The usual conversion of serine to glycine may be available to the normal tissues and the resistant tumor, and for this reason asparaginase affects these tissues very little. The importance of glycine in purine synthesis may explain why the lack of glycine which occurs only in the susceptible tumor is more damaging to cells than the lack of asparagine which occurs in all tissues after asparaginase treatment.

WAYNE L. RYAN
HALVOR C. SORNSON
Department of Biochemistry,
University of Nebraska,
College of Medicine,
Omaha 68105

References and Notes

1. J. G. Kidd, *J. Exp. Med.* **98**, 565 (1953).
2. J. D. Broome, *ibid.* **118**, 99 (1963).
3. L. T. Mashburn and J. C. Wriston, *Arch. Biochem. Biophys.* **105**, 451 (1964).
4. J. D. Broome, *Trans. N.Y. Acad. Sci.* **30**, 690 (1968).
5. W. L. Ryan and J. E. Dworak, *Cancer Res.*, in press.
6. L. T. Mashburn, E. A. Boyse, H. A. Campbell, L. J. Old, *Proc. Soc. Exp. Biol. Med.* **124**, 568 (1966).
7. D. H. Spackman, *Amino Acid Analyzer Manual* (Beckman Instruments, Stanford Industrial Park, Palo Alto, Calif. (1962).
8. J. B. Broome, *J. Exp. Med.* **118**, 121 (1963).
9. L. Borella, L. J. Journey, R. Hiramoto, *Cancer Res.* **26**, 2451 (1966).
10. T. Ohnuma, F. Bergel, R. C. Bray, *Biochem. J.* **103**, 238 (1967).
11. A. Meister, H. A. Sober, S. V. Tice, R. E. Fraser, *J. Biol. Chem.* **197**, 319 (1952).
12. Supported in part by a grant from the Hartford Foundation.

8 December 1969; revised 12 January 1970

Heterozygous Beta Thalassemia: Balanced Globin Synthesis in Bone Marrow Cells

Abstract. *In two patients with heterozygous beta thalassemia the rates of synthesis of the alpha and beta chains of hemoglobin were equal in nucleated red cell precursors, although beta chain synthesis was reduced in peripheral blood reticulocytes. This finding suggests a relative instability of beta chain messenger RNA in beta thalassemia.*

A marked imbalance of globin chain synthesis occurs in the immature red cells of the peripheral blood of patients with homozygous beta thalassemia (Cooley's anemia) (*1*). Production of normal beta chains is decreased or absent, resulting in a relative excess of normal alpha chains. Reduced synthesis of beta chains also occurs in the peripheral blood of patients heterozygous for beta thalassemia (A_2 thalassemia) (*1*). Defective control of globin synthesis in thalassemia can be shown in experiments involving incubation of peripheral blood red cells with radioactive amino acids and measurement of radioactivity incorporated into the separated globin chains. In nonthalassemic controls with reticulocytosis, equal amounts of radioactivity are incorporated into alpha and beta chains, and the specific activities (radioactivity per absorbance unit) of the two chains are also equal. In patients heterozygous for beta thalassemia, both radioactivity and specific activity of beta chains are approximately one-half those of alpha chains. The difference in uptake of radioactivity between the globin chains indicates that beta chain synthesis proceeds at a slower rate than alpha chain synthesis in peripheral blood reticulocytes of these patients. Studies by Bank have failed to reveal evidence of a significant excess of alpha chains in hemolyzates from patients with heterozygous beta thalassemia (*2*). The relative decrease in synthesis of beta chains in reticulocytes is thus not accompanied by a corresponding disproportion in total amounts of beta and alpha chains in the peripheral blood red cells, indicating a difference between relative net synthesis of alpha and beta chains in nucleated red cells and reticulocytes in heterozygous beta thalassemia. My study was designed to compare the synthesis of globin chains of bone marrow with that of peripheral blood in this disorder.

The relative amounts of globin chains in the peripheral blood reflect mainly synthesis of globin by nucleated red cells in the bone marrow and any premature unequal removal of globin chains. The rates of synthesis of alpha and beta chains may be approximately equal in the bone marrow of patients with heterozygous beta thalassemia during the major period of globin synthesis, with the observed decrease in beta chain synthesis appearing late in red cell maturation. Alternatively, if the decreased rate of synthesis of beta chains in the peripheral blood were also present to the same degree in the nucleated red cell precursors, an intramedullary loss of approximately one-half of the alpha chains produced would be necessary for equal amounts of the chains to be present in the peripheral blood. In the homozygous condition (Cooley's anemia), loss of alpha chain occurs both in vivo and in incubated cells by precipitation and attachment to the membrane (*3*). The cells with inclusions are preferentially destroyed, presumably because of membrane damage (*4*). Those cells that are able to make significant amounts of gamma chain are better able to survive, since alpha chain precipitation is prevented by the formation of hemoglobin F($\alpha_2\gamma_2$). A comparison of globin synthesis in nucleated precursors and reticulocytes in Cooley's anemia has been attempted (*5*) but is difficult to interpret because of the selective destruction of cells with the most significant thalassemia defect in this disorder. A similar comparison in heterozygous thalassemia would be more critical, particularly in patients without intracellular inclusions in the marrow and with normal amounts of hemoglobin F. The following experiments compare globin synthesis in the peripheral blood and marrow of two such patients. Both patients had mild anemia and reticulocytosis, hypochromia, microcytosis, and elevated levels of Hb A_2.

Peripheral blood samples were incubated with L-[^{14}C]leucine (uniformly labeled) for 2 hours. The red cells were washed and lysed, and the alpha and beta chains were separated by chromatography on carboxymethyl cellulose in 8*M* urea (*6*). The absorbance of each fraction was determined at 280 nm, and radioactivity was measured in a liquid scintillation spectrometer.

Table 1. Synthesis of alpha and beta chains in reticulocytes and nucleated red cells from patients heterozygous for beta thalassemia.

Subject	Ratio of beta to alpha	
	Specific activity (count/min per O.D.)	Total radioactivity (count/min)
	Peripheral blood	
C.D.	0.55	0.57
G.H. No. 1	0.62	0.55
	Bone marrow	
C.D.	0.89	0.94
G.H. No. 1	0.94	1.02
G.H. No. 2	0.94	0.98

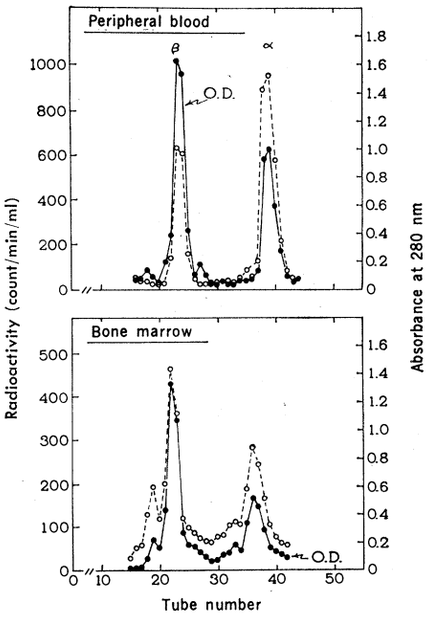

Fig. 1. Separation of globin chains from peripheral blood and bone marrow (No. 1) of patient G.H. by chromatography on carboxymethyl cellulose in 8*M* urea at *p*H 6.7. Dotted lines, counts per minute per milliliter, solid lines, optical density.

From the peak tubes for each chain, the specific activity was expressed by dividing the number of counts per minute by the absorbance, after correction for the difference in extinction coefficients of the chains (*6*) (see Table 1).

Bone marrow samples were obtained from each patient. Two volumes of 3 percent dextran were added to 3.5 ml of marrow (*5*). The cells were allowed to settle for 1 hour. The supernatant suspension was centrifuged to recover the nucleated cell fraction. An enrichment of nucleated red cells compared to nonnucleated red cells of at least 22-fold was obtained by the sedimentation process. The cells were added to 3 ml of the patient's plasma containing 2 mg of glucose per milliliter and incubated with L-[^{14}C]leucine for 2 hours in the same manner as the peripheral blood samples (*6*). A 3-ml sample of centrifuged peripheral red cells from the same patient was added at the end of the incubation. The globin chains were separated and processed as described for peripheral blood. Figure 1 illustrates one such experiment (patient G.H.).

A similar experiment with bone marrow from a nonthalassemic subject resulted in a ratio of specific activities of beta to alpha chains of 1.03. In the peripheral blood the ratio of the specific activities of beta to alpha chains for this same subject was 1.09. In a second experiment on G.H. (No. 2), the nucleated and carrier cells were added directly to a mixture of acetone and 12*N* HCl (90 : 1) without the preliminary hemolysis and separation of red cell membranes. Results of both experiments from the same marrow were similar, an indication of a lack of differential removal of globin chains with the red cell ghosts.

The balanced production of globin chains in the nucleated red cell precursors, indicated by the radioactivities of the chains (Table 1), differs from the decreased beta chain synthesis in the reticulocytes of these patients. The ratios of specific activities of beta to alpha for the marrows, with the use of peripheral red cells from the same patients as carrier, are within the normal range of values obtained in our laboratory on peripheral blood, 0.99 ± 0.05 (1 S.D.) and are similar to the results on peripheral blood reported by others (*1*). The relative amounts of globin chain synthesized in marrow cells compared with the amounts of globin in mature cells in the peripheral blood suggests that little or no selective destruction of globin chain has occurred during cell maturation. The ratios of specific activities of beta to alpha chains in the peripheral blood are within the range for A_2 thalassemia heterozygotes in our laboratory, 0.57 ± 0.09 (1 S.D.). These findings indicate that globin synthesis in the marrow of patients heterozygous for beta thalassemia is balanced, and that a significant relative decrease in beta chain synthesis must occur as the nucleated cell matures into the reticulocyte.

The nature of the inherited defect resulting in reduced synthesis of globin in beta thalassemia is unknown. The polyribosomes in thalassemic cells do not seem to be rate limiting for protein synthesis, since they respond normally to an artificial messenger RNA (mRNA) (*7*). The speed of beta chain synthesis and termination is normal, once translation is initiated (*8*). The defect would therefore appear to lie in a deficiency in the amount of beta mRNA being actively translated, either because of increased rate of decay of mRNA, decreased synthesis of mRNA, or delay in initiation of translation. The data presented here are not consistent with the last two possibilities, since the production of alpha and beta chains is equal in the bone marrow cells. There have been no direct measurements of the rates of decay of specific normal messenger RNA in erythroid cells. However, comparison of hemoglobin synthesis in bone marrow with that in reticulocytes in nonthalassemic subjects suggests that there is a decreased rate of synthesis of Hb F and Hb A_2 relative to Hb A in the older cells (*9*). These findings support the hypothesis that mRNA for gamma or delta chain decays more rapidly than mRNA for beta chain. There are experiments indicating that the mRNA for the beta chain of sickle hemoglobin (Hb S) is less stable than mRNA for beta chain of Hb A in heterozygotes for Hb S and Hb A (*10*). My results are compatible with an instability of beta chain synthesis in beta thalassemia, and with an unusually rapid decay of beta chain mRNA relative to that of alpha chain. The nucleated red cell of the heterozygote appears to be able to compensate fully for this defect, whereas that of the homozygote cannot. At the reticulocyte stage the capacity for compensation is lost, and a relative decrease in beta chain synthesis becomes apparent.

ELIAS SCHWARTZ
Cardeza Foundation for Hematologic Research and *Department of Pediatrics, Jefferson Medical College, Philadelphia, Pennsylvania 19107*

References and Notes

1. D. Heywood, M. Karon, S. Weissman, *Science* **146**, 530 (1964); D. J. Weatherall, J. B. Clegg, M. A. Naughton, *Nature* **208**, 1061 (1965); A. Bank and P. A. Marks, *ibid.* **212**, 1198 (1966); F. Conconi, A. Bargellesi, S. Pontremoli, V. Vigi, S. Volpato, D. Gaburro, *ibid.* **217**, 259 (1968).
2. A. Bank, *J. Clin. Invest.* **47**, 860 (1968).
3. Ph. Fessas, *Blood* **21**, 21 (1963); ———, D. Loukopoulos, A. Kaltsoya, *Biochim. Biophys. Acta* **124**, 430 (1966); A. Bargellesi, S. Pontremoli, C. Menini, F. Conconi, *Eur. J. Biochem.* **3**, 364 (1968).
4. D. G. Nathan and R. F. Gunn, *Amer. J. Med.* **41**, 815 (1966).
5. A. S. Braverman and A. Bank, *J. Mol. Biol.* **42**, 57 (1969).
6. J. B. Clegg, M. A. Naughton, D. J. Weatherall, *Nature* **207**, 945 (1967); Y. W. Kan, E. Schwartz, D. G. Nathan, *J. Clin. Invest.* **47**, 2515 (1968).
7. A. Bank and P. A. Marks, *J. Clin. Invest.* **45**, 330 (1966).
8. J. B. Clegg, D. J. Weatherall, S. Na-Nakorn, P. Wasi, *Nature* **220**, 664 (1968).
9. T. F. Necheles, M. Steiner, M. Baldini, *Blood* **25**, 897 (1965); R. F. Rieder and D. J. Weatherall, *J. Clin. Invest.* **44**, 42 (1965); R. M. Winslow and V. M. Ingram, *J. Biol. Chem.* **241**, 1144 (1966); H. M. Kazazian and H. A. Itano, *ibid.* **243**, 2048 (1968).
10. S. H. Boyer, P. Hathaway, M. D. Garrick, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 333 (1964).
11. Supported in part by PHS grant AM 12896. I thank Mrs. Veronica Watson for technical assistance.

23 December 1969