(Garrow, 1968) probably heals as well as one whose labile protein pool overflows with an intake of more than 100 g/day. However, we must agree with Clark (1967) that there is no evidence as yet to show whether moderate protein depletion affects the convalescence of a patient.

I am most grateful to Sisters Brett, Lacey, More and Murphy for their help in assessing the rate of wound healing.

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The control of haemoglobin synthesis: factors controlling the output of α and β chains

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Analysis of the effects of amino acid starvation in reticulocytes is comparatively simple compared with similar analysis in other tissues of whole organisms. This is

mainly because of the absence of RNA synthesis in reticulocytes, but also because the bulk of the protein being synthesized is haemoglobin, a protein whose structure is completely known. The absence of RNA synthesis eliminates complications that would otherwise arise through RNA-mediated control mechanisms which in turn might mask the effects of amino acid starvation on the protein synthetic machinery in the cells (Munro, 1969). Consequently reticulocytes have been used to study the effect of amino acid starvation on the actual process of protein synthesis and assembly.

The existence of polysomes, structures containing several ribosomes moving in sequence along the same messenger RNA, is a consequence of the fact that it takes a ribosome longer to move down the mRNA than the time between successive additions of ribosomes to the mRNA. If the rate of addition of amino acids to the growing polypeptide chain is constant then the ribosomes will, on average, be evenly distributed on the mRNA (Fig. 1a). On the other hand, if there exists a single point at which the time taken for the addition of the next amino acid is unduly long then a queue of ribosomes will form behind this point (Fig. 1c, d). Hori, Fisher & Rabinovitz (1967) have measured the sucrose density gradient profiles of polysomes from reticulocytes starved of particular amino acids and their findings have been interpreted in the following manner. In the cases where there is little or no change in the profile, it has been suggested that there are sufficient points of retarded synthesis to permit the number of ribosomes on each mRNA molecule to remain approximately the same (Fig. 1b). The breakdown of polysomes accompanying tryptophan starvation is thought to be due to the fact that the tryptophans in the α and β chains of rabbit globin occur within the thirty-five most N-terminal amino acids (Hori *et al.* 1967). This leads to a situation of the type shown in Fig. 1c. In a similar way inhibition of incorporation of isoleucine by *O*-methyl threonine leads to the formation of abnormally large polysomes carrying nascent β chains (Hori & Rabinovitz, 1968; Kazazian & Freedman, 1968). This is thought to be due to a distribution of ribosomes like that shown in Fig. 1d, since isoleucine occurs only near the C-terminal end of the β chain.

It is possible to measure directly the distribution of ribosomes on the mRNA. This is achieved by estimating the relative amounts of the tryptic peptides derived from the nascent chains on the ribosomes. In practice this involves measuring the specific activity of the tryptic peptides prepared from cells labelled with a radioactive amino acid (Hunt, Hunter & Munro, 1968*a*). Cells were incubated under the conditions of Lingrel & Borsook (1963), who used amino acid concentrations two to five times serum levels, and under these conditions the distribution of ribosomes on the mRNA appears to be random. When the cells were incubated in a medium lacking tryptophan, the distribution of ribosomes along the mRNA was no longer uniform and showed a bunching of ribosomes towards the beginning of the mRNA. This confirms the hypothesis advanced by Hori *et al.* (1967) to explain the effects on polysome profiles observed during tryptophan starvation. It is important to note that under conditions of adequate supply of amino acids there appears to be no single rate-limiting step in the passage of ribosomes down the mRNA, although such single rate-limiting steps have previously been postulated (Winslow & Ingram,

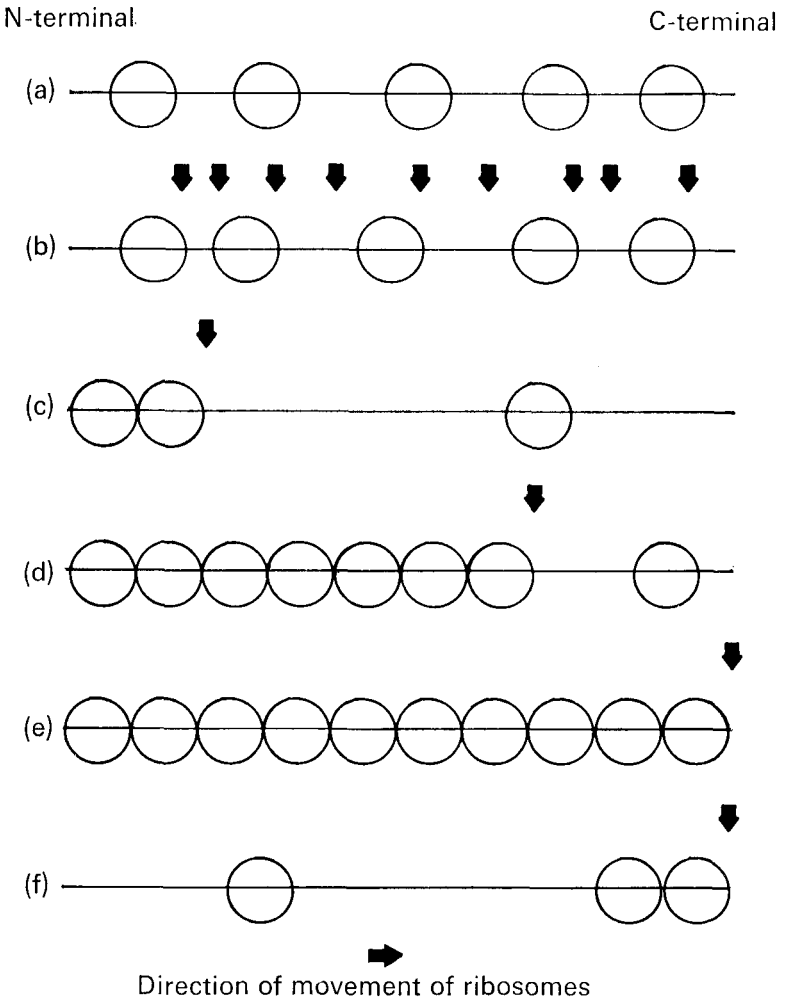

Fig. 1. Diagrammatic representation of possible distributions of ribosomes along messenger RNA.
(a) A random distribution of ribosomes along the mRNA with no rate-limiting steps.
(b) A random distribution brought about by a large number of randomly distributed rate-limiting steps.
(c) The ribosomes are distributed mainly at the N-terminal end of the mRNA, because there is a single rate-limiting step near the beginning of the mRNA.
(d) A single rate-limiting step near the end of the mRNA causes the ribosomes to queue to the beginning of the mRNA. Polysomes of this sort would be larger than average.
(e) A single rate-limiting step in the process of chain termination could cause the ribosomes to queue along the entire length of the mRNA and hence would lead to a random distribution of ribosomes.
(f) In a special case of (e) if the ribosomes did not join the mRNA quickly enough then only a small queue of ribosomes would be formed from the end of the mRNA. Such polysomes would be smaller than those found in (e).

1966; Itano, 1966). A random distribution of ribosomes could be a consequence of complete packing of the mRNA (Fig. 1e) with ribosomes queuing from the termination step of protein synthesis. If this were the case then small polysomes would arise when the mRNA was not completely packed with ribosomes and they would

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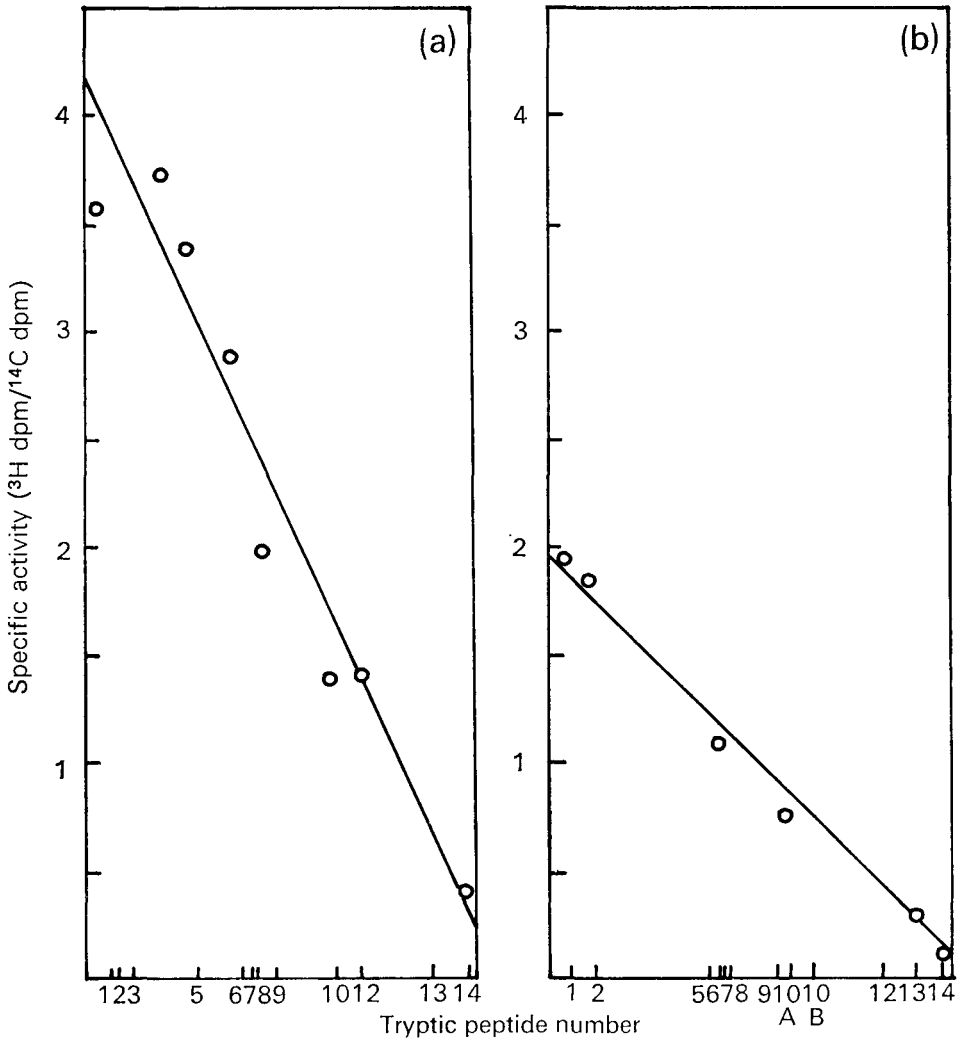

Fig. 2. Specific activity plots from the small polysomes of reticulocytes incubated under normal conditions.

Polysomes were isolated from reticulocytes incubated with [^{3}H]lysine for 20 min at 37° by pH 5 precipitation followed by fractionation on sucrose density gradients (Hunt *et al.* 1968*a*). Polysomes larger than trimers were discarded and the remainder were analysed. The specific activities of the tryptic peptides derived from the nascent chains of these small polysomes were determined as described by Hunt *et al.* (1968*a*), and are plotted against their respective tryptic peptide number for each chain.

	(a) α chain peptides	
slope	−0·027	SD 9%
intercept	4·11	SD 6%
	(b) β chain peptides	
slope	−0·0102	SD 5%
intercept	1·635	SD 4%

The number of ribosomes making a particular chain is proportional to the specific activity of the N-terminal peptide (Hunt *et al.* 1968*a*). Small polysomes therefore contain more ribosomes synthesizing α chains than β chains (Hunt *et al.* 1968*b*). In the same experiment it was shown by sampling the whole population of ribosomes that there are roughly equal numbers of ribosomes synthesizing α and β chains in reticulocytes. The finding of a random distribution of ribosomes on small polysomes rules out the explanation shown in Fig. 1(e),(f) for an overall random distribution of ribosomes in all classes of polysomes.

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have a distribution of ribosomes like that shown in Fig. 1f. This idea is incorrect, as small polysomes show a random ribosome distribution (Fig. 2). It follows that the termination process of protein synthesis does not constitute a rate-limiting step, although control at this point has previously been suggested (Colombo & Baglioni, 1966; Baglioni & Campana, 1967).

The analysis of the nascent chains showed that α and β chains were made on different sizes of polysome. The average number of ribosomes (n) attached to a single mRNA in a particular class of polysome, the average assembly time of the protein made by that class (A), and the average time between successive addition of ribosomes to the mRNA (i) are related by the expression $n=A/i$. By taking advantage of the difference in amino acid composition of each chain, it has been possible to measure the detailed size distribution of polysomes making α and β chains (Hunt, Hunter & Munro, 1968*b*). On the whole β chains are made on larger polysomes than α chains. From the expression cited above, this implies that there is either a difference in the rate of movement of ribosomes down each class of mRNA or a difference in the rate of attachment of ribosomes to the different classes of mRNA molecule.

Analysis of the average rate of movement of the ribosomes down the mRNA shows that the α chain is made faster than the β chain (Hunt, Hunter & Munro, 1969; Table 1). This difference is sufficient to account for the difference in the size of the polysomes making each chain. There are two ways of accounting for the difference in the rates of translation of α and β chains. Either the protein synthetic machinery for the two chains is not completely identical so that synthesis of the two chains can occur at different rates, or else the rate of translation of a mRNA is

Table 1. *The translation times and relative outputs of α and β chains under different conditions*

Incubation conditions	α chain translation time (A^α)	β chain translation time (A^β)	A^β/A^α	N^β/N^α	$Output^\alpha/Output^\beta$
(1) Control	140±26	191±21	1·36	1·1	1·24
(2) Minus threonine	199±22	216±43	1·08	1·2	0·90

The translation times are given in sec ± the number of sec to give the upper and lower 5% fiducial limits. The full details of the experiments used to measure the rates of translation of α and β chains are described elsewhere (Hunt *et al.* 1969). Reticulocytes from the same rabbit were used to obtain the values shown in the table. Cells were incubated under the conditions of Lingrel & Borsook (1963) except that the cells used in line 2 were incubated in medium containing no threonine for 10 min before the start of the experiment and that the incubation temperature in both cases was 20°. The relative number of ribosomes making α and β chains (N^β/N^α) was measured as described by Hunt *et al.* (1968*a*). (A) is defined as the average assembly time and (N) as the total number of ribosomes translating a particular mRNA. Using the experimentally determined values of A and N it is possible to calculate the relative outputs of the two chains (N/A = output/unit time) and these values are quoted in the table.

The difference between the rates of translation of α and β chains in line 1 is considered significantly different as each lies outside the 5% fiducial limits of the other. Similarly the α chain translation times in lines 1 and 2 are considered significantly different, but the difference between the β chain times is not significant.

The table shows that the gross outputs of α and β chains are normally not balanced and an excess of α chains is made. Threonine starvation affects α chain synthesis more than β chain synthesis and consequently alters the ratio of α and β chain gross outputs to such an extent that under these conditions excess β chains are made.

dependent on the nucleotide sequence of that mRNA and consequently can be different for two different types of mRNA molecule. There is no reason to favour the former possibility, while it is easy to envisage molecular mechanisms that would operate to control the rate of translation through the nucleotide sequence. For instance, the supply of a particular charged tRNA molecule could be the rate-limiting factor in the passage of ribosomes down the mRNA. Of course this could not apply to a tRNA used only once or infrequently on the mRNA, as this would lead to an uneven distribution of ribosomes. Although there are marked differences in the amino acid composition of the two chains of globin, the degeneracy of the genetic code makes it impossible to implicate a particular amino acid as the cause of the differential rates of translation of the α and β chains. This line of argument, however, does suggest that differential effects on the rate of translation of the two chains would be achieved by starving cells of amino acids which occur more frequently in one chain than in the other. Consequently we have measured the rate of translation of α and β chains in cells starved of the amino acid threonine, which occurs eleven times in the α chain and four times in the β chain. The overall rate of protein synthesis was decreased by 20% under these conditions. The smallness of the decrease is probably due to a partial supply of the amino acid through protein turnover (Schweiger, Rapoport & Scholzel, 1956). Table 1 shows that the effect on the rate of translation of the α chains is, as predicted, more marked than that on the β chain.

In order to compare the relative production of α and β chains under these and normal conditions, it is important to measure gross output as molecules synthesized per unit time. It is not sufficient to compare the incorporation of radioactivity into globin, as this net synthesis differs from the gross output since it takes into account the turnover of protein in the cell. It is possible to calculate the rate of production of protein chains from the number of ribosomes making the protein at any moment (N) and the average time it takes for ribosomes to translate the mRNA molecules (A): the output per unit time is N/A. In our case it is possible to measure the relative numbers of ribosomes making α and β chains (Hunt *et al.* 1968*a*) and to calculate the corresponding relative rates of production. The results of such a calculation are shown in Table 1. It is apparent that under normal conditions there is an over-production of α chains. This at first sight would seem surprising but is wholly compatible with the finding of a pool of free α chains in these cells (Shaeffer, Trostle & Evans, 1967; Tavill, Grayzel, London, Williams & Vanderhoff, 1968) and the recognized instability of free α chains (Baglioni, Campana & Colombo, 1966; Huehns, Dance, Shooter & Beaven, 1962). When the cells are starved of threonine it not only takes longer to make each α chain but there are fewer α chains made. This finding, if confirmed in cells starved of threonine for long periods of time, suggests that the balance of synthesis of α and β chains is not self-correcting in reticulocytes but is predetermined by the number of mRNA molecules remaining in the cells and the differential rates of translation of these molecules. This type of imbalanced synthesis, caused by amino acid starvation, will occur in other cell types but is probably masked by RNA-mediated control mechanisms working in conjunction with the nucleus.

So far we have discussed situations which affect the process of the passage of ribosomes down the mRNA. Alterations in the rate of addition of ribosomes to the mRNA molecules will also influence the polysome profile and the gross output of protein even if there has been no change in the rate of translation of mRNA. For example, if ribosomes were added at a slower rate, but the rate of translation remained the same, then the number of ribosomes on each mRNA would diminish and there would appear to be a breakdown of polysomes. Since in this case there will be fewer ribosomes making protein, even though each one makes it at the same rate, the amount of protein synthesized will be less. These are the effects observed in reticulocytes starved of haem or iron. Under these conditions it has been shown that there is a decrease of protein synthesis and an apparent breakdown of polysomes (Bruns & London, 1965; Waxman & Rabinovitz, 1966). In preliminary experiments we have shown that iron deficiency does not decrease the rate of movement of ribosomes down the mRNA. This is wholly compatible with iron or haem controlling globin synthesis through the process of addition of ribosomes to mRNA to start a new round of synthesis.

We conclude that the time taken for a ribosome to move down a particular mRNA is affected by the nucleotide sequence of the mRNA and the supply of amino acids. The gross output of protein is a function of the number of synthetic units and the time required to make each chain. Amino acid starvation affects the gross output mainly through alteration of the assembly time. On the other hand, iron deficiency decreases the gross output by reducing the number of synthetic units.

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