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N^5-Methyltetrahydrofolate-Homocysteine Cobalamin Methyltransferase Activity in Chronic Erythremic Myelosis (Di Guglielmo Syndrome) (39831)[1]

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In the present study, the activity of N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase was determined in sonicates of marrow obtained from patients with chronic erythremic myelosis (DiGuglielmo syndrome), severe untreated pernicious anemia and various other types of marrows that served as controls.

Materials and methods. Bone marrow was obtained by sternal or posterior iliac puncture in heparinized syringes at the time of diagnosis from five patients with chronic erythremic myelosis (DiGuglielmo syndrome). Clinical and hematological findings in these patients fulfilled currently accepted diagnostic criteria for this preleukemic disorder (1-3). All patients had splenomegaly, anemia with hemoglobins ranging from 7-9 g%, leukopenia, thrombocytopenia, reticulocytopenia, and marked megaloblastoid erythroid hyperplasia of the marrow. Approximately 50% of erythroid precursors were proerythroblasts, and 40% were megaloblastoid intermediate macronormoblasts. Ringed sideroblasts and PAS (periodic acid-Schiff) positivity of the majority of erythroid precursors were observed in all marrows. All patients had serum vitamin B_{12} levels greater than 800 pg/ml at the time of diagnosis, although none of them had been treated with vitamin B_{12} for at least 8 months prior to diagnosis.

Marrow was also obtained from three patients with severe untreated pernicious anemia who had hemoglobins ranging from 3-5 g%, leukopenia, thrombocytopenia, marked megaloblastic erythroid hyperplasia of the marrow with approximately 50% proerythroblasts and 40% intermediate megaloblasts, and serum B_{12} levels less than 60 pg/ml. As additional controls, marrow was obtained from two patients with severe untreated autoimmune hemolytic anemia, who had marked normoblastic erythroid hyperplasia of the marrows with approximately 20% proerythroblasts and 70% intermediate normoblasts, and from two patients with myelomonocytic leukemia (95% or greater leukemic blasts) as examples of high cell turnover of nonerythroid tissue. Marrow was also obtained from eight presumed normal persons undergoing evaluations for medical illnesses that did not ultimately disclose hematological abnormalities. These normal marrows contained approximately 30-40% erythroblasts.

Marrow flecks were washed in phosphate-buffered saline, pH 7.4, and approximately 100-300 mg of marrow particles was recovered. Because of sample limitations in a diagnostic marrow aspiration and the predominantly erythroid character of marrows from patients with chronic erythremic myelosis and pernicious anemia, further fractionations of marrow particles were not attempted. Marrow flecks were suspended in 0.4-2.0 ml (depending on the amount of marrow available) of 80 m*M* K_2PO_4 buffer, pH 7.4, and were subjected to two 5-sec microprobe sonications using a Branson sonicator. Marrow sonicates were dialyzed overnight in phosphate buffer at 2-5° and were assayed for N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase activity (4). In all instances, approximately 1.0 mg of protein was used for the assays, and nonenzymatic controls were included. Since previous experiments demonstrated substantial loss of enzymatic activity after freezing, in contrast to the findings of Taylor *et al.* (4), the present assays were performed on fresh undialyzed sonicates

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and on dialyzed sonicates. Nonradioactive substrate reagents and methylcobalamin (99% pure by spectroscopy and chromatography) were purchased from Sigma, St. Louis, Mo. [^{14}C]Methyltetrahydrofolate (specific activity: 53 μCi/μmole) was obtained from Amersham/Searle. All reactions were carried out in the dark for 3 hr at 37° under hydrogen gas. Enzymatic activity was expressed as picomoles of [^{14}C]-methionine per milligram of protein per hour.

In addition to the complete system, effects of omission of various essential substrates in the enzymatic reaction using pathological and normal marrows were studied (4). Increased concentrations of essential substrates such as *S*-adenosylmethionine, homocysteine, and methyltetrahydrofolate were added to the point of and in excess of saturation to ascertain their effects on enzymatic activity. Aqueous solutions of methylcobalamin at a final concentration of 50 μM were added to marrow sonicates in an effort to determine whether N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase in chronic erythremic myelosis exists as the apoenzyme as in untreated pernicious anemia (4), or primarily as the holomethyltransferase. In sonicates of marrows from patients with untreated pernicious anemia, various concentrations of methylcobalamin were added to obtain apoenzyme activation curves.

Results. Table 1 demonstrates marrow N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase activity in five patients with chronic erythremic myelosis. Compared to activity in untreated pernicious anemia and normal marrows, the activity of the enzyme in four of five patients was increased, ranging from 8701 to 13,548 pmole of [^{14}C]methionine/mg of protein/hr and, in some instances, was almost four times greater than normal. In one patient, enzymatic activity was lower than in normals and the other four patients with chronic erythremic myelosis. Omission of *S*-adenosylmethionine or $FMNH_2$ (flavin mononucleotide, reduced) + DTT (dithiothreitol) led to a substantial reduction of enzymatic activity. Increased *S*-adenosylmethionine, homocysteine, or methyltetrahydrofolate to saturation levels did not increase enzymatic activity appreciably. Likewise, addition of methylcobalamin had no significant effect on activity of N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase.

Table 2 shows the effects of methylcobalamin added to sonicates of marrow from patients with untreated pernicious anemia. In all instances, activity increased substantially. Figure 1 demonstrates the effects of various concentrations of methylcobalamin on N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase in sonicates of marrow from two patients with severe untreated pernicious anemia. These apparent apoenzyme activation curves indicate formation of active B_{12} holomethyltransferase. In incubation mixtures containing methylcobalamin, omission of *S*-adenosylmethionine or $FMNH_2$ + DTT led to marked reduction of enzymatic activity. Increased concentration of substrates did not affect activity of the enzyme appreciable.

Table 3 demonstrates N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase activity in control marrows. In

TABLE 1. N^5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE COBALAMIN METHYLTRANSFERASE SPECIFIC ACTIVITIES IN MARROWS FROM PATIENTS WITH CHRONIC ERYTHREMIC MYELOSIS.[a]

Patient	Activity	Activity with added methylcobalamin (50 μM)
1	1,934	1,840
2	8,701	9,993
3	8,575	6,785
4	10,641	10,828
5	13,548	13,764

[a] Activity is expressed as picomoles of [^{14}C]methionine per milligram of protein per hour.

TABLE 2. N^5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE COBALAMIN METHYLTRANSFERASE SPECIFIC ACTIVITIES IN MARROWS FROM THREE PATIENTS WITH SEVERE UNTREATED PERNICIOUS ANEMIA.[a]

Patient	Serum B_{12} (pg/ml)	Activity	Activity with added methylcobalamin (50 μM)
6	58	458	1465
7	43	528	1222
8	14	931	3054

[a] Activity is expressed as picomoles of [^{14}C]methionine per milligram of protein per hour.

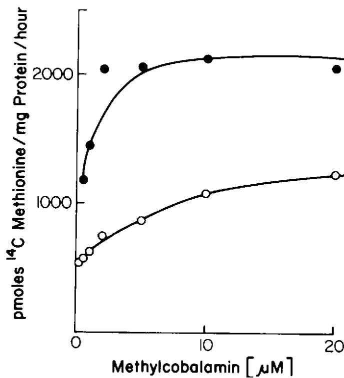

FIG. 1. Increase in N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase activity occurring as the concentration of methylcobalamin is increased in sonicates of marrows obtained from two patients with severe untreated pernicious anemia. Serum B_{12} concentration of patient 7 (open circles) was 43 pg/ml, and, in patient 8 (solid circles), was 14 pg/ml. These findings reflect progressive saturation of the apoenzyme by increasing increments of methylcobalamin, causing increased enzymatic activity as a result of formation of active B_{12} holomethyltransferase.

TABLE 3. N^5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE COBALAMIN METHYLTRANSFERASE SPECIFIC ACTIVITY IN CONTROL MARROWS.[a]

Type of marrow	Activity
Normal	
9	2684
10	2917
11	3540
12	4022
13	4302
14	4448
15	4536
16	5038
Autoimmune hemolytic anemia	
18	6393
19	7894
Myelomonocytic leukemia	
20	4479
21	4854

[a] Activity is expressed as picomoles of [^{14}C]-methionine per milligram of protein per hour.

normal persons enzymatic activities ranged from 2684 to 5038 pmole of [^{14}C]-methionine/mg of protein/hr. In the two patients with autoimmune hemolytic anemia, enzymatic activities were higher than in normals, but slightly lower than those encountered in four of five marrows from patients with chronic erythremic myelosis. In myelomonocytic leukemia enzymatic activities were within the range found in normal marrows. Reduction in enzymatic activity occurred when *S*-adenosylmethionine or $FMNH_2$ + DTT was omitted (4, 5), and addition of methylcobalamin to control marrow sonicates did not significantly alter their enzymatic activity. In all nonenzymatic controls, enzyme activity was significantly and reproducibly lower than in the complete systems. There were no significant differences in enzymatic activity in dialyzed compared to undialyzed samples.

Discussion. Observations on patients with pernicious anemia confirm those of Taylor *et al.* (4) and extend their findings by demonstrating progressive formation of active B_{12} holomethyltransferase after addition of increasing amounts of methylcobalamin to marrows containing primarily apoenzyme. Methyltransferase activities in normal marrows were considerably higher than those reported by Taylor *et al.* (4). One factor that may account in part for this discrepancy was the handling of marrow samples prior to assay. Although Taylor *et al.* stated that freezing (up to 1 year) did not affect enzymatic activity in their experiments (4), in the present studies freezing and storage caused marked reduction in activity, necessitating the use of fresh undialyzed and dialyzed marrow sonicates for assays.

Although reported earlier as being low in chronic erythremic myelosis (6), N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase activity measured in an atmosphere more highly anaerobic than that used previously and with different patients was found to be higher than in marrows obtained from normal persons. The relatively low enzymatic activity in one patient with chronic erythremic myelosis who clinically and hematologically resembled the other four patients with this disorder remains unexplained. Inability of added methylcobalamin to increase N^5-methyltransferase activity in chronic erythremic myelosis marrows suggests that, in this disorder, the enzyme probably exists as the holomethyltransferase.

High cell turnover may be among the more important mechanisms accounting for increased B_{12}-methyltransferase activity in chronic erythremic myelosis. Similar mechanisms may have accounted for high enzy-

matic activities in other disorders known to have high cell turnover, such as autoimmune hemolytic anemia in the present study and perhaps in marrows described as having "erythroid hyperplasia" by Taylor *et al.* (4). Recent compelling evidence suggests that N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase is a "log phase" enzyme, whose activity is a direct function of the proliferating state rather than the potential of any given tissue (8). Accordingly, increased activity of this enzyme may well be related to high proliferative potential and increased cell turnover, as reflected by rapid cellular division and intramedullary cell death (2, 8, 9).

Summary. Activity of N^5-methyltetrahydrofolate-homocysteine cobalamin methyltransferase was determined in sonicates of bone marrow obtained from five patients with chronic erythremic myelosis (DiGuglielmo syndrome), three patients with severe untreated pernicious anemia, and eight normal persons. In four of the five patients with chronic erythremic myelosis, enzymatic activity was higher than in normals, and addition of methylcobalamin did not affect enzymatic activity, suggesting that the enzyme exists as the holomethyltransferase. Increasing essential substrates such as *S*-adenosylmethionine, homocysteine, and methyltetrahydrofolate to fourfold the level used in the standard assay did not affect enzymatic activity significantly. Increased activity of this enzyme in chronic erythremic myelosis may relate to the high proliferative capacity reflected by increased cell turnover and intramedullary cell death.

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