Regulation of Hemoglobin Synthesis

EQUAl RATES OF TRANSLATION AND TERMINATION OF $\alpha$- AND $\beta$-GLOBIN CHAINS*

(Received for publication, January 3, 1972)

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SUMMARY

We find no difference in the rate of translation of $\alpha$- and $\beta$-globin mRNA in cell-free extracts of rabbit reticulocytes (about 200 s per chain at 25°) nor in the rate of release of completed globin chains from polyribosomes (about 15 s per chain). In these reactions, as well as incubation of intact reticulocytes, each $\beta$-globin mRNA contains 30 to 40% more ribosomes than each $\alpha$-mRNA. These results imply that each $\beta$-mRNA initiates synthesis of about 40% more $\beta$-globin than each $\alpha$-mRNA initiates synthesis of $\alpha$ chains. Hence, reticulocytes must contain 30 to 40% more $\alpha$-mRNA than $\beta$ in order to synthesize equal numbers of the two hemoglobin chains. This conclusion is consistent with results of a previous indirect measure of the relative amounts of $\alpha$- and $\beta$-globin mRNA (Lodish, H. F. (1971) J. Biol. Chem. 246, 7131).

Rabbit reticulocytes are an important system for studying regulation of protein synthesis at the levels after fabrication of messenger RNA. These cells have no nucleus, and make no DNA or RNA. By contrast, reticulocytes synthesize large amounts of hemoglobin and, more importantly, they are regulated so as to make essentially equal amounts of $\alpha$ and $\beta$ chains (1-3).

The recent findings of Hunt et al. suggest that the regulation of synthesis of the two globin chains is complex; they observed that, in intact cells, $\beta$ chains are made on polyribosomes which contain 30 to 40% more ribosomes than do those synthesizing $\alpha$ chains (4). We have confirmed this result for intact human (5) and rabbit reticulocytes (see Fig. 5) and for rabbit bone marrow; and also for crude lysates from rabbit reticulocytes which synthesize hemoglobin at a linear rate for long periods of time (6). These results mean that each $\beta$-globin mRNA is associated in polysomes containing 30 to 40% more ribosomes than bound to each $\alpha$-mRNA.

* This research was supported by Grant AI-08814 from the National Institutes of Health.
† A recipient of a Research Career Development Award 1-K4-GM-50, 176-01 from National Institutes of Health.

We recently published experiments, utilizing translation inhibitors, which indicated that this difference was due solely to differences in the rate of initiation of globin chains: each $\beta$-mRNA initiates protein synthesis about 40% more efficiently than each $\alpha$-mRNA. Since each $\alpha$-mRNA would synthesize more $\beta$ chain than each $\alpha$-mRNA would make $\alpha$, the cell would have to have about 40% more $\alpha$-mRNA than $\beta$. A corollary of this work is that the rate of translation of the two chains be the same (6).

By contrast, however, Hunt et al. (7) with the method of Knopf and Lamirou (8) to measure the rate of translation concluded that $\alpha$ chains are translated 30 to 70% faster than $\beta$. They noted that this difference in translation rate is sufficient to account for the difference in polysome size and suggested that the rate and mechanism of initiation is the same for the two chains.

In this paper we use a different method to measure the translation rates in crude lysates of rabbit reticulocytes. Under conditions where $\beta$ chains are made on 40% larger polysomes than $\alpha$, we find no differences in the rate of elongation of the two chains. Further, we find that the time required for release of the completed proteins from polysomes is the same for both chains (10 to 15 s at 25°). For reasons discussed later in detail, our method makes fewer assumptions and is more accurate than that of Hunt et al. (7).

These results support our previous contention that there is a difference in rates of initiation of the two globin chains and, hence, that reticulocytes contain more $\alpha$-globin mRNA (6).

MATERIALS AND METHODS

Reagents—Cycloheximide was purchased from Nutritional Biochemicals and $[35]S$-methionine from Amersham-Searle Corp. All other radioactive amino acids were purchased from New England Nuclear Corp., and used at specific activities indicated in the individual experiments. Creatine phosphate and creatine phosphokinase were obtained from Calbiochem; trypsin treated with L-1-tosylamido-2-phenylethylchloromethylketone (TPCK) was purchased from Worthington.

Cell-free Protein Synthesis—Preparation of reticulocytes and their lysates, and conditions for cell-free protein synthesis have been detailed previously (6, 9, 10). Each extract was tested in detail for the optimum concentration of magnesium acetate (1.5 mM for all lysates used here) and for hemin (10 mg per ml for all lysates). Reactions generally contained 19 nonradioactive
FIG. 1. Autoradiogram of tryptic digest of [14C]tyrosine-labeled rabbit α- and β-globin chains. Globin was prepared from a 0.5-ml cell-free reaction labeled with [14C]tyrosine; [14C]tyrosine-labeled α and β chains were separated by chromatography on carboxymethylcellulose with a pyridine-formic acid gradient (6, 12). Fractions containing α and β chains, respectively, were pooled, lyophilized, resuspended in water, and then digested with trypsin as described under "Materials and Methods." Duplicate samples of each digest, freed of salt by repeated lyophilization, were subjected to paper ionophoresis at pH 6.5 (pyridine-acetic acid buffer) on Whatman No. 3MM paper for 3 hours at 40 volts per cm. Autoradiography of the dried paper was for 6 days with Kodak Royal Blue x-ray film.

Preparation of Protein for Analysis—The method for measuring translation rate depends on accurate measure of the rate of increases in 3H radioactivity in particular tryptic peptides in both released and total reaction products. This can be measured easily by use of an internal standard of [14C]- or [35S]-labeled globin. These uniformly labeled proteins were prepared by incubating a cell-free reaction, containing [14C]tyrosine or [35S]methionine, for 40 min at 25°; over 98% of the radioactivity incorporated into polypeptides is found in protein (globin) released from the ribosomes.2

Aliquots of 400 μl were taken from reactions containing [3H]-tyrosine or [3H]methionine, at times indicated in the individual experiments, into a tube containing 2.0 ml of ice-cold Buffer B (0.01 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.0; 0.06 M KCl, 0.0015 M magnesium acetate, 50 μg of cycloheximide); incorporation of radioactive amino acids is stopped instantly. To each of these reactions was added the same volume of a 40-min reaction, containing [14C]tyrosine or [35S]methionine globin; the amount of reaction added was calculated to give a ratio of [3H]- to [14C]tyrosine or [3H]- or [35S]-methionine of about 6:12 at the midpoint of the incorporation.

Half of each time sample was used immediately to prepare globin (total reaction); half was layered on top of 4 ml of 20% sucrose (in Buffer B) and centrifuged 3 hours at 4° and 45,000 rpm in the Spinco SW 50.1 or SW 65 Beckman rotor to pellet ribosomes. The red top quarter of the tube was aspirated and from it globin was immediately prepared (released chains).

To prepare globin (11) the sample was added dropwise with rapid stirring to 25 ml of acid acetone (0.06 M HCl in acetone) at -20°; after 10 min sitting at 0° the globin precipitate was re-
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T3H) TYROSINE PEPTIDES

A

Fig. 3. Specific activity of [3H]tyrosine tryptic peptides. A, peptides αT4 and βT4: ○, αT4 in total reaction product; Δ, βT4 in total reaction product; ●, αT4 in released globin; ▲, βT4 in released globin. B, peptides αT15 and βT15: ○, αT15 in total reaction product; Δ, βT15 in total reaction product; ●, αT15 in released globin; ▲, βT15 in released globin. The specific activities of the above peptides are plotted against time. Table I shows the calculation of translation times for each chain with the procedure outlined in Fig. 1. The translation rates (seconds per amino acid residue) are 1.49 for the α chain (obtained from αT4) and 1.48 for the β (obtained from βT4). Table IV shows the calculation of the time required to release a complete globin chain from the ribosome.

Peptides αT15 and βT15 were usually resolved from each other by the initial pH 6.5 ionophoresis. When they were not, the region of paper corresponding to both bands was excised and sewn onto a new strip of paper, and subjected to ionophoresis at pH 6.5 for twice the time of the first separation; this procedure always resolved the two peptides. Each αT15 and βT15 peptide was also purified by descending paper chromatography in the solvent 1-butanol-acetic acid-pyridine-water (30:6:20:24).

In all cases peptides were identified by radioautography, which measures the 14C radioactivity common to all peptides in an experiment. Peptide βT15 generally was not studied. Identification of the peptides was made by (a) the ionophoretic mobility of [14C]tyrosine peptides isolated from purified α and β chains expected from their amino acid composition (13, 14). (For instance, αT4 has a negative charge at pH 6.5 and a positive charge at pH 3.5, as expected.) (b) a partial amino acid composition; labeling of purified peptides with reactions containing [14C]lysine or [14C]arginine or [14C]isoleucine was as expected from the peptide amino acid composition (13, 14).

Separation of Methionine Tryptic Peptides—Rabbit α and β chains each yield one tryptic peptide which contains methionine, and these are completely resolved by paper ionophoresis at pH 6.5 as described in Fig. 2; all six tyrosine-containing peptides were generally completely resolved from each other. The region of paper from the different samples corresponding to Peptide αT4 was excised, sewn onto a new sheet of paper, and subjected to paper ionophoresis at pH 3.5. Over 95% of the radioactivity again migrated in a single band, and this was considered to be radiochemically pure αT4 peptide. Similarly, Peptides αT6 and βT6 were repurified by paper ionophoresis at pH 1.9 and 3.5, respectively. Peptides αT15 and βT15 were usually resolved from each other by the initial pH 6.5 ionophoresis. When they were not, the region of paper corresponding to both bands was excised and sewn onto a new strip of paper, and subjected to ionophoresis at pH 6.5 for twice the time of the first separation; this procedure always resolved the two peptides. Each αT15 and βT15 peptide was also purified by descending paper chromatography in the solvent 1-butanol-acetic acid-pyridine-water (30:6:20:24).

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Separation of Methionine Tryptic Peptides—Rabbit α and β chains each yield one tryptic peptide which contains methionine, and these are completely resolved by paper ionophoresis at pH...
Data taken from Fig. 3A, and calculated with only the data points at 6.5, 7.0, 7.5, and 8.0 min. The slopes of the lines αT4 (total) and βT4 (total) were not significantly different if all data points are used. x and y represent, respectively, the horizontal and vertical coordinates of Fig. 3. This method of presenting data shows that of Hunt et al. (7). Note that in the calculation of translation rate the time for release of completed chains (Table IV) is neglected.

### Table I

**Translation rate determined with tyrosine peptides αT4 and βT4**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>With tyrosine peptides</th>
<th>With methionine peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>αT4</td>
<td>βT4</td>
<td>αT4</td>
</tr>
<tr>
<td>Mean of y,  y</td>
<td>13.75 5.52</td>
<td>13.51 5.74</td>
</tr>
<tr>
<td>Mean of x,  x</td>
<td>7.25 7.25</td>
<td>7.25 7.25</td>
</tr>
<tr>
<td>Gradient m (min⁻¹)</td>
<td>2.05 2.07</td>
<td>2.02 2.03</td>
</tr>
<tr>
<td>Mean of m,  m</td>
<td>3.01</td>
<td>2.97</td>
</tr>
<tr>
<td>Vertical distance Δg</td>
<td>8.23</td>
<td>7.77</td>
</tr>
<tr>
<td>Δz in seconds = Δg/μ</td>
<td>164 (αT4 to COOH terminus)</td>
<td>157 (βT4 to COOH terminus)</td>
</tr>
</tbody>
</table>

### Table II

**Translation rates from experiments with [14C]tyrosine and [3H]methionine**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>αT4</th>
<th>βT4</th>
<th>αT5</th>
<th>βT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of y,  y</td>
<td>4.26</td>
<td>3.06</td>
<td>4.66</td>
<td>3.59</td>
</tr>
<tr>
<td>Mean of x,  x</td>
<td>10.25</td>
<td>10.25</td>
<td>10.25</td>
<td>10.25</td>
</tr>
<tr>
<td>Gradient m (min⁻¹)</td>
<td>0.467</td>
<td>0.507</td>
<td>0.514</td>
<td>0.543</td>
</tr>
<tr>
<td>Mean of m,  m (min⁻¹)</td>
<td>0.488</td>
<td>1.20</td>
<td>0.529</td>
<td>1.07</td>
</tr>
<tr>
<td>Vertical distance Δg</td>
<td>149 (αT5 to COOH terminus)</td>
<td>122 (βT5 to COOH terminus)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table III

**Translation rate determined with methionine peptides αT5 and βT5**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>αT5</th>
<th>βT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of y,  y</td>
<td>1.47</td>
<td>1.41</td>
</tr>
<tr>
<td>Mean of x,  x</td>
<td>207</td>
<td>206</td>
</tr>
</tbody>
</table>

3.5 (6). Peptide αT5 was repurified by ionophoresis at pH 1.9, and βT5 by ionophoresis at pH 6.5.

**Counting of Peptides from Paper**—Regions of paper corresponding to the purified peptides were placed on the bottom of glass scintillation vials; sodium hydroxide (2 ml of 0.1 M) was added and the samples were shaken gently for 1 hour at 37°C. Acetic acid (1.8 ml of 0.3 M) and then 12 ml of Aquasol (New England Nuclear Corp.) were added, and 1H and 14C or 35S radioactivity were counted in a two-channel Beckman LS-233 or LS-230 scintillation counter.

### Results

**Measurement of Elongation and Termination Rate**—When a radioactive amino acid is added to a cell or cell-free system synthesizing globin, radioactivity appears first in the nascent chain, then, only after a lag, in released protein. The radioactive label will not appear in an NH₂-terminal amino acid in completed, released globin chains until a ribosome has traversed the mRNA from the NH₂- to COOH-terminal end. The translation time for a mRNA, strictly, the elongation plus termination time, is taken as the time interval required for the specific activity of the NH₂-terminal residue in released protein to reach the specific activity that the same residue had in the total (nascent plus released) protein at the beginning of the time interval (Fig. 2). If, instead, one measures the COOH-terminal residue one obtains the time required for release of the completed globin chain from the ribosome.

As the rate of ribosome movement down the globin mRNA's is apparently uniform (15), it is not necessary to use the most NH₂-terminal peptides for this type of analysis. In separate determinations, we have used both tyrosine and methionine containing tryptic peptides. We used the tyrosine tryptic Peptides αT4, αT6, and βT4 which are, respectively, 110, 90, and 106 residues from the COOH termini of the respective chains and the methionine-containing Peptides αT5 and βT5 (101 residues from the α-COOH terminus and 87 residues from the β-COOH terminus, respectively). In this type of experiment the “position” of the radioactive tyrosine or methionine is considered to be the position of the lysine or arginine at the COOH terminus of the relevant tryptic peptide; when analyzing the nascent chains we will recognize radioactivity in a particular residue only if the chain is extended to the point where the nascent chains, when digested, would yield the radioactive residue in the appropriate authentic peptide. To study the rate of chain termination, we utilize the COOH-terminal Peptides αT5 and βT5, both of which contain tyrosine.

As there are only 3 tyrosine and 1 methionine residue per chain, the separation of the labeled peptides is simplified and contamination by nonradioactive peptides will not interfere with the results.
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Termination rate determined with tyrosine peptides αT15 and βT15 (calculated from data shown in Fig. 3B)

The mean of μ, μ, is different for the αT15 and βT15 samples since in this experiment (Fig. 3B) the 54-min samples for the latter peptides were lost.

<table>
<thead>
<tr>
<th></th>
<th>αT15</th>
<th>βT15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Released</td>
<td>Total</td>
</tr>
<tr>
<td>product</td>
<td>chains</td>
<td>product</td>
</tr>
<tr>
<td>Mean of μ, μ</td>
<td>7.28</td>
<td>5.75</td>
</tr>
<tr>
<td>Gradient m (min⁻¹)</td>
<td>2.96</td>
<td>2.95</td>
</tr>
<tr>
<td>Mean of m, μ</td>
<td>2.02</td>
<td>0.73</td>
</tr>
<tr>
<td>Vertical distance AG</td>
<td>0.73</td>
<td>0.75</td>
</tr>
<tr>
<td>Δx = Δy/m in seconds (time for release of completed chains)</td>
<td>15.0</td>
<td>15.1</td>
</tr>
</tbody>
</table>

(Table II, Line 2). A repeat of the experiment with a different lysate also gave essentially the same rate of translation of the two chains.

Rate of Chain Termination—The tyrosine-containing carboxyl-terminal Peptides αT15 (tyrosylarginine) and βT15 (tyrosylhistidine) were utilized in the same type of experiment to determine the rate of release from the ribosomes of completed globin chains (Fig. 3B). For each chain the plots of specific activity of the peptides in total and released product, versus time, yield parallel straight lines, the horizontal displacement of the two curves is the time required to release the completed globin chain containing the COOH-terminal arginine or histidine residue from the ribosome. Table IV, summarizing the data from Fig. 3B, shows that the rate of termination of the two chains is the same. This time required for release is about 15 s at 25°C. In a repeat of the experiment (same reaction as in Experiment 3, Table II) the termination time was 9.0 s for α and 10.3 s for β globin. Thus, the termination time represents about one-twentieth of the time required to translate a complete globin chain (about 300 s at 25°C) in our lysates. Hence, one would expect that, of the nascent globin chains on polysomes, 5% would be completed; this estimate agrees with the direct measurement by Hunt et al. (15) and by ourselves.²

Absolute Rate of Chain Elongation—As noted above, the calculation of translation rates in Tables I to III neglects the time required to release the completed globin chain from the polyribosomes. If one subtracts the time required for release of completed α and β chains (15 s, Table IV) from the time required to translate from αT4 or βT4 to the end of the chain and release the completed chain (164 s for α, 157 s for β) determined in the same experiment, this yields a more exact measure of the time required to add a single amino acid residue to the growing chain: 1.35 s for α and 1.34 s for β.

Size of Polysomes Synthesizing α and β Chains—Since all of our experiments were done in cell-free systems, it was essential to confirm that β chains were indeed synthesized on larger polysomes than α. All of our extracts satisfied this; a typical example is shown in Fig. 5A. β Chains are made predominantly on clusters of five ribosomes, while α are made on polysomes containing three to four ribosomes. Fig. 5B shows that essentially the same result was obtained by incubation of intact reticulocytes.
DISCUSSION

Use of Cell-free Systems—All of our experiments on the measurement of translation rate utilized crude lysates of rabbit reticulocytes. This was done for several reasons: (a) to eliminate problems caused by pools in the cells of nonradioactive amino acids and to eliminate reduction of the specific activities of the radioactive amino acids by nonradioactive amino acids in the medium; (b) to permit many control experiments to be done with the same frozen extract from the same reticulocytes; (c) to provide a direct comparison with our previous work on effects of translation inhibitors on cell-free globin synthesis (6). For several reasons we believe our results are applicable to whole intact reticulocytes. (a) our lysates, supplemented with hemin, synthesize protein at a linear rate for at least 20 min at 25°. (b) the lysates, as do the intact cells, synthesize equal amounts of α and β chains (6). (c) the time to translate a globin chain, about 200 s at 25°, is within a factor of 2 that determined by previous workers with intact reticulocytes (7, 8). (d) the lysates synthesize β chains on polysomes which are significantly larger than those producing α chains, exactly as do the intact cells (Fig. 5).
All of our experiments were conducted at 25°; the rate of protein synthesis at 25° is about one-sixth that at 37°, so measurements of elongation and release rates are far more accurate at the lower temperature. That the use of this temperature, in itself, should not affect our conclusions is supported by two unpublished experiments: (a) at both temperatures cell-free extracts (and whole cells) synthesize equal amounts of both globin chains. (b) results virtually identical with those of Fig. 5, A and B, were obtained after incubation at 37°. This latter result implies that, in going from 37–25° elongation and initiation of protein synthesis are slowed proportionately.

Validity of Our Method for Determining Elongation and Termination Rates—In our study of termination rates, we measured directly the time required to release a completed α- or β-globin chain from the ribosome (Fig. 3B). By contrast, the elongation rate is calculated from the time required for a ribosome to synthesize from residues 31 (Peptide αT4) or 56 (Peptide αT6) to the carboxyl terminus of the α chain, or from residue 40 (Peptide βT4) to the carboxyl terminus of the β chain, and to release the completed chain from the ribosome. In order to calculate the elongation rate of the entire chain from these data, it is necessary to assume that the rate of peptide synthesis is uniform along the chain or, equivalently, that the distribution of ribosomes along the mRNA is uniform. The results of Hunt et al. (15) and Luppis et al. (16) strongly indicate that this is the case. However, neither of these experiments nor our own eliminate the very unlikely possibility that there be a rate-limiting step in synthesis of one of the chains after the initiation step but before the position corresponding to the carboxyl end of the first tryptic peptide (for instance, the incorporation of the third amino acid).

Regulation of Globin Synthesis—In our system the time required to synthesize a complete polypeptide chain (Tables II and III) and the time needed to release the complete chain from the ribosome (Table IV) is the same for α- and β-globin chains. Nonetheless β-mRNA contains approximately 30% more ribosomes than α-mRNA (Fig. 5, A and B). The β-globin chain is only slightly larger than the α (146 residues to 141); this, by itself, would affect the relative sizes of the two polyribosomes by less than 4%. Hence our results imply that each β-mRNA initiates protein synthesis at a 30% greater rate than each α-mRNA; hence, over a long period of time each β-mRNA would produce about 30% more globin chains than would each α-mRNA. Since these extracts synthesize equal amounts of the two chains (Table III of Reference 6), such a result implies, as discussed in the introduction, that the cells must contain about 30% more functional α-mRNA.

This finding is in agreement with our earlier indirect measurement of the relative amounts of functional α- and β-mRNA, in the reticulocyte.

These experiments (6) utilized seven antibiotics which, at concentrations which inhibit protein synthesis in reticulocyte extracts 60 to 80%, all reduced the rate of propagation of nascent globin chains, but did not directly inhibit initiation of new chains. This resulted in accumulation of large polyribosomes, in which the mRNA molecules were saturated with ribosomes. Under these conditions protein synthesis was not limited by chain initiation and the relative amounts of the two globin chains produced should be proportional to the relative amount of the mRNA; indeed we found that all drugs induced a change in the ratio of α- to β-globin synthesized from the normal 1.0 to about 1.4.

Also, we showed that in the presence of any of the seven translation inhibitors, both classes of globin chains were made on the same classes of very large polyribosomes, and that these ribosomes contained relatively more nascent α chains than β.

Comparison with Previous Work—The present results differ markedly from those of Hunt et al. (7) who, with the method of Knopf and Lamfrom (8) to measure translation rates, concluded that the α chain was translated up to 70% faster than the β chain. They compared the rate of labeling in the released globin of two peptides per chain, one NH2-terminal, the other COOH-terminal. The translation time of a mRNA was taken as the time interval required for the specific activity of the NH2-terminal amino acid (in released chains) to reach the specific activity that the COOH-terminal amino acid had at the beginning of the time interval. For such an analysis to be valid it is essential that, following addition of radioactive amino acid, the rate of labeling of these two peptides in the nascent chains (or equivalently in the total product: nascent plus released chains) be identical. Their experiments contained no such controls. In our system using cell-free synthesis this requirement is clearly met: comparing Fig. 3, A and B, it is apparent that whereas, in the total reaction product, Peptides αT4, βT4, and βT15 are labeled at essentially the same rate, an additional 15 to 20 s is required for Peptide αT15 to reach an equivalent specific activity. This same result was obtained also with the two other extracts studied (data not shown). Likewise, there is reproducibly a different rate of labeling of the two methionine-containing Peptides αT5 and βT5 in the total reaction product (Fig. 4). Such results are certainly unexpected. They could easily be explained however, if, for instance, a different tRNA Tyr was required to transfer tyrosine into the αT5 peptide than into the others. Since the lysates initially contain nonradioactive aminocetyl-tRNA, it might take different periods of time for the different tRNA Tyr's to transfer their (nonradioactive) tyrosine into protein and be recharged with the radioactive tyrosine.

In fact, we can obtain a result similar to Hunt et al. (7), apparent faster "translation" of α chains, if we perform their type of calculation, comparing in released chains Peptides αT4, αT15,
\(\beta T_4\), and \(\beta T_{15}\) (Table V). We emphasize again, however, that one of the critical assumptions for this type of calculation is simply not valid in our system, and that such an apparent difference is a consequence only of the abnormal labeling pattern of Peptide \(\alpha T_{15}\). Note, though, that the time required for translation of the entire \(\alpha\) chain is considerably less, using this type of calculation than obtained with the procedure outlined in Fig. 2 and Tables I and IV. A marked difference between the labeling of Peptides \(\alpha T_{15}\) and \(\beta T_{15}\) (in released globin chains) is also apparent in the studies of Hunt et al. (7) on intact reticulocytes.

Acknowledgments—We acknowledge again the many useful discussions with Doctors D. Nathan, D. Housman, and S. Penman.

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