PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 459–462 (1975)

The Dependence of the Hemoglobin $(\beta+\gamma)/\alpha$ Chain Synthetic Ratio on the Degree of Anemia in β-Thalassemia (38561)

FARAMARZ ISMAIL-BEIGI AND MANSOUR HAGHSHENASS
(Introduced by J. N. Loeb)

Department of Internal Medicine and Pahlavi Medical Research Unit, Pahlavi University, Shiraz, Iran

The fundamental molecular defect in thalassemia appears to be an inherited disturbance in the rate of synthesis of one of the hemoglobin polypeptide chains which results in an imbalance of globin chain production (1, 2). In homozygous β-thalassemia (Cooley's anemia), there is underproduction of hemoglobin β chains relative to α chains both in bone marrow and in reticulocytes of affected patients, and this imbalance leads to a $(\beta + \gamma)/\alpha$ chain synthetic ratio of less than unity (3–6).

Despite the observation that hemoglobin chain synthetic ratios are highly variable from patient to patient, it has not been determined whether, in a given patient, this ratio can vary according to different circumstances. In view of the possibility that α chain synthesis and β chain synthesis are subject to independent regulation, the following study was performed to determine whether the peripheral blood hemoglobin chain synthetic ratio in a given β-thalassemic subject depends upon his degree of anemia.

Materials and Methods. Three subjects (ages 16, 17, and 20 yr) with Cooley's anemia were studied. The diagnosis was established on clinical and paraclinical grounds in both the subjects and their family members. None of the subjects were clinically affected to a severe degree. All had marked hypochromia with anisocytosis and poikilocytosis, elevation of fetal hemoglobin (45–65% hemoglobin F), and bone marrows which stained positively for iron. The subjects all had impressive splenomegaly, and two of them underwent splenectomy at the conclusion of the study with clinical improvement. All subjects were observed and treated with adequate folic acid for at least a month prior to initiation of the studies. Hematocrit determinations and reticulocyte counts were done on alternate days throughout the study.

Following an initial determination of the peripheral blood $(\beta + \gamma)/\alpha$ chain synthetic ratio, the patients were transfused with blood according to the schedules shown in Figs. 1–3. Hemoglobin chain synthetic ratios were determined 4–6 days after each transfusion so that the synthetic activity of the patients' own reticulocytes would be measured. Following splenectomy the synthetic ratio was measured 2 wk postoperatively. For the determination of the peripheral blood hemoglobin chain synthetic ratios, 10 μCi of ^{14}C-labelled protein hydrolysate (Amersham Co.), 0.10 ml of 1:3-diluted Amigen (Baxter Co.), and 1.0 μmole of $FeSO_4$ were incubated with 2.5 ml of reticulocyte-rich blood (hematocrit adjusted to 25%) for 90 min at 37° in a metabolic shaker. The previously described procedures for isolation of globin, for chain separation, and for radioactive counting were followed (4, 5). Since our techniques did not permit a complete separation of β and γ chains, the two peaks were pooled and counted as one ("$\beta + \gamma$"). By this technique the $(\beta + \gamma)/\alpha$ synthetic ratio of blood from 6 normal subjects was found to be 0.97 ± 0.02.

Results. Figure 1 shows the results of peripheral blood hemoglobin chain synthetic ratio determinations in the first subject at various intervals following stepwise raising of his hematocrit by transfusion. Hematocrits and reticulocyte counts (percent) are both plotted on the ordinate. It is evident that the $(\beta + \gamma)/\alpha$ chain synthetic ratio did not remain constant, but rather that it increased towards unity as the hematocrit was increased and the reticulocyte count fell. Following splenectomy, the hematocrit continued to rise without subsequent transfusion, and a high $(\beta + \gamma)/\alpha$ ratio was maintained.

Similar results of studies performed on two additional subjects are shown in Figs. 2 and 3. As in the first subject, the decrease in reticulocyte count induced by stepwise trans-

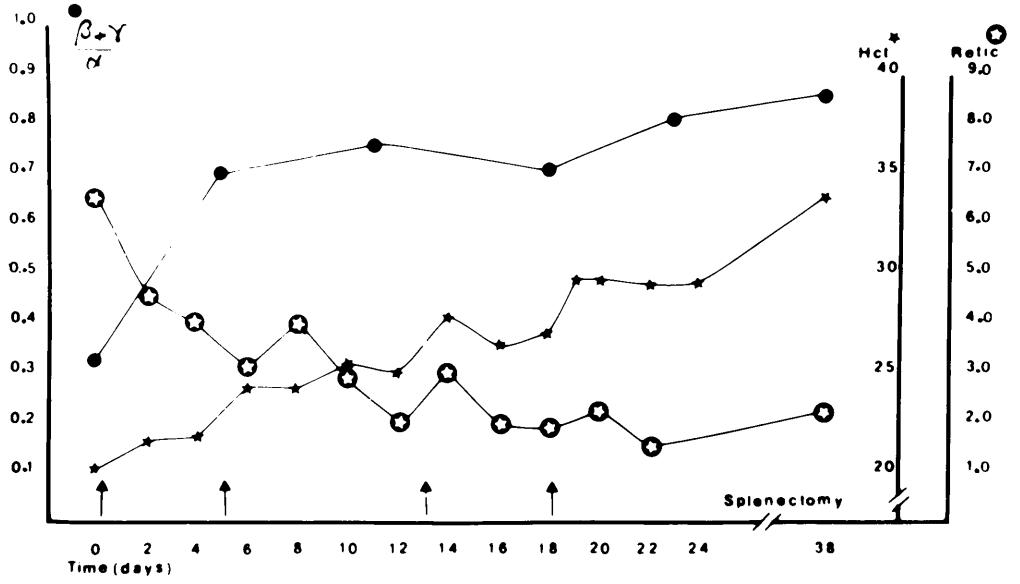

FIG. 1. Subject 1. Effects of transfusions (100 ml of packed red cells at times marked with arrows) on hematocrit reticulocyte count, and $(\beta + \gamma)/\alpha$ synthetic ratio.

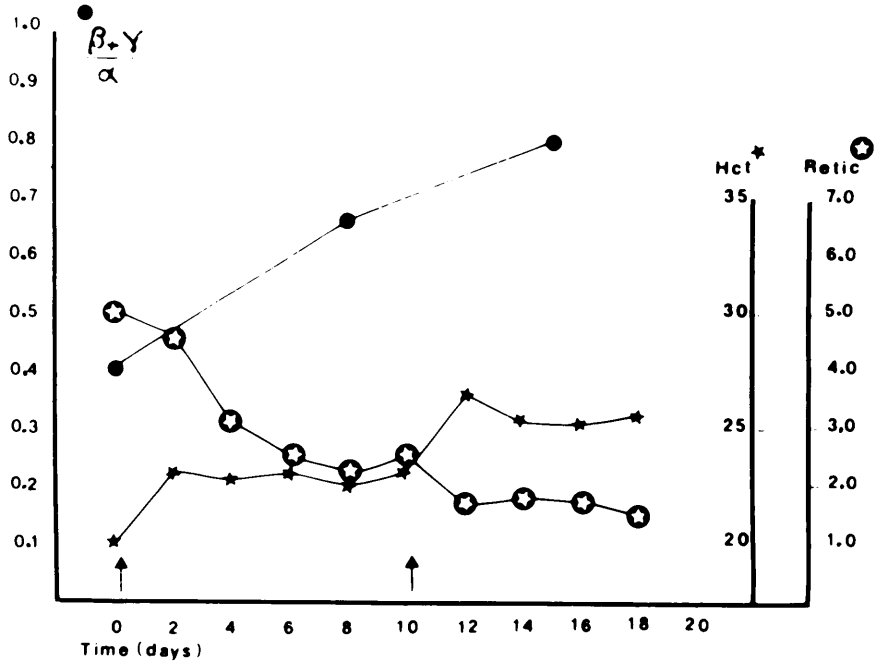

FIG. 2. Subject 2. Effects of transfusions (150 ml of packed red cells at times marked with arrows) on hematocrit, reticulocyte count, and $(\beta + \gamma)/\alpha$ synthetic ratio.

fusion was associated with a striking rise in the chain synthetic ratio towards unity.

Discussion. The data summarized in Figs. 1–3 show that the hemoglobin $(\beta + \gamma)/\alpha$ chain synthetic ratio is not necessarily constant in a given patient, but rather that it can vary as a function of his hematocrit. In all three patients hemoglobin chain synthesis

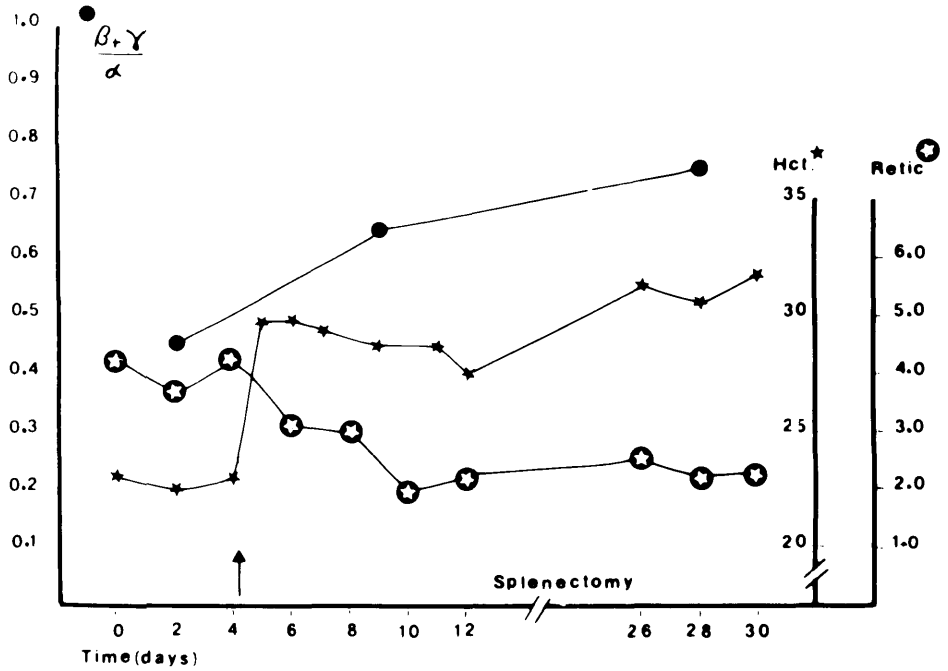

FIG. 3. Subject 3. Effects of transfusion (250 ml of packed red cells at time marked with arrow) on hematocrit, reticulocyte count, and $(\beta + \gamma)/\alpha$ synthetic ratio.

became more balanced as the stimulus for erythropoiesis was decreased by transfusion.

Although the molecular mechanisms in thalassemic disorders remain uncertain, the available evidence indicates that the defect leading to decreased rate of β chain relative to α chain synthesis in β-thalassemia is most probably at the level of transcription of genetic information (2, 5, 7–9). Recent evidence furthermore shows that one of the effects of erythropoietin is to stimulate hemoglobin synthesis by increasing the quantity of globin mRNA (10, 11). If one postulates that the β (as opposed to the α) "regulator site" in β-thalassemic subjects is relatively insensitive to the high degree of erythropoietic stimulation induced by severe anemia, or that it is maximally (though ineffectually) stimulated at even mild degrees of anemia, one would predict that the globin chain synthetic ratio should become more balanced as the erythropoietic stimulus is decreased. The data presented here show a return towards more balanced chain synthesis as the drive for erythropoieses is decreased, and would be consistent with such a mechanism.

While our observations are compatible with a selective decrease in α chain synthesis as the hematocrit is raised by transfusion, they do not specifically exclude the possibility that, instead, β chain synthesis *increases*. Whether under these conditions the absolute rate of β chain synthesis remains relatively invariant is unknown, and thus the determination of absolute rates of globin mRNA and hemoglobin chain synthesis at various degrees of anemia will be of importance in future experiments.

Summary. The peripheral blood hemoglobin $(\beta + \gamma)/\alpha$ chain synthetic ratio in three homozygous β-thalassemic subjects was shown to increase towards unity as their hematocrits were raised by stepwise transfusion. The observation that the chain synthetic ratio can vary in a given individual is discussed in relation to current understanding about the regulation of hemoglobin synthesis.

This work was supported by The Iran Foundation, Pahlavi University Research Council Grant No. 51-MD-24, W.H.O. Grant No. G3/181/79, and The Ministry of Higher Education of Iran. The authors wish to thank Dr. John N. Loeb for helpful criticism and Miss Nasrin Mohammadi for able technical assistance.

1. Pauling, L., Harvey Lect. **49,** 216 (1954).
2. Weatherall, D. J., and Clegg, J. B., "The Thalassaemia Syndromes," Second Ed. Blackwell Scientific Publications, Oxford (1972).
3. Heywood, J. D., Karon, M., and Weissmann, S., Science **146,** 530 (1964).
4. Weatherall, D. J., Clegg, J. B., and Naughton, M. A., Nature (London) **208,** 1061 (1965).
5. Bank, A., and Marks, P. A., Nature (London) **212,** 1198 (1966).
6. Bank, A., Braverman, S., O'Donnell, J. V., and Marks, P. A., Blood **31,** 226 (1968).
7. Marks, P. A., Burka, E. R., and Rifkind, R. A., Medicine **43,** 769 (1964).
8. Nienhuis, A. W., Laycock, D. G., and Anderson, W. F., Nature (London) **231,** 205 (1971).
9. Clegg, J. B., Weatherall, D. J., Na-Nakorn, S., and Wasi, P., Nature (London) **220,** 664 (1968).
10. Stohlman, F., Jr., Semin. Hematol. **4,** 304 (1967).
11. Terada, M., Cantor, L., Metafora, S., Rifkind, R. A., Bank, A., and Marks, P. A., Proc. Nat. Acad. Sci. U.S.A. **69,** 3575 (1972).

Received September 24, 1974. P.S.E.B.M. 1975, Vol. 148.