Congenital Familial Megaloblastic Anemia

By Beatrice C. Lampkin, Allan Pyesmany, Carol B. Hyman, and Denman Hammond

Two sisters with a previously unreported megaloblastic anemia unassociated with a deficiency of either folic acid or vitamin B₁₂ are described. Deficiencies of these vitamins were ruled out by standard studies. All other previously reported forms of megaloblastic anemia not secondary to a vitamin deficiency, such as orotic aciduria, were also excluded by appropriate studies. Optimal hemoglobin responses were obtained after the administration of large amounts of both vitamin B₁₂ and folic acid. Because of this hemoglobin response, the conversion of deoxyuridine-5-monophosphate to deoxythymidine-5-monophosphate in vitro was examined in bone marrow samples from both patients using a modification of a method described by Killmann. This preliminary step in DNA synthesis was found to be normal. The results of this test and the optimal hemoglobin response after administration of both vitamins suggest that both folic acid and vitamin B₁₂ may be necessary at some other preliminary step in DNA synthesis.

Megaloblastic anemia most frequently arises as a result of a deficiency of folic acid or vitamin B₁₂. Such vitamin deficiency may result from inadequate ingestion or malabsorption of the vitamin. Only an occasional child has been reported to have congenital megaloblastic anemia unassociated with a deficiency of either folic acid or vitamin B₁₂. The
clinical course and laboratory findings in two sisters with a previously undescribed form of congenital megaloblastic anemia unassociated with a deficiency of either vitamin are reported in this paper.

**METHODS AND SPECIAL LABORATORY TESTS**

All routine hematologic studies were done by standard methods. The amount of formiminoglutamic acid excreted in the urine after a loading dose of 5 g of L-histidine was measured using the method reported by Meiss et al. Urine was analyzed for orotic acid by column chromatography. Urinary amino acids were quantitated by paper chromatography. The serum vitamin B12 levels were measured by microbiologic assay using *Lactobacillus leichmannii* and by the radioisotope method using coated charcoal. The serum folate level was measured by microbiologic assay using *Lactobacillus casei*. The absorption of vitamin B12 was evaluated by the method described by Schilling, with the exception that 0.5 μCi of 57Co-labeled vitamin B12 was used instead of 2 μCi of 60Co-labeled vitamin B12.

The ability of the patients’ marrow cells to convert deoxyuridine-5-monophosphate (dUMP) to deoxythymidine-5-monophosphate (dTMP) in vitro was evaluated because it has been reported that folic acid is necessary directly and vitamin B12 indirectly for the synthesis of dTMP from dUMP. A modification of a method described by Killmann was used for this evaluation. In this modified test, 1 ml aliquots of heparinized samples of marrow were transferred to bottles containing 0.2 ml of saline (0.85%) or 0.2 ml of deoxyuridine (1.2 μM). All bottles were placed in a water bath at 37°C and were constantly agitation. After incubation for 1 hr, 0.2 ml of tritiated thymidine containing 2 μCi (specific activity, 1.9 Ci/m mole) was added to each bottle. The specimens were allowed to incubate at 37°C with constant agitation for an additional 50 min and were then transferred to disposable sedimentation tubes and centrifuged at 1200 g for 10 min. The myeloid-erythroid layer and a small amount of plasma were carefully removed with a Pasteur pipette, transferred to a watch glass and mixed well with the same pipette. Cover slip smears were made and autoradiographs were prepared with Kodak AR 10 stripping film. After an exposure period of from 13 to 14 days at 4°C, the films were developed and the slides stained with Wright’s stain. All slides of a particular sample of marrow were placed under film at the same time and were incubated for the same period of time. Three to six slides from each aliquot of marrow were examined.

To check the validity of this test, 24 samples of normal marrow, samples of marrow from five patients with proven vitamin B12-deficiency, and eight patients with acute lymphoblastic leukemia who were receiving methotrexate, an inhibitor of the synthesis of dTMP, were studied.

Figure 1A is a photomicrograph of a saline preincubated aliquot of marrow from a normal child. Heavy labeling with tritiated thymidine is seen. Figure 1B is a photomicrograph of a deoxyuridine-preincubated aliquot from the same sample. A striking decrease in the intensity of labeling is seen, indicating that deoxyuridine had been normally converted to deoxythymidine which prevented significant incorporation of labeled thymidine.

In contrast, little or no change was seen in the intensity of labeling in the saline and deoxyuridine-preincubated aliquots of samples of marrow from the patients with a vitamin B12 deficiency or the patients who were receiving methotrexate. After obtaining these results, this test was done on samples of marrow from our two patients when their marrows were megaloblastic and they were receiving no therapy.

**CASE REPORTS**

**Sibling 1**

The first sibling, a white girl, was born at full term. Her birth weight was 5 lb, 9 oz. Pregnancy and delivery were uncomplicated. At 7 wk of age she was admitted to The Children’s Hospital of Los Angeles because of pallor, lethargy, and several brief staring
Fig. 1.—Incubation of a sample of normal marrow with saline (A) or deoxyuridine (du) (B) and tritiated thymidine (\(^{3}\)HT). Note the striking decrease in labeling with \(^{3}\)HT in the du preincubated aliquots as compared to the saline preincubated aliquot.

episodes. On physical examination the infant was a small, poorly nourished, pale, and irritable girl who appeared to be neurologically normal. Laboratory data on admission were as follows: hemoglobin, 3.2 g/100 ml; hematocrit, 11%; red blood cell count, 1,000,000/cu mm. The red cell indices were macrocytic and the reticulocyte count was 3.5%. White blood cell count was 23,000/cu mm with 67% granulocytes. Platelets were normal on smear. Anisocytosis and poikilocytosis, macrocytes, slight polychromasia, on occasional megaloblastic nucleated red cell and hypersegmentation of the neutrophils were seen in a blood smear. Marked megaloblastic changes were seen in a bone marrow aspirate that confirmed the diagnosis of megaloblastic anemia.

After a small blood transfusion which raised the hemoglobin to 7.4 g/100 ml, 100 \(\mu\)g of folic acid were given intramuscularly. A repeat bone marrow aspirate 48 hr later was still megaloblastic. An additional 200 \(\mu\)g of folic acid were administered intramuscularly and, upon examination of the bone marrow 48 hr later, there were minimal morphologic changes in the marrow towards normal.

On the 23rd hospital day, pneumococcal meningitis was diagnosed and antibiotic therapy was begun. Following 10 days of therapy for meningitis, the infant was discharged home on no therapy. Hemoglobin at time of discharge was 8.0 g/100 ml.

When the child was 6 months old, the hemoglobin level was still 8.0 g/100 ml, and the marrow was strikingly megaloblastic. One-hundred \(\mu\)g of vitamin \(B_{12}\) were administered intramuscularly every other day for 26 days. There was no reticulocytosis, but the hemoglobin increased from 8.0 to 11.4 g/100 ml. Vitamin \(B_{12}\) was stopped. One and one-half months later, when the child was 8 months old, she was readmitted to the hospital because of bronchopneumonia. Microcephaly and mental retardation were evident during that admission. Hemoglobin on admission was again 8.0 g/100 ml. A bone marrow aspirate was markedly megaloblastic. The pneumonia was treated with antibiotics.
and the patient was placed on 100 \( \mu g \) of vitamin \( B_12 \) and 15 mg of folic acid intramuscularly daily (Fig. 2). There was a rapid reticulocyte response which peaked at 16.5% on the ninth day of therapy. Subsequently, folic acid was continued orally in the same dose and vitamin \( B_{12} \) in a dose of 1000 \( \mu g \) intramuscularly twice weekly. On this regimen the hemoglobin increased to 14 g/100 ml. The bone marrow was normal with the exception of the presence of giant metamyelocytes. Hypersegmentation of neutrophils persisted on the blood smear and the red cell indices were minimally macrocytic.

Vitamin \( B_{12} \) was discontinued but the same daily dose of folic acid continued. As seen in Fig. 3, the hemoglobin dropped to 10.5 g/100 ml over a period of 215 days after stopping vitamin \( B_{12} \); 1000 \( \mu g \) of vitamin \( B_{12} \) were reinstituted intramuscularly weekly and the hemoglobin returned to a normal value. Folic acid was discontinued but vitamin \( B_{12} \) continued. The decrease in hemoglobin after stopping folic acid but continuing vitamin \( B_{12} \) is shown in Fig. 4. Fifteen mg of folic acid were restarted daily by mouth, and again with the administration of both vitamins, a normal hemoglobin level was obtained.

Both vitamins were then discontinued. Approximately 160 days later the hemoglobin had decreased to 8.0 g/100 ml. At this time the marrow was markedly megaloblastic, 2750 \( \mu g \) of folinic acid were given intramuscularly; 96 hours later there was no change in the morphology of the marrow.

After discontinuing all therapy for 7% months and when the patient was anemic and the marrow markedly megaloblastic, the patient was given 1 mg of folic acid orally daily. The reticulocyte and hemoglobin responses to this dose of folic acid are seen in

![Fig. 2.—Response to large doses of folic acid and vitamin \( B_{12} \), sibling 1.](image)

![Fig. 3.—Decrease in hemoglobin after stopping vitamin \( B_{12} \), sibling 1.](image)
CONGENITAL FAMILIAL MEGALOBLASTIC ANEMIA

Fig. 4.—Decrease in hemoglobin after stopping folic acid, sibling 1.

Fig. 5.—Response to folic acid alone, sibling 1.

For the past 2 yr she has been receiving 15 mg of folic acid orally daily and 1000 μg of vitamin B<sub>12</sub> intramuscularly once a week. Her hemoglobin remains normal on this therapy. She is now 10 yr old and is severely mentally retarded.

Sibling 2

The second sibling was also born at full term and weighed 6 lb, 5 oz. She was first seen when she was 3 wk old because of a hemoglobin of 8.7 g/100 ml. Macrocytes and hypersegmented neutrophils were seen on a blood smear. Megaloblastic anemia was confirmed by a bone marrow examination. This sibling’s response to therapy with folic acid and vitamin B<sub>12</sub> was similar to that of the first sibling. She also has been receiving 15 mg of folic acid daily and 1000 μg of vitamin B<sub>12</sub> intramuscularly once a week for the past 2 yr and has a normal hemoglobin. She is now 7-yr old and has an I. Q. of 100.

Family History

There was no other family history of anemia or history of consanguinity. Both parents have normal hemoglobin concentrations, red cell indices, reticulocyte counts, and morphology of red cells.
RESULTS OF SPECIAL LABORATORY TESTS

In Tables 1 and 2 are seen results of studies obtained in both siblings during periods of anemia and while the marrows were megaloblastic. As seen in Table 1, neither patient had orotic aciduria, abnormal excretion of amino acids in the urine, or abnormal karyotypes of the blood or bone marrow. There was no hyperuricemia. The hemoglobin electrophoresis was normal, as was the Schilling test in both patients.

As seen in Table 2, the serum vitamin B₁₂ levels were repeatedly normal in the first sibling following prolonged periods of no therapy and following periods of therapy with folic acid. Likewise, the serum vitamin B₁₂ level was normal in the second sibling while she was receiving folic acid therapy. However, the serum vitamin B₁₂ level was increased 14 months after stopping all therapy and while she was receiving vitamin B₁₂ parenterally. The following results were found in both siblings: The serum folate levels were at the upper limits of normal while receiving no therapy but were increased during periods of folic acid therapy. The red cell folate levels were normal or increased while receiving folic acid. Urinary formiminoglutamic acid excretion after 5 g of L-histidine was increased while on no therapy and also after several months of therapy with 1 mg of folic acid daily by mouth. The excretion of formiminoglutamic acid was normal after receiving 15 mg of folic acid daily for 3–6 months or after receiving a small amount of vitamin B₁₂ in addition to 1 mg of folic acid daily.

There was no abnormality in the in vitro synthesis of deoxythymidine-5-monophosphate from deoxyuridine-5-monophosphate as measured by autoradiography, after 14 months of no therapy and when the samples of marrow from both patients were markedly megaloblastic.

DISCUSSION

Most megaloblastic anemias are secondary to either a dietary deficiency of folic acid or vitamin B₁₂ or a deficiency secondary to malabsorption of either vitamin. A deficiency of either vitamin B₁₂ or folic acid was excluded in our patients by normal serum levels while they were receiving no therapy. The normal or increased folate level in the red cells while receiving folic acid indicates that there was no intracellular deficiency of folic acid. The lack of response to folinic acid indicates our patients are able to reduce folic acid normally.⁸

<table>
<thead>
<tr>
<th>Table 1.—Studies Obtained When Marrows Were Megaloblastic and Patients Not on Therapy</th>
<th>Sibling 1</th>
<th>Sibling 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary orotic acid</td>
<td>Negative × 2</td>
<td>Negative × 2</td>
</tr>
<tr>
<td>Urinary amino acids</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Chromosomal analysis blood and marrow</td>
<td>Normal karyotype</td>
<td>Normal karyotype</td>
</tr>
<tr>
<td>Serum uric acid (m/100ml)</td>
<td>4.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Hemoglobin electrophoresis</td>
<td>A/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Schilling test urinary excretion of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>⁵⁷Co-vitamin B₁₂</td>
<td>28.4%</td>
<td>39.9%</td>
</tr>
</tbody>
</table>
Table 2.—Studies Obtained While Patients Had Evidence of Megaloblastic Anemia

<table>
<thead>
<tr>
<th>Normal Range Therapy Given</th>
<th>Duration</th>
<th>Serum Vitamin B&lt;sub&gt;12&lt;/sub&gt; (µg/ml) 90-630</th>
<th>Serum Folate (µg/ml) 5-21</th>
<th>Red Cell Folate (µg/ml) 186-640</th>
<th>Formiminoglutamic Acid Excretion (mg/8 hr) 1-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid (15 mg daily)</td>
<td>9 days</td>
<td>Sib 1: 1800</td>
<td>Sib 1: 61.2</td>
<td>Sib 1: 747</td>
<td></td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (1000 µg wk)</td>
<td>3 months</td>
<td>Sib 2: 610</td>
<td>Sib 2: 747</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (15 mg daily)</td>
<td>6 months</td>
<td>Sib 1: 530</td>
<td>Sib 1: 2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>550</td>
<td>Sib 2: 550</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No therapy</td>
<td>7 months</td>
<td>Sib 1: 320,420</td>
<td>Sib 1: 16.4</td>
<td>Sib 1: 55,33</td>
<td>Sib 2: 25.2</td>
</tr>
<tr>
<td>No therapy</td>
<td>14 months</td>
<td>Sib 1: 920</td>
<td>Sib 1: 17.6</td>
<td>Sib 1: 55,33</td>
<td>Sib 2: 25.2</td>
</tr>
<tr>
<td>Folic acid (1 mg daily)</td>
<td>2 months</td>
<td>Sib 1: 360</td>
<td>Sib 1: 250</td>
<td>Sib 1: 36</td>
<td>Sib 2: 20</td>
</tr>
<tr>
<td>No vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>9 months</td>
<td>Sib 1: 59</td>
<td>Sib 1: 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (1 mg daily)</td>
<td>3 months</td>
<td>Sib 1: 240</td>
<td>Sib 1: 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>10 months</td>
<td>Sib 1: 240</td>
<td>Sib 1: 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (1 mg daily)</td>
<td>3 months</td>
<td>Sib 1: 240</td>
<td>Sib 1: 5.9</td>
<td>Sib 1: 7.8</td>
<td></td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (2 µg i.m.)</td>
<td>2 days prior</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rarely megaloblastic anemias have been reported in children which were not due to a deficiency of folic acid or vitamin B12. Such anemias have been associated with orotic aciduria, formiminotransferase deficiency, N5 methyltetrahydrofolate transferase deficiency, or an increased demand for thiamine. The anemias associated with these conditions are unresponsive to folic acid or vitamin B12. In contrast the anemia in our patients was responsive to these two vitamins. In addition, orotic aciduria was not found.

The morphologic aberration seen in megaloblastic anemia is thought to result from a decreased rate of DNA synthesis. Because suboptimal hemoglobin levels were obtained in our patients after they were given either folic acid or vitamin B12, but optimal hemoglobin responses were obtained by giving them both vitamins together, we suspected that both vitamins were necessary in the synthesis of DNA at some common point. In order for DNA synthesis to occur, all four deoxyribonucleotides must be present in equimolar ratios. It has been well established that folic acid is essential for the conversion of dUMP to dTMP, one of the four necessary deoxyribonucleotides. In 1964 Killmann, and more recently Metz and co-workers, demonstrated that vitamin B12 may be indirectly necessary for this preliminary step in DNA synthesis. We, therefore, suspected that our patients had a block in the conversion of dUMP to dTMP. However, this conversion was normal in vitro, indicating there is no block in this step. The absence of orotic aciduria would indicate that the block in DNA synthesis occurs after the formation of orotidine-5-monophosphate (OMP). Therefore, it would appear that vitamin B12 and folic acid are necessary at some step in DNA synthesis after the formation of OMP but before the synthesis of dTMP.

Although the anemia was corrected by simultaneous administration of folic acid and vitamin B12, giant metamyelocytes remained in the marrow and hypersegmentation of the neutrophils and occasional macrocytes were present in the blood. These observations would suggest that some defect in DNA synthesis persists, even though the anemia is corrected.

The increase in the hemoglobin level and normal excretion of formiminoglutamic acid after therapy with large daily doses of folic acid indicate that these patients have an aberration in folic acid metabolism. The normal excretion of formiminoglutamic acid after a small dose of folic acid plus vitamin B12, and the attainment of a normal hemoglobin value after the simultaneous administration of folic acid and vitamin B12 indicate that vitamin B12 is necessary for the proper utilization of folic acid.

ACKNOWLEDGMENT

The authors would like to thank Dr. Alvin Mauer for his encouragement and help, Mrs. Julie Alamin for her technical assistance, and Miss Betty Powell for typing the manuscript.

REFERENCES

CONGENITAL FAMILIAL MEGALOBLASTIC ANEMIA


Congenital Familial Megaloblastic Anemia

BEATRICE C. LAMPKIN, ALLAN PYESMANY, CAROL B. HYMAN and DENMAN HAMMOND