Globin Chain Synthesis in the Marrow and Reticulocytes of Beta Thalassemia, Hemoglobin H Disease, and Beta Delta Thalassemia

By Mordechai Shchory and Bracha Ramot

α, β, and γ globin chain synthesis in bone marrow and peripheral blood reticulocytes were studied in two patients with thalassemia major, two with thalassemia intermedia, one with thalassemia minor, one with Hb H disease, and one with homozygous βδ-thalassemia. Nine nonthalassemic patients served as controls. In thalassemia major, a marked imbalance of α-to-β chain synthesis was found in the bone marrow as well as in reticulocytes. The imbalance, however, was slightly more evident in the latter. In the patients with thalassemia intermedia and minor the α-to-β-globin chain ratios in the reticulocytes were of the same order of magnitude, despite the marked clinical differences between thalassemia intermedia and minor. A balanced synthesis was found in the bone marrow of the patient with thalassemia minor. The bone marrow globin synthesis in thalassemia intermedia was not studied. Contrary to that in Hb H disease and βδ-thalassemia, the imbalance was more apparent in the bone marrow. In the latter, no evidence for imbalance was detected in the reticulocytes. These results point out the need for further studies on globin chain synthesis in the bone marrow and reticulocytes of patients with the various thalassemia syndromes and the effect of the free globin chain pool on those results.

Thalassemic syndromes classically display ineffective hematopoiesis. Such factors as intramedullary death of erythroblasts, as shown by the unaccountable amount of urobilinogen excreted by such patients, as well as the presence of inclusion bodies, a result of free globin chain precipitation, portray some of the evidence for it.

Considerable data have been accumulated to show an imbalanced synthesis of globin chains in the reticulocytes of α- and β-thalassemia. However, there is little information concerning globin synthesis in the bone marrow of the various thalassemic syndromes. Schwartz has stressed the fact that in two cases heterozygous for β-thalassemia, balanced chain synthesis was observed in the bone marrow. Braverman and Bank have shown that the imbalance in synthesis increases as red cell precursors mature. No data are available on globin chain synthesis in the bone marrow of other thalassemic syndromes.

The purpose of this report is to present our findings regarding globin chain synthesis in reticulocytes and bone marrow of hemoglobin H disease, β-thalassemia major, intermedia, and minor, and homozygous βδ-thalassemia.
MATERIALS AND METHODS

The diagnosis and pertinent clinical data on the patients examined are given in Table 1. Nine individuals with a reticulocytosis due to acute bleeding served as controls. Approximately 10 ml of peripheral blood and 2.5 ml of bone marrow material were washed three times with cold isotonic NKM solution (0.13 M NaCl, 0.005 M KCl, .008 M MgCl₂), and the buffy coat was discarded. The packed cells were suspended in an equal volume of Krebs-Henseleit solution.₈ Iron and a mixture of L-amino acids in the ratios described by Borsook et al.,⁹ excluding valine or leucine, were added. The incubations were carried out at 35-36°C with continuous shaking. After a 5-min period of preincubation, 2–4 μCi/ml of L-valine-₁⁴C or L-leucine-₁⁴C (specific activity 260–350 mCi/mM) were added, and the incubation continued for 90 min. At the end of the incubation the cells were centrifuged and washed three times with NKM solution. They were then lysed by 4 volumes of 5 mM MgCl₂, followed 60 sec later by the addition of one-half volume of 1.5 M KCl to restore the solution to isotonicity. The lysate was centrifuged at 27,000 g for 30 min at 4°C to remove stroma and white cells. Globin was prepared from the clear hemoglobin solution by cold acid-acetone precipitation.¹⁰ After evaporation of the acetone, the samples were dialyzed overnight against 200 volumes of distilled water, lyophilized, and stored at 4°C.

The globin chains were separated by column chromatography on carboxymethyl cellulose (CMC) at pH 6.7, according to the method of Clegg et al.,¹¹ with slight modifications. The starting buffer contained 8 M deionized urea, 0.005 M Na₂HPO₄, and 0.045 M β-mercaptoethanol adjusted to pH 6.7 with 10% phosphoric acid. Forty to seventy milligrams of globin were dissolved in 2 ml of the starting buffer and dialyzed against the same buffer for 3 hr. The dialyzed globin was loaded on a 1 X 12 cm CMC column (Bio-Rad, exchange capacity 0.66 meq/g) previously equilibrated with the starting buffer. The globin was eluted with a linear gradient system using a gradient mixing apparatus. The first chamber contained 120 ml of the starting buffer, and the second held 120 ml of the same buffer but with a concentration of 0.5 M Na₂HPO₄. The flow rate was maintained by a peristaltic pump at 0.05 ml/min, and 4-ml fractions were collected during continuous uv recording of the eluate. The optical density of each tube was determined at 280 nm in a Gilford 2400 spectrophotometer. To aliquots of 1 ml of each fraction, 0.5 mg of bovine serum albumin was added as a carrier and then precipitated by trichloroacetic acid in a final concentration of 15%. The precipitates were heated at 90°C for 20 min and filtered on Millipore discs 2.5 mm in diameter. The dried discs were put into vials containing 10 ml of scintillation fluid (5 g PPO and 0.5 g POPOP/liter toluene) and counted in a Packard Tri-Carb liquid scintillation counter at a counting efficiency of 65%. The calculations were performed only on the two peak tubes, and the specific activities are reported as cpm/OD.

The OD values for the various chains were corrected according to Kan et al.¹² When ¹⁴C-valine was used, the cpm values for the β-chain were divided by 18/13 and those for the α-chain by 12/13, according to the valine contents of each chain.

### Table 1. Hematologic Data

<table>
<thead>
<tr>
<th>Patient and Diagnosis</th>
<th>Hemoglobin (g/100 ml)</th>
<th>Hb A₂ (%)</th>
<th>Hb F (%)</th>
<th>Hb H (%)</th>
<th>Hb Bart (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M.S.* Thalassemia major</td>
<td>7.5</td>
<td>1.5</td>
<td>46</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2. Z. G. Thalassemia major</td>
<td>8.5</td>
<td>3.0</td>
<td>29</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3. Ch.H, Thalassemia intermedia</td>
<td>7.2</td>
<td>7.3</td>
<td>4.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4. A.M.* Thalassemia intermedia</td>
<td>6.9</td>
<td>6.6</td>
<td>3.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5. B.R. Thalassemia minor (β)</td>
<td>10.1</td>
<td>4.3</td>
<td>1.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6. K.A. Hb H disease (6)</td>
<td>7.5–9.5</td>
<td>1</td>
<td>—</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>7. A.R.I. β-thalassemia</td>
<td>12.3</td>
<td>—</td>
<td>&gt;90</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Postsplenectomy.
GLOBIN CHAIN SYNTHESIS

The bone marrow aspirate, obtained from the posterior iliac crest, and peripheral blood samples were mixed with brilliant cresyl blue, and smears were examined for the presence of inclusion bodies and reticulocytes.

Fig. 1. Globin chain separation by chromatography on CMC column of patient No. 1, homozygous beta thalassemia. First peak represents beta chains and the second alpha chains. Absorbancy and radioactivity values are corrected as described in text.
Fig. 2. Globin chain separation by chromatography on CMC column of patient No. 7, homozygous beta delta thalassemia. First peak represents gamma chain and the second alpha chains. Absorbancy and radioactivity values are corrected according to text.

RESULTS

A definite imbalance in the $\alpha$ to $\beta$ or $\alpha$ to $\gamma$ globin chain ratios was found in the various types of thalassemia. The pattern of imbalance in the bone marrow and in the peripheral blood was different in the various types of thalassemia, as shown in Table 2 and Figs. 1 and 2.

In the two cases (No. 3 and 4) of $\beta$-thalassemia intermedia and one case (No. 5) of $\beta$-thalassemia minor, very similar $\alpha$-to-$\beta$-globin chain ratios were observed in the peripheral blood. Of special interest is the finding of a balanced globin chain synthesis in the bone marrow of the patient with $\beta$-thalassemia minor, while the peripheral blood reticulocytes showed a marked imbalance, of the order found in the two patients with thalassemia intermedia.

In contrast, in Hb H disease (case 6) the globin chain synthesis in the bone marrow cells was more markedly impaired than in the peripheral blood.

In the case of $\beta\delta$-thalassemia (case 7 Fig. 2) there was in the bone marrow a preponderance of labeled $\alpha$-chains relative to $\gamma$-chains ($\alpha:\gamma$ ratio of 1.53), but no excess of $\alpha$-chain synthesis was found in the peripheral blood. In effect, a slightly higher ratio of $\gamma$-relative to $\alpha$-chains was noted ($\alpha:\gamma$ ratio of 0.78). The accuracy of the ratio obtained for the peripheral blood might be questioned, since it is based on low counts. However, the ratio of 0.78 was obtained on two separate experiments, furthermore, inclusion bodies were observed only in the bone marrow normoblasts in the case of $\beta\delta$-thalassemia, while
none was seen in the peripheral blood. This is in contrast to the cases of \(\beta\)-thalassemia and Hb H disease in which inclusion bodies were found in both the bone marrow and in peripheral blood erythroid cells.

**DISCUSSION**

There is abundant evidence to indicate the existence of a defect in the synthesis of the various globin chains in thalassemia, as demonstrated in studies on reticulocytes of thalassemia patients.\(^{12-15}\) However, the precise nature of this defect has not yet been elucidated.\(^{16,17}\) Data on hemoglobin synthesis in the bone marrow of such patients are scanty, and to the best of our knowledge, no information on globin chain synthesis in the bone marrow of \(\beta\beta\)-thalassemia\(^{7,18-20}\) or Hb H disease has been published.

It should be noted that although reticulocytes were not separated from bone marrow cells in our studies, this contamination is negligible, as the synthetic capacity of reticulocytes is much lower than that of the bone marrow cells.

Our findings indicate a definite imbalance of \(\alpha\)- to \(\gamma\)-chain synthesis in the bone marrow of a patient homozygous for beta delta thalassemia, where an excess of \(\alpha\)-chain synthesis relative to that of \(\gamma\)-chain synthesis was demonstrated. The observed imbalance is consistent with the finding of inclusion bodies in the bone marrow normoblasts, but not in the peripheral blood, where the ratio of \(\alpha:\gamma\) was close to normal.

The imbalance between \(\alpha\)- and \(\beta\)-chain synthesis in Hb H disease is also somewhat greater in the bone marrow than in the peripheral blood reticulocytes. We have no explanation for this finding, since the inclusions in Hb H disease are larger in the peripheral blood cells than in the normoblasts. The possibility of isotope dilution in a larger pool of free \(\beta\)-chains in the reticulocytes of Hb H disease was considered. This, however, could not be confirmed by comparing the ratio of the total amounts of globin chains in peripheral blood and bone marrow preparations. It is obvious that more information is needed to clarify this point.

Our results in two patients homozygous for \(\beta\)-thalassemia (one after splenectomy) are in accordance with previously published findings.\(^3\)

Of special interest are the results in two patients with thalassemia inter-

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**Table 2. Specific Activities of the Various Globin Chains and their Ratios in the Different Thalassemia Syndromes**

<table>
<thead>
<tr>
<th>Case No. and Diagnosis</th>
<th>Peripheral Blood Specific Activity (cpm/OD 100)</th>
<th>Bone Marrow Specific Activity (cpm/OD 100)</th>
<th>Ratio</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\alpha)</td>
<td>(\beta)</td>
<td>(\gamma)</td>
<td>(\alpha/\text{non-}\alpha)</td>
</tr>
<tr>
<td>1. Thalassemia major</td>
<td>9581</td>
<td>2270</td>
<td>—</td>
<td>4.22</td>
</tr>
<tr>
<td>2. Thalassemia minor</td>
<td>352</td>
<td>70</td>
<td>—</td>
<td>4.45</td>
</tr>
<tr>
<td>3. Thalassemia major</td>
<td>4300</td>
<td>2059</td>
<td>—</td>
<td>2.09</td>
</tr>
<tr>
<td>4. Thalassemia major</td>
<td>7647</td>
<td>3400</td>
<td>—</td>
<td>2.25</td>
</tr>
<tr>
<td>5. Thalassemia minor ((\beta))</td>
<td>862</td>
<td>334</td>
<td>—</td>
<td>2.58</td>
</tr>
<tr>
<td>6. Hb H disease</td>
<td>1162</td>
<td>1694</td>
<td>—</td>
<td>0.68</td>
</tr>
<tr>
<td>7. (\beta\beta)-Thalassemia</td>
<td>181</td>
<td>—</td>
<td>232</td>
<td>0.78</td>
</tr>
<tr>
<td>8. Normal controls</td>
<td>Mean 0.97</td>
<td>SD 0.07</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
media and in one with thalassemia minor. In spite of the striking clinical differences between them very similar α- to β-chain ratios were found in their peripheral blood samples, while a balanced synthesis was observed in the bone marrow of the patient with thalassemia minor (Table 2). The latter finding confirms the observations of Schwartz in two cases of β-thalassemia minor. The latter finding confirms the observations of Schwartz in two cases of fi-thalassemia minor.4

The poor correlation between the severity of the imbalance in globin chain synthesis by the methods used could suggest either that the imbalance in synthesis and chain precipitation is not the determining factor in the development of the anemias or that the pool size of free chains in the various patients varies very markedly. Free chains should, therefore, be separated prior to globin precipitation in various thalassemia syndromes in order to solve this problem, as indicated by White et al.21 in sideroblastic anemias.

Since a wide range of α- to β-chain ratios in normal marrow have been reported by various investigators,4,5 it seems obvious that more work is also needed to determine the range of normal values.

REFERENCES


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