Congenital Methylmalonic Aciduria–Homocystinuria With Megaloblastic Anemia: Observations on Response to Hydroxocobalamin and on the Effect of Homocysteine and Methionine on the Deoxyuridine Suppression Test

By Ralph Carmel, Antranik A. Bedros, John W. Mace, and Stephen I. Goodman

Congenital methylmalonic aciduria–homocystinuria, a disorder with an incompletely defined cobalamin abnormality, has not been accompanied by megaloblastosis in most of the initially described cases. This has raised questions about the exact role of cobalamin in relation to megaloblastic anemia. Therefore, we present our observations on a patient with this syndrome whose manifestations conformed to current concepts of cobalamin function and on her response to hydroxocobalamin. In addition to the megaloblastic anemia (and other hematologic abnormalities) she developed severe neurologic impairment shortly after birth. Hydroxocobalamin injections diminished her methylmalonic acid and homocysteine excretion and corrected her megaloblastosis, though mild neutrophil nuclear hypersegmentation has persisted even after a year. Clinical status also improved, particularly physical growth and neurologic development. However, she remains neurologically impaired following an unexplained clinical catastrophe. We documented her megaloblastic process by demonstrating abnormal deoxyuridine suppression test results in her initial bone marrow aspirate and normal results after treatment. Furthermore, the abnormal deoxyuridine suppression was corrected in vitro by folic acid, but not by methylenetetrahydrofolic acid, cyanocobalamin, adenosylcobalamin, methylcobalamin, or methionine. Homocysteine slightly worsened the deoxyuridine suppression pattern. Subsequent studies showed that, in contrast to previous reports, homocysteine had no beneficial effect in other normoblastic or megaloblastic marrows either. The findings in our patient are compatible with the "methyltetrahydrofolate trap" hypothesis. Why this syndrome presents such a wide spectrum of manifestations is not entirely clear, nor is it clear why it should differ so from other disorders of cobalamin. These aspects are reviewed. Severity of the defect, differences in tissue susceptibility, or possibly even other factors such as ability to retain cobalamin intracellularly may influence the manifestations of this metabolic disorder.

The two end-products excreted in congenital methylmalonic aciduria-homocystinuria reflect intermediates normally metabolized by the only known cobalamin-dependent reactions in man. Fenton and Rosenberg recently reviewed the current state of knowledge of this syndrome and hypothesized that the defect involves cob(III)alamin reductase, which reduces the charge of the cobalt of cobalamin from +3 to +2. This reduction normally occurs after cobalamin enters the cell and appears to be the final step common to the production of both adenosylcobalamin and methylcobalamin. The latter two are coenzymes, respectively, for methylmalonyl-CoA mutase and 5-methyltetrahydrofolate:homocysteine methyltransferase (Fig. 1, reaction 3), whose impaired activities cause methylmalonic acid and homocysteine to accumulate in this disorder.

Thus far, six cases have been described. Interestingly, no two have had identical clinical pictures. In fact, biochemical and genetic complementation studies indicate that the biochemical expression represents at least two different genetic entities. These have been termed cobalamin C and cobalamin D mutations, the latter representing a milder biochemical and clinical defect than the former.

A puzzling feature of the disorder has been the fact that only two of the patients demonstrated megaloblastic anemia. Such anemia theoretically should always accompany this metabolic error, given the invariable megaloblastosis of acquired cobalamin deficiency and the role of cobalamin in the "methyltetrahydrofolate trap" hypothesis. The generally accepted hypothesis states, in brief, that in cobalamin deficiency folate is sequestered as methyltetrahydrofolate due to the lack of methylcobalamin (Fig. 1, reaction 3). As a result, methylenetetrahydrofolate eventually becomes depleted and unavailable for thymidylate synthesis (Fig. 1, reaction 1), and megaloblastic anemia ensues.

Therefore, we present our observations in a patient with methylmalonic aciduria-homocystinuria who demonstrated megaloblastic anemia. Furthermore, therapy with hydroxocobalamin improved the megaloblastic anemia, the excretion of both metabolites, and at least temporarily the clinical status. Such response has not been previously documented. In addition, studies with the deoxyuridine suppression test led to revised observations on the effect of homocysteine on this test.
METHYLMALONIC ACIDURIA-HOMOCYSTINURIA

CASE REPORT

D. S. was born on 6/12/78, the 2780-g product of a full-term uncomplicated pregnancy to a gravida 6, para 6 34-yr-old Mexican-American woman. Family history was unremarkable except for a maternal grandmother with a seizure disorder since the age of 4 mo. The patient’s five siblings (three of whom were by her father) and her parents were in good health. The patient was feeding well after birth and was given RhoGam because her blood type was A- and her mother’s was A+.

First admission (Loma Linda University Medical Center, 6/17/78) at the age of 5 days was for fever of 2-day duration. Weight was 2700 g and head circumference 33.5 cm. Neurologic examination, hemoglobin level (18 g/dl), mean corpuscular volume (MCV, 93 fl), and platelet, white blood cell, and differential counts were normal. Retrospective review of the peripheral blood smear, however, revealed hypersegmented neutrophils, 3% of which had 6 or more nuclear lobes. She also had a total serum bilirubin of 8.9 mg/dI, of which 1.2 mg was direct reacting. Extensive work-up for her fever was negative.

Second admission (LLUMC, 7/27/78) at 6.5 wk of age was for evaluation of seizures occurring during the previous week. These consisted of several daily episodes of fluttering of eyelids and jerking of the arms lasting a few minutes and followed by irritability. She had not yet started to smile or to follow with her eyes. Significant physical findings were 34.6 cm head circumference and 2920 g weight (both below 5th percentile), greatly increased cranial translumination (especially bifrontally), severe head lag, and perirectal excoriations.

Hemoglobin level was 8.3 g/dl, MCV 93 fl, WBC count 4900/µl with 18% neutrophils, 78% lymphocytes and 4% monocytes; 8% of the neutrophils had 6 or more nuclear lobes. The red cells were normocytic, with occasional macrocytes and several fragmented cells. The hospital course was marked by hypothermia, bradycardia, lethargy, and hypotonia. After the first week acidosis developed, probably resulting from diarrhea. Anion gap was unremarkable. She also later developed transient prerenal azotemia. Echoencephalogram and computerized tomography suggested the presence of small subdural hematomas but repeated subdural taps were negative. Electroencephalogram showed nonspecific abnormalities, and nerve conduction studies showed equivocal slowing. Electrocardiogram, echocardiogram, chest x-ray, and clinical course raised the question of myocardopathy. A range of tests were done and, with minor exceptions (lactate dehydrogenase of 367 U), were normal. Throughout her course she had hyperkalemia (5.3-8.8 meq/liter), which was treated but was felt to be primarily a spurious result of suspected hemolysis.

During the first week, hemoglobin level fell to 5.1 g/dl with reticulocyte counts of 0.4% or less (Fig. 2). The cause of this presumed hemolysis or of the sharp fall in platelet count from 151,000 to 19,000/µl was never clearly established. Bone marrow aspirated on 8/9 was hypercellular with 2:1 myeloid-erythroid ratio, adequate megakaryocytes, and 4+ iron stores; it was profoundly megaloblastic. Special studies thereupon established the diagnosis...
of congenital methylmalonic aciduria-homocystinuria (see Results Section). A bone marrow examination on 8/17 again showed megaloblastic changes, but red cell hypoplasia was now also evident. The thrombocytopenia had remitted, possibly spontaneously, but a peculiar in vitro gelling of plasma persisted for some time. Quick prothrombin time, partial thromboplastin time, thrombin time, plasma protamine test, and antithrombin III assay were normal when tested later in her hospitalization.

The patient was given 10 µg hydroxocobalamin on 4 days (Fig. 2), with no change in bone marrow appearance. Injections of 1000 µg hydroxocobalamin were begun on 8/29. Methylmalonic acid and homocystine excretion decreased thereafter, and megaloblastosis in the bone marrow, done on 9/7, improved markedly. At this time too, marrow erythroid activity reappeared (myeloid/erythroid ratio of 2:1), iron stores fell from 3-4+ to 0-1+, and reticulocytosis occurred. However, megaloblastic changes in the white cell series were still present. A final bone marrow examined on 10/3 showed normoblastic erythroid hyperplasia. Mild myeloid megaloblastic changes persisted. The patient began to gain weight and show considerable neurologic improvement. Head circumference, which was 34 cm as late as 9/7, increased thereafter. Feedings were switched from Isomil to Nutramigen on 9/30 and then Probanate, both of which contain more methionine than does Isomil (0.65 and 0.93 g/liter versus 0.4 g/liter). At discharge on 10/23, weight was 4400 g and head circumference was 39 cm. She was felt, however, still to be functioning below age level. Medications at discharge included Mycolog cream for monilial perianal rash, phenobarbital for seizures, and multivitamins with iron.

**Third admission** (Bernalillo County Medical Center, Albuquerque, 10/24/78) immediately followed her trip home from Loma Linda. She developed breathing difficulties and vomiting on the airplane, and seizures and hypotonia on disembarking. Physical examination revealed tachypnea with rhonchi and inspiratory wheezing, especially in the left lung, a liver palpable 5 cm below the costal margin, flaccid muscle tone, and intermittent focal seizures with twitching of her right leg. Furthermore she had blood in stool and urine. Hemoglobin was 11.2 g/dl, WBC 9100/µl with 82% neutrophils, 16% lymphocytes and 2% monocytes; hypersegmented neutrophils were noted. Platelet count was 74,000/µl, Quick prothrombin time 62 sec (12 sec control), partial thromboplastin time 64 sec (39 sec control), and fibrinogen level was only 25 mg/dl. Serum glutamate oxaloacetate transaminase (SGOT) was 2192 U (normal <40) and alkaline phosphatase 350 U (normal <150 for age). Bilirubin, initially normal, later rose to 1.9 mg/dl, of which 1.4 mg/dl was direct-reacting. Blood urea nitrogen (BUN) was 49 mg/dl, creatinine 1.1 mg/dl, potassium 5.7 meq/liter, arterial pH 7.46, and chest x-ray showed right middle lobe infiltrate. Left-sided atelectasis developed later.

Treatment included fresh frozen plasma and antibiotics. After a stormy course, her biochemical and coagulation abnormalities improved. However, she still had a liver palpable 2 cm below the rib margin on discharge on 11/9 with a serum bilirubin of 1.2 mg/dl. Computerized tomography showed mild prominence of the interhemispheric fissures and of the suprasellar cistern.

**Fourth and fifth admissions** (St. Vincent's Hospital, Santa Fe, 11/10 and 11/15/78) were for seizures, fever, and “fussiness.” Work-up of her fever was unrevealing though she continued to have some Candida in her left ear. Her right-sided pulmonary infiltrate continued to clear and her electroencephalogram showed rare spike activity. Blood counts and laboratory tests were unremarkable except for elevated lactate dehydrogenase, y-glutamyltransferase, alkaline phosphatase, and SGOT levels.

**Sixth admission** (BCMC, 2/5/79) was for better control of her seizure disorder. She had been getting 1000 µg hydroxocobalamin for 3 consecutive days every 2 wk. Her head circumference was 40 cm with an unusual shape due to inadequate anterior and lateral skull growth. She was irritable and her eyes tended to wander purposelessly with occasional rapid downward jerks. Her head control was poor and muscle tone was increased in the extremities. Mild peripheral “salt and pepper” retinal degeneration was noted but the retina was functionally intact. She was considered to have cortical blindness and also appeared to be deaf. Liver was palpable 1–2 cm below the rib. Her blood count and red cell indices were entirely normal as were BUN and SGOT. She continued to have frequent myoclonic jerks that had no electrical correlates on electroencephalogram.

Since discharge myoclonus has continued. In July 1979 her blood count was completely normal with hemoglobin level of 14.5 g/dl. However, neutrophil hypersegmentation has persisted. She continues to get 3 hydroxocobalamin injections every 2 wk.

**MATERIALS AND METHODS**

Amino acids in urine were measured on a Beckman model 121 M amino acid analyzer (Beckman Instruments, Palo Alto, Calif.) using lithium citrate buffers. Chromatography of organic acids in urine was performed as previously described. Methylmalonic acid was identified by combined gas chromatography–mass spectroscopy only in initial samples, when the diagnosis was being established. Later quantitation was by gas chromatography using the technique of internal standardization; the internal standard was phenylacetic acid.

Serum cobalamin was measured by radioassay, initially using saliva as the binder and later by a modification of the assay of Kolhouse et al. using partially purified intrinsic factor. Serum unsaturated cobalamin-binding capacity and transcobalamin fractionation were determined by Sephadex G-200 gel chromatography. Serum folate was assayed microbiologically. Reticulocyte uptake of 57Co-labeled cyanocobalamin (specific activity 167 µCi/µg, Amersham-Searle Corp., Arlington Heights, Ill.) was tested by the method of Retief et al. Uptake of 57Co-cyanocobalamin by the patient's bone marrow aspirate cells was performed in a similar manner. The heparinized aspirated cells were washed with Tris-buffered Hank's salt solution, pH 7.3, and passed twice through a 25-gauge needle to break up clumps and particles. The cells were then washed twice with the buffered Hank's solution, and 1-ml aliquots of resuspended marrow cells were incubated at 37°C in duplicate tubes with 0.1 ml of the patient's or a control subject's serum, 100 pg of 57Co-cyanocobalamin, and 1.0 ml of 0.9% NaCl–10 mM CaCl2. After 20 min, the reaction was terminated with cold 0.9% NaCl. The cells were centrifuged, washed twice, and their radioactivity was counted.

The deoxyuridine suppression test followed the technique of Metz et al. with minor modifications as previously described. The principle of the test is that folate or cobalamin deficiency, by virtue of the resultant lack of methylenetetrahydrofolate, leads to impairment of subsequent methyl donors to thymidine in DNA. In this test, hydroxocobalamin was converted to deoxyuridylic acid, is not effectively methylated to thymidylic acid; it is therefore incapable of normally suppressing incorporation of subsequently added 3H-thymidine into DNA.) Iron was added to all tubes to circumvent the effect of iron deficiency on this test. All testing was done in triplicate. Because of the small amounts of marrow available, we initially dispensed with deoxyuridine-free controls for each added vitamin (but not for the added amino acids); instead, all deoxyuridine-suppressed results were compared to the single baseline deoxyuridine-free result without additives. Deoxyuridine suppression was calculated as percent residual 3H-thymidine incorporated into DNA in the presence of deoxyuridine as compared to the deoxyuridine-free incorporation; normally, residual incorporation is <10%, meaning effective suppression.

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**References**

2. Carmel et al. (1976) Blood 48: 572
5. Carmel et al. (1975) Blood 46: 572
7. Carmel et al. (1975) Blood 46: 572
11. Carmel et al. (1975) Blood 46: 572
13. Carmel et al. (1975) Blood 46: 572
15. Carmel et al. (1975) Blood 46: 572
17. Carmel et al. (1975) Blood 46: 572
23. Carmel et al. (1975) Blood 46: 572
25. Carmel et al. (1975) Blood 46: 572
27. Carmel et al. (1975) Blood 46: 572
29. Carmel et al. (1975) Blood 46: 572
31. Carmel et al. (1975) Blood 46: 572
33. Carmel et al. (1975) Blood 46: 572
Table 1. Studies Demonstrating That D.S. Serum Mediates Cyanocobalamin Uptake by Reticulocytes Normally and That D.S. Marrow Takes up Cyanocobalamin Normally

<table>
<thead>
<tr>
<th>Uptake of $^{57}$Co-cyanocobalamin by</th>
<th>Reticulocyte-Rich RBC</th>
<th>D.S. Bone Marrow Aspirate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediated by</td>
<td>Net cpm</td>
<td>Ratio to Buffer-Mediated Uptake</td>
</tr>
<tr>
<td>Buffer</td>
<td>122</td>
<td>—</td>
</tr>
<tr>
<td>D.S. serum</td>
<td>857</td>
<td>7.0</td>
</tr>
<tr>
<td>Normal serum</td>
<td>1052</td>
<td>8.6</td>
</tr>
<tr>
<td>High transcobalamin I serum</td>
<td>193</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Because of the instability of the various cobalamins to light, all incubations were in the dark. Cyanocobalamin, methylcobalamin, adenosylcobalamin, methyltetrahydrofolic acid, folic acid (pteroylglutamic acid) and d,l-homocysteine were obtained from Sigma Chemical Co., St. Louis, Mo., and L-methionine from Nutritional Biochemical Co., Cleveland, Ohio.

RESULTS

Cobalamin, Transcobalamin, and Folate Studies

Initial serum cobalamin level was normal (610 ng/liter), but serum folate level was >50.0 μg/liter (normal = 5–25). Red cell folate was greatly elevated (1559 μg/liter) during her third admission. At the age of 1 yr, serum cobalamin was >3000 ng/liter (following a recent hydroxocobalamin injection), and serum folate was 43.5 μg/liter.

Serum unsaturated cobalamin-binding capacity before treatment was 830 ng/liter, of which 639 ng/liter was transcobalamin II, and cerebrospinal fluid cobalamin-binding capacity was 100 ng/liter, 60 ng/liter being transcobalamin II. All of these were within normal limits. Her serum transcobalamin II was functionally normal. Like normal serum, it greatly enhanced cyanocobalamin uptake by a control subject’s reticulocytes when compared to buffer- or transcobalamin-I-mediated uptake (Table 1). The patient’s bone marrow uptake of $^{57}$Co-cyanocobalamin was enhanced by transcobalamin II, as expected (Table 1).

Family Study

Her parents and three full siblings all had normal blood counts and serum cobalamin levels. None excreted methylmalonic acid or homocystine. Curiously, two of her siblings had elevated serum folate levels (30.3 and 39.1 μg/liter).

Amino Acid and Methylmalonic Acid Studies

At presentation, urinary excretion of methylmalonic acid (0.47–4.98 mg/mg creatinine), homocystine (0.16–0.36 mg/mg creatinine), and cystathionine (0.08–0.12 mg/mg creatinine) was elevated. Normally, the first two should be undetectable, while normal urinary cystathionine at that age is 0.006–0.021 mg/mg creatinine. Plasma amino acid analysis showed decreased methionine (0.007 μM/ml; normal = 0.01–0.02). Levels of threonine, asparagine, proline, valine, isoleucine, leucine, and tyrosine were also low, while aspartic acid, serine, glutamic acid, glycine, cystathionine, homocystine, ornithine, and histidine levels were increased. However, the blood sample was not obtained in a fasted state.

The course of her methylmalonic aciduria and homocystinuria, and their relationship to therapy and hematologic status, are shown in Fig. 2. In April 1979, urinary methylmalonic acid was 0.087 mg/mg creatinine and homocystine was 0.02 mg/mg creatinine. In June 1979, homocystine was not detectable in plasma and methionine was 0.01 μM/ml.

Deoxyuridine Suppression Test (Table 2)

The patient’s bone marrow of 8/29 was megaloblastic not only morphologically but also by deoxyuridine suppression (Table 2). After hydroxocobalamin therapy deoxyuridine suppression became normal (marrowsof 9/7 and 10/3).

The initial marrow was not improved with small in vitro doses of various cobalamins (1 μg/tube), indicating that mere deficiency of cobalamin was not the problem. Furthermore, cyanocobalamin actually aggravated the defect. That the initial marrow’s deoxyuridine suppression pattern was not improved by methyltetrahydrofolic acid (50 μg/tube) but was improved by folic acid (50 μg/tube) is compatible with a cobalamin-related abnormality in the “methyltetrahydrofolate trap” hypothesis. In fact, methyltetrahydrofolic acid seemed to aggravate the abnormality not only in the megaloblastic marrow but also slightly worsened the behavior of her subsequent normoblastic marrow specimens.

Effect of Homocysteine and Methionine on Deoxyuridine Suppression

Homocysteine (100 μg/tube) added in vitro in the final marrow experiment (Table 2) had a mildly but
Table 3. Effect on the Deoxyuridine Suppression Test of Different Concentrations of Homocysteine and Methionine Added to Normoblastic Marrow 2 from Table 2, With Special Attention to Effects on $^3$H-Thymidine Incorporation Into DNA With and Without Added Deoxyuridine

<table>
<thead>
<tr>
<th>In Vitro Additions</th>
<th>None</th>
<th>Cyanocobalamin</th>
<th>Methylcobalamin</th>
<th>Adenosylcobalamin</th>
<th>Folic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.S. marrow</td>
<td>45%</td>
<td>56%</td>
<td>46%</td>
<td>46%</td>
<td>16%</td>
</tr>
<tr>
<td>8/29</td>
<td>66%*</td>
<td>46%</td>
<td>46%</td>
<td>46%</td>
<td>16%</td>
</tr>
<tr>
<td>9/7</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
<td>8%</td>
<td>5%</td>
</tr>
<tr>
<td>11%*</td>
<td>15%*</td>
<td>10%*</td>
<td>12%*</td>
<td>5%</td>
<td>10%*</td>
</tr>
<tr>
<td>10/3</td>
<td>14%†</td>
<td>11%†</td>
<td>8%</td>
<td>5%</td>
<td>10%*</td>
</tr>
</tbody>
</table>

Control marrows

<table>
<thead>
<tr>
<th>None</th>
<th>Cyanocobalamin</th>
<th>Folic Acid</th>
<th>Methylererythrofolic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoblastic</td>
<td>6%</td>
<td>4%</td>
<td>9%</td>
</tr>
<tr>
<td>Normoblastic</td>
<td>7%†</td>
<td>4%†</td>
<td>9%†</td>
</tr>
<tr>
<td>Normoblastic</td>
<td>12%‡</td>
<td>5%‡</td>
<td>16%‡</td>
</tr>
<tr>
<td>Normoblastic</td>
<td>9%</td>
<td>10%</td>
<td>8%</td>
</tr>
<tr>
<td>Normoblastic</td>
<td>11%‡</td>
<td>11%‡</td>
<td>16%‡</td>
</tr>
<tr>
<td>Normoblastic</td>
<td>39%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Normoblastic</td>
<td>37%†</td>
<td>18%†</td>
<td></td>
</tr>
<tr>
<td>Normoblastic</td>
<td>51%‡</td>
<td>19%‡</td>
<td></td>
</tr>
<tr>
<td>Normoblastic</td>
<td>41%</td>
<td>25%</td>
<td>22%</td>
</tr>
<tr>
<td>Normoblastic</td>
<td>46%†</td>
<td>36%†</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as percent residual $^3$H-thymidine incorporation into DNA when deoxyuridine had been added. Normal marrow values are <10%; megaloblastic marrow usually has >20% incorporation.

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consistently deleterious effect on deoxyuridine suppression (producing 14% residual $^3$H-thymidine incorporation, compared to 7% without homocysteine and 8% with methionine). Because this finding was surprising, we then studied two normoblastic and two megaloblastic adult subjects. These showed that methionine (100 $\mu$g/tube) aggravated the suppression defect in one normoblastic marrow and in the folate-deficient marrow, as previously reported, though not in the cobalamin-deficient marrow (Table 2, bottom half). In none of the four, however, did homocysteine improve deoxyuridine suppression. In fact, in the folate-deficient marrow, it appeared to interfere with the corrective effect of folic acid. The reason for the apparent discrepancy between our results and previous ones was clarified by dose-effect studies in the second normoblastic marrow experiment. Table 3 shows that 1 mg of homocysteine, as used in previous studies, impairs $^3$H-thymidine incorporation into DNA whether or not deoxyuridine

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Table 3. Effect on the Deoxyuridine Suppression Test of Different Concentrations of Homocysteine and Methionine Added to Normoblastic Marrow 2 from Table 2, With Special Attention to Effects on $^3$H-Thymidine Incorporation Into DNA With and Without Added Deoxyuridine

<table>
<thead>
<tr>
<th>$^3$H-Thymidine Incorporated Into DNA (cpm)*</th>
<th>Percent Residual Incorporation (B / A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Without Deoxyuridine</td>
<td>(B) With Deoxyuridine</td>
</tr>
<tr>
<td>+ o,l-homocysteine</td>
<td>7,798</td>
</tr>
<tr>
<td>+ 100 $\mu$g</td>
<td>7,272</td>
</tr>
<tr>
<td>+ 1 mg</td>
<td>3,703</td>
</tr>
<tr>
<td>+ 2 mg</td>
<td>3,107</td>
</tr>
<tr>
<td>+ l-methionine</td>
<td>9,470</td>
</tr>
<tr>
<td>+ 100 $\mu$g</td>
<td>8,584</td>
</tr>
<tr>
<td>+ 1 mg</td>
<td>3,073</td>
</tr>
</tbody>
</table>

*Average of triplicate tubes.
Table 4. Comparison of Clinical Features in Patient D.S. and in Previously Published Cases of Congenital Methylmalonic Aciduria-Homocystinuria

<table>
<thead>
<tr>
<th>Cobalamin mutation</th>
<th>DS</th>
<th>EM*</th>
<th>MM†</th>
<th>XR</th>
<th>SB*</th>
<th>JR**</th>
<th>MR†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at presentation</td>
<td>5 wk</td>
<td>4 wk</td>
<td>6 mo</td>
<td>3 yr</td>
<td>Birth</td>
<td>14 yr</td>
<td>(2½ yr)†</td>
</tr>
<tr>
<td>Age at death</td>
<td>—</td>
<td>7½ wk</td>
<td>7 yr</td>
<td>—</td>
<td>4 mo</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

| Anemia | Yes | Yes | Yes | No | Yes | No |
| Megaloblastosis | Yes | Yes | Yes | No | Yes | No |
| Red cell hypoplasia, ? hemolysis | Yes | Yes | Yes | No | Yes | No |
| Intermittent reticulocytosis | — | — | — | — | — | — |
| Neurologic disorder | Yes | Yes | Yes | Yes | Yes | Yes |
| Cobalamin therapy | OH-Cbl | CN-Cbl§ | CN-Cbl|| | OH-Cbl** | CN-Cbl|| | OH-Cbl |
| Improvement of Methylmalonic aciduria | Yes | No | No | Yes | Yes | Yes |
| Homocystinuria | Yes | Yes | — | Yes | Yes | Yes |
| Anemia | Yes | No | ?†† | — | No | — |
| Clinical status | Yes | No | — | Yes | Yes | ? |

OH—Cbl, hydroxocobalamin; CN—Cbl, cyanocobalamin; Ado—Cbl, adenosylcobalamin.

*Cobalamin C presumed but not documented.
†Patient asymptomatic; discovered on family study.
§Bone marrow not examined.
||Single injections of 50 µg and 25 µg.
†Five 1000-µg injections.
*Five 1000-µg injections.
**Daily 1000-µg injections.
††Hemoglobin level rose but marrow remained megaloblastic.

Methionine did not produce noticeable worsening of deoxyuridine suppression in marrow 2 at our 100-µg dose level but clearly did so when 1 mg was added. Methionine seemed to slightly enhance 3H-thymidine incorporation into DNA. As with homocysteine, but in the opposite direction, comparison to methionine controls without deoxyuridine produces different suppression results than comparison to the amino-acid-free baseline. Nevertheless, results in the adult marrows in Table 2 indicate that methionine has an “antifolate” effect even at the 100-µg dose level.

DISCUSSION

Our patient had methylmalonic aciduria, homocystinuria, cystathioninuria, and low plasma methionine in the face of normal serum cobalamin and transcobalamin II levels, all typical findings of the syndrome of congenital methylmalonic aciduria-homocystinuria. Her marrow cells were able to take up cyanocobalamin normally in vitro, though we did not establish whether cobalamin entered the cell. Complementation studies using her fibroblasts (H. Willard and L. Rosenberg, unpublished data) identified her as a cobalamin C mutant.

Table 4 compares our patient’s clinical picture to that of the six other published cases, and several generalizations may be made about this disorder. The disturbing heterogeneity of the manifestations can be considerably simplified by separating away the two brothers with the cobalamin D mutation, which represents a different genetic defect involving the cobalamin pathway.1 In keeping with their milder biochemical abnormality, those brothers had mild or absent clinical manifestations and had no megaloblastic anemia.1

The four or five* patients now constituting the cobalamin C group show a greater, though not total, clinical homogeneity. They tend to present at a very
early age and to have a stormy clinical course and poor growth. The chief sequelae have been neurologic: microcephaly, mental retardation, an atypical seizure disorder, and hypotonia. Post-mortem examination of the brain has been variable. In one case,2 histologic changes were felt to resemble those of pernicious anemia,23 but a second case2 showed arteriolar changes thought to resemble those of homocystinemia due to cystathionine synthetase deficiency,24 and a third had no histologic abnormalities in the brain although he did have arterial and arteriolar changes elsewhere, suggesting to the authors the possibility of thrombotic microangiopathy.6

None of the patients had the clinical spinal cord involvement usually seen in older patients with cobalamin deficiency, but spinal cord was not examined post-mortem. Some patients, including ours, have had slowed nerve conduction.5 While nerve biopsy was negative in one case,5 neural fatty acid changes have been described in another.29

In addition to the neurologic problems, severe, poorly understood complications occurred in three patients. These included, in varying combinations, presumed hemolysis with red cell fragmentation, red cell hypoplasia, myeloid hyperplasia, neutropenia, thrombocytopenia, bleeding, possible disseminated intravascular coagulation (postulated but never confirmed), congestive heart failure, apparent myocardiopathy, prerenal azotemia, hepatic disease, and episodes of acidosis apparently unrelated to the organic aciduria. Our patient has recovered from these various complications, though she is left with serious neurologic disability; the other two succumbed.26

Whether the vascular changes noted on post-mortem examination in those cases caused the complications is unclear. The possible contributory role of homocysteine itself also remains an open question.6,26 The peculiar gelling of our patient's blood in vitro may conceivably be related to these problems.

The anemia in this disorder has generated particular interest. Megaloblastic anemia was uncommon among the first few cases described, a paradox that, among other things, cast some doubt on the validity of the "methyltetrahydrofolate trap" hypothesis. Now only two of the cobalamin C mutants remain without megaloblastic anemia, though the question remains why those two, and the cobalamin D mutants, had no megaloblastosis. One patient was not even anemic and had no bone marrow examination.5 She was diagnosed at a much later age than the others, had a relatively benign course, and thus seems to fit somewhere between the typical cobalamin C and cobalamin D pictures. She may represent a milder biochemical subtype or have some mitigating factor. Perhaps it is relevant that the two patients with megaloblastic anemia so studied had not only the decreased tissue levels of methylcobalamin and adenosylcobalamin that one expects in this disorder but also low total cobalamin content,4,22 whereas a patient without megaloblastosis had fairly normal tissue total cobalamin content (with low adenosylcobalamin fraction).2

Furthermore, the finding that one patient demonstrated less markedly abnormal tissue cobalamins in her erythrocytes and possibly spleen than in her liver, kidney, and brain6 may be relevant. Hematopoietic tissue may be less susceptible to the defect of this disorder than other tissues, at least in young children, and thus may become megaloblastic only in the more severe cases. A recent review30 emphasized that differences in methyltransferase and other enzyme activities occur between different organs as well as between adult and fetal tissues. Comparisons of enzyme and cobalamin levels in bone marrow and other tissues of the patients would help resolve the issue. In some ways, too, the occasional absence of megaloblastic anemia in patients with congenital methylmalonic aciduria-homocystinuria is reminiscent of the consistent failure to produce such anemia in animal models of cobalamin deficiency. This too may provide clues for further investigation.

Several features of the megaloblastic anemia are worth emphasizing. Both our patient and patient S.B.6 had normal blood counts at birth and became anemic only some weeks later. The key to early recognition of the megaloblastic process is hypersegmentation of neutrophil nuclei, which appeared in both cases within a few days of birth. Others have commented on the importance of this morphological clue in the diagnosis of megaloblastic anemia in children.27 Also interesting our patient’s neutrophil hypersegmentation was persisting for a year despite the correction of bone marrow erythroid morphology and deoxyuridine suppression pressure. Myeloid changes normally are the last to be corrected in treated megaloblastic anemia,28 but their persistence in our patient is remarkable. Neutrophil abnormality appears to be unusually resistant to therapy in this syndrome.

Macrocytosis, in contrast, is an inconstant development. Although some macrocytes appeared in our patient, her MCV remained normal. Furthermore, added causes contributed to and perhaps predominated in the development of the anemia itself in her and several others. Our patient and patient S. B.6 may have had hemolysis. She and patient E. M.2 also had red cell hypoplasia, which occurs as a transient phenomenon in children29 and has also been described in several malnourished adults with megaloblastic anemia.30
Our bone marrow deoxyuridine suppression results established several points. Biochemical megaloblastosis was identified. Its correction by folic acid but not by methyltetrahydrofolate acid in vitro is compatible with the “methyltetrahydrofolate trap” hypothesis and fits with the role of cobalamin established in acquired cobalamin deficiency. Furthermore, with hydroxocobalamin therapy, the deoxyuridine suppression test reverted to normal. The mild megaloblastosis remaining was limited to nondividing myeloid cells, hence presumably the normal deoxyuridine suppression result.

Unresponsiveness of our patient’s marrow to a small amount of cyanocobalamin or adenosylcobalamin added in vitro was not surprising. Why methylcobalamin produced no response, even though it was not inhibitory like cyanocobalamin, is unclear. Cobalamin-deficient bone marrow is usually exquisitely responsive to methylcobalamin in this test system. Perhaps the unresponsiveness reflects the fact that in vitro studies have shown incomplete methyltransferase holoenzyme formation when methylcobalamin was added, even though the basic defect seems to be lack of appropriate cobalamin coenzymes. The finding is also compatible with the possibility that retention or degradation of the cobalamins may be abnormal in this disease.

The amino acid results in our deoxyuridine suppression test were unexpected. Methionine added in vitro is said to worsen deoxyuridine suppression (i.e., to have an antifolate or anticobalamin effect). Homocysteine reportedly has a beneficial effect on the test, though such a finding is not readily compatible with the “methyltetrahydrofolate trap” hypothesis. We studied these phenomena in our patient only after she had already been treated. Methionine had little effect, perhaps in line with previous observations and our own confirmation here that methionine affected cobalamin-deficient marrow much less than folate-deficient marrow. However, homocysteine caused a small, but definitely deleterious effect. Our subsequent experiments with control marrows produced variable results. Methionine usually but not always demonstrated “antifolate” activity. Homocysteine usually had little clear-cut effect, but at no time was it beneficial.

This discrepancy with previously reported data appears traceable to the fact that we used lower concentrations of amino acids (100 μg, whereas the earlier studies used 1 mg amino acid per tube). Another factor may be the need for individual deoxyuridine controls for each additive to detect its possible direct effect on 3H-thymidine incorporation into DNA as previously noted. Homocysteine in concentrations of 1 mg/tube appears to have inhibited 3H-thymidine incorporation by mechanisms unrelated to deoxyuridine suppression. While we have not excluded the possibility that this inhibition of 3H-thymidine incorporation reflects enhancement by homocysteine of conversion of endogenous, previously blocked deoxyuridine, such an explanation would be unlikely. Homocysteine at this high dose level has been shown to impair both deoxyuridine and thymidine incorporation into DNA and, in fact, to be cytotoxic. Thus, homocysteine seems to have no beneficial effect on deoxyuridine suppression, and its activity is best reflected by comparison to the controls containing homocysteine shown in Table 3. Our methionine data do not contradict previous results, although 1 mg/tube concentrations may exaggerate methionine effect. It still remains possible that even our concentrations were too great. Further studies are underway to reassess the effects of the various amino acids.

The general theoretical approaches to therapy in methylmalonic aciduria-homocystinuria have been reviewed. Our patient was the first in whom cobalamin therapy not only decreased methylmalonic acid and homocysteine excretion but also corrected megaloblastic anemia. The therapy also produced clinical improvement, notably the growth of the head. However, the megaloblastic process was not totally obliterated, since hypersegmentation of neutrophil nuclei has persisted, and methylmalonic aciduria and homocystinuria have never totally disappeared. More intensive hydroxocobalamin and/or dietary therapy might possibly achieve such results. It is impossible to tell whether the catastrophic events of her third hospitalization and the subsequent neurologic deterioration were directly related to cobalamin metabolism or not.

Finally, among the many puzzles posed by this entity there is also the intriguing question of why the various acquired and hereditary cobalamin disorders differ in their manifestations when all have the final outcome of impaired cobalamin activity. These differences have been alluded to previously and are outlined in Table 5. One striking feature is the diversity of the neurologic abnormalities, if one sets aside the background of listlessness, irritability, and failure to thrive common to most children with any cobalamin abnormality. Possibly, the specific neurologic manifestations depend primarily on the developmental state of the central nervous system: the younger the child the greater the likelihood of predominantly cerebral and mental abnormality, while the typical adult spinal cord involvement does not occur or cannot be recognized.

However, the usual lack of any neurologic abnormalities in transcobalamin II deficiency contrasts particularly dramatically with the severe neurologic
manifestations of congenital methylmalonic aciduria-homocystinuria. To further highlight the contrast between these two entities, the former is dominated by severe and often brittle megaloblastic anemia, whereas the latter does not manifest megaloblastic anemia in all cases. One likely explanation may be that transcobalamin II is crucial for delivering cobalamin to bone marrow but is less crucial for other organs, such as liver (hence also the frequent lack of methylmalonic aciduria and homocystinuria in transcobalamin II, such as transcobalamin III, deficiency) and brain. As suggested in a study using rabbits, other proteins besides transcobalamin II may be capable of delivering cobalamin to liver.47 In contrast, hematopoietic tissue may be less susceptible than brain, liver, and other tissues to the defect in congenital methylmalonic aciduria-homocystinuria. Only the more severely affected individuals with congenital methylmalonic aciduria-homocystinuria, or those not possessing some mitigating mechanisms, thus, would develop megaloblastic anemia.

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