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**5-METHYLTETRAHYDROFOLATE-DEPENDENT METHYLTRANSFERASE ACTIVITY IN CHRONIC ERYTHREMIC MYELOSIS AND OTHER TYPES OF ANEMIAS**

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Thus far, two vitamin B-12-dependent enzymes have been identified in man [1]. One is the 5'-deoxyadenosylcobalamin-dependent methylmalonyl CoA mutase that catalyzes the conversion of L-methylmalonyl CoA to succinyl CoA. The other is the methylcobalamin-dependent N<sup>5</sup>-methyltetrahydrofolate-homocysteine methyltransferase that, along with S-adenosylmethionine in a flavin-reducing system, catalyzes the conversion of homocysteine to methionine. Recently the methylcobalamin-dependent methyltransferase enzyme has been identified in extracts of normal human bone marrows [2] and in the bone marrow of patients with iron deficiency and leukemia [2]. In two cases of vitamin B-12 deficiency, the enzyme was found to exist primarily in the form of the apoenzyme [2]. In the present studies, attention was focused on patients with several types of disorders of erythropoiesis characterized by marked erythroid hyperplasia of the bone marrow, in an effort to detect differences in the methyltransferase activity of the marrow extracts obtained from these individuals.

Bone marrow was obtained by needle aspiration from the sternum or iliac crest at the time of diagnosis from two patients with severe untreated autoimmune hemolytic anemia; five patients with chronic erythremic myelosis (DiGuglielmo syndrome), a refractory macrocytic anemia that may be the forerunner of acute leukemia in certain individuals [3—5]; and from two patients with severe untreated pernicious anemia. According to stringent human subject regulations, the marrows were aspirated by the "second-pull" technique following the initial diagnostic aspirate, thereby permitting only small quantities of marrow to be obtained (10—20 mg). Similar ethical limitations did not permit the obtaining of bone marrow from normal, healthy individuals at the time of this study. Consequently, only pathological marrows characterized by marked erythroid hyperplasia were utilized. Because of the necessity of utilizing marrow extracts rich in protein for the enzymatic assay [2], only duplicate assays of the methyltransferase could be performed in the present

experiments, and various additives as described later could not be tested as a result of these limitations. The particles of marrow were washed five times in iced Hank's solution (BBL, Cockeysville, Md.), and homogenized in 1.0–1.5 ml iced 0.34 M sucrose solution for 45 min. The homogenate was dialyzed overnight at 4°C in 1.5 M potassium phosphate buffer and protein determined by the method of Lowry et al. [6].

The procedure for determination of the 5-methyltetrahydrofolate-dependent methyltransferase was essentially that described by Loughlin et al. [7]. Reagents included N<sup>5</sup>-[<sup>14</sup>C]methyltetrahydrofolic acid (61.1 Ci/mol, Amersham Searle), N<sup>5</sup>-methyltetrahydrofolic acid, homocysteine [8], FADH<sub>2</sub> [7], and S-adenosylmethionine. The latter four reagents were obtained from Sigma Chemical Co., St. Louis, and prepared freshly each time. Homogenate preparations from the patient's marrows were used as the source of the methyltransferase enzyme in an assay system with a final volume of 1.0 ml containing 0.1 μmol potassium phosphate buffer pH 7.2, N<sup>5</sup>-[<sup>14</sup>C]methyltetrahydrofolic acid 0.528 μmol (20 Ci/mol), 10 μmol homocysteine, 1.0 μmol S-adenosylmethionine, 0.2 μmol FADH<sub>2</sub>, 0.3 ml of the marrow homogenate and 0.1 ml distilled water. Assay mixtures were incubated for 2 h at 37°C in a hydrogen atmosphere and the reaction was terminated by immersion of the reaction tubes in an ice bath. Control tubes were run for each experiment, and included omission of essential reactants, such as homogenate and S-adenosylmethionine and other types of temperature controls as described by Loughlin et al. [7]. Following termination of the reaction, residual labelled and unlabelled methyltetrahydrofolate were removed from the assay mixture by passage of this mixture through a microcolumn (0.5 × 4.0 cm) of Dowex-1 chloride [9]. The eluates from experimental and control samples were lyophilized and stored at -20°C. N<sup>5</sup>-[<sup>14</sup>C]methyltetrahydrofolate and [<sup>14</sup>C]methionine were separated in an descending paper chromatography system containing isopropanol/water/1 M HCl (160:40:8, v/v/v). Known standards of methionine (Sigma) were used in each chromatogram. Amino acid spots were identified with ninhydrin/hydridantin reagent [10] cut from the paper chromatograms, placed in scintillation vials containing Toluene/Triton X-100/PPO/POPOP and counted in a Nuclear Chicago model 725 liquid scintillation counter.

Since all of the control samples contained only background radioactivity at the site of methionine, the activity of the 5-methyltetrahydrofolate methyltransferase was expressed as the difference between measured radioactivity of the [<sup>14</sup>C]methionine spot of the incubation mixture containing all of the reactants, minus the background radioactivity seen in the methionine area in all of the controls, divided by the total protein of the homogenate. Enzymatic activity was expressed as pmol [<sup>14</sup>C]methionine/mg protein/h.

Table I illustrates the results of the assays experiments. In the various disorders of erythropoiesis studied, the two patients with autoimmune hemolytic anemia had the highest values. In the five cases of chronic erythremic myelosis, methyltransferase activity was unusually low compared to those in the cases of autoimmune hemolytic anemia, and in one patient with chronic erythremic myelosis, no methyltransferase activity could be ascertained. In the

TABLE I

5-METHYLTETRAHYDROFOLATE-DEPENDENT METHYLTRANSFERASE ACTIVITY IN DISORDERS OF ERYTHROPOIESIS CHARACTERIZED BY MARROW ERYTHROID HYPERPLASIA

Disorder	Patient	[ <sup>14</sup> C]Methionine (pmol/mg protein/h)
Autoimmune hemolytic anemia	1	258
	2	152
Chronic erythremic myelosis	3	108
	4	21
	5	18
	6	12
	7	0
Untreated pernicious anemia	8	0
	9	0

two patients with severe untreated pernicious anemia and in all of the control samples, including those where various essential reactants were omitted, methyltransferase activity could not be detected.

The present studies have examined 5-methyltetrahydrofolate-homocysteine methyltransferase activity in marrow extracts from patients with erythroid disorders typified by marrow erythroid hyperplasia. Evidence for defective methionine generation was obtained in the cases of vitamin B-12 deficiency, as noted by others [2,11]. In addition, unusually low values for methyltransferase were found in patients with chronic erythremic myelosis (DiGuglielmo syndrome), a disorder often regarded as a preleukemic condition [3-5] and associated with other enzymatic abnormalities [12-14].

Although in all instances lower than in the two cases of autoimmune hemolytic anemia, variations in the enzymatic activity of patients with chronic erythremic myelosis are difficult to explain at this point. One individual (Patient 4) whose methylcobalamin methyltransferase activity was 21 pmol/mg protein/h subsequently developed subacute myelomonocytic leukemia [15] approximately a year after the diagnosis of chronic erythremic myelosis was made. Up to the time of leukemic transformation, the clinical course and severity of disease in this patient was identical to the other patients with chronic erythremic myelosis. Patient 7, whose bone marrow did not demonstrate detectable methylcobalamin methyltransferase activity, has not as yet shown evidence of leukemic transformation, although he has had an established diagnosis of chronic erythremic myelosis for 5 years and in all hematologic and clinical respects resembles the other patients with this disorder. Therefore, it is not possible at this point to predict which patients may have a greater likelihood of ultimately developing acute myeloblastic or myelomonocytic leukemia on the basis of certain methylcobalamin methyltransferase values of their marrow erythroid precursors. Studies of additional patients with chronic erythremic myelosis combined with prolonged observations of these patients may permit such correlations to be made in the future.

Because of the small amounts of marrow available, and the necessity of utilizing maximal amounts of marrow protein to perform methyltransferase assays, in the present experiments we were unable to explore some of the reasons why the methyltransferase activity was low in chronic erythremic myelosis, including the possibilities of abnormalities of the apoenzyme or

the presence of a metabolic inhibitor. When sufficient quantities of marrow become available, it will be important to perform the methyltransferase assays in the presence of methylcobalamin and to mix the chronic erythremic myelosis marrow extracts with normal marrow extracts prior to methyltransferase assays, to gain further information regarding some of the mechanisms that might be responsible for the results obtained in the present study.

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