Autonomous Synthesis of $\alpha$ and $\beta$ Hemoglobin Chains in Rabbit Erythroid Cells

By P. P. Dembure and M. D. Garrick

Hemoglobin $\beta$-chain synthesis by rabbit erythroid cells was tested for dependence on availability of complementary $\alpha$-chains. Reticulocytes and bone marrow cells were obtained from variant rabbits that have hemoglobin with isoleucine in $\alpha$-chains but not in $\beta$-chains. This characteristic permits the use of L-o-methylthreonine, a specific isoleucine antagonist, to inhibit selectively the synthesis of hemoglobin $\alpha$-chains without directly affecting that of $\beta$-chains. Study of hemoglobin synthesis by bone marrow cells presents two problems that require careful management: (A) the fragility of the globin-synthesizing apparatus and (B) the isolation of globin from the various proteins made by the mixture of nucleated cells. Disruption of synthetic activity was minimized by collecting the bone marrow in autologous plasma then removing fat and connective tissue while the cells were suspended in this medium. Purification involved gel filtration of hemoglobin and globin then CM-cellulose chromatography of globin chains. Absence of radioactive isoleucine in $\beta$-chains demonstrated the efficacy of this scheme in removing isoleucine-containing proteins that otherwise elute with $\beta$-chains on CM-cellulose columns. In reticulocytes, when synthesis of $\alpha$-chains is inhibited by 30%–80%, that of $\beta$-chains is stimulated by 20%–60%, but in marrow cells, incorporation into $\beta$-chains stays at control level when $\alpha$ incorporation is inhibited. The data indicate that $\beta$-chain synthesis is independent of the availability of complementary $\alpha$-chains.

To account for coordination of globin synthesis in mammalian systems, regulatory mechanisms have been proposed that render the synthesis of one globin chain type dependent on the other.$^1$ The validity of such mechanisms may be tested by specifically inhibiting incorporation into selected globin chain types without directly affecting their complementary hemoglobin subunits. L-o-Methylthreonine, a synthetic isoleucine isostere in which the methylene group has been replaced by oxygen, permits selective inhibition of the synthesis of isoleucine-containing peptides. The compound competes with isoleucine for the aminoacyl-tRNA synthetase but is not attached to the tRNA.$^4$ Hence, it lowers the concentration of Ile-tRNA and slows down peptide chain extension at isoleucyl residues.$^5$

Human fetal hemoglobin contains isoleucine residues in the $\gamma$-chain but not in the $\alpha$-chain, a characteristic that permits the use of L-o-methylthreonine to inhibit $\gamma$-chain synthesis selectively. Honig et al.$^6$ and Garrick et al.$^8$ observed that $\alpha$-chain synthesis by human fetal reticulocytes proceeds independently of the synthesis of complementary non-$\alpha$-globin chains. Existence of an $\alpha$-chain-dependent mechanism for $\beta$-chain synthesis can be tested in rabbit erythroid systems since hemoglobin from variant rabbits contains isoleucine in $\alpha$-chains but not in $\beta$-chains, permitting selective inhibition of $\alpha$-chain synthesis by L-o-methylthreonine. In the $\beta$-chain of these rabbits, the single isoleucine residue at position 112 has been replaced by valine,$^9,10$ and this trait is inherited in a mendelian fashion.$^{11}$

Studies with rabbit reticulocyte lysates$^{12,13}$ suggested that synthesis of non-$\alpha$-globin chains may depend in part on the presence or concomitant synthesis of $\alpha$-chains. However, these studies have not been repeated elsewhere, and they were done with cell-free systems that are more sensitive to disturbance than intact cells. Our previous studies$^{14}$ confirmed earlier reports of Wolf et al.$^{15}$ and Rabinovitz et al.$^{16}$ which showed that $\beta$-chain synthesis in intact rabbit reticulocytes proceeds autonomously when $\alpha$-chain synthesis is inhibited by L-o-methylthreonine. Our data and those of Wolf et al.$^{15}$ differed in that we detected stimulation of $\beta$-chain synthesis with L-o-methylthreonine, while in their studies $\beta$-chain synthesis was not affected. When we reduced the proportion of cells in the incubation mixture, our results reproduced their results. Hence, the different observations were accounted for completely by the proportion of cells present in the incubation mixture used in the respective studies.

Rabbit reticulocytes contain a pool of uncombined $\alpha$-chains that may obscure the demonstration of an $\alpha$-chain-dependent mechanism for $\beta$-chain synthesis. Bone marrow cells are convenient for examining the interaction of $\alpha$ and $\beta$-globin synthesis, since they do not contain a detectable pool of free $\alpha$-globin chains.$^{15,17,18}$ In addition, because erythroid marrow cells are nucleated, they may have mechanisms for regulation of $\alpha$ and $\beta$-chain synthesis that are lost.

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during reticulocyte formation. Wolf et al.\textsuperscript{15} observed an apparent depression of \( \beta \)-globin chain synthesis with the isoleucine antagonist, interpreted as a partial dependence on the availability of complementary \( \alpha \)-chains. Bone marrow cells, however, synthesize a variety of nonhemoglobin proteins, and Wolf et al.\textsuperscript{15} did not demonstrate the efficiency of their purification procedure in removing contaminating proteins. This point is particularly important in view of the fact that apparent inhibition of \( \beta \)-chain synthesis decreased with increased purity of the marrow product. Also, our previous reticulocyte studies\textsuperscript{14} raised the possibility that excessive cell dilution could contribute to these results. We report here data indicating that \( \beta \)-chain synthesis by bone marrow cells is independent of availability of \( \alpha \)-chains.

**MATERIALS AND METHODS**

Reticulocytes were obtained from New Zealand white rabbits and incubations carried out as previously described. For bone marrow studies, rabbits were exsanguinated, and flesh was cleaned off the humeri, femora, and tibiae before cutting the bone ends. The marrow was forced out by air pressure and flushing with autologous heparinized plasma. The plasma suspension was placed in 50-mi centrifuge tubes and vortexed for 2 mm to release single cells. After centrifugation at 1200 g for 5 min, the top fat layer was removed and the cells were washed once with the incubation medium containing amino acids at concentrations optimal for hemoglobin synthesis.\textsuperscript{14}

Occasionally, the bone marrow cells were prepared in a manner similar to that described by Wolf et al.\textsuperscript{15} In this case, the marrow was initially suspended in 0.9% saline, homogenized gently in a glass tube with a Teflon pestle, and then treated as above with saline replacing plasma.

Bone marrow incubations were otherwise under the same conditions as those previously described for reticulocytes.\textsuperscript{14} The incubations were terminated by rapid chilling on ice and centrifugation for 5 min at 1200 g to remove the incubation medium. The cells were lysed by addition of 10 volumes of water plus 2 freeze-thaw cycles.

Carrier hemoglobin was added to bone marrow hemolysates and the mixture placed on a 2.5 x 120 cm column of Sephadex G-100. Hemoglobin was eluted at 0.32 ml/min with Tris-HCl, pH 7.4, containing 10 mM KCN. Globin was prepared from the purified hemoglobin\textsuperscript{14} and also passed through a Sephadex G-100 column but eluted with a solution containing 0.02 M pyridine and 0.2 M formic acid. The \( \alpha \) and \( \beta \)-globin chains were separated on CM-cellulose as previously described.\textsuperscript{14} For each fraction from gel filtration and chain separation columns, an aliquot was taken for radioactivity measurement in a Beckman LS-230 liquid scintillation counter.

Radiochemicals were purchased from the respective vendors as follows: l-[4,5-\( ^3 \text{H} \)] lysine, 43–60 Ci/m mole and l-[\( ^3 \text{H} \)] isoleucine, 8 Ci/m mole from Schwarz/Mann, Orangeburg, N.Y.; l-\([U-\text{\( ^14 \text{C} \)}] \) lysine, 290 mCi/m mole from ICN, Cleveland, Ohio; and

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**Fig. 1.** Relation between method of preparation for bone marrow cells and incorporation into hemoglobin. Bone marrow cells were incubated with 40 \( \mu \)Ci/ml of l-[\( ^3 \text{H} \)]lysine for 30 min at 37\(^\circ\)C. Hemolysates were subjected to gel filtration on Sephadex G-100. The void volume was at fraction 37; the peak for hemoglobin at 60. (A) Cells isolated in autologous plasma. (B) Cells isolated in saline. (C) Cells (from marrow) isolated in saline in the presence of peripheral blood cells from a nonanemic rabbit. (O——O) Absorbance at 280 nm; (●—●) tritium.
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\( t \)-[Me-\(^{1}H\)] methionine, 11. 5 Ci/mmole and \( t \)-[\(^{35}S\)] methionine, 381.25 Ci/mmole from New England Nuclear Corporation, Boston, Mass.

RESULTS

Isolation of Bone Marrow Cells

Preparation of bone marrow cells for the study of hemoglobin synthesis in vitro requires that the cells be freed from fat and connective tissue then dispersed in an appropriate medium. Morrell et al.\(^{2}\) have noted that repeated saline washes of marrow cells by centrifugation yielded marked cytologic alterations and decreased synthetic activity. Morphological integrity was best maintained by isolating marrow cells in autologous plasma. We therefore examined amino acid incorporation into hemoglobin and larger nonheme proteins as a function of how the marrow cells were prepared (Fig. 1).

When cells were isolated in autologous plasma (Fig. 1A), nonheme proteins represented 30% of the incorporation, while the remainder eluted with hemoglobin. Physiologic saline as the isolation medium (Fig. 1B) resulted in both a drastic decrease for total incorporation and a relative increase in the fraction eluting before the hemoglobin (51% of the total). Nonanemic erythrocytes were also added in the saline (Fig. 1C), purportedly to protect marrow erythroid cells as suggested by others.\(^{15}\) This treatment improved overall integration; unfortunately, however, hemoglobin synthesis responded unfavorably with 78% of the incorporation eluting prior to the hemoglobin.

Isolation of Globin From Nonglobin Proteins

One problem in the study of hemoglobin synthesis by bone marrow cells is purifying globin chains from the variety of proteins arising from the nucleated erythroid and nonerythroid cells. When selective inhibition of \( \alpha \)-chain synthesis is to be accomplished by an isoleucine analogue, any isoleucine containing protein that elutes along with \( \beta \)-chains on CM-cellulose columns will produce results that may be falsely interpreted to indicate that \( \beta \)-chain synthesis is also inhibited. We have, therefore, relied first on passing hemolysates through Sephadex G-100 columns to separate hemoglobin from proteins of higher or lower molecular weight. Globin was then prepared from the partially purified hemoglobin and also passed through Sephadex G-100 columns to resolve the globin from any proteins that had coeluted with the hemoglobin. Radioactive isoleucine was used to demonstrate the efficacy of the purification scheme in removing isoleucine-containing proteins that otherwise would coelute with \( \beta \)-chains on CM-cellulose columns. Contaminating proteins do overlap \( \beta \)-chains in an unpurified sample, and the purification scheme employed is satisfactory in removing these isoleucine-containing proteins (Fig. 2). When, however, bone marrow cells were prepared in physiologic saline,\(^{15}\) the relative contribution of isoleucine-containing contaminants was sufficiently increased that this purification procedure was inadequate (data not shown).

Hemoglobin Synthesis by Rabbit Bone Marrow Cells

The bone marrow preparation and globin purification scheme described above provide suitable conditions for examining whether an \( \alpha \)-chain-dependent mechanism for \( \beta \)-chain synthesis exists. We therefore examined whether inhibition of \( \alpha \)-chain production is accompanied by increased, decreased, or unaltered \( \beta \)-chain synthesis. Table 1 summarizes the effects of \( t \)-o-methylthreonine on globin synthesis by bone marrow cells. The data are similar to our earlier data for reticulocytes\(^{14}\) in that \( \alpha \)-chain synthesis may be inhibited without also inhibiting \( \beta \)-chain synthesis. They differ, however, in that \( \beta \)-chain synthesis increased when \( \alpha \)-chain production was inhibited in reticulocytes, while in marrow, \( \beta \)-chain synthesis proceeds unaltered. We, therefore, examined several explanations for this difference. One possible explanation is that the gel filtration of hemoglobin needed for marrow samples removes excess uncombined \( \beta \)-chains. To test this possibility, we analyzed lysates from 4-hr reticulocyte incubations, comparing results for aliquots that were not purified to results after employing the purification scheme for marrow lysates. Retic-
plasma were incubated for L-[3H]-lysine. Independently, reticulocyte-rich blood was incubated for 2 hr with 100 μCi of L-[14C]-lysine/ml of blood. Incorporation into acid-precipitable material from the reticulocyte incubation was determined and an aliquot containing approximately 40,000 cpm was added to each bone marrow sample as a standard before Islying. The mixtures were then purified by successive gel filtration of hemoglobin and globin on Sephadex G-100 columns. Values in parentheses are percent incorporation relative to the control.

### Table 1. Effects of L-o-Methylthreonine on Globin Synthesis by Bone Marrow Cells

<table>
<thead>
<tr>
<th>Exp.</th>
<th>L-o-Methylthreonine (mM)</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>5.82 (100)</td>
<td>6.54 (100)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.08 (36)</td>
<td>6.68 (102)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>3.25 (100)</td>
<td>4.67 (100)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.19 (36)</td>
<td>4.50 (96)</td>
</tr>
</tbody>
</table>

Bone marrow cells from each of two rabbits isolated in autologous plasma were incubated for 30 min at 37°C with 167 μCi/ml of L-[3H]-lysine. Independently, reticulocyte-rich blood was incubated for 2 hr with 100 μCi of L-[14C]-lysine/ml of blood. Incorporation into acid-precipitable material from the reticulocyte incubation was determined and an aliquot containing approximately 40,000 cpm was added to each bone marrow sample as a standard before Islying. The mixtures were then purified by successive gel filtration of hemoglobin and globin on Sephadex G-100 columns before chain separation on CM-cellulose columns. Values in parentheses are percent incorporation relative to the control.

### Table 2. Effect of Purification Procedure for Marrow on Stimulation of β-Chain Synthesis in Reticulocytes

<table>
<thead>
<tr>
<th>L-o-Methylthreonine (mM)</th>
<th>Prior Purification</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>6.69 (100)</td>
<td>6.69 (100)</td>
</tr>
<tr>
<td>25</td>
<td>No</td>
<td>3.26 (40)</td>
<td>9.90 (148)</td>
</tr>
<tr>
<td>0</td>
<td>Yes</td>
<td>6.18 (100)</td>
<td>6.01 (100)</td>
</tr>
<tr>
<td>25</td>
<td>Yes</td>
<td>2.61 (42)</td>
<td>8.64 (144)</td>
</tr>
</tbody>
</table>

Reticulocyte-rich cells were incubated at 33% cell volume for 2 hr with 20 μCi of L-[3H]-lysine/ml incubation mixture. About 160,000 cpm of hemoglobin previously labeled with L-[14C]-lysine were added to 40 mg of the H-lysine hemolysates. For each mixture, 60% was purified by Sephadex G-100 gel filtrations first of hemolysate, then of globin, while the remaining 40% was stored at −20°C. The α and β chains were separated from each aliquot and αH/14C ratios were determined.

decreases total incorporation with little if any differential effect on α and β-globin synthesis. Twenty-five millimolar L-o-methylthreonine inhibits incorporation into α-chains increasingly with increasing cell dilution, probably because the internal pool of isoleucine is also diluted. This inhibition is accompanied by a stimulation of β-globin synthesis except when the proportion of cells to volume is no more than 16%. Reticulocytes from seven different rabbits compared for this response had β-globin synthesis = 146 (±10% standard deviation) relative to the control value when cell volume is 33% of an incubation mixture containing 25 mM L-o-methylthreonine and β incorporation = 104 (±11%) for incubations at 16% cell volume. Thus, increased incorporation into β-globin chains when α incorporation is inhibited by L-o-methylthreonine critically depends on cell concentration when reticulocyte hemoglobin synthesis is examined.

When marrow is examined, it is difficult to have exact control of the erythroid cell dilution because nonerythroid cells and debris are also present. We therefore examined whether mature red blood cells (i.e., those no longer making globin) would demonstrably contribute to the cell proliferation effect. If so, these nonincorporating erythrocytes could also be used to examine the effect of cell dilution in marrow incubations. Surprisingly, however, after 6 days of refrigerated storage, nonanemic rabbit peripheral red blood cells retained sufficient protein synthetic activity to make a significant contribution to total incorporation (Fig. 3B). Repeated removal of less dense cells13 did not entirely eliminate incorporation (not shown), but preincubation at 37°C overnight did (Fig. 3A).

When added to reticulocytes, 6-day-old cells restored increased synthesis of β-chains that otherwise does not occur in incubations with a low proportion of

### Table 3. Effects of Cell Dilution on Globin Synthesis in Reticulocytes

<table>
<thead>
<tr>
<th>Cell Volume (%)</th>
<th>L-o-Methylthreonine (mM)</th>
<th>Counts - min−1 Amax Unit−1</th>
<th>α</th>
<th>β</th>
<th>α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0</td>
<td>53,800 (100)</td>
<td>53,800 (100)</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>21,700 ( 40)</td>
<td>45,200 (163)</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0</td>
<td>75,900 (100)</td>
<td>48,500 (100)</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>0</td>
<td>16,300 ( 21)</td>
<td>66,800 (138)</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>65,000 (100)</td>
<td>37,200 (100)</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>25</td>
<td>11,400 ( 18)</td>
<td>40,900 (110)</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>25</td>
<td>36,800 (100)</td>
<td>21,000 (100)</td>
<td>1.77</td>
<td></td>
</tr>
</tbody>
</table>

Reticulocytes were obtained from a phenylhydrazine-treated rabbit and were incubated for 2 hr with 20 μCi of L-[14C]-lysine/ml of incubation mixture. The incubation mixtures contained the same amount of cells and the proportion of cells was altered by addition of the incubation medium without altering the molar concentration of the other constituents. α/β ratios for controls were determined by integrating total counts.
cells (Table 4). In contrast, addition of peripheral blood cells to bone marrow did not result in stimulation of β-chain synthesis when L-o-methylthreonine inhibits α-chain formation (Fig. 4). These results, in combination with data showing that the stimulation of β-globin synthesis in reticulocytes requires a substance that enters and leaves cells, indicate that excessive cell dilution makes the postulated substance less available to reticulocytes. Presumably, the diffusible substance interacts with nucleated cells differently than with reticulocytes.

Degradation of excess β-chains is another way to account for this absence of increased β-chain synthesis in marrow. If degradation occurs rapidly, its effects would be least at shorter incubation times and increase with longer incubations. During a 60-min incubation, L-o-methylthreonine inhibited the synthesis of α-chains to 22% of control incorporation, while throughout this period, β-chain synthesis proceeded at

| Table 4. Effects of 6-Day-Old Cells on Stimulation of β-Chain Synthesis in Reticulocytes |
|-----------------|-------------|-----------------|-----------------|
| Cells           | L-o-Methylthreonine (mM) | Counts · min \(^{3}H\) | Unit \(^{14}C\) (% Control) |
| Reticulocytes   | 0           | 43,500 (100)    | 28,700 (100)    |
| Reticulocytes   | 25          | 3,840 (106)     | 30,100 (106)    |
| Reticulocytes and old cells | 0           | 80,500 (100)    | 50,100 (100)    |
| Reticulocytes and old cells | 25          | 23,300 (170)    | 85,600 (170)    |

Peripheral blood cells from a nonanemic rabbit were aged then washed three times with saline, as described in the legend of Fig. 3. Two milliliters of these cells were added to 1 ml of reticulocyte-rich cells obtained from a phenylhydrazine-treated rabbit. One volume of the mixed cells was added to 2 volumes of incubation medium and incubated for 2 hr with 20 μCi of L-[^3]H-lysine/ml of incubation mixture. For comparison, reticulocyte-rich cells were incubated separately with[^3]H-lysine in mixtures containing 11% cells by volume.
the same rate as the control (Fig. 4). There are two reasons to expect that, in bone marrow cells incubated with L-o-methylthreonine, newly made excess β-chains should be uncombined. First, these cells do not have a detectable pool of free α-globin chains to combine with excess β-chains. Second, we have previously shown that even in reticulocytes after 30-min incubation with 25 mM L-o-methylthreonine, the free α-chain pool has disappeared. Because β-chains made in the presence of inhibitor survive as well as those of the control over the time period examined in marrow cells (Fig. 4), degradation of excess β-chains is unlikely to be the reason for lack of increased β-chain synthesis in marrow preparations. Otherwise, a very peculiar time course for degradation of excess β-globin chains would have to be postulated. In particular, the percentage of β-globin incorporation compared to the control would have to decrease from about 145% (the value typically found in reticulocytes) to 100% with a half-life of no more than 5 min, while the half-life for a decline from 100% (the value found in marrow) to about 25% (the value found for α-chain incorporation in inhibited marrow) would have to be at least 4 hr to fit the data obtained. An explanation involving such a discontinuity in kinetics of degradation is unnecessarily complex and requires that there be two classes of uncombined globin chains; hence, it is highly improbable. Moreover, our observations are more consistent with the finding that excess β-globin chains in hemoglobin-H disease are removed with a half-life of 33 hr, a rate of destruction that would not have any effect in our 1-hr incubation.

DISCUSSION

Our data are in disagreement with those of Wolf et al. who observed an apparent modulation of β-chain synthesis by α-chains in their bone marrow studies. When we prepared bone marrow cells in saline, according to Wolf et al., amino acid incorporation was increased in nonglobin proteins including those that coelute with β-chains on CM-cellulose columns. When subjected to the purification scheme, these samples exhibited negligible globin synthesis in our hands.

Our data reveal an interesting difference between reticulocytes and bone marrow cells with regard to the effect of L-o-methylthreonine on β-chain synthesis. Stimulation of β-chain synthesis with L-o-methylthreonine occurs in reticulocytes but is not observed in bone marrow cells. Increased β-chain synthesis when α-chain production is reduced is interpreted as resulting from a relief of competition between α and β RNAs for a limited translational capacity. Competition between α and β mRNA has previously been demonstrated in reticulocyte lysates.

Several possible reasons for failure to observe stimulation of β-chain synthesis with L-o-methylthreonine in bone marrow cells have been examined. When applied to reticulocytes, the purification does not eliminate stimulation (Table 2). Degradation of excess β-chains is not a likely explanation in view of the time course (Fig. 4). Cell dilution also does not appear to account for the difference (Table 4). If the purification scheme, degradation of excess β-chains, and cell dilution are eliminated, what possibilities remain to account for the discrepancy between reticulocytes and nucleated erythroid cells? Synthesis of hemoglobin by the latter may be under more direct nuclear control. Alternatively, in the bone marrow, some other component of the translational apparatus, distinct from the one competed for by mRNAs in reticulocytes, may be rate limiting for β-chain synthesis. If mRNAs, for example, are rate limiting in bone marrow cells, then β mRNA is already being translated at its maximal rate in this tissue. Because they are nucleated, bone marrow cells have the capacity to replenish mRNAs as well as the various translational components. Messenger RNAs would be rate limiting if they were synthesized at a lower rate than the rate of replenishment for the translational capacity.

Our goal in these studies was to determine how β-globin synthesis responds in marrow erythroid cells when α-globin incorporation is specifically inhibited. Experiments comparing this relationship to the interaction we previously found in reticulocytes provided two intriguing observations concerning nonanemic rabbit erythrocytes and rabbit reticulocytes. First, that even after 6 days storage at 4°C, rabbit erythrocytes still have noteworthy incorporation of amino acids into globin chains, and second, that nonincorporating erythrocytes can provide a diffusible substance to reticulocytes that is required for stimulation of β-globin synthesis when α synthesis is reduced by L-o-methylthreonine. The characterization of this substance was not the goal of this set of studies and its nature, mode of action, and relation to a diffusible substance that similarly affects β-globin synthesis in the Ferrara form of β-thalassemia remain unexplained.

Our data indicate that the synthesis of β-chains is independent of the availability of complementary α-chains in bone marrow cells during short-term incubations. The data also suggest that bone marrow cells have mechanisms that reduce the importance of competition for limited translational components as a means of determining the rate of synthesis of each globin chain type.
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