Failure of Response to N⁵-methyltetrahydrofolate in Combined Folate and B₁₂ Deficiency

Evidence in Support of the "Folate Trap" Hypothesis

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A patient with combined folate and B₁₂ deficiency due to tropical sprue failed to respond to the administration of N⁵-methyltetrahydrofolate when given by mouth or intravenously. After the injection of 1 µg of B₁₂ intravenously the patient became asymptomatic and laboratory tests returned to normal. Failure of response to N⁵-methyltetrahydrofolate in the presence of B₁₂ deficiency is evidence in favor of the "folate trap" hypothesis.

To date, only two B₁₂-dependent enzymes have been demonstrated in mammalian species, methylmalonyl-CoA mutase and N⁵-methyltetrahydrofolate-homocysteine methyl transferase (1). The latter enzyme is required for transfer of a methyl group from N⁵-methyltetrahydrofolate to homocysteine. This results in the formation of tetrahydrofolate and methionine. Tetrahydrofolate is then available to enter into further one-carbon transfers necessary for DNA synthesis (2).

To explain the occurrence of megaloblastic anemia in B₁₂ deficiency states, it has been suggested that N⁵-methyltetrahydrofolate cannot be converted to tetrahydrofolate, thereby leading to decreased DNA synthesis and consequent megaloblastosis. This theory is known as the "methyltetrahydrofolate trap" or "folate trap" hypothesis (3, 4).

The patient with tropical sprue is frequently deficient in both B₁₂ and folic acid, and indeed, the disease has been shown to respond to therapy with oral or parenteral crystalline folic acid and parenteral vitamin B₁₂ (5).

The availability of a patient with tropical sprue, deficient in both folic acid and vitamin B₁₂, provided an opportunity to test the response of bone marrow and intestinal mucosal cells to N⁵-methyltetrahydrofolate in the presence of B₁₂ deficiency.

CASE REPORT

RM (Lincoln Hospital #38-95-63), a 33-year-old Puerto Rican female, entered Lincoln Hospital on January 30, 1971, with a chief complaint of weakness and dizziness for 3 months accompanied by diarrhea and a 24-pound weight loss. She had last lived in Puerto Rico in 1961. One year prior to admission, she had been treated elsewhere for weakness with multivitamin therapy.

Physical examination was unremarkable except for cachexia. Laboratory examination revealed: hematocrit 20%, WBC 3900, platelet count 167,000; the smear was macrocytic with hypersegmented polymorphonuclear cells. Bone marrow was megaloblastic with giant bands and 3+ iron stores. Serum folate (6) (L. casei): 1.2 × 10⁻⁹ g/ml (normal range in our laboratory 4.5 to 14 × 10⁻⁹ g/ml; serum B₁₂ by isotope dilution (7): 100 µg/ml (normal 200 to 900 µg/ml); serum haptoglobin was not detectable.

Prothrombin time: 18 seconds with 14 seconds control; calcium 8.4 mg%; total protein 6.5 g%; albumin 4.3 g%; se-
COMBINED FOLATE AND B₁₂ DEFICIENCY

Fig 1. Small bowel biopsy at the level of the ligament of Treitz. Subtotal villous atrophy and round cell infiltration of the lamina propria are present.

rum carotene was not detectable. D-xylose excretion (25-g dose) was 0.9 g/5 hr (normal greater than 5 g); 72-hr stool fat, 12 g/24 hr.

Small intestinal biopsy taken at the ligament of Treitz revealed subtotal villous atrophy with round cell infiltration of the lamina propria compatible with tropical sprue (Figure 1).

The patient was maintained on a 2500 calorie hospital diet for 3 weeks. This diet contains 100 to 125 μg free folate (L casei) and 300 to 400 μg of folate activity after exposure to chick pancreas. When the patient failed to improve, informed consent was obtained, and she was given N⁵-methyltetrahydrofolate.

METHODS

DL-5-Methyltetrahydrofolate* was dissolved in isotonic saline containing 2% ascorbate and assayed for folate activity using L casei (6) and S faecalis (8). No activity for S faecalis was found to be present. On the basis of the L casei activity, this material was divided into aliquots containing 100 μg of active material. By microbiologic assay, the commercial material was found to contain 40 to 45% of its stated content of N⁵-methyltetrahydrofolate by weight. The aliquots were passed through a 0.22 micron sterile filter (Millipore Filter Corporation, Bedford, MA), drawn into 20 ml sterile plastic syringes, and frozen. The filtered material was bacteriologically sterile on blood agar and thioglycollate broth and was nonpyrogenic in both guinea pigs and human volunteers.

The patient was begun on 100 μg of N⁵-methyltetrahydrofolate orally, given each morning on an empty stomach. Serum folate, serum B₁₂, hematocrit, and reticulocyte counts were monitored.

At the conclusion of 10 days of oral therapy, the patient was given 100 μg of N⁵-methyltetrahydrofolate intravenously. Serum folate levels were monitored every 15 minutes for 4 hours and then daily for 10 days. At the conclusion of this period, 1 ml of vitamin B₁₂ (Vitarine Corporation, New York, NY) containing 1000 μg of B₁₂ was diluted in 1000 ml of sterile isotonic saline. One ml (containing 1 μg) was withdrawn and injected intravenously.

RESULTS

The results described are illustrated in Figure 2. The administration of 100 μg/day of active N⁵-methyltetrahydrofolate in ascorbate for 10 days did not result in an increase in serum folate.