Influence of Amino Acid Supply on Ribosomes and Protein Synthesis of Perfused Rat Liver

By L. S. JEFFERSON* AND A. KORNER†

Department of Biochemistry, University of Cambridge, and Department of Physiology, Vanderbilt University, Nashville, Tenn. 37203, U.S.A.

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1. The livers of rats were perfused *in situ* with medium containing mixtures of amino acids in multiples of their concentration in normal rat plasma. The incorporation of labelled amino acid into protein of the liver and of the perfusing medium increased with increasing amino acid concentration. During 60 min. perfusions, labelling of liver protein reached a plateau, and labelling of medium protein was inhibited when the initial concentration of the amino acid mixture was more than ten times the normal plasma value. 2. Examination of polysome profiles derived from livers perfused without amino acids in the medium showed that the number of large aggregates was decreased and the number of small aggregates, particularly monomers and dimers, was increased with time of perfusion. The addition of amino acids to the perfusion medium reversed this polysome shift to an extent that was dependent on the initial concentration of amino acids. Polysome profiles derived from livers perfused for 60 min. with ten times the normal plasma concentration of amino acids were essentially the same as the polysome profiles of normal non-perfused livers. 3. The ability of ribosome preparations from perfused livers to incorporate amino acids into protein *in vitro* decreased with increasing time of perfusion when no amino acids were added to the medium, but increased as the concentration of amino acids in the perfusion medium was increased. 4. The ability of cell sap from perfused livers to support protein synthesis *in vitro* was not influenced by the amino acid concentration of the perfusion medium. 5. Livers were perfused for 60 min. with medium containing amino acid mixtures at ten times the normal plasma concentration but deficient in one amino acid. Maximal incorporation of labelled amino acid into liver protein, the stability of the polysome profile and the ability of ribosome preparations to incorporate amino acids into protein were found to depend on the presence of 11 amino acids: arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine. A mixture of these 11 amino acids, at ten times their normal plasma concentration, stimulated the incorporation of labelled amino acid into liver protein, stabilized the polysome profile and increased the ability of ribosome preparations to incorporate amino acids into protein to the same extent as the complete mixture. 6. It is concluded that the availability of certain amino acids plays an important role in the control of protein synthesis, possibly by stimulating the ability of ribosomes to become, and to remain, attached to messenger RNA.

Jefferson & Korner (1967) noted that, when rat livers were perfused *in situ*, a higher concentration of amino acids than that found in rat plasma enhanced the protein-anabolic effect of growth

hormone, and suggested that one or more amino acids might become limiting during perfusion. Protein biosynthesis is, naturally, dependent on the availability of amino acids (for review see Christensen, 1964) and this is particularly true of liver, an organ in which protein metabolism is very active.

Incorporation of labelled amino acids into protein of liver slices is increased by the presence of elevated

*Present address: Department of Physiology, Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, Pa. 17033, U.S.A.

†Present address: School of Biological Sciences, University of Sussex, Brighton BN1 9QG.

concentrations of single essential amino acids (Simpson & Tarver, 1950; Hanking & Roberts, 1965a), and the amino acid-incorporating activity of ribosomes and pH 5-enzyme preparations isolated from liver slices incubated with a single essential amino acid has been shown to vary with the concentration of that amino acid (Hanking & Roberts, 1965b). Studies in which rats and mice were fed with either a complete amino acid mixture or a mixture with one amino acid omitted (Fleck, Shepherd & Munro, 1965; Wunner, Bell & Munro, 1966; Staehelin, Verney & Sidransky, 1967; Sidransky, Bongiorno, Sarma & Verney, 1967; Wunner, 1967) showed the amino acid-incorporating activity of liver microsomes and the degree of aggregation of liver polysomes to be affected by the supply of a single amino acid. This view is supported by the 'revival' of a liver incorporating system *in vitro* and the resynthesis of polysomes occasioned by an amino acid mixture (Baliga, Pronczuk & Munro, 1968). These reports indicate that protein synthesis by the liver is influenced by the supply of amino acids because of changes in the activity of subcellular components engaged in protein synthesis.

In the present study, we investigated the influence of the supply of each of the amino acids on hepatic protein synthesis in perfused livers of rats, and studied the changes caused by amino acids in polysome profiles and incorporating ability *in vitro* of ribosomes and cell sap of perfused liver.

MATERIALS AND METHODS

Animals. Male rats of the Wistar and Sprague-Dawley strains maintained on Purina laboratory chow and weighing 100–120 g. were kept without food for 18–22 hr. before use.

Chemicals. DL-[1-¹⁴C]Phenylalanine (44.2 mc/m-mole) was obtained from The Radiochemical Centre (Amersham, Bucks.). L-[U-¹⁴C]Phenylalanine (366 mc/m-mole) and L-[U-¹⁴C]lysine (220 mc/m-mole) were obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.). Bovine serum albumin fraction V was purchased from Armour Pharmaceutical Co. Ltd. (Eastbourne, Sussex) and from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.). The disodium salts of ATP and GTP were purchased from the Sigma Chemical Co. (St Louis, Mo., U.S.A.). Solutions of these nucleotides were made up in glass-distilled water and the pH was adjusted to 7.6 with tris-HCl buffer before use. The commercial sucrose used was free of significant ribonuclease activity.

Amino acids purchased from British Drug Houses Ltd. (Poole, Dorset) and Mann Research Laboratories Inc. (New York, N.Y., U.S.A.) were used. All amino acids (L-isomers), except L-tyrosine (which is insoluble at this concentration), were dissolved in 0.9% NaCl at 200 times the normal rat plasma concentration (Scharff & Wool, 1964; Schimassek & Gerok, 1965) for each amino acid, and the pH was adjusted to 7.4 with N-NaOH. A portion of this solution as well as a weighed amount of crystalline L-tyrosine was added to the

perfusion medium to give the desired initial concentration of amino acids. The labelled amino acid, when added, was present in the amino acid solution at a constant specific radioactivity.

Perfusion of livers. Livers were perfused as described by Jefferson & Korner (1967) by the technique of Mortimore (1961). Briefly, this entails anaesthetization of the rat with an intraperitoneal injection of sodium pentobarbital (50 mg./kg. body wt.) followed by perfusion of the liver *in situ*. The perfusion medium was Krebs-Henseleit bicarbonate buffer (Krebs & Henseleit, 1932) containing 3 g. of bovine serum albumin powder and 150 mg. of glucose/100 ml., and sufficient saline-buffer-washed erythrocytes (Exton & Park, 1967) to give a haematocrit of 20%. The initial recirculating volume of medium was 50 ml. and the flow rate was 7.0 ml./min.

At the end of perfusion experiments designed to study the incorporation of a labelled amino acid into protein, the livers were removed and homogenized as described by Jefferson & Korner (1967). Samples of the homogenized liver and samples of medium were analysed for radioactivity incorporated into protein (Jefferson & Korner, 1967). In other experiments livers were removed for the preparation of polysomes and ribosomes. In this case the liver was rapidly removed into ice-cold 20 mM-tris-HCl buffer, pH 7.6, containing 0.25 M-sucrose, 10 mM-magnesium acetate, 40 mM-NaCl, 100 mM-KCl and 6 mM-mercaptoethanol, cut finely, rinsed twice and homogenized in 4 vol. of the same medium as described by Munro, Jackson & Korner (1964).

Preparation of polysomes, ribosomes and cell sap. The liver homogenate was centrifuged for 10 min. at 10000g at 0° to remove mitochondria, nuclei and debris. The postmitochondrial supernatant, treated with Triton X-100 at a final concentration of 1% (Hunter & Korner, 1966), was used for the preparation of polysomes and ribosomes. Polysomes (Wettstein, Staehelin & Noll, 1963) were prepared essentially as described by Munro *et al.* (1964) with the slight modification of a 1.0 M-sucrose step replacing the 0.5 M-sucrose step. Ribosomes were prepared as described by Munro *et al.* (1964). After aspirating of the supernatant fluid and rinsing of the inside of the tubes and the surface of the pellets, the polysomes and ribosomes were stored at -20°. No loss of activity was noted on storage.

Cell sap was prepared by centrifuging the postmitochondrial supernatant for 1.5 hr. at 50000 rev./min. in the Te 50 rotor of the Spinco model L2-50 ultracentrifuge and passing the supernatant through a column of Sephadex G-25 to remove inhibitors of protein synthesis (Munro *et al.* 1964). Unless otherwise noted, cell sap was prepared from the postmitochondrial supernatant obtained from non-perfused livers of normal male rats.

Analysis of polysomes by sucrose-density-gradient centrifugation. Polysome pellets were suspended in 20 mM-tris-HCl buffer, pH 7.6, containing 5 mM-magnesium acetate, 40 mM-NaCl and 100 mM-KCl to give a concentration of approx. 200 E_{260} units/ml. A more uniform suspension could be obtained by incubating the polysomes for 30 min. at 37° (A. R. Hunter & L. S. Jefferson, unpublished work). Portions of suspended polysomes (50 μ l.) were layered on 15–30% (w/v) sucrose density gradients (5 ml.) in the suspension medium and centrifuged at 50000 rev./min. for 30 min. in the SW50 rotor of the Spinco model L2-50 ultracentrifuge. Gradients were prepared and centrifuged at 0°. After centrifugation the E_{260} pattern on the gradient

was recorded automatically with a ratio-recording spectrophotometer (Beckman model DK-2A) with the flow-cell attachment.

Ribosome preparations as an amino acid-incorporating system. Samples of ribosome preparations (0.15 mg. of RNA) were incubated at 37° in a total volume of 1 ml. containing 20 mM-tris-HCl buffer, pH 7.6, 10 mM-magnesium acetate, 40 mM-NaCl, 100 mM-KCl, 6 mM-mercaptoethanol, 5 mM-ATP, 0.1 mM-GTP, 0.5 µC of [¹⁴C]phenylalanine, 1 µM of each of the other L-amino acids, and cell sap (5 mg. of protein). Incubation was stopped by the addition of 5 ml. of 0.5 N-HClO₄ containing unlabelled L-phenylalanine. The resulting precipitate was freed of amino acids, RNA and lipid, and the radioactivity in protein was determined by the procedure of Munro *et al.* (1964). Corrections were made for the radioactivity of the zero-time controls, background counts and self-absorption. Results are reported as counts/min. incorporated into 1 mg. of protein/mg. of particle RNA incubated.

Determination of RNA and protein. RNA was determined by the method of Fleck & Munro (1962) as described by Munro *et al.* (1964). Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Results of experiments designed to determine the optimum supply of amino acids required to support protein synthesis in rat liver during a 60 min. perfusion are shown in Fig. 1. Amino acids were added to the perfusion medium in multiples of their concentration in normal rat plasma and [¹⁴C]phenylalanine was present in each experiment at a constant specific radioactivity. The labelling of liver protein

increased almost linearly as the initial concentration of amino acids in the perfusion medium was raised. Incorporation into liver protein reached a plateau when the amino acid concentration was eight to 12 times the normal plasma value. Also, the labelling of protein released into the medium also increased linearly with increasing initial concentration of amino acids in the perfusion medium. Either the labelling of this fraction was inhibited at concentrations of amino acids higher than ten times the normal plasma value or, alternatively, the release of labelled proteins by the liver was inhibited by high concentrations of amino acids. The optimum initial concentration of amino acids to support protein synthesis by the perfused liver for 60 min. under these conditions is clearly ten times the normal rat plasma value.

Table 1. Influence of deficient amino acid mixtures on the incorporation of labelled amino acid into protein of perfused livers

Livers were perfused for 60 min. with a medium containing all amino acids, except the deficient one, at ten times their normal plasma concentration. The results for each deficient amino acid mixture are expressed as a percentage of the amount of radioactive amino acid incorporated into protein with the complete mixture. The labelled amino acid was [¹⁴C]phenylalanine, except in the phenylalanine-deficient mixture, when [¹⁴C]lysine was used. Group II is a mixture of the following 11 amino acids at ten times their normal plasma concentration: arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine. Each value is the mean of three experiments.

Fig. 1. Influence of various concentrations of amino acids on the incorporation of [¹⁴C]phenylalanine into liver protein (●) and proteins in the medium (○) after 60 min. perfusion with recirculating medium. Each point represents the mean of two experiments. The specific radioactivity of phenylalanine was kept constant.

Amino acid omitted	Radioactivity incorporated into protein (% of that with complete mixture)
Alanine	110.2
Arginine	75.6
Asparagine	56.2
Aspartic acid	97.2
Cysteine	101.6
Glutamic acid	104.4
Glutamine	100.5
Glycine	97.9
Histidine	95.5
Isoleucine	74.1
Leucine	78.2
Lysine	65.6
Methionine	53.8
Phenylalanine	68.0
Proline	64.4
Serine	92.5
Threonine	65.0
Tryptophan	57.0
Tyrosine	108.0
Valine	64.0
Group II amino acids added; others omitted	95.2

To determine which of the amino acids are limiting for protein synthesis in the perfused liver system, experiments were carried out in which the initial concentration of the amino acids was ten times the normal plasma value but in each experiment one amino acid was omitted from the amino acid mixture. In Table 1 the results for each deficient amino acid mixture are expressed as a percentage of the amount of label incorporated with the complete mixture. The labelled amino acid was [^{14}C]phenylalanine, except in the phenylalanine-deficient mixture, when [^{14}C]lysine was used. Perfusion with amino acid mixtures deficient in any one of 11 amino acids (arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine) resulted in a substantial decrease in the incorporation of labelled amino acid into protein. The results in Table 1 also show that addition of a mixture of these 11 amino acids ('group II') at ten times their normal plasma concentration to the perfusion medium resulted in essentially the same amount of labelled amino acid incorporation as that observed with the complete amino acid mixture.

During perfusion, degradation of liver proteins

occurs with consequent dilution of the added radioactive amino acid. It is possible that high concentrations of amino acids in the perfusing medium could diminish the extent of protein degradation, cause less dilution of the radioactive amino acid used in the experiment described in Fig. 1 and lead to the appearance without the reality of increased protein synthesis. Livers were therefore perfused with one or ten times the plasma concentration of amino acids, but to avoid dilution the medium was not recirculated but was passed through the liver only once. The results show that [^{14}C]phenylalanine, at the same specific radioactivity in both cases, was incorporated to a greater extent into protein of livers perfused with the high concentration of amino acids (79.2 ± 0.9 counts/min./mg.) than into protein of livers perfused with a low concentration of amino acids (35.7 ± 2.3 counts/min./mg.) ($P < 0.001$ for these two values).

The mean specific radioactivity of the phenylalanine of the liver and the medium was determined during a 60 min. recirculating-perfusion experiment such as that shown in Fig. 1. These measurements were used to calculate the amount of phenylalanine incorporated into protein. This calculation and the

Fig. 2. Sucrose-density-gradient analysis of polysomes prepared from livers after various times of perfusion with no amino acids added to the medium. Patterns (b), (c) and (d) are profiles obtained after 15, 30 and 60 min. perfusion respectively. The pattern of polysomes from non-perfused livers (a) is shown for comparison. Polysome suspensions were prepared as described in the Materials and Methods section; 12 E_{260} units (0.05 ml.) of each polysome suspension was layered on a 15–30% (w/v) sucrose gradient (5 ml.) containing 20 mM-tris-HCl buffer, pH 7.6, 5 mM-magnesium acetate, 40 mM-NaCl and 100 mM-KCl, and centrifuged at 50000 rev./min. for 30 min. at 0° in the SW50 rotor of the Spinco L2-50 ultracentrifuge. E_{260} was recorded automatically with a ratio-recording spectrophotometer with the flow-cell attachment. The top of the gradient is indicated and the peaks of ultraviolet-absorbing material are marked by the numbers on the abscissa to indicate the number of ribosomes aggregated in polysomes.

results of the non-recirculating experiment described above show that part of the increased radioactive amino acid incorporation obtained with high concentrations of perfusing amino acids (Fig. 1) can be attributed to diminished dilution of the radioactive amino acid, but part is the result of increased protein synthesis.

It is possible that the effect on protein synthesis of high concentrations of amino acids might have occurred by alterations in the subcellular components engaged in protein synthesis. We therefore investigated the effect of amino acid supply on the polysome profile and amino acid-incorporating activity of ribosome preparations from perfused liver.

Fig. 2 illustrates the sucrose-density-gradient pattern of polysomes prepared from livers after various times of perfusion when no amino acids were added to the medium. Fig. 2(a) shows a typical pattern of polysomes derived from non-perfused livers in which the major portion of the ultraviolet-absorbing material was found in the

region of the larger ribosomal aggregates. Figs. 2(b)–2(d) show that, with increasing time of perfusion, there was progressive breakdown of the polysomes. This shift in ribosome distribution, the loss of large ribosomal aggregates and the gain of monomers and dimers, was observed within 15 min. of perfusion. After 30 min. of perfusion little further change was observed. The area under the curve of the polysome tracings was measured with a polar planimeter and the distribution of ribosomal aggregates calculated. The amount of ultraviolet-absorbing material in the combined monomer and dimer fractions increased from 18% of the total polysome profile in the non-perfused livers to 40%, 52% and 56% of the total after 15, 30 and 60 min. of perfusion respectively.

Ribosomes were prepared from livers perfused for various times and their incorporating ability was analysed under conditions giving optimum amino acid incorporation into protein *in vitro* (Munro *et al.* 1964). The capacity of the ribosome preparations to incorporate [14 C]phenylalanine into protein *in vitro* (Fig. 3) decreased with increasing time of perfusion of livers from which the ribosomes were derived. The ability of ribosome preparations from non-perfused livers to incorporate amino acids into protein is shown for comparison. The change in the ability of ribosome preparations to synthesize protein occurred mainly during the first 30 min. of perfusion, with little further change up to 60 min.

The changes in polysome profiles (Fig. 2) and amino acid-incorporating activity of ribosome preparations (Fig. 3) from livers after various times of perfusion might reflect changes resulting from amino acid deficiency. To determine if amino acid supply could influence these changes, livers were perfused for 60 min. with various concentrations of amino acids. The polysome profiles (Fig. 4) show that the shift from heavy aggregates toward monomers and dimers was reversed by supplying amino acids. The extent of the breakdown of the heavier aggregates to smaller aggregates is dependent on the initial concentration of amino acids in the perfusing medium. Indeed, with ten times the normal plasma concentration of amino acids present initially, the polysome profiles from livers perfused for 60 min. are indistinguishable from those from non-perfused livers (compare Figs. 2a and 4e). The combined monomer and dimer fractions accounted for 18% of the total ultraviolet-absorbing material in both instances.

The amino acid-incorporating activity of ribosome preparations from livers perfused for 60 min. also depended on amino acid supply. As shown in Fig. 5, the ability of ribosome preparations to incorporate [14 C]phenylalanine into protein was influenced by the concentration of amino acids initially present in the perfusion medium. The

Fig. 3. Amino acid incorporation into protein by ribosomes prepared from livers perfused with no added amino acids for 15 min. (△), 30 min. (□) or 60 min. (●). The ability of ribosome preparations from non-perfused livers to incorporate amino acids into protein is shown for comparison (○). Ribosome preparations (0.15 mg. of RNA) were incubated at 37° in a total volume of 1 ml. containing 20 mM-tris-HCl buffer, pH 7.6, 10 mM-magnesium acetate, 40 mM-NaCl, 100 mM-KCl, 6 mM-mercaptoethanol, 5 mM-ATP, 0.1 mM-GTP, 0.5 μ C of [14 C]phenylalanine, 1 μ M of each of the other L-amino acids and cell sap (5 mg. of protein). Incubation was stopped by the addition of 5 ml. of 0.5 N-HClO₄ containing unlabelled phenylalanine. Each curve represents the mean of three experiments.

Fig. 4. Sucrose-density-gradient analysis of polysomes prepared from livers after 60 min. of perfusion with medium containing no added amino acids (a), the normal plasma concentration of each amino acid (b), or three times (c), five times (d) and ten times (e) the normal plasma concentration of each amino acid. Details of the gradient are given in the legend of Fig. 2.

capacity of the ribosome to incorporate amino acids into protein *in vitro* increased as the supply of amino acids to the perfused liver was increased.

The ability of cell sap from perfused livers to support protein synthesis *in vitro* was not influenced by the amino acid supply. The results in Table 2 show that cell sap from livers perfused for 60 min. with no amino acids and with five times the normal plasma concentration did not differ in their ability to support incorporation of [^{14}C]phenylalanine into protein when incubated with ribosomes *in vitro*. The effect of amino acid supply was apparent only on the ribosome preparations.

We investigated the effects of deficiency in a single amino acid on the pattern of polysomes and the amino acid-incorporating capacity of ribosome preparations derived from livers perfused for 60 min. with all amino acids, except one, present initially at ten times their normal plasma concentration. The polysome profiles from livers perfused with deficient amino acid mixtures, each lacking in one amino acid, followed two patterns (Figs. 6a and 6b). Pattern A (Fig. 6a) resembles polysome profiles

from non-perfused livers or livers perfused with a complete mixture of amino acids (Fig. 4e) and pattern B (Fig. 6b) resembles polysome profiles from livers perfused for 60 min. with no amino acids added to the medium (Fig. 2d). Livers perfused with amino acid mixtures deficient in any one of the following amino acids yielded polysome profiles of the A-type pattern: alanine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, serine and tyrosine (group I); whereas livers perfused with amino acid mixtures deficient in any one of the following amino acids yielded polysomes having profiles of the B-type pattern: arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan or valine (group II). Livers perfused with a mixture of the group II amino acids yielded polysome profiles of the A-type pattern. These results are summarized in Table 3.

It was also found that the amino acid-incorporating ability of ribosome preparations from livers perfused with deficient amino acid mixtures gave two types of results (Fig. 7). Curve A illustrates

the results obtained with ribosome preparations derived from livers perfused with amino acid mixtures deficient in any one of the group I amino acids. There was no apparent difference in the ability of ribosome preparations from these livers to incorporate [¹⁴C]phenylalanine into protein *in vitro*. Curve *B* illustrates the results obtained with ribosome preparations derived from livers perfused with amino acid mixtures deficient in any one of the group II amino acids. The deficiency of any one of these amino acids in the

perfusion medium resulted in the ribosome preparation being less capable of incorporating [¹⁴C]phenylalanine into protein *in vitro*. Livers perfused with a

Fig. 5. Amino acid incorporation into protein by ribosomes prepared from livers after 60 min. of perfusion with medium containing no added amino acids (○), the normal plasma concentration of each amino acid (△), or three times (□), five times (●) and ten times (▲) the normal concentration of amino acids. Details of the incubation conditions are given in the legend of Fig. 3. Each curve represents the mean of three experiments.

Fig. 6. Sucrose-density-gradient analysis of polysomes. (a) Typical sucrose gradient of polysomes prepared from livers after 60 min. of perfusion with medium containing amino acid mixtures (each amino acid present, initially, at ten times its normal plasma concentration) deficient in any one of the following amino acids: alanine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, serine or tyrosine (group I). (b) Typical sucrose gradient of polysomes prepared from livers after 60 min. of perfusion with medium containing amino acid mixtures (each amino acid present, initially, at ten times its normal plasma concentration) deficient in any one of the following amino acids: arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan or valine (group II). Livers perfused with medium containing a mixture of the last 11 amino acids (group II) (present, initially, at ten times their normal plasma concentration) yielded polysomes with a profile similar to that illustrated in (a). Details of the gradient are given in the legend of Fig. 2.

Table 2. Amino acid incorporation into protein by ribosomes and cell sap prepared from perfused livers

Livers were perfused for 60 min. with medium containing either no amino acids (0 × aa) or all amino acids at five times their normal plasma concentration (5 × aa). Details of the incubation conditions are given in the legend of Fig. 3. Each value is the mean of three experiments.

Initial amino acid concentration of perfusing medium of livers yielding component		Time (min.) ...	Radioactivity incorporated into protein (counts/min./mg. of protein/mg. of RNA)		
Component ...			10	20	40
	Ribosomes	Cell sap			
	5 × aa	5 × aa	754	1265	2030
	5 × aa	0 × aa	796	1175	1945
	0 × aa	5 × aa	566	888	1400
	0 × aa	0 × aa	566	866	1290

Fig. 7. Amino acid incorporation into protein by ribosomes prepared from livers perfused for 60 min. with medium containing amino acid mixtures (each amino acid present, initially, at ten times its normal plasma concentration) deficient in any one of the group I amino acids (○, curve A) or deficient in any one of the group II amino acids (●, curve B). The list of group I and group II amino acids are given in the legend of Fig. 6. Details of the incubation conditions are given in the legend of Fig. 3.

mixture of the group II amino acids yielded ribosome preparations that gave results similar to those depicted by curve A in Fig. 7. These results are summarized in Table 3.

DISCUSSION

Taken together our experiments show that, in a perfused rat liver system, the incorporation of a radioactive amino acid into liver protein and into protein released into the perfusing medium, the pattern of distribution of ribosomes into polysomes and the ability of isolated ribosomes to incorporate amino acids into protein *in vitro* all depend on the supply of a sufficiency of 11 amino acids. A concentration of these 11, or a mixture of all amino acids, at ten times the normal plasma concentration seemed to be optimum for the perfused liver system. Higher concentrations caused only a slight increase in the incorporation of amino acids into liver protein and caused an inhibition of labelling of proteins released into the medium. High concentrations of amino acids *in vitro* are reported to inhibit incorporation of radioactive amino acids into diaphragm and reticulocyte protein (Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1952).

Table 3. Summary of the effects of deficient amino acid mixtures on the polysome profiles and protein-synthesizing ability of ribosomes

Ribosomes were prepared from livers perfused for 60 min. with medium containing all amino acids, except the deficient one, at ten times their normal plasma concentration. Details of the experiments are given in legends to Figs. 6 and 7.

Amino acid omitted	Type of polysome profile pattern (Fig. 6)	Type of amino acid-incorporation curve of ribosomes (Fig. 7)
Alanine	A	A
Arginine	B	B
Asparagine	B	B
Aspartic acid	A	A
Cysteine	A	A
Glutamic acid	A	A
Glutamine	A	A
Glycine	A	A
Histidine	A	A
Isoleucine	B	B
Leucine	B	B
Lysine	B	B
Methionine	B	B
Phenylalanine	B	B
Proline	B	B
Serine	A	A
Threonine	B	B
Tryptophan	B	B
Tyrosine	A	A
Valine	B	B
Group II amino acids added; others omitted	A	A

The 11 amino acids that seem to be needed for the perfused liver system are arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine. This is similar to, but not identical with, the list of amino acids essential for rats (Rose, 1938). Asparagine and proline are present in our 11 in place of histidine in the list of essential amino acids. The concentration of these 11 amino acids is low in liver intracellular water (Schimassek & Gerok, 1965), but this is not a characteristic unique to this group of amino acids. Perfused liver is capable of releasing into the medium the complete range of amino acids normally found in plasma without alteration of intracellular concentrations (Schimassek & Gerok, 1965); however, even after 3 hr. of perfusion, the concentration of these 11 amino acids, except for valine, isoleucine and leucine (tryptophan not given), was much lower than that found in normal plasma (Schimassek & Gerok, 1965). It seems likely that the need for high concentrations of amino acids is to prevent degradation of the protein of perfused rat liver (G. E.

Mortimore, personal communication) and to supply those whose concentration is limiting for protein synthesis. The particular 11 required probably reflect limited availability from protein degradation and from synthesis from other amino acids. The list of amino acids needed for optimum protein synthesis may depend on the system studied, so that it is not very surprising that Sidransky & Bongiorno (1967) found, in mice, that amino acid mixtures deficient in threonine, methionine or isoleucine could cause shifts to heavy polysomes in liver, but that a tryptophan-deficient mixture could not.

Our results show a parallel between the state of ribosomal aggregation (the polysome profile) and protein-synthetic ability in liver and in isolated ribosomes. A change in the size of polysomes can occur, broadly, through two different kinds of mechanism: a change in the rate of synthesis or degradation or the size of the messenger RNA, or a change in the number of ribosomes attached to the messages. It seems unlikely that the drastic degradation of polysomes noted when livers were perfused without added amino acids and the rapid reversal of this effect when amino acids were added to the perfusing medium could have occurred by alterations in the rate of synthesis or degradation of messenger RNA, despite the facts that elevation of the amino acid concentration of the perfusing medium stimulates the labelling of RNA with orotic acid (Jefferson & Korner, 1967) and that giving amino acids to rats stimulates RNA synthesis (F. Labrie & A. Korner, unpublished work). Our objections to this mechanism are that any change in rates of messenger RNA synthesis or degradation might result in alterations in the number of polysomes but is unlikely to alter their size distribution (Villa-Trevino, Farber, Staehelin, Wettstein & Noll, 1964), for the types of protein synthesized are not greatly altered by altering the rate of RNA synthesis. We therefore suggest that the change in polysome size brought about by amino acids is because of alterations in the number of ribosomes attached to the messages.

It might be argued that lack of an amino acid could halt the movement of ribosomes along the message because of lack of that particular aminoacyl-transfer-RNA. A queue of ribosomes would form before the codon of the absent amino acid, but those ribosomes beyond it could continue to translate the message, become detached from it and leave the polysomes smaller than before. Such a situation has been shown to occur when reticulocytes are incubated in the absence of tryptophan (Hori, Fisher & Rabinovitz, 1967). However, this explanation would only apply when the deficient amino acid occurs only once or, at most, occasionally in the polypeptide and not too near the C-terminus of it. It was shown with the reticulocyte system that lack

of amino acids occurring often in haemoglobin does not result in polysome breakdown; blocks present at several points on the message would prevent most ribosomes from rolling off it. The mixed population of proteins synthesized in liver would not have compositions such that lack of one amino acid would result in the change in polysome profiles found.

It might be suggested that a general deficiency of amino acids would slow down the whole translation process because ribosomes would have to await the arrival of aminoacyl-transfer-RNA for a longer period than when amino acids are abundant. But slowing down of the translation process without increased rate of detachment of ribosomes would result in an increase and not a decrease in the number of ribosomes attached to the message.

It could be argued that lack of ribosomes on the message could render it more susceptible to cleavage either *in vivo* or during preparation of the polysomes. This would result in the formation of smaller polysomes, some of which would lack a proper terminating codon and others of which would lack a proper initiating codon. The former would maintain their number of ribosomes, which would be unable to be detached from the message, whereas the latter would lose their ribosomes, but would not be able to reinitiate protein synthesis. This view does not resolve the question how lack of amino acids in the perfusing medium could have depleted the polysomes of ribosomes in the first instance. We suggest that amino acids must be able, directly or indirectly, to stimulate the ability of ribosomes to become attached to and to remain attached to the message.

This suggestion allows us to explain our results from incorporation experiments *in vitro*. We found a correlation between the polysome profile and the incorporating ability of ribosomes assayed *in vitro* in the presence of adequate amounts of amino acids. The extent of amino acid incorporation measured *in vitro* is a reflection of the number of ribosomes remaining attached to the message in each of the preparations of polysomes. Since the results are presented as specific radioactivity/mg. of ribosomal RNA present, the specific radioactivity obtained *in vitro* should be independent of the number of ribosomes attached to the messages, but will, however, be influenced by the number of ribosomes present in the preparation that are not attached to the message or that are easily detached from it. We can explain our finding that the specific radioactivities obtained with ribosome preparations *in vitro* are proportional to the number of ribosomes remaining on the message by arguing that there are more free ribosomes in preparations from livers perfused without amino acids and that the free ribosomes *in vitro* (as *in vivo*) are less able to be

attached to the message but more able to be detached from it before completion of the synthesis of the protein.

Other situations are known in which the ability of ribosomes to be attached to, or to translate, a message is altered. Hypophysectomy of the rat decreased, and treatment with growth hormone increased, the ability of liver ribosomes to attach to and translate natural or synthetic messages (Korner & Gumbly, 1966) and a similar situation was described for ribosomes from muscle of diabetic and insulin-treated animals (Stirewalt, Wool & Cavicchi, 1967). It has also been shown (Wool & Kurihara, 1967) that the number of ribosomes that are active in preparations from muscle of diabetic rats is about half the number that are active in preparations from normal rats.

We therefore conclude that amino acids can influence the ability of ribosomes to be attached to, to remain attached to or to translate messenger RNA. The mechanism by which this alteration in ribosomal activity can occur is not known.

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