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Methylated Arginines in Chronic Erythremic Myelosis[1] (37929)

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This report describes the occurrence of methylated arginines in hydrolysates of histones obtained from bone marrow erythroid precursors of five patients with chronic erythremic myelosis (DiGuglielmo's syndrome). Methylated arginines such as those to be described were not found in similar histone hydrolysates obtained from patients with a variety of other disorders of erythropoiesis.

Materials and Methods. Sternal or iliac bone marrow was obtained in a heparinized glass syringe immediately after the diagnostic aspiration from five patients with chronic erythremic myelosis. All of these patients had refractory macrocytic anemia with splenomegaly and intense megaloblastoid erythroid hyperplasia of the bone marrow. Four of the patients had at least 20% proerythroblasts in the marrow, and one patient had as high as 40% proerythroblasts. A PAS (periodic acid–Schiff) stain on the marrows revealed PAS-positive material within the cytoplasm of proerythroblasts, and a Prussian blue stain disclosed many ringed sideroblasts and abundant stainable iron. Samples of bone marrow were also obtained from three patients with untreated classical pernicious anemia, from three patients with pernicious anemia 20 hr after their initial dose of vitamin B12, and from two patients with autoimmune hemolytic anemia. Since all of the bone marrows were composed largely (up to 90%) of erythroid precursors and because the sample amounts were restricted to that obtainable during a routine aspiration, no efforts to fractionate marrow samples were made. From 60–70 mg of marrow flecks, lysine-rich and arginine-rich histones were extracted by methods described previously (1), and approximately 400 μg of each type of histone was obtained.

The histones were hydrolyzed for 24 hr in 6 *N* HCl and evaporated to dryness in a rotary evaporator. Each sample of histone yielded approximately 300 μg total amino acids and 100–200 μg were used for each electrophoretic run. Amino acid electrophoresis was performed as described elsewhere (2). Methylated derivatives of lysine and arginine were used as standards. These included *N*-epsilon-methyl-L-lysine hydrochloride, N^G-monomethyl-L-arginine, N^G-N'^G-dimethyl-L-arginine, and N^G-dimethyl-L-arginine (Calbiochem, San Diego, CA). Electrophoretic patterns were developed in collidine stain as described previously (2), photographed, and read in a Beckman microzone densitometer model R-110 (Beckman Instruments, Fullerton, CA).

Results. As shown in Fig. 1, a typical bone marrow from a patient with chronic erythremic myelosis demonstrated marked hyperplasia of megaloblastoid intermediate macronormoblasts. Proerythroblasts were also increased in number. Figure 2 illustrates typical electrophoretic patterns of amino acids obtained from the histones of these erythroid precursors. In particular, one and in several instances two methylated arginines were found. These correspond to the N^G-dimethyl-L-arginine and N^G-N'^G-dimethyl-L-arginine standards. Methylated arginines were observed in the pH 1.0 histone

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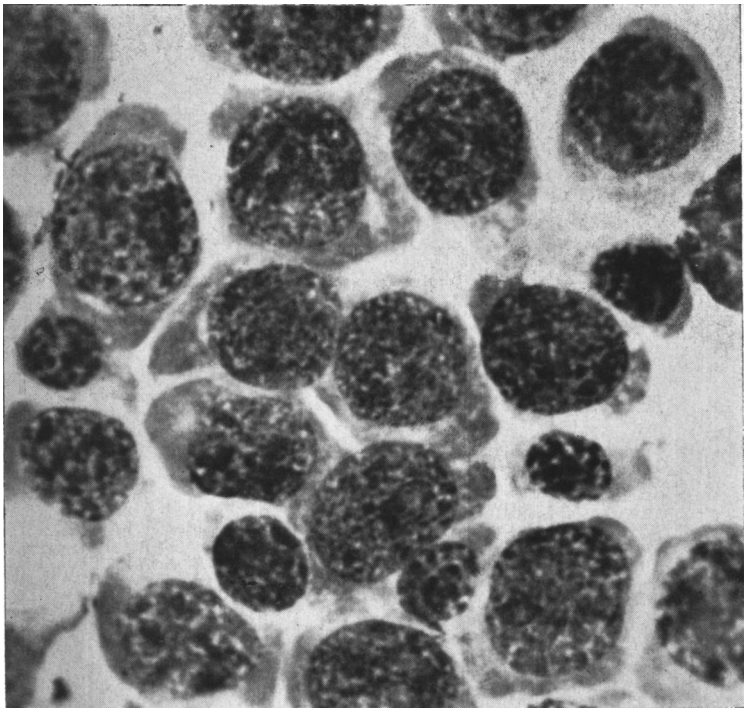

FIG. 1. Typical large megaloblastoid intermediate macronormoblasts from marrow of patient with chronic erythremic myelosis (×1200).

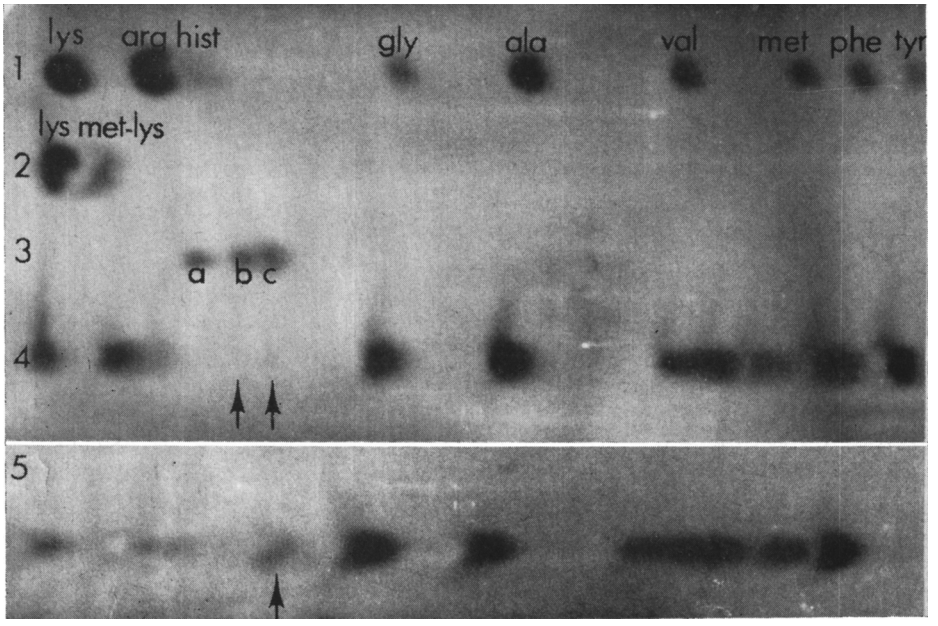

FIG. 2. Typical electrophoretic separations of amino acids which were obtained by acid hydrolysis of arginine-rich histones extracted from bone marrow erythroid precursors of two different patients with chronic erythremic myelosis. The arrows represent methylated arginine(s) in the sample. Row 1 = known amino acid standard. Row 2 = L-lysine and methyl-L-lysine standards. Row 3 = methylated arginine standards; a = N^G-monomethyl-L-arginine, b = N^G-dimethyl-L-arginine, c = N^G-N'^G-dimethyl-L-arginine. Rows 4 and 5 = electrophoretic patterns of amino acids obtained from histones of two different patients with chronic erythremic myelosis (arrows indicate methylated arginine).

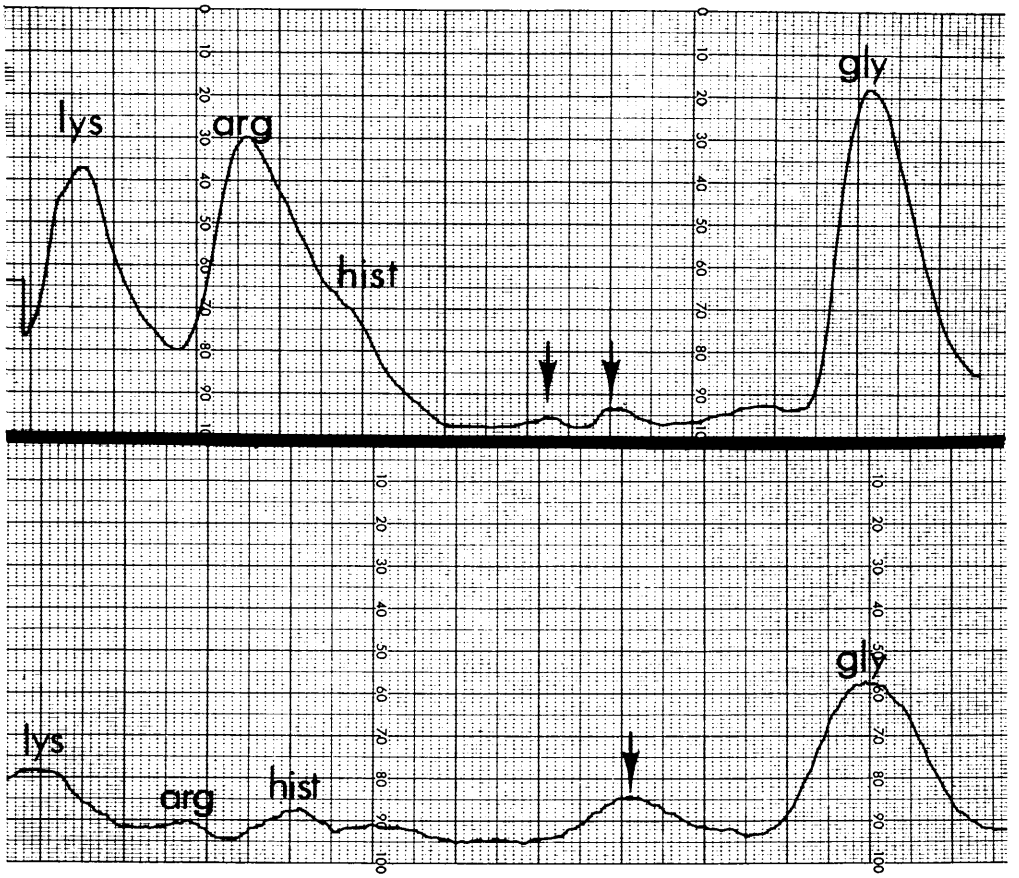

FIG. 3. Section of microdensitometer pattern of electrophoretic strips illustrated in Fig. 2, rows 4 and 5. Arrows indicate methylated arginines migrating between L-histidine and glycine.

fraction (arginine rich) in all instances, and in pH 1.8 histone fraction (lysine rich) in one case. Typical densitometer patterns of these electrophoretic strips are shown in Fig. 3. Methylated arginines were not observed in the electrophoretic patterns of amino acids obtained from erythroid histones in the various other types of anemia studied.

Discussion. These studies demonstrate the occurrence of methylated arginines in acid hydrolysates of histones obtained from patients with chronic erythremic myelosis. Several previous studies (3, 4) have also suggested that there may be an abnormality of arginine-rich histones in this disorder.

Currently the pathogenesis of the megaloblastoid-type erythropoiesis in chronic erythremic myelosis is unknown. Megaloblastoid chromatin pattern is characterized by coarse chromatin strands, block-like aggregates of chromatin, and fenestrated appearance superficially resembling that of the megaloblast. Arginine-rich histone is believed to play a role in the condensation of chromatin (5), and methylation of histones may be involved in the tight coiling of chromosomes into the compact masses called chromatin (6). It is conceivable that, in erythremic myelosis, histones containing methylated arginines may exert an even greater aggregating influence on chromosomes than histones containing only L-arginine. This might cause chromosomes to form large block-like aggregates characteristic of megaloblastoid maturation.

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