and soil aeration. For example, oxygen release from roots may be a factor in sustaining the "rhizosphere effect," the abundant microbial activity often observed on the surface of plant roots in soil.

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Hemoglobins A and F: Formation in Thalassemia and Other Hemolytic Anemias

Abstract. *Rates of synthesis of hemoglobin A by erythroid cells from thalassemic subjects are markedly decreased. Formation of hemoglobin F, however, proceeds at similar rates in cells from subjects with thalassemia and other types of hemolytic anemias. A mechanism is suggested regarding the altered patterns of hemoglobin synthesis under conditions of erythropoietic stimulation in subjects with and without thalassemia.*

The thalassemia syndromes include a group of inherited diseases which are characterized by a marked decrease in the concentration of hemoglobin A and, in many instances, an increase in the concentration of hemoglobin F. Various theories have been advanced to explain the mechanism of this disorder. Recently, hypotheses which presume a primary defect in the rates of synthesis of the globins (*1–4*) have been emphasized. Itano (*1*) and Ingram and Stretton (*2*) postulated that the defect in hemoglobin A synthesis in thalassemia is associated with the formation of a structurally abnormal hemoglobin which is made at a rate slower than normal. However, analysis of the amino acid content of individual tryptic peptides of hemoglobin from the thalassemias have as yet revealed no structural abnormalities (*5*). An alternative proposal (*1, 2*) is that the defect in thalassemia involves the genetic regulatory system resulting in an altered rate of synthesis of structurally normal hemoglobin A.

Ribosomes characterized by sedimentation coefficients greater than 100*S* (polyribosomes) are the site of protein synthesis in erythroid cells of both thalassemic and nonthalassemic subjects (*4*). However, polyribosomes in cells from thalassemic patients have a significantly lower capacity to incorporate leucine than polyribosomes in cells from nonthalassemic persons (*4*). Leucine is in all peptide chains of hemoglobins A and F. Isoleucine, which is in hemoglobin F but not in hemoglobin A, is incorporated by polyribosomes in cells of thalassemic and nonthalassemic patients in at least comparable amounts. These initial observations suggested that in thalassemia the defect in protein synthesis involves a selective impairment in the capacity of polyribosomes to form hemoglobin A. In populations of erythroid cells synthesizing hemoglobin A and hemoglobin F, this hypothesis could be tested further by determining the rate of formation of these proteins.

Blood was obtained from patients with thalassemia major, sickle cell anemia, and hemolytic anemias without primary abnormalities in hemoglobins. Cells were separated from the blood, suspended in a modified Krebs-Ringer bicarbonate medium (*6*) that contained C^{14}-leucine (17.1 μc/μmole) and C^{14}-valine (20.3 μc/μmole), and incubated at 37°C. At intervals up to 80 minutes, portions of the cell suspension were removed to determine the amount of radioactivity incorporated into acid-insoluble material (*7*). At 80 minutes the cells were recovered from the cell suspension by centrifugation and then lysed by a short exposure to a hypotonic solution (*7*). The hemolysate was made free of ribosomes by centrifugation at 200,000*g* for 2 hours. Hemoglobin concentrations were determined and ribosomes were analyzed (*4*). Hemoglobin fractions were separated from ribosome-free hemolysates by using an amberlite IRC-50 synthetic cation-exchange resin with developer No. 2 (*8*). The amount of nonheme protein contaminating the hemoglobin fractions was determined by DEAE (diethylaminoethyl) chromatography (*9*).

The proportion of the hemoglobin which was hemoglobin F was generally higher in the subjects with thalassemia major than in the patients with sickle cell anemia or chronic hemolytic anemia (Table 1). In all subjects studied, however, the concentrations of hemoglobin were similar, and the percentage of hemoglobin F was above normal (Table 1). The normal concentration of hemoglobin F is below 2 percent.

The rate of incorporation of amino acid into hemoglobin A by cells from thalassemic subjects was strikingly lower than that by cells from subjects with the other types of hemolytic anemia (Table 1). By contrast, the incorporation into the fraction containing hemoglobin F was not markedly different in cells from the thalassemic compared with the nonthalassemic subjects. In no experiment did the incorportation of radioactive amino acid into nonheme protein exceed 6 percent of the total amino acid incorporated. The decreased rate of amino acid incorporation into hemoglobin A cannot be attributed to a failure of saturation of the ribosomes in thalassemic cells. In all instances examined, the radioactivity associated with ribosomes reached a plateau value within 5 minutes. The incorporation of the C^{14}-amino acids into the supernatant protein continued to increase at a linear rate during the 80-minute period of incubation. In three patients with thalassemia (Table 1, thalassemia subjects 1, 3, and 4) the relative concentration of hemoglobin F to hemoglobin A was smaller than the ratio of amino acid incorporated into these fractions, respectively. A possible explanation for this finding is that prior to these studies these patients were regularly transfused. The other patient with thalassemia (Table 1, thalassemic subject 2) had never been transfused. In this case, the relative concentration of hemoglobins F to A was higher than the ratio of their rates of synthesis. This could be due to a lower rate of turnover of hemoglobin F in vivo, as suggested by Gabuzda *et al.* (*10*).

The present results indicate a selective decrease in the capacity of ribosomes of erythroid cells of thalassemic subjects to synthesize hemoglobin A, which is composed of two alpha- and two beta-peptide chains. The rate of amino acid incorporation into hemoglobin F, composed of two alpha- and 2 gamma-peptide chains, was similar in cells of both thalassemic and nonthalassemic subjects. Therefore the primary defect in these subjects with thalassemia major may be a decreased capacity to synthesize beta chains. In normal subjects, more than 90 percent of the protein formed by erythroid cells is

Table 1. Hematological data on individual subjects and amino acid incorporated into hemoglobin.

Peripheral blood *		Ribosomes recovered ($mg/10^{10}$ reticulocytes)	Hemoglobin fractions † (mg/ml)		Amino acid incorporation ‡ (mμmoles/mg ribosomes)		
Hemoglobin (g/100 ml blood)	Reticulocyte (%)		F	A	Total soluble protein	Fractions F	Fractions A
		Thalassemia					
7.1	11	1.9	27	41	51.8	23.0	20.8
7.7	12	2.2	59	22	46.2	19.6	19.0
6.5	2	3.4	21	60	17.0	6.6	8.8
6.4	11	3.5	38	46	35.0	17.2	13.8
		Sickle cell anemia					
6.5	7	3.4	14	85	352.0	33.2	296.8
7.4	9	0.9	18	83	332.0	63.0	269.0
6.7	9	1.7	8	79	137.0	10.2	114.4
		Chronic hemolytic nonspherocytic anemia					
7.1	43	2.7	10	71	433.4	40.2	403.8
9.0	11	0.8	6	77	428.6	28.6	341.4

* Hemoglobin concentration and reticulocyte count on peripheral blood on day of study. † Hemoglobin content of fractions F and A are expressed in milligrams per milliliter of incubation mixture used for determination of rates of amino acid incorporation into proteins. Fractions F and A correspond to fractions A_I and A_{II} of Allen, Schroeder, and Balog (*8*). The components of fraction F include hemoglobin F and nonheme protein. In the studies on patients with Sickle cell anemia fraction A represents hemoglobin S. ‡ Amino acid incorporated into total soluble protein is based on determinations of total radioactivity in the fraction insoluble in hot trichloracetic acid of ribosome-free hemolysates prepared from cells after incubation for 80 minutes (*7*). Amino acid incorporation is expressed as the millimicromoles of leucine plus valine incorporated per milligram of total ribosomes recovered from the hemolysates of cells prepared from the incubation mixture after 80 minutes of incubation at 37°C.

hemoglobin A. That erythroid cells from patients with thalassemia may have a more than 80 percent decrease in the rate of amino acid incorporation into soluble protein suggests that there is a decrease in the rate of formation of alpha as well as of beta chains. The decrease in alpha-chain synthesis could be secondary to impaired beta-chain formation if, for example, beta chains are necessary for the release of alpha chains from the ribosomes. It has been observed that an excess of beta-chain formation may occur in thalassemia syndromes in which, on the basis of genetic analyses, the primary defect appears to involve alpha-chain synthesis (*3, 5, 11*). In most thalassemia syndromes, including those we studied, genetic analyses are consistent with the interpretation that the primary defect involves beta-chain formation. In such subjects uncombined alpha chains have been detected in hemolysates, but in concentrations of only 0.5 percent or less of the total hemoglobin content (*12*). The possibility exists that a larger amount of alpha chains is formed, but they are rapidly destroyed and, therefore, are not detectable as soluble protein in hemolysates.

A selective defect in the rate of formation of structurally normal beta chains could be due to an alteration in the RNA directing hemoglobin synthesis (messenger RNA). Such an alteration in messenger RNA might reflect a decrease in the amount of normal messenger RNA synthesized, or such a modification in the messenger RNA that, though it is produced in normal or near normal amounts and can associate with ribosomes, it leads to a decrease in the rate of hemoglobin A synthesis at the ribosome level.

The finding that the synthesis of hemoglobin F proceeds at a similar rate in cells from thalassemic and nonthalassemic subjects suggests that the beta thalassemia gene is not associated with a specific compensatory mechanism by which a decrease in beta-chain synthesis leads to an increase in gamma-chain formation. Observations in this study indicating that thalassemia is associated with a selective defect in globin formation are not incompatible with the evidence of enzymatic defects in heme synthesis in these disorders (*10, 13*). A failure in globin synthesis could lead to an excess of heme which results in turn, by a negative "feedback" mechanism, in a decrease in the activity of specific enzymes taking part in heme formation.

A mechanism for hemoglobin formation, based in part on earlier hypotheses advanced by various workers (*1–3*), can be proposed as follows. Among the early precursor erythroid cells in normal adults there are cells that form beta or gamma chains or both. Under normal circumstances of erythropoiesis, as these cells mature their ribosomes lose the capacity for gamma-chain synthesis when the cells have made only 1 to 2 percent of their final complement of hemoglobin. Under conditions of erythropoietic stress, the maturation of erythroid cells is so altered that circulating reticulocytes are derived directly from early erythroid forms (*14*) and a greater fraction of hemoglobin is produced by cells retaining the capacity for gamma-chain synthesis. This could explain an increase in hemoglobin F in any type of hemolytic anemia. If, in addition to the accelerated maturation, there is a defect in beta-chain synthesis, as in thalassemia syndromes, the result would be a greater increase in the proportion of hemoglobin F. This proposal accounts for the marked decrease in the formation of hemoglobin A and our rather unexpected result that synthesis of hemoglobin F in thalassemic cells may be similar to that in cells of certain other hemolytic anemias not associated with impaired beta-chain formation. We consider that the normal mechanism of conversion from fetal to adult hemoglobin involves a different mechanism, such as control of the rate of messenger RNA synthesis at the level of the gene.

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