Variation in the Amount of Hemoglobin S in a Patient with Sickle Cell Trait and Megaloblastic Anemia

By Paul Heller, Vincent J. Yakulis, Robert B. Epstein and Sigmund Friedland

The relative amount of hemoglobin S in individuals with the sickle cell trait is generally presumed to be constant throughout life, but variations in this proportion from one family to the next have been detected by Neil, Wells and Itano. According to the latter investigators, these variations follow a bimodal distribution pattern with modes around 34-36 per cent and 40-42 per cent. The lowest value in their study was 22.3 per cent. To our knowledge values lower than this have not been reported except for the individual in whom Singer and Fisher found 5 per cent of hemoglobin S. This was an erroneous finding as the fraction believed to be hemoglobin S was actually A2 which had not yet been recognized as a minor fraction of hemoglobin at the time of that report.

According to present concepts, not only the molecular structure but also the rate of synthesis of the polypeptide chains of hemoglobin is under genetic control and environmental factors do not significantly alter the relative proportion of hemoglobin S in individuals with the sickle cell trait. The case reported here appears to be an interesting exception to this general rule.

Report of Case

A 56 year old Negro was admitted to the hospital because of severe generalized weakness. As the patient was somewhat confused and tended to confabulate, no adequate history could be obtained. His relatives stated that the patient had been a heavy drinker and had been eating "very little" for several months prior to admission. His diet consisted mainly of soups and chili and was completely devoid of fresh vegetables and fruit. The weakness, especially of the lower extremities, had gradually increased and 3 days prior to admission he was almost unable to walk. He had not taken any drugs or vitamins and had received no blood transfusions.

Positive physical findings included a poor nutritional state, neglected oral hygiene, moderately severe pallor, slight hepatomegaly, generalized muscular weakness, normal to hyperactive deep tendon reflexes and moderate diminution of the vibratory sense.

Among the initial laboratory values were the following abnormalities: hematocrit 21.5 per cent, hemoglobin 7.4 Gm. per cent, red cell count 1.8 million/cu. mm., white cell count 8400/cu. mm. There was macrocytosis, poikilocytosis, leptocytosis, polychromasia and several hypersegmented leukocytes. Bromsulphalein retention was 23 per cent in 45 minutes. Total bilirubin was 2.3 mg. per cent with 0.7 mg. conjugated. Serum albumin was 3.2 Gm. and globulin 3.4 Gm. per cent. The level of serum lactic dehydrogenase was markedly elevated. The bone marrow showed marked erythroid hyperplasia with approximately 50 per cent of the erythroid precursor cells being megaloblastic. Many giant

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Fig. 1.—Agar gel electrophoresis, Tris-EDTA-boric acid-buffer, pH 8.9, 1 hour. 20 V cm., unstained. 1) Normal hemolysate; 2) A-S hemolysate (patient's daughter); 3) patient's hemolysate (bone marrow megaloblastic); 4) patient's hemolysate (after recovery).

metamyelocytes with bizarre nuclei were noted. The vitamin B₁₂ level was 1340 μg./ml. (normal 710 ± 130 μg./ml.) as measured by the Lactobacillus leichmanni assay. The urinary excretion of formiminoglutamic acid was markedly increased, even without histidine loading. The folic acid level was 2.8 μg./ml. (normal 7.75–15.9 μg.) according to the technic of Herbert. The initial test for sickling gave equivocal results and the electrophoregram performed on agar gel and starch gel showed a minor abnormal fraction of the migratory characteristics of hemoglobin S (figs. 1 and 2). This fraction was present in a concentration of 10.6 per cent and A₁ in a concentration of 1.7 per cent as measured by direct scanning of the unstained agar gel electrophoregram in a Spinco Analytrol (with interference filter for 420 mμ). This result was confirmed on two separate blood samples obtained within 48 hours following admission. Fetal hemoglobin was 0.6 per cent. Solubility of the reduced hemolysate in 2.24 M phosphate buffer was 98 per cent. In view of these unusual findings the possibility of the presence of a rare hemoglobin, especially hemoglobin Lepore, was suspected.

When the tests were repeated 2 weeks later the patient had improved on a dietary regimen alone. The bone marrow was now normoblastic and the hemoglobin value had increased to 11 Gm. per cent within 2 weeks. The serum level of vitamin B₁₂ had dropped to 500 μg./ml. and the folic acid level had increased to 10 μg./ml. The test for sickling became unequivocally positive and the proportion of the abnormal hemoglobin fraction was 38.5 per cent (figs. 1 and 2). Solubility of patient's hemolysate in 2.24 M phosphate buffer was now 66 per cent, consistent with the sickle cell trait. In the meantime the patient's daughter was examined and was found to have A-S hemoglobin and a positive sickle cell phenomenon.

The megaloblastic anemia was considered to be due to folic acid deficiency. The initial high serum level of vitamin B₁₂ was compatible not only with this abnormality but also with the probably present hepatic cirrhosis.

**DISCUSSION**

In megaloblastic anemia the synthesis of ribonucleic acid and deoxyribonucleic acid, especially the latter, is disturbed. It is therefore possible that
the genetic determinants for hemoglobin synthesis are affected in this disease. Nathan and Gardner\textsuperscript{14} have recently obtained suggestive evidence that the maturation time of the primitive megaloblast is prolonged and that there might be a decreased rate of globin synthesis in the primitive megaloblastic cells. The explanation usually given\textsuperscript{15} for the inequality of the amounts of the two hemoglobins in the simple heterozygous state (A-S) is a slower rate of synthesis of hemoglobin S than of hemoglobin A. While this hypothesis has to our knowledge not yet been experimentally proved, it best agrees with the available facts. Another possible explanation is the unequal distribution of S-hemoglobin among the erythrocytes of the A-S heterozygote, thus conveying to them varying survival rates depending on their content of hemoglobin S. To our knowledge no evidence in support of this hypothesis is available.

The findings in the patient of the present report suggest that the synthesis of hemoglobin S in megaloblastic marrow is inhibited to a greater degree than that of hemoglobin A. The dramatic change of the relative amount of hemoglobin S from approximately 10 per cent to the usual 40 per cent after the normoblastic conversion of the bone marrow had taken place is in favor of this possibility.

Minor variations in the relative proportion of fetal hemoglobin\textsuperscript{16} and hemoglobin A\textsubscript{2}\textsuperscript{17} have been found in acquired disease, but, to our knowledge, such variations have not been reported for the major hemoglobin fractions.

**Summary**

A patient with sickle cell trait and nutritional megaloblastic anemia was found to have a much smaller proportion of hemoglobin S during the megaloblastic phase than after recovery. This observation suggests preferential synthesis of hemoglobin A by megaloblastic bone marrow in the presence of the A-S trait.

**Summario in Interlingua**

In un patiente con le character genetic de cellulas falciforme e con megal-
oblascic anemia nutritional, il essese trovate que le proportion de hemoglobina S essese molto plus basse durante le phase megaloblastic que post le re-stablimento. Iste observation suggere le occurrentia de un synthese preferential de hemoglobina A per megaloblastic medulla ossee in le presentia del character genetic A-S.

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REFERENCES
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