through induced changes in the (tertiary) protein structure, or by "polymerization," or by alteration of the "water structure," or by a combination of these factors.

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Adenosylmethionine Elevation in Leukemic White Blood Cells

Abstract. *White blood cell preparations from patients with chronic myelocytic leukemia have more* S-*adenosylmethionine, an important metabolic intermediate, than normal peripheral white cells or thoracic-duct lymphocytes. The previously reported elevation of cyanocobalamine (vitamin* B_{12}*) in the serum of patients with this disease is corroborated. The possible usefulness of methylation antimetabolites in cancer chemotherapy is suggested.*

Although many biochemical and metabolic studies have been reported (*1, 2*), biological transmethylation has not been evaluated extensively in relation to leukemia. The chemistry of methyl transfers in relation to cyanocobalamine (B_{12}) and folic acid metabolism has been reviewed (*3, 4*). Folic acid is increased in leukemic cells (*5*), and antifolate agents have been used for chemotherapy in leukemia (*6*). Patients with chronic myelocytic leukemia have increased amounts of B_{12} in their serums (*7*).

In our study we assayed *S*-adenosylmethionine (SAMe), an important donor of methyl groups in transmethylation reactions. We studied white cell preparations from blood of chronic myelocytic leukemia patients and normal (control) patients. In several instances the B_{12} in serum of patients and controls was also determined.

A total of eight determinations in seven untreated patients with chronic myelocytic leukemia were made at the National Cancer Institute. The diagnosis was established by hematologic and cytogenetic examinations of peripheral blood and marrow. Total peripheral white counts ranged from 67,100 to 195,000 cells/mm³ (Table 1).

All the patients had a much higher proportion of immature cells (younger than myelocytes) (6 to 31 percent, median 20 percent) in their peripheral blood than normal individuals, who have none, as determined by differential white cell counts of peripheral blood smears (Table 1). The concentration of B_{12} in the serum of four patients (475, 560, 690, 1080 $\mu\mu$g/ml) was much higher than normal (100 to 300 $\mu\mu$g/ml). The B_{12} was determined by the method of Mendelsohn and Watkin (*7*).

Whole blood (50 ml) or white-cell-rich plasma (20 ml) obtained by plasmapheresis was subjected to dextran centrifugal sedimentation and osmotic stress at 4°C by the method of Fallon *et al.* (*8*). The concentrated white cells were stored at −20°C until assayed for SAMe, usually within 24 hours. Microscopic study of the cell preparations confirmed that they contain white blood cells, platelets, and a minimum of debris (*8*).

Similar white cell preparations from four normal blood donors were obtained by plasmapheresis and the same sedimentation technique. These samples served as controls in the assay of leukemic blood cells. Comparisons were made with lymphocytes prepared by dextran sedimentation of lymph, obtained at thoracic duct cannulation, from patients with isolated, solid, nonhematogenous visceral tumors. These preparations were found, by microscopy, to consist entirely of small (98 percent) and large (2 percent) lymphocytes.

The fractionation of leukemic blood by differential centrifugation allowed us to obtain a preparation rich in immature cells. In this preparation more than 50 percent of the cells were "blast" cells and more than 60 percent of the cells were younger than myelocytes (Table 1), as shown by microscopy.

The white-blood-cell preparations were weighed, homogenized, and assayed by a method (*9*) in which melatonin-H^3-methoxy-C^{14} is synthetized enzymatically from *N*-acetylserotonin-H^3, SAMe-methyl-C^{14}, and hydroxyindole-*O*-methyl transferase (from bovine pineal gland) in the presence of tissue extracts containing unlabeled endogenous SAMe. The ratio, H^3 to C^{14}, is determined in melatonin-H^3-C^{14}, the only radioactive product extracted and counted. Tissue SAMe reduces the specific activity (radioactivity per mole) of the SAMe-C^{14} and so leads to an elevated $H^3 : C^{14}$ ratio. The change in this ratio bears a theoretical and experimentally demonstrated linear relation to the amount of tissue SAMe added to the incubation mixture, and

Table 1. Hematologic findings and *S*-adenosylmethionine in white blood cells of eight patients with chronic myelogenous leukemia.

Total white cells (cells/mm³)	Cells younger than myelocytes (%)	SAMe (μg/g)
	Controls	
2500–8500	0	1.0–3.0
	"Blast"-cell preparation	
	> 60	7.0
	Patients	
137,000	31	6.1
136,000	27	17.3
144,000	24	8.2
195,000	22	3.9
194,000	19	7.1
156,000	8	4.5
67,100	6	13.9

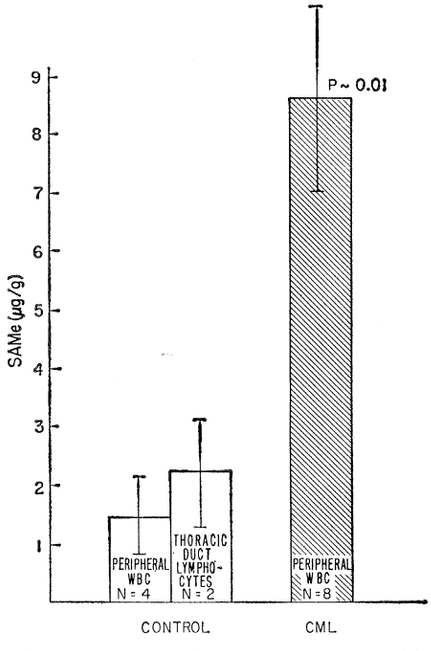

Fig. 1. Concentration of *S*-adenosylmethionine in the white blood cells of normal individuals and those of patients with chronic myelocytic leukemia (*CML*). *N*, number of patients.

thus provides a sensitive, specific assay method. The results obtained for normal peripheral white blood cells, lymphocytes, and peripheral white cells from our patients are presented (Fig. 1).

Normal white blood cells and the thoracic-duct lymphocytes (nonleukemic cells) had measurable amounts of the methyl donor (each about 2 μg per gram of wet tissue). Leukemic cells had much more SAMe (8.6 $\pm$ 1.6 μg/g), the difference being statistically significant. The increased amounts of white-cell SAMe coincide with increased serum B_{12} and white-cell folic acid (*9*). All of these substances are important in the chemical transfer of methyl groups, but the physiological significance of these findings is not evident.

The increase in the adenosylmethionine is associated with a high proportion of immature cells in leukemic blood smears. Thus the question arises whether the elevations are unique to leukemic white blood cells, or merely correlate with metabolic immaturity in general. Minor elevations (about twice the adult concentrations) occur in the liver and brain of the newborn rat (*10*). On the other hand, there is a striking lack of direct correlation between SAMe content and the degree of immaturity of cells in the blood smears of our patients (Table 1).

The measurement of adenosylmethionine in normal marrow, while theoretically important, would not be comparable to preparations of peripheral blood cells from leukemic patients, since marrow contains immature red as well as white cells. In addition, the amounts of normal human marrow needed for assay could neither be obtained readily, nor without significant dilution with peripheral blood cells. A study of this compound in animal marrow and blood cells might provide adequate amounts of material, but would be of questionable comparability with human leukemic cells. The study of white blood cell preparations from patients with acute leukemia would be useful, since they contain high proportions of immature cells.

While there is no known direct relation of adenosylmethionine to nucleic acid metabolism, methyl transfer is important in the synthesis of the methylated pyrimidine, thymine (*2, 3*). Therefore, it would be well to search for methylation antimetabolites useful in therapy. There is information indicating that certain methyl acceptors such as pyrogallol, and certain potent monoamine oxidase inhibitors such as pargyline, can lower the amounts of SAMe in rat tissues strikingly (*10*). That such a lowering agent might inhibit tumor growth is suggested.

To one fasted patient with chronic myelocytic leukemia, 50 mg of pargyline was given orally during a 24-hour period, before and after which blood samples were taken, white cell preparations were made, and assays for the adenosylmethionine were performed. The patient was clinically unchanged and ate normally throughout the experimental period. The amount of SAMe in the cells before treatment was 6.2 μg/g, and after treatment it fell to 1.8 μg/g; however, this single finding cannot be interpreted until further data are available.

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Abnormal Immune Mechanism in Allogeneic Radiation Chimeras

Abstract. *Mice were subjected to x-rays (950 roentgens) and injected with isogeneic (isologous) or allogeneic (homologous) bone marrow. Six to 8 months later these chimeras were injected with* Salmonella typhi *flagellar antigen, and the formation of antibodies resistant and sensitive to destruction by treatment in vitro with 2-mercaptoethanol was determined. The allogeneic chimeras showed almost normal amounts of serum antibody after a third injection of antigen but a relative defect in their ability to synthesize antibody resistant to 2-mercaptoethanol. Apparently control of antibody formation becomes abnormal in the presence of the immunologic tolerance existing between the host and the foreign hematopoietic graft.*

The immunologic recovery of lethally irradiated mice treated with isogeneic (*1*) bone marrow was nearly normal when tested two or more months later with particulate antigens (*2*). In contrast, the immune status of irradiated mice treated with allogeneic (homologous) or xenogeneic (heterologous) marrow remained subnormal well beyond the critical period of secondary disease that these mice experience 20 to 40 days after irradiation. This reduced capacity in chimeras injected with foreign bone marrow is difficult to reconcile with the current opinion that a state of immunologic tolerance exists between the transplanted graft and foreign host. In contrast to the preceding findings, Garver *et al.* (*3*) reported a normal agglutinin response in allogeneic chimeras stimulated with multiple injections of human red-cell antigens 8 months after irradiation and marrow treatment. Vos and Weyzen (*4*) reported normal reactivity of allogeneic chimeric lymph-node cells against transplantation antigens not present in the chimeric host or grafted tissues. Thus, chimeric lymph-node cells injected into an irradiated mouse of a strain unrelated to either host or chimeric donor showed a killing effect comparable to lymph-node cells obtained from normal mice.

The immune response following antigen injection in many species of animals consists of the sequential formation of two major molecular forms of antibody: the 19*S* macroglobulins and the 7*S* γ-globulins (*5*). Depending upon the species of animal and the type and dosage of antigen, the antibody formed during the first and second weeks after antigenic stimulation is usually the 19*S* variety, and it