamin was prepared by methods previously described in detail.36

Total vitamin B₁₂ binding capacity and IF content of gastric juice were measured by the charcoal method of Gottlieb et al.33 Since human IF stimulates uptake of vitamin B₁₂ by guinea-pig ileum,14 IF was also assayed by measurement of in vitro uptake of *Co-labeled cyanocobalamin by brush borders prepared from guinea-pig ileum. The procedures used to prepare guinea-pig brush borders and to measure tissue uptake were similar to those previously described for the hamster.34

Serum vitamin B₁₂ level18 and the unsaturated vitamin B₁₂ binding capacity of serum15 were determined by charcoal radioassay. The proportions of serum binding due to transcobalamin I and transcobalamin II were estimated by DEAE-cellulose chromatography.77 Serum specimens were also tested for the presence of "blocking"10 and "binding"78 antibodies against IF and for ability to inhibit attachment of IF-B₁₂ to brush borders.19

After he was fully informed about the purposes of the procedure and its possible hazards, Case 1 consented to personal intubation to obtain mucosal biopsies from the terminal ileum. A hydraulic biopsy tube28 was passed under fluoroscopic control into the right colon and was then withdrawn until it was located in the terminal ileum a few centimeters from the ileocecal valve as determined by injection of radiopaque contrast material.

Five biopsies were taken. Two of the biopsies were carefully oriented on monofilament nylon mesh and divided. Half of each biopsy was fixed in Bouin’s solution, embedded in paraffin and serially sectioned for light microscopy. The other half was fixed in chrome-osmium,79 embedded in Epoxy resin and prepared for electron microscopy as previously described.28 Thin sections were examined with a Philips EM-300 electron microscope.

Three of the biopsies were immediately homogenized with a ground-glass homogenizer in cold 5 mM EDTA at pH 7.5. After centrifugation, the homogenate was washed in Krebs-Ringer bicarbonate solution, recentrifuged and suspended in 3.2 ml of the solution. Aliquots of the homogenate were then taken for estimation of protein content80 and for studies of vitamin B₁₂ uptake.80 Two nanograms of *Co-labeled cyanocobalamin, either free or bound to IF, were added to each aliquot. After incubation at room temperature for 30 minutes, the homogenate was centrifuged and washed twice in the Krebs-Ringer solution, and tissue radioactivity was determined in a Packard Autogamma counter. Ileal-mucosa biopsies were also obtained from two consenting patients with normal Schilling tests who had had ileostomies for ulcerative colitis and who understood the investigative nature and possible hazards of the procedure. Biopsies were obtained by passage of a multipurpose biopsy tube28 15 cm into the ileostomy stoma. Studies of vitamin B₁₂ uptake with homogenized biopsies were carried out as described above.

Results

Vitamin B₁₂ Absorption Tests

As shown in Table 1, the three patients had impaired vitamin B₁₂ absorption even when *Co-labeled cyanocobalamin was bound to IF derived from pooled human gastric juice (IF-B₁₂). In contrast, the three healthy siblings and both parents had normal Schilling tests under these conditions. When Case 1 was given tetracycline, 1 g daily for five days, the Schilling test performed with IF-B₁₂ remained abnormally low. At the same time, administration of tetracycline for five days increased IF-B₁₂ absorption in a patient with small-bowel bacterial overgrowth associated with scleroderma. When IF-B₁₂ was administered to Case 1 together with pancreatic extract (Cotazym) there was no improvement in vitamin B₁₂ absorption. Nevertheless, the same quantity of Cotazym markedly increased IF.

Table 1. Vitamin B₁₂ Absorption Tests (per Cent Urinary Excretion).

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>IF-B₁₂</th>
<th>IF-B₁₂</th>
<th>IF-B₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TETRACYCLINE</td>
<td>PANCERATIC</td>
<td>EXTRACT</td>
</tr>
<tr>
<td>Patients:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>2.8</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Case 2</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siblings:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother</td>
<td>12.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother</td>
<td>6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sister</td>
<td>14.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>10.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient with scleroderma</td>
<td>1.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Patient with pancreatic insufficiency</td>
<td>2.0</td>
<td>11.0</td>
<td></td>
</tr>
</tbody>
</table>

*Co-cyanocobalamin bound to pooled human gastric juice.

Figure 1. Light Micrograph of an Ileal Biopsy from Case 1 (Hematoxylin and Eosin Stain X200 before Reproduction). The histology of the ileum is entirely normal.
**Figure 2. Electron Micrographs of Apical Cytoplasm of a Typical Ileal Absorptive Cell from Case 1.**

In A the apical cytoplasm, microvilli (M) and glycoprotein surface coat (S) appear normal (X22,000 before reproduction). In B (X75,000 before reproduction) a normal-appearing trilaminar membrane (short arrows) delimits the microvilli. Filaments of surface coat (long arrows) appear attached to the outer leaflet of the microvillous membrane.

$B_{12}$ absorption in a patient with pancreatic insufficiency due to cystic fibrosis.

In a patient with well documented pernicious anemia, the urinary excretion of radioactive label was only 2.3% per cent when free $^{57}$Co-labeled cyanocobalamin was given but increased to 13.0% per cent when the vitamin was bound to pooled human gastric juice. When this patient was tested with radioactive cyanocobalamin bound to gastric juice obtained from Cases 1 and 2, urinary excretion of radioactive label was 13.2% and 10.7% per cent respectively.

**Gastric Juice and Serum Measurements**

All three patients secreted acid gastric juice with a total vitamin $B_{12}$ binding capacity (44.5 to 69.9 ng per milliliter of gastric juice) that was well within the range of normal values. Of the total binding capacity, 40.8 to 61.2 ng of vitamin $B_{12}$ per milliliter was specifically due to IF as determined by measurements with "blocking" IF antibody present in the serum of a patient with pernicious anemia. In addition, gastric juice from each of the patients promoted uptake of $^{57}$Co-labeled cyanocobalamin by guinea-pig brush borders to the same extent as pooled gastric juice from healthy volunteers and gastric juice obtained from one of the siblings with normal vitamin $B_{12}$ absorption.

Serum vitamin $B_{12}$ levels obtained in the three patients while they were receiving monthly injections of the vitamin were within normal limits. Total unsaturated vitamin $B_{12}$ binding capacities of these sera (744 to 1924 pg per milliliter) were also normal. Serum binding due to the "beta" binding fraction, which contains transcobalamin II, ranged from 618 to 1429 pg of vitamin $B_{12}$ per milliliter, well within the range of previously described
Figure 3. Uptake of Free and IF-Bound Vitamin B₁₂ by Homogenates of Ileal-Mucosa Biopsies from Two Volunteers and from Case 1. IF was obtained from a single pool of normal human gastric juice (open circles) and from gastric juice (triangle) obtained from the patient (Case 1).

normal values.³³⁻³⁵ Serums from the three patients contained no “blocking” or “binding” antibodies against IF. Preincubation of guinea-pig brush borders with undiluted serums from all three patients failed to inhibit subsequent tissue uptake of IF-B₁₂.

**Ileal Mucosal Biopsies**

Figure 1 is a light micrograph of a mucosal biopsy obtained from the terminal ileum of Case 1. At the light microscopical level, mucosal architecture was entirely normal. Moreover, the histologic appearance of the ileal epithelium and the lamina propria was also normal. Figure 2A is an electron micrograph of the apical third of a representative ileal absorptive cell from the junction of the middle and upper thirds of a villus. The morphology of the absorptive surface of this and other absorptive cells appeared normal. High-resolution micrographs of the microvilli were also normal and showed the intact trilaminar apical plasma membrane, an intact microvillus core and a fibrillar surface coat (Fig. 2B). Moreover, the fine structure of the epithelial-cell cytoplasm was normal, as was the morphology of the basolamina and basal plasma membrane.

As shown in Figure 3, IF from pooled normal human gastric juice markedly stimulated in vitro uptake of ⁵⁷Co-labeled cyanocobalamin by homogenized ileal-mucosa biopsies from two volunteers who had ileostomies and normal vitamin B₁₂ absorption. A similar IF-mediated increase in tissue uptake was observed with homogenized ileal-mucosa biopsies obtained from Case 1. This striking increase occurred when both pooled normal human gastric juice and the patient’s own gastric juice were used as the source of IF.

**Discussion**

The three brothers described above have all the clinical and laboratory features previously observed in patients with familial vitamin B₁₂ malabsorption.¹⁸ Each had anemia responsive to parenteral vitamin B₁₂ during childhood, and each has markedly impaired absorption of vitamin B₁₂ even when the vitamin is given with IF. Tests on one of the brothers documented that neither tetracycline nor pancreatic extract corrected the malabsorption of IF-B₁₂. On the other hand, all three absorb xylose and dietary fat normally, and none have clinical or radiologic evidence of small-bowel disease. Furthermore, as has been observed in most cases, all three brothers have persistent and mild proteinuria, with no other evidence of renal-function impairment.

The absorptive defect in patients with familial selective vitamin B₁₂ malabsorption and proteinuria has not been defined. Physiologic absorption of the vitamin can be divided into three stages.³⁶ Binding of the vitamin by IF to form a macromolecular IF-B₁₂ constitutes the first step. Next, the IF-B₁₂ attaches to specific receptor sites on the surface of ileal absorptive cells. Finally, the vitamin appears to be transferred by an energy-requiring active transport process across the intestinal cell. How vitamin B₁₂ normally enters and leaves the cell is poorly understood, but it is generally agreed that once the vitamin emerges, it becomes bound to transcobalamin II for transport via the portal blood.³⁷

As in previous reports, the present findings indicate that binding of the vitamin by IF proceeds normally in patients with familial selective vitamin B₁₂ malabsorption. Not only did normal human gastric juice fail to improve absorption, but in addition all three brothers secreted acid gastric juice that contained adequate quantities of IF when assayed with anti-IF antibody. This IF was fully capable of binding vitamin B₁₂, enhancing tissue uptake of radioactive cyanocobalamin by guinea-pig brush borders and correcting vitamin B₁₂ malabsorption in a patient with pernicious anemia. None of the patients had “blocking” or “binding” antibodies to IF in their sera. Thus, these patients clearly differ from previously described cases of juvenile pernicious anemia resulting from lack of IF²⁸ or from the presence of abnormal IF²⁹.

Although experimental evidence was lacking, it seemed reasonable to propose that lack of specific ileal receptor sites for IF-B₁₂ constitutes the fundamental defect in patients with selective vitamin B₁₂ malabsorption.⁵⁰ In the present study, however, uptake of IF-B₁₂ by homogenized ileal biopsies obtained from one of the patients was normal (Fig. 3) when compared to uptakes by biopsies from two control subjects. Moreover, the observed uptakes of vitamin B₁₂ in our study were similar to those previously reported by Carmel et al.³⁸ who used ileal-mucosa homogenates obtained at surgery.

Since in vitro uptake of IF-B₁₂ by homogenized mucosa reflects attachment of the complex to microvillous-membrane receptors,³⁵ the observed normal uptake argues strongly against lack of ileal...