Congenital Pernicious Anemia with Coexistent Transitory Intestinal Malabsorption of Vitamin B₁₂

By Beatrice C. Lampkin and Alvin M. Mauer

SELECTIVE MALABSORPTION of vitamin B₁₂ in the ileum and lack of intrinsic factor in the stomach are major causes of vitamin B₁₂ deficiency in children.¹ ³ The purpose of this report is to present studies in a child in whom congenital pernicious anemia was coexistent with transitory selective intestinal malabsorption of vitamin B₁₂.

METHODS

Red and white blood cell counts were done by an electronic particle counter.¹ Hæmolobin determinations were done by the cyanmethemoglobin method⁷ and the volume of packed red cells was measured by a microhæmatocrit technic.⁸ Platelet and reticulocyte counts were done by the method of Dameshek.⁷ The serum vitamin B₁₂ level was measured by microbiologic assay using Lactobacillus leichmannii⁹ and the serum folate level was measured by microbiologic assay using Lactobacillus casei.⁹ The absorption of vitamin B₁₂ was evaluated by the method described by Schilling with the exception that 0.5 µ of Co²⁷ labeled vitamin B₁₂ was used instead of 2 µ of Co²⁷ labeled vitamin B₁₂.¹⁰ Each time the second part of the Schilling test was done, one capsule containing 1 N.F. unit of hog intrinsic factor (Squibb) was administered, except on one occasion when 100 ml. of neutralized normal human gastric juice was given. Dr. Victor Herbert, the Mount Sinai Hospital, New York, N. Y., assayed the patient’s gastric juice for intrinsic factor and her serum for antibodies to intrinsic factor by a coated charcoal adsorption technic, described by his group.¹¹ The serum of the patient was examined for antibodies against parietal and thyroid cells by an immunofluorescent technic, described by Taylor and co-workers and Doniach and co-workers.¹²,¹³ Biopsies of the stomach and jejunum were obtained with a Crosby capsule by Dr. William K. Schubert.

The amount of haptoglobin present before and after vitamin B₁₂ therapy was estimated by immunoelectrophoresis,¹⁴ and the level of lactic acid dehydrogenase before and after vitamin B₁₂ therapy was measured by the method described by Wroblewski and LaDue.¹⁵

The D-xylose excretion test was done by administering 15 Gm./M² of D-xylose orally and collecting all urine over a 5-hour period.¹⁶ The amount of xylose present in the urine was measured using the method reported by Roe and Rice.¹⁷ After the patient had been given a 50 Gm. fat diet for 1 week, all stools were collected for a 3-day period, while she was still receiving the same diet, and the stools were analyzed for fat as described by Van deKamer and co-workers.¹⁸

Urinary protein determinations were measured by the method described by Goa, and urinary aminoacids were quantitated by paper chromatography.¹⁹,²⁰

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CASE REPORT

The patient, a 2%-year-old Caucasian girl, was referred to Children's Hospital because of anemia and a suspected diagnosis of acute lymphoblastic leukemia. For the 8 months before admission she had been pale, irritable, less active, and sleeping more than usual. Seven months before admission she had had an episode of fever. On examination no abnormality was found and no specific therapy was given. The fever disappeared after a few days. The other symptoms progressed in severity until admission to the hospital.

There was no history of bleeding or excessive bruising, and the stools had been normal in number and appearance. The dietary history was excellent but the mother was concerned about poor weight gain. The patient weighted 4 lb. 9 oz. at birth after 7 months gestation. On review of the family history, no significant related illnesses were found.

On admission the child was pale and irritable. She weighed 2234 lb. and was 32 in. tall. Both of these values were below the third percentile according to a Harvard percentile chart. The palate was high and arched with a small anterior partial cleft. The tongue was smooth but not sore or inflamed. The liver and spleen were both palpable 2 cm. below the costal margins. The neurologic examination was within normal limits.

The hemoglobin was 5.4 Gm. per cent; hematocrit, 16 per cent, and red blood cell count 1.5 million/mm. The red blood cell indices were calculated to be: MCV, 106 cu.m; MCHC, 34 per cent; and MCH, 36 μg. The white blood cell count was 8400/mm. with 20 per cent neutrophils, 2 per cent basophils, 76 per cent lymphocytes, 1 per cent metamyelocytes, and 1 per cent myelocytes. On blood smear there were macrocytes, microcytes, occasional nucleated red blood cells which were megaloblastic, and giant platelets. The platelet count was 190,000/mm. and the reticulocyte count was 3.4 per cent. The diagnosis of megaloblastic anemia was confirmed on examination of the bone marrow aspirate.

The hospital course is shown in Figure 1. A small transfusion of packed red cells was given on admission and increased the hemoglobin level to 7 Gm. per cent. Before the first part of the Schilling test, 2 μg. of vitamin B₁₂ were given intramuscularly on two consecutive days. A bone marrow sample was obtained 60 hours after the first dose. Some decrease in the megaloblastic features had occurred but not enough to confirm the diagnosis of a vitamin B₁₂ responsive anemia. A single injection of 5 μg. vitamin B₁₂ was given, and 48 hours later complete conversion to normoblastic hyperplasia was found in the aspirated bone marrow sample. Reticulocytosis began two days after the first injection of vitamin B₁₂ and was maximal on the sixth day. A progressive increase in hemoglobin concentration to normal values occurred thereafter. She has been receiving 1000 μg. vitamin B₁₂ intramuscularly each month since diagnosis.

RESULTS

Before therapy, the serum vitamin B₁₂ level was 50 pg./ml. and the serum folic acid level was 10 ng./ml. Stool examinations for ova and parasites were negative. The serum protein-bound iodine concentration was 6.6 μg. per cent. The serum calcium concentration was 11.0 mg. per cent and serum protein 5.7 Gm. per cent. Measurement of lactic acid dehydrogenase activity before treatment was 3032 units/ml. of serum, and after treatment it returned to a normal level of 126 units/ml. of serum. Haptoglobin was absent from serum on immunoelectrophoresis before treatment but was present after correction of the anemia.

Studies done to determine the cause of the vitamin B₁₂ deficiency are shown in Table 1. Three determinations for intrinsic factor by in vitro assay were negative. Of particular interest is that on two occasions, 4 and 6 weeks after beginning therapy with vitamin B₁₂, deficient absorption of vitamin B₁₂ was found when radioactive labeled vitamin B₁₂ was given with an intrinsic factor.
preparation of known potency. However, 6 months after therapy was started, normal absorption was obtained with both human and hog intrinsic factors. Deficient absorption still was found without intrinsic factor. No antibodies to intrinsic factor or parietal or thyroid cells were found.

The studies done to evaluate intestinal absorption and renal function are shown in Table 2. All values were found to be within the normal ranges and no evidence for either generalized intestinal malabsorption or impaired renal function was found.

Studies done on members of the patient's family are shown in Table 3. No abnormalities were found.

**DISCUSSION**

Unless intestinal malabsorption is coexistent with pernicious anemia, the deficiency of vitamin B₁₂ caused by malabsorption in the ileum can be separated easily from that due to a lack of intrinsic factor by the Schilling test or other such assays for absorption of vitamin B₁₂. When there is absence of intrinsic factor without associated intestinal malabsorption, a significant increase in absorption of vitamin B₁₂ occurs after the administration of intrinsic factor.²²

Generalized intestinal malabsorption can be separated from selective intestinal malabsorption of vitamin B₁₂. In patients with selective malabsorption, the xylose excretion test, fecal fat excretion test, contrast roentgen study of the intestines, and intestinal biopsy are normal.³ If the result of an absorption test for vitamin B₁₂ indicates intestinal malabsorption, then coexistent pernicious anemia can be excluded by demonstration of intrinsic factor in gastric fluid.²¹

The absorption of vitamin B₁₂ in our patient with the administration of hog intrinsic factor of known potency, as determined by the Schilling test, was abnormal 4 and 6 weeks after starting therapy. Since other studies for intestinal
Table 1.—Studies to Determine the Cause of the Vitamin B₁₂ Deficiency

<table>
<thead>
<tr>
<th>State of Vitamin B₁₂ Therapy</th>
<th>Schilling Test</th>
<th>Gastric Studies</th>
<th>Antibody Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Co⁷⁺ Excreted without Intrinsic Factor</td>
<td>% Co⁷⁺ Excreted with Hog Intrinsic Factor</td>
<td>% Co⁷⁺ Excreted with 100 cc. Human Gastric Juice</td>
<td>Anti-Intrinsic Factor</td>
</tr>
<tr>
<td>Before</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks after</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks after</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months after</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6½ months after</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 months after</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

malabsorption were normal, the abnormal Schilling tests were indicative of selective malabsorption of vitamin B₁₂ in the ileum. The consistent lack of intrinsic factor in gastric fluid indicated the patient had pernicious anemia. The age of the patient, presence of HCl in gastric fluid, normal gastric biopsy, and absence of antibodies to intrinsic factor in parietal cells clearly establish that she has congenital pernicious anemia.²⁴ Six months after therapy was started intestinal malabsorption of vitamin B₁₂ was no longer present, but the absence of intrinsic factor persisted, as shown by the abnormal Schilling test without intrinsic factor.

Transitory selective intestinal malabsorption of vitamin B₁₂ coexistent with congenital pernicious anemia has not been reported since Co⁷⁺ labeled vitamin B₁₂ has been available for evaluation of the absorption of that compound. However, both transitory selective malabsorption and generalized malabsorption of vitamin B₁₂ have been reported in a few adults with pernicious anemia.²³,²⁵ The results of studies in these patients were indicative that both selective and generalized intestinal malabsorption may be caused by vitamin B₁₂ deficiency and in some cases reversed by the administration of the vitamin. The reversibility may depend upon the extent of the lesion before therapy is started. The cytologic abnormalities of buccal mucosa and neurologic signs in patients may be wholly or in part reversible with replacement therapy.²⁶,²⁷ Possibly selective malabsorption may progress to generalized malabsorption in some patients in whom therapy is not begun soon enough.

Twenty children have been reported in whom there was persistent selective malabsorption of vitamin B₁₂ by the small bowel. Intrinsic factor was present
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Table 2.—Studies to Evaluate Intestinal Absorption and Renal Function

<table>
<thead>
<tr>
<th>Intestinal Absorption Studies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>D-xylose excretion test</td>
<td>21.6% excretion</td>
</tr>
<tr>
<td>72-hour fecal fat determination</td>
<td>5.8% excretion</td>
</tr>
<tr>
<td>Jejunum biopsy</td>
<td>Normal</td>
</tr>
<tr>
<td>Barium enema</td>
<td>Normal</td>
</tr>
<tr>
<td>Upper GI series</td>
<td>Normal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal Function Studies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IVP</td>
<td>Normal</td>
</tr>
<tr>
<td>Urinary protein</td>
<td>Negative 6X</td>
</tr>
<tr>
<td>Routine analysis</td>
<td>1st time: 22 mg.</td>
</tr>
<tr>
<td>24-hour urine determinations</td>
<td>2nd time: 12 mg.</td>
</tr>
<tr>
<td>Urinary aminoacids</td>
<td>Normal</td>
</tr>
<tr>
<td>BUN</td>
<td>15 mg%</td>
</tr>
<tr>
<td>Urinary specific gravity</td>
<td>1.030</td>
</tr>
</tbody>
</table>

in their gastric fluids and 18 of the 20 patients had albuminuria at the time of diagnosis or subsequently. Initially a combined defect of absent intrinsic factor and defective absorption was considered in our patient. For this reason the renal function was evaluated. The absence of significant proteinuria made this possibility unlikely. The later demonstration of normal absorption of vitamin B₁₂ with intrinsic factor after 6 months of therapy was indicative of a transitory defect.

There is a strong familial occurrence of both selective malabsorption of vitamin B₁₂ and congenital pernicious anemia. These two defects have not been shown conclusively, however, to be present in the same family or the same patient. In 1964 Pearson reported studies in a 32-month-old male child in whom absent intrinsic factor was found by an in vitro assay. Three years after starting vitamin B₁₂ therapy, urinary excretion of Co⁵⁷ labeled vitamin B₁₂ did not increase as expected after administration of intrinsic factor. The results of tests for generalized malabsorption were normal. Since the megaloblastic anemia had been diagnosed 15 months before treatment with vitamin B₁₂, intestinal changes may have been irreversible. Although it is possible that this patient had 2 congenital defects, the absence of proteinuria and lack of a demon-

Table 3.—Family Studies

<table>
<thead>
<tr>
<th>Family Member</th>
<th>Age (years)</th>
<th>Hemoglobin (Gm.%</th>
<th>Peripheral Blood Smear</th>
<th>Schilling Test</th>
<th>Parietal Cell Antibodies</th>
<th>Serum Vitamin B₁₂ Level (pg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>31</td>
<td>13.7</td>
<td>Normal</td>
<td>12.1</td>
<td>Negative</td>
<td>680</td>
</tr>
<tr>
<td>Mother</td>
<td>28</td>
<td>13.0</td>
<td>Normal</td>
<td>18.6</td>
<td>Negative</td>
<td>570</td>
</tr>
<tr>
<td>Brother</td>
<td>6½</td>
<td>11.9</td>
<td>Normal</td>
<td>13.0</td>
<td>Negative</td>
<td>—</td>
</tr>
<tr>
<td>Paternal aunt</td>
<td>21</td>
<td>13.1</td>
<td>Normal</td>
<td>—</td>
<td>Negative</td>
<td>—</td>
</tr>
</tbody>
</table>
strated defect in selective malabsorption for vitamin B_{12} in other family members makes this dual defect seem unlikely.\(^1\)

In the study of a child with vitamin B_{12} responsive anemia it is necessary to assay gastric fluid for intrinsic factor if abnormal absorption results after administration of intrinsic factor to determine if coexisting defects are present. Other tests of intestinal absorption should also be done. If defective absorption is found, the studies should be repeated after several months of treatment.

**SUMMARY**

Pernicious anemia with coexistent transitory intestinal malabsorption of vitamin B_{12} has been established in adults. In this report a child with congenital pernicious anemia was documented to have had transitory selective intestinal malabsorption of vitamin B_{12}. The diagnosis of congenital pernicious anemia was established by the age of the patient, absence of intrinsic factor in gastric fluid, lack of antibodies to intrinsic factor and parietal cells, presence of HCl in gastric fluid, and normal gastric biopsy. The xylose excretion test, 72-hour fecal fat determination, upper gastrointestinal series, and biopsy of the jejunum were normal, but the Schilling test was abnormal with hog intrinsic factor of known potency on two occasions, indicating selective malabsorption of vitamin B_{12}. Seven months after therapy the Schilling test was still abnormal without intrinsic factor, but was normal with both human and hog intrinsic factor. The normal absorption with intrinsic factor after therapy is indicative that the selective malabsorption which was originally present was probably a consequence of the vitamin B_{12} deficiency resulting from lack of intrinsic factor. In patients with abnormal radioactive vitamin B_{12} absorption tests with administration of intrinsic factor, coexistent pernicious anemia must be excluded by demonstration of intrinsic factor in gastric fluid.

**SUMMARIO IN INTERLINGUA**

Le occurrentia de anemia perniciose in coexistentia con transiente malabsorption intestinal de vitamina B_{12} es un facto establite quanto a patientes adulte. In le presente reporto provas es presentate pro le occurrentia in un patiente pediatric con congenite anemia perniciose de transiente selective malabsorption intestinal de vitamina B_{12}. Le diagnose de congenite anemia perniciose esseva establite a base del etate del patiente del absentia de factor intrinsec in le liquido gastric, del absentia de anticorpore anti factor intrinsec e cellulas parietal, del presentia de HCl in le liquido gastric, e del normalitate del biopsia gastric. Le test del excretion de xylosa, le determination de grassia fecal pro 72 horas, le serie de roentgenopelliculas superogastrointestinal, e le biopsia del jejuno eseva normal, sed anormal responsas eseva notate in due occasiones in le test de Schilling con le uso de porcin factor intrinsec de cognoscite potentia, lo que indicava un selective malabsorption de vitamina B_{12}. Septe menses post le therapia, le test de Schilling eseva ancora anormal sin le uso de factor intrinsec sed normal con le uso de factor intrinsec tanto human como etiam porcin. Le absorption normal con factor intrinsec post le therapia indica que le malabsorption selective que eseva presente originalmente eseva probabilemente un consequentia del carentia de vitamina B_{12} occassionate per le manco de factor intrinsec. In patientes in qui le test de absorption de radioactive vitamina B_{12} es anormal etiam post le administration de factor intrinsec, le coexistentia de anemia perniciose debe esser exclusite per le demonstration de factor intrinsec in le liquido gastric.

**ACKNOWLEDGMENTS**

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REFERENCES


24. Herbert, V., Streiff, R. R., and Sullivan,


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