2. The glucosyl, maltosyl and lactosyl derivatives of 6-APA were prepared as their sodium salts and some of their properties examined.

3. These derivatives of 6-APA are stable to both staphylococcal and *Bacillus cereus* penicillinase, but are readily hydrolysed to the parent compounds by acid.

4. Compounds behaving like those prepared from 6-APA and sugars were identified in both the culture filtrates and the mycelium of a culture of *Penicillium chrysogenum* grown under conditions in which it produced 6-APA.

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The Function of High-Molecular-Weight Ribonucleic Acid from Rabbit Reticulocytes in Haemoglobin Biosynthesis

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There is now considerable evidence for the existence, in bacteria, of a fraction of RNA which is specifically involved in determining the amino acid sequence in protein biosynthesis. This so-called messenger RNA (m-RNA*) appears to have a DNA-like base composition (Volkin & Astrachan, 1956; Volkin, Astrachan & Countryman, 1956; Hayashi & Spiegelman, 1961; Nomura, Hall & Spiegelman, 1962) and is synthesized at a faster rate than either the ribosomal RNA or the s-RNA (Brenner, Jacob & Meselson, 1961; Gros *et al.* 1961). The m-RNA appears to be heterogeneous, as indicated by sedimentation coefficients ranging from about 8 to 30s (Hayashi & Spiegelman, 1961; Ishihama, Mizuno, Takai, Ohtaka & Osawa, 1962; Monier, Naono, Hayes, Hayes & Gros, 1962).

* Abbreviations: m-RNA, messenger RNA; s-RNA, transfer RNA.

In cell-free preparations from *Escherichia coli*, m-RNA isolated by fractionation on sucrose density gradients has been shown to stimulate the incorporation of amino acids into protein (Monier *et al.* 1962), but evidence for the RNA-directed synthesis of specific protein has so far been obtained only with RNA from the f2 coliphage and related viruses. On the addition of such RNA preparations to a cell-free system consisting of ribosomes and amino acid-activating enzymes from *E. coli*, amino acid incorporation into virus proteins has been demonstrated (Nathans, Notani, Schwartz & Zinder, 1962; Ohtaka & Spiegelman, 1963). Tobacco-mosaic-virus RNA also stimulates amino acid incorporation by cell-free preparations from *E. coli*, but protein resembling tobacco-mosaic-virus protein is at most only 10% of the total synthesized

(Tsugita, Fraenkel-Conrat, Nirenberg & Matthaei, 1962).

There is relatively little information about the existence of m-RNA in animal cells. A rapidly labelled nuclear-RNA fraction has been isolated from liver, but its base composition did not closely resemble that of DNA (Hiatt, 1962). Nuclear RNA (Barondes, Dingman & Sporn, 1962) and ribosomal RNA (Maxwell, 1962) from liver stimulate amino acid incorporation by *E. coli* ribosomes *in vitro*, but since the proteins synthesized were not characterized these results may not be conclusive evidence for a specific messenger effect.

The effect of high-molecular-weight RNA on amino acid incorporation into protein has now been studied with a cell-free preparation derived from rabbit reticulocytes and high-molecular-weight RNA isolated from reticulocyte ribosomes. By fractionating the ribosomes, a cell-free system has been obtained which responds well to both polynucleic acid and high-molecular-weight RNA from reticulocyte ribosomes, and evidence is presented that one of the proteins synthesized is haemoglobin. Some of the results have been briefly reported in two communications to The Biochemical Society (Arnstein, Cox & Hunt, 1962*b*; Arnstein & Cox, 1963).

EXPERIMENTAL

Materials. ATP (dipotassium salt) and GTP (sodium salt) were obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.) and Sigma Chemical Co. (St Louis, Mo., U.S.A.) respectively.

A solution of potassium phosphoenolpyruvate was prepared by acidifying a suspension of the silver-barium salt (C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany) in water at 4° with *N*-HCl and *N*-H₂SO₄, removing the precipitate by centrifuging and neutralizing the solution with *N*-KOH. For use in the cell-free system, an aqueous solution of phosphoenolpyruvate (100 μ moles/ml.), ATP (5 μ moles/ml.) and GTP (1.25 μ moles/ml.) was prepared, the pH was adjusted to 7.6 with *N*-KOH and the mixture was stored frozen at -18°.

The following materials were obtained from commercial sources: glutathione (C. F. Boehringer und Soehne, or Roche Products Ltd., Welwyn Garden City, Herts.), pyruvate kinase (C. F. Boehringer und Soehne), polynucleic acid (Miles Chemical Corp., Elkhart, Ind., U.S.A.) and uniformly ¹⁴C-labelled L-amino acids (The Radiochemical Centre, Amersham, Bucks.).

Yeast s-RNA was given by Dr G. L. Brown, M.R.C. Biophysics Research Unit, King's College, London, and rabbit-liver s-RNA was given by Dr B. A. Askonas, National Institute for Medical Research. Reticulocyte s-RNA was prepared by phenol extraction of (a) the cell sap after removing amino acid-activating enzymes and ribosomes at pH 5, or (b) the pH 5-enzyme fraction after removing ribosomes by centrifugation at 105 000*g* for 2 hr. (see below).

High-molecular-weight rabbit-reticulocyte RNA was isolated from reticulocyte ribosomes (H₆₀ fraction) by extraction with 5*M*-guanidinium chloride as described by Cox & Arnstein (1963). The RNA was dissolved in 0.01 *M*-EDTA at pH 7 in the presence of bentonite (1 mg./ml.), the solution was dialysed at 0° against 0.01 *M*-KCl for several hours and the bentonite was then removed by centrifuging. The solutions contained usually about 5 mg. of RNA/ml. and were stored at -18°.

Estimation of protein and ribonucleic acid. Proteins and RNA were estimated by measuring the ultraviolet absorption at 260 and 280 μ of portions diluted with sufficient 0.1 *M*-phosphate buffer, pH 7, to give an extinction at 260 μ of between 0.3 and 1. The concentration of protein and RNA was then estimated by means of a nomograph based on the extinction coefficients of enolase and nucleic acid (California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.; cf. Warburg & Christian, 1942). Since rabbit-reticulocyte ribosomes contain 50% of RNA (Ts'o & Vinograd, 1961), the amount of ribosomes in a suspension could be calculated from the observed RNA content.

Isolation of reticulocytes and preparation of subcellular fractions. (a) Standard method. Rabbits (sandy lop strain; approx. 2.5–3 kg. body wt.) were made anaemic by repeated injections of neutralized 2.5% (w/v) phenylhydrazine hydrochloride, usually in daily doses of 0.3 ml./kg. for 6 or 7 days. On the eighth day the animals were injected intravenously with 1% (w/v) heparin (1 ml.) and Nembutal (0.8 ml.) and further anaesthetized with ether. Blood was collected from the heart and immediately cooled in ice, all subsequent operations being carried out at below 4°. The cells were sedimented by centrifuging at 1200*g* for 10 min., and washed three times with 0.154 *M*-NaCl–0.01 *M*-sodium phosphate buffer, pH 7. The washed cells (approx. 20 ml. from one animal) were lysed by suspension in 4 vol. of 0.005 *M*-MgCl₂. After about 5 min., 1.5 *M*-sucrose–0.15 *M*-KCl (1 vol.) was added and the lysate was centrifuged at 10 000*g* for 10 min. to remove cell debris and leucocytes, which were not lysed by this procedure, as found by cell counts. The supernatant was decanted carefully and centrifuged at 105 000*g* for 60 min. to sediment about 80% of the total ribosomes. The ribosomes were washed by resuspension in medium A₁ (0.25 *M*-sucrose–0.025 *M*-KCl–0.001 *M*-MgCl₂–0.05 *M*-tris, pH 7.6) to a concentration of about 10 mg./ml., an equal volume of medium A₂ (medium A₁ made 0.005 *M* with respect to MgCl₂) was added and the suspension was centrifuged at 105 000*g* for 1 or 2 hr. This fraction is referred to below as 'heavy' or H₆₀-ribosomes and contains almost all the polyribosomes (see Fig. 1*a*).

The supernatant, after removal of the H₆₀-ribosomes, was acidified to pH 5.1 with *N*-acetic acid and centrifuged at 1800*g* for 20 min., when a precipitate consisting of the amino acid-activating enzymes and residual ribosomes (about 20% of the total ribosomes) was obtained. The supernatant cell sap was decanted and, if required for an incubation experiment, neutralized to pH 7.6 with *N*-KOH. The precipitate was rinsed with a small volume of medium A₂ and dissolved in 10 ml. of medium A₅, 0.1 ml. of *N*-KOH being added to neutralize the solution to pH 7.6. This solution was centrifuged first at 10 000*g* for 10 min. to remove traces of insoluble material, then at 105 000*g* for 2 hr. to isolate the ribosomes. This ribosome fraction apparently consisted largely of 80 s ribosomes (Fig. 1*b*) and is referred

to below as 'light' or L_{60} -ribosomes. The yield of ribosome-free pH 5-enzyme solution was usually 3–5 mg. of protein/ml. of cells. The total amount of ribosomes was about 3 mg./ml. of cells.

Both pH 5 enzymes and ribosomes were usually freshly prepared for use in the cell-free system, but these fractions were sometimes stored at -18° , the enzyme solution being first quickly frozen in solid CO_2 . It has been found that storage of the pH 5 enzymes for more than a few days tended to decrease or abolish the response of the cell-free system to high-molecular-weight RNA and was therefore avoided. Ribosomes did not deteriorate at -18° or even when kept at 4° for 24 hr.

(b) Variations of the standard method. (1) The debris-free lysate was centrifuged at 105 000g for 20 min., when a pellet containing only about 50% of the total ribosomes was obtained. The ribosomes were washed by resuspension as in the standard method and resedimented. This fraction is referred to below as H_{20} -ribosomes. After removing the H_{20} -ribosome fraction the supernatant was acidified to pH 5.1 with N -acetic acid and centrifuged at 1800g for 20 min. The light ribosomes (L_{20} -ribosomes) and pH 5 enzymes were then isolated as in the standard method. (2) In some experiments, the final 2 hr. centrifugation at 105 000g was omitted and the solution of L -ribosomes and pH 5 enzymes was used as such. This unfractionated mixture is referred to below as an unfractionated L_{60} - (or L_{20} -) ribosomes-pH 5 enzymes mixture.

Amino acid incorporation by the cell-free system. The cell-free system described by Schweet, Lamfrom & Allen (1958) was used with several modifications, as follows. Phosphoenolpyruvate and pyruvate kinase were substituted as the ATP-generating system, the amount of ribosomes was decreased and the ratio of pH 5 enzymes to ribosomes was increased. In general, the incubation mixture (0.5–1.0 ml.) contained 1–2 mg. of ribosomes, 3–6 mg. of pH 5 enzymes, 50–100 μg . of pyruvate kinase, 5 or 10 μmoles of phosphoenolpyruvate with ATP and GTP in the proportion of 0.05 and 0.0125 $\mu\text{mole}/\mu\text{mole}$ of phosphoenolpyruvate respectively, 5 μmoles of glutathione (neutralized with KOH), 50 μmoles of KCl/ml., 5 μmoles of MgCl_2 /ml., 25–50 μmoles of tris buffer (pH 7.6)/ml. and a ^{14}C -labelled amino acid (approx. 0.01 μmole) in a mixture (0.05–0.1 ml.) of unlabelled amino acids. The unlabelled amino acid mixture contained either the reticulocyte amino acids in half the amounts given by Borsook, Fischer & Keighley (1957) or equimolar amounts (2 $\mu\text{moles}/\text{ml}$.) of each of the 20 protein amino acids, but in each case omitting the unlabelled amino acid corresponding to the radioactive one. As ribosomes and pH 5 enzymes were always added in either medium A_1 or medium A_5 , variable amounts of sucrose were also present. Since the amount of haemoglobin precipitated with the pH 5-enzyme fraction was slightly variable, the cell-free system was usually reconstituted from L -ribosomes and pH 5 enzymes in the same proportion as present in the cell rather than in any definite ratio by weight. The composition of the incubation mixture used in different experiments is given in Table 1. Unless otherwise stated, all incubations were at 37° for 1 hr.

Isolation of protein and estimation of radioactivity. Except where otherwise stated, the following procedure was used. After incubation, tubes were cooled in ice, the same volume of supernatant cell sap was added to each tube as carrier, usually to give about 20 mg. of total

protein, and the protein was precipitated by the addition of 0.5 vol. of 30% (w/v) trichloroacetic acid. The precipitate was isolated by centrifuging, washed twice with 10% (w/v) trichloroacetic acid at 90° , redissolved in N -NaOH (1 ml.) and reprecipitated from 10% trichloroacetic acid. After washing with water, ethanol-ether (3:1, v/v) and ether, the protein was air-dried at room temperature and counted on 1 cm.² polythene disks. At least 1000 counts were collected, giving a statistical accuracy of $\pm 3\%$. Since amino acid incorporation is proportional to the amount of ribosomes and it has been found that the dilution of the labelled amino acid by free amino acids in the cell-free preparation is negligible (Table 2), all results have been corrected for differences in the quantity of ribosomes and are expressed as μmole -moles of amino acid incorporated into protein/mg. of ribosomes.

Release of soluble protein. In Expt. 18 (Table 1) carrier supernatant cell sap (0.5 ml.; 34 mg. of protein) and H_{60} -ribosomes (1 ml.; 10.5 mg.) were added to the incubated tubes after cooling in ice and the ribosomes were re-isolated by centrifuging at 105 000g for 2 hr. The supernatants were decanted, 1 ml. portions from each sample (i.e. 18% of the total) were acidified with 30% trichloroacetic acid (0.5 ml.) and protein was isolated for counting in the usual way. The remaining portions of the supernatants were stored at -18° for subsequent chromatography on CM-cellulose (see below). The ribosome pellets were homogenized in 1 ml. of water, and the protein was isolated by the standard method and counted.

In Expt. 19 (Table 1) the same procedure was followed except that 24 mg. of supernatant cell sap and 5.7 mg. of H_{60} -ribosomes were added as carrier, and the mixture was stored at -18° for 1 day before re-isolating the ribosomes by centrifugation at 105 000g for 1 hr.

Density-gradient fractionation of incubated ribosomes. In Expt. 18 (Table 1) incubation of some of the tubes was terminated after 10 min. by freezing. The incubated preparation was stored at -18° , thawed, and a portion (3 ml.) was layered on a 15–30% (w/v) sucrose gradient (Warner, Rich & Hall, 1962), made 0.01 M with respect to MgCl_2 , and centrifuged for 3.5 hr. at 25 000 rev./min. (Spinco SW 25 rotor). Fractions were collected with a siphon and the extinctions were measured with a Uvispek spectrophotometer (Hilger and Watts Ltd., London). Radioactivity was assayed by the procedure of Mans & Novelli (1961).

Chromatography of haemoglobin. The method of Huisman, Martis & Dozy (1958) was used, and all operations were carried out at 4° unless otherwise stated.

The stored ribosome-free incubation mixtures (Expt. 18 in Table 1) were dialysed against 0.001 M-phosphate buffer, pH 7, for 24 hr. and the haemoglobin was converted into the cyanmet derivative by adding 2 drops of 5% (w/v) potassium ferriocyanide. After 15 min., 2 drops of 2% (w/v) KCN were added. The samples were dialysed for at least 24 hr. against a buffer prepared by mixing 0.01 M- Na_2HPO_4 -0.0015 M-KCN, pH 9.8, with sufficient 0.01 M- NaH_2PO_4 -0.0015 M-KCN, pH 4.4, to obtain pH 7.0.

A column (40 cm. $\times$ 2.2 cm. diam.) of CM-cellulose (Peterson & Sober, 1951) was prepared and equilibrated with the above-mentioned buffer at pH 7.0. The samples were chromatographed by using a gradient from pH 7.0 to pH 8.5 (with 500 ml. of eluent), followed by sufficient buffer at pH 8.5 to elute the haemoglobin peak. Elution was then continued with 0.1 N-NaOH. Fractions (3–9 ml.)

Table 1. *Composition of incubation mixtures used for testing amino acid incorporation by cell-free systems*

The isolation of ribosomes and pH 5 enzymes and the composition of the amino acid mixtures are given in the Experimental section. Each tube also contained 5 μ moles of glutathione (20 μ moles in Expt. 18), pyruvate kinase and phosphoenol pyruvate, ATP, and GTP in the molar proportions 80:4:1. The amount of phosphoenol pyruvate was usually 5 μ moles/tube, but 6 μ moles were used in Expt. 1, 10 μ moles in Expts. 2-5, 8 and 13, and 20 μ moles in Expt. 18. Pyruvate kinase was used at 100 μ g./tube, except for Expts. 6 and 10 (40 μ g.), 9, 12, 14 and 16 (50 μ g.), 15 and 17 (60 μ g.), 11 (130 μ g.) and 18 (500 μ g.). Additional details are given either in the text or in the appropriate Tables and Figures.

Expt. no.	Final vol. (ml.)	Amino acid mixture (ml.)	¹⁴ C-labelled amino acid			Ribosomes (mg.)	pH 5 enzymes (mg.)	Sucrose (μ moles)	Concn. of salts and buffer (μ moles/ml.)			High-molecular-weight reticulocyte RNA used (prep. no.)
			(μ C)	(μ C)	(μ g.)				KCl	MgCl ₂	Tris	
1	1.0	0.1	Phe	0.167	0.61	H ₆₀	2.1*	42	50	10	12	—
2	0.8	0.1	Phe	0.4	1.0	H ₆₀	3.0	60	50	Variable	25	80
3	0.8	0.1	Phe	0.4	1.0	L ₆₀	0.5†	100	50	5.0	25	80
4	1.0	0.1	Phe	0.4	1.0	H ₆₀ or L ₆₀	1.9	100	45	4.6	30	80
5	0.8	0.1	Phe	0.4	1.0	L ₆₀	1.6	63	45	4.6	30	80
6	0.6	0.06	Phe	0.25	0.63	L ₆₀	3.0†	60	50	5.0	25	80
7	0.8	0.04‡	Phe	0.4	1.0	L ₆₀	0.8§	73	50	5.0	25	H 129
8	0.8	0.1	Phe	0.4	1.0	L ₆₀	2.2	63	50	5.0	25	{ 80
9	1.0	0.05‡	Leu	0.1	1.3	H ₆₀ or L ₆₀	1.0	200	50	5.0	30	{ 97
10	0.6	0.06	Phe	0.25	0.67	L ₆₀	1.0	63	50	5.0	25	80
11	0.8	0.05	Val	0.5	2.3	L ₆₀	0.8§	125	50	5.0	50	80
12	0.8	0.05‡	Leu	0.1	1.3	H ₆₀ or L ₆₀	0.8	75	50	5.0	25	H 130
13	0.8	0.1‡	Phe	1.0	Variable	L ₆₀	0.8	100	50	5.0	30	97/115
14	0.6	0.05	Val	0.5	2.3	L ₆₀	1.4§	75	50	4.5	40	80
15	1.0	0.05‡	Leu	0.45	1.3	L ₆₀	0.8	75	40	4.0	25	H 130
16	0.4	0.05	See Table 9			L ₆₀	0.8†	40	50	5.0	25	80
17	0.6	0.05	See Table 9			L ₆₀	5.0	75	50	4.5	40	80
18	4.0	0.2	Val	2.0	9.3	H ₆₀ or L ₆₀	5.2	420	50	5.0	25	80
19	0.5	0.05	Leu	0.5	1.5	L ₆₀	5.4	50	40	5.0	25	80

* Enzyme stored for 4 weeks at -18°, ribosome suspension prepared from pellets stored for up to 5 months at -18°.

† Present in the pH 5 enzyme fraction (see the text).

‡ This amino acid mixture contained 2 μ moles of each of 19 unlabelled amino acids/ml.

§ Enzyme stored for 1 day at -18°, ribosomes stored for 1 day either at -18° (Expt. 11) or at 4° (Expts. 7 and 13).

were collected, 1 ml. samples from alternate tubes being assayed for radioactivity by precipitating the protein on to paper strips according to the method of Mans & Novelli (1961). The washed proteins were then counted on the paper by using a Tri-Carb liquid-scintillation counter (Packard Instrument Co. Inc., LaGrange, Ill., U.S.A.) with approx. 60% efficiency.

End-group analysis of haemoglobin. The fractions containing haemoglobin (main peak) were pooled and made 0.05 M with respect to HCl, and 1% (v/v) HCl in acetone (10 vol.) was added. The globin was centrifuged, washed with acetone and ether and dried *in vacuo* over P_2O_5 and NaOH. Portions of protein (10 mg.) were dissolved in 8 M-urea (5 ml.) and treated with 1-fluoro-2,4-dinitrobenzene (0.1 ml.) in an autotitrator at pH 8.5 at 37° for 2 hr. when the consumption of alkali reached a plateau. The DNP-protein solution was washed with ether, made 1 N with respect to HCl, an equal volume of N-HCl was added to dilute the urea and the protein was isolated by centrifuging. The precipitate was washed three times with water (10 ml.), acetone and peroxide-free ether, and dried *in vacuo* over P_2O_5 and NaOH.

The dried protein was heated at 110° for 23 hr. in a sealed tube with 5 ml. of constant-boiling HCl (twice distilled from stannous chloride). The hydrolysate was diluted to 25 ml. with water and extracted three times with peroxide-free ether (10 ml.). The ether layer was separated and washed twice with water (5 ml.). The ether extract and the extracted hydrolysate were evaporated to dryness. The residue from the aqueous phase was dissolved in water (10 ml.), that from the ether extract in ethanol (1 ml.). Three 0.2 ml. portions from each fraction were transferred to Whatman no. 1 filter-paper circles stuck on aluminium disks (3 cm.²) and dried under infrared lamps with a constant flow of air from a hair-drier. The radioactivity was determined with a low-background counter with a Micromil window (Nuclear-Chicago Organisation, Des Plaines, Ill., U.S.A.), the efficiency of this counter for ^{14}C being about 15%. Chromatography of the ether extract on paper with 2-methylbutan-2-ol saturated with 0.1 M-phthalate buffer, pH 6, followed by radioautography, showed that all of the radioactivity was associated with the DNP-valine spot.

RESULTS

Fractionation of ribosomes. The ribosomes present in lysed reticulocytes are in the form of monomers (of sedimentation coefficient 80s) and polysomes (aggregates with sedimentation coefficients > 100s) (Warner *et al.* 1962). The method of fractionation used in our experiments yields heavy-ribosome pellets (H) which are rich in polysomes (Fig. 1a) by centrifugation of the lysate at 100 000g for either 20 min. (H_{20}) or 60 min. (H_{60}). The residual ribosomes (L_{20} or L_{60} respectively) were recovered from the supernatant by acidification at pH 5.1, as described in the Experimental section. This L-ribosome fraction was found to consist of mainly 80s particles (Fig. 1b).

The H_{20} - or H_{60} -ribosome fractions were considerably more active than the corresponding L_{20} - or L_{60} -ribosomes in incorporating amino acids into

polypeptide. When either polyuridylic acid or high-molecular-weight RNA from reticulocyte ribosomes was added to the cell-free system, there was an increase in the incorporation of phenylalanine into peptide linkage (Tables 2 and 3). With RNA, this increase (in terms of μ m-moles of phenylalanine incorporated/mg. of ribosomes) was about 2-3 times as great for the L-ribosome as for the H-ribosome fractions, but with polyuridylic acid the difference was somewhat less. In view of the smaller background incorporation and the greater response to added polynucleotide, the cell-free system utilizing L-ribosomes provides a more sensitive and reliable measure of the 'messenger'

Fig. 1. Sedimentation properties of ribosomes fractionated by the standard method. The H_{60} - and L_{60} -ribosomes were obtained from lysed reticulocytes by the standard method (see the Experimental section). The ribosome preparations (0.1 mg./ml. of medium A_1) were centrifuged at 33 450 rev./min. at 20° in the analytical ultracentrifuge (Spinco model E). Photographs were taken at 4 min. intervals and the extinctions of the films were estimated with a Joyce-Loebl microdensitometer. The tracings refer to (a) H_{80} -ribosomes and (b) L_{80} -ribosomes.

Table 2. *Response of protein synthesis by fractionated ribosomes to ribonucleic acid and polyuridylic acid*

The ribosomes were fractionated by centrifuging the lysate at 105000*g* for 20 min. to sediment the H₂₀-ribosomes (variation 1 of standard method), L₂₀-ribosomes being obtained from the supernatant. The responses of the cell-free system containing ribosome-free pH 5 enzymes and either H₂₀- or L₂₀-ribosomes, or an unfractionated mixture of L₂₀-ribosomes and pH 5 enzymes (variation 2 of the standard procedure), to high-molecular-weight reticulocyte RNA or polyuridylic acid were compared under the conditions described in Table 1 (Expt. 3).

Expt. no.	Ribosomes	Polynucleotide added (mg./mg. of ribosomes*)	Incorporation of phenylalanine into protein (μm-moles/mg. of ribosomes)	
			Total	Increase with polynucleotide
3a	H ₂₀	None	0.488	—
	H ₂₀	RNA (0.12 mg.)	0.554	0.066
	H ₂₀	RNA (0.23 mg.)	0.500	0.012
	H ₂₀	Polyuridylic acid (0.026 mg.)	0.849	0.361
3b	L ₂₀	None	0.308	—
	L ₂₀	RNA (0.12 mg.)	0.542	0.216
	L ₂₀	RNA (0.23 mg.)	0.572	0.264
	L ₂₀	Polyuridylic acid (0.026 mg.)	1.16	0.63
3c	Unfractionated L ₂₀ + pH 5 enzymes	None	0.451	—
	Unfractionated L ₂₀ + pH 5 enzymes	RNA (0.12 mg.)	0.803	0.352
	Unfractionated L ₂₀ + pH 5 enzymes	RNA (0.23 mg.)	0.912	0.461
	Unfractionated L ₂₀ + pH 5 enzymes	Polyuridylic acid (0.026 mg.)	1.51	1.06
3d	None	None	0.002	—
	None	RNA (0.12 mg.)	0.003	—
	None	RNA (0.23 mg.)	0.002	—
	None	Polyuridylic acid (0.026 mg.)	0.003	—

* The amount of polynucleotide added/tube was in the absence of ribosomes (Expt. 3d) the same as in the other experiments.

Table 3. *Response of protein synthesis by fractionated ribosomes to polynucleotides*

The ribosomes were fractionated by the standard method, H₆₀-ribosomes being sedimented by centrifuging the lysate at 105000*g* for 1 hr. and L₆₀-ribosomes obtained from the supernatant. The incubation mixture had the composition given in Table 1 (Expt. 4). The RNA was the same preparation of high-molecular-weight reticulocyte RNA as in Expt. 3 (see Table 2).

Expt. no.	Ribosomes	Polynucleotide added (mg./mg. of ribosomes*)	Incorporation of phenylalanine into protein (μm-moles/mg. of ribosomes)	
			Total	Increase with polynucleotide
4a	H ₆₀	None	0.470	—
	H ₆₀	RNA (0.15 mg.)	0.677	0.207
	H ₆₀	RNA (0.30 mg.)	0.573	0.103
	H ₆₀	Polyuridylic acid (0.08 mg.)	4.15	3.68
4b	L ₆₀	None	0.0645	—
	L ₆₀	RNA (0.15 mg.)	0.481	0.416
	L ₆₀	RNA (0.30 mg.)	0.637	0.572
	L ₆₀	Polyuridylic acid (0.08 mg.)	5.42	5.36
4c	None	None	0.0039	—
	None	RNA (0.15 mg.)	0.0074	0.0035
	None	RNA (0.30 mg.)	0.0066	0.0027
	None	Polyuridylic acid (0.08 mg.)	0.0888	0.0849

* The amount of polynucleotide added/tube in the absence of ribosomes (Expt. 4c) was the same as in Expts. 4a and 4b.

activity of polynucleotides than that containing unfractionated ribosomes.

Amino acid incorporation by the cell-free system. It was found in preliminary experiments that the incorporation of amino acids into protein was dependent on a supply of ATP and was proportional to the amount of ribosomes, as reported by Schweet *et al.* (1958) and Allen & Schweet (1962). When ribosomes were omitted, there was little or no incorporation either in the absence or in the presence of polynucleotides. The concentrations of magnesium chloride and potassium chloride for optimum incorporation were 5 and 50 mM respectively in the absence of polynucleotide (cf. Allen & Schweet, 1962). A similar Mg^{2+} ion concentration optimum was found for stimulation by

Fig. 2. Effect of Mg^{2+} ion concentration on the stimulation of the reticulocyte cell-free system by RNA. Each tube contained 2 mg. of H_{60} -ribosomes, a mixture of pH 5 enzymes (3 mg.) and L_{60} -ribosomes (approx. 0.5 mg.), L-[^{14}C]phenylalanine (0.4 μ C, 1.0 μ g.) and other constituents as described in Table 1 (Expt. 2). The amount of amino acid incorporated into protein in the absence (○) and in the presence (Δ) of high-molecular-weight RNA (0.22 mg./mg. of ribosomes) was determined at various Mg^{2+} ion concentrations.

RNA (Fig. 2), but the effect of K^+ ions was not investigated. When polyuridylic acid was included in the incubation mixture, the Mg^{2+} ion concentration was increased to 7–10 mM (cf. Arnstein, Cox & Hunt, 1962a).

All results have been expressed as μ m-moles of amino acid incorporated/mg. of ribosomes on the basis that the pool of free amino acid can be neglected. Thus variations in the amount of labelled amino acid added to the incubation mixture (Table 4) had only a slight effect on the amino acid incorporation and no apparent effect on the polyuridylic acid-induced stimulation of the incorporation of phenylalanine.

Stimulation of fractionated ribosomes by high-molecular-weight reticulocyte ribonucleic acid and polyuridylic acid. The capacity of the L_{60} -ribosome fraction to respond to increasing amounts of three different preparations of high-molecular-weight reticulocyte RNA is shown in Fig. 3. Amino acid incorporation increases linearly up to a dose of 0.2–0.3 mg. of RNA/mg. of ribosomes, when a plateau is reached. Very high concentrations of RNA appear to give only sub-optimum stimulation.

The effect of high-molecular-weight RNA appears to be specific, since amino acid incorporation by L-ribosomes was not greatly stimulated by the addition of supernatant cell sap, by increasing the amount of pH 5 enzyme, or by adding both these fractions (Figs. 4a and 4b).

Within a limited range, the amount of pH 5 enzyme had little effect on the response of the cell-free system to high-molecular-weight RNA (Table 5). In Expt. 10 the stimulation of phenylalanine incorporation by RNA was slightly better with 2.5 mg. of pH 5 enzymes/mg. of ribosomes than with 4.2 mg./mg. but in Expt. 11 the stimulation of valine incorporation was better with the larger amount. The use of frozen preparations of pH 5 enzymes (e.g. in Expt. 11), however, always resulted in a decreased response of the cell-free system to

Table 4. Stimulation of the incorporation of phenylalanine by polyuridylic acid with various substrate concentrations

Each tube contained L-[^{14}C]phenylalanine (0.167 μ C; 0.61 μ g.) and various amounts of unlabelled phenylalanine, 1.8 mg. of H_{60} -ribosomes and 2.1 mg. of pH 5 enzymes. For other constituents see Expt. 1 in Table 1.

Unlabelled phenylalanine added (μ g.)	Total phenylalanine present (μ g.)	Polyuridylic acid added (μ g.)	Incorporation of phenylalanine into protein (per mg. of ribosomes)		
			Total		Increase with polyuridylic acid (μ m-moles)
			(μ mC)	(μ m-moles)	
None	0.61	None	14.5	0.318	—
None	0.61	55	102	2.25	1.93
1.3	1.91	None	6.1	0.418	—
1.3	1.91	55	33.9	2.33	1.91
3.25	3.86	None	3.6	0.501	—
3.25	3.86	55	17.1	2.37	1.87

RNA, even though the normal incorporation by either H- or L-ribosomes was not greatly affected (H. R. V. Arnstein & K. G. Nair, unpublished work). When L-ribosomes were washed by resuspension in medium A in the same way as H-ribosomes, a similar dependence of amino acid incorporation on pH 5 enzymes was obtained (Table 6). This experiment also shows that the response of L-ribosomes to high-molecular-weight reticulocyte RNA is approximately the same before and after washing.

The amount of labelled phenylalanine added to the cell-free system has no effect on the stimulation of amino acid incorporation by RNA (Table 7), in accordance with the results obtained with polyuridylic acid (Table 4). s-RNA from yeast, reticulocytes or liver had little or no effect on the incorporation of amino acids either in the absence or in the presence of high-molecular-weight RNA (Table 8), presumably because the pH 5-enzyme fraction contains sufficient s-RNA. The slight activity of s-RNA from the pH 5-enzyme fraction of reticulocytes may have been due to contamination of this fraction by traces of L-ribosomes.

Kinetics of amino acid incorporation and its stimulation by high-molecular-weight ribonucleic acid.

A comparison of the amount of labelled amino acid incorporated into ribosomes and released as soluble protein after various periods of incubation up to 1 hr. (Fig. 5) shows that there is an initial lag of 5–10 min. in the response to RNA, but eventually L-ribosomes with RNA synthesize about the same amount of soluble protein as do H-ribosomes. The addition of RNA also results in an increased amino acid incorporation into the ribosomes, suggesting the formation of new active ribosomes.

Fractionation of the ribosomes isolated from a system which had actively synthesized protein from

Fig. 3. Response of protein synthesis by light ribosomes to different doses of RNA. The cell-free systems were as follows: (a) An unfractionated mixture of L_{80} -ribosomes and pH 5 enzymes, prepared by variation 2 of the standard method, and RNA prep. no. 80 (see Expt. 5 in Table 1) (●). (b) L_{80} -ribosomes prepared by the standard method and RNA prep. no. 80 (see Expt. 6 in Table 1) (▲). (c) L_{80} -ribosomes prepared by the standard method, but the pH 5 enzymes were stored frozen at -18° and the ribosomes were kept at 4° , both for 1 day, and RNA prep. no. H 129 (see Expt. 7 in Table 1) (■). (d) An unfractionated mixture of L_{20} -ribosomes and pH 5 enzymes, prepared by variation 2 of the standard method (see Expt. 8 in Table 1), with either RNA prep. no. 80 (○) or RNA prep. no. 97 (△). (e) The result of Expt. 10 (Table 1), taken from Table 5, lines 1 and 2 (□).

Fig. 4. Effect of pH 5 enzymes and cell sap on protein synthesis by L_{80} - and H_{80} -ribosomes. Each tube contained L- $[^{14}\text{C}]$ leucine ($0.1\ \mu\text{C}$, $1.3\ \mu\text{C}$) in 1.0 ml. and 1 mg. of either L_{80} -ribosomes (●, ▲, ■) or H_{80} -ribosomes (○, △, □) prepared by the standard method, the amount of pH 5 enzymes or supernatant cell sap, or both, being varied. For other constituents see Expt. 9 in Table 1. (a) Effect of pH 5 enzymes in the presence (△, ▲, 11 mg./mg. of ribosomes; □, ■, 22 mg./mg. of ribosomes) or absence (○, ●) of cell sap. (b) Effect of cell sap in the absence of pH 5 enzymes.

Table 5. *Effect of pH 5 enzyme concentration on stimulation of protein synthesis by ribonucleic acid*

The cell-free system (for details see Table 1) contained pH 5 enzymes and L₆₀-ribosomes prepared by the standard method. In Expt. 11 both these fractions were stored frozen at -18° for 1 day. The high-molecular-weight RNA preparation was prep. no. 80.

Expt. no.	¹⁴ C-labelled amino acid present	pH 5 enzymes (mg./mg. of ribosomes)	RNA added (mg./mg. of ribosomes)	Amino acid incorporation into protein (μm-mole/mg. of ribosomes)
10	Phenylalanine	2.5	None	0.167
		2.5	0.44	0.754
		4.2	None	0.184
		4.2	0.44	0.599
11	Valine	2.6	None	0.065
		2.6	0.26	0.200
		2.6	0.52	0.170
		5.2	None	0.064
		5.2	0.26	0.396
		5.2	0.52	0.370

Table 6. *Effect of washing procedure on protein synthesis by L₆₀-ribosomes and on its stimulation by ribonucleic acid*

The L₆₀-ribosomes were isolated by the standard procedure and washed once by resuspension as described in the Experimental section for H₆₀-ribosomes. The cell-free system was then reconstituted by using either washed or unwashed L₆₀-ribosomes with or without pH 5 enzymes. Details of the incubation mixture are given in Table 1 (Expt. 12). High-molecular-weight reticulocyte RNA prep. no. H130 was used.

Ribosomes	pH 5 enzymes (mg./mg. of ribosomes)	RNA added (mg./mg. of ribosomes)	Incorporation of leucine into protein (μm-mole/mg. of ribosomes)
L ₆₀	None	None	0.115
L ₆₀ , washed	None	None	0.024
L ₆₀	3.75	None	0.200
L ₆₀ , washed	3.75	None	0.177
L ₆₀	3.75	0.3	0.330
L ₆₀ , washed	3.75	0.3	0.315

Table 7. *Effect of amino acid concentration on the stimulation of amino acid incorporation by ribonucleic acid*

Experimental details are given in Table 1 (Expt. 13).

L-[¹⁴ C]-Phenylalanine (μmole/tube)	RNA added (mg./mg. of ribosomes)	Incorporation of phenylalanine into protein (μm-mole/mg. of ribosomes)	
		Total	Increase with RNA
0.015	None	0.026	—
0.015	0.14	0.118	0.092
0.1	None	0.057	—
0.1	0.14	0.155	0.098

labelled valine for 10 min. indicates that the monomeric 80s particles are not active in protein synthesis and that stimulation of protein synthesis by RNA is accompanied by an increase in the labelling of the polysome fraction (Fig. 6).

Characterization of the product synthesized in the presence of high-molecular-weight ribonucleic acid. The incorporation of valine, leucine, phenylalanine and isoleucine by L-ribosomes with or without addition of reticulocyte RNA is compared in Table 9. The utilization of all four amino acids for protein synthesis is stimulated by RNA and the proportion incorporated relative to valine is similar to the occurrence of these amino acids in haemoglobin. Except for leucine, the agreement between the observed incorporation and the theoretical value based on the amino acid composition of haemoglobin was better in the presence of RNA than in its absence. In view of the errors likely to be caused by the dilution of the labelled amino acids by small but not insignificant pools of free amino acids, as well as by unlabelled amino acids attached to the s-RNA, the values in Table 9 are in reasonable agreement with those expected if most of the protein synthesized were similar to haemoglobin in amino acid composition.

When ribosomes and soluble protein were separated by ultracentrifugation of the cell-free system after incorporation of labelled valine, the percentages of labelled soluble protein released by H- and L-ribosomes were found to be similar. The effect of RNA on the cell-free system seems to consist of stimulating amino acid incorporation into both ribosomes and soluble protein to about the same extent when L-ribosomes were used (Table 10), but in this experiment the addition of RNA to H-ribosomes caused an inhibition of protein synthesis. Chromatography of the soluble protein (Table 11 and Fig. 7) shows that there is a considerable increase in the radioactivity of the haemoglobin peak when RNA is added, although the synthesis of other proteins (peaks 1 and 3 in Table 11) is also stimulated. Almost 60 % of the radioactivity in the soluble protein was associated with the haemoglobin fractions from the cell-free

Table 8. *Comparison of stimulation of protein synthesis by light ribosomes with transfer ribonucleic acids or high-molecular-weight reticulocyte ribonucleic acid*L₆₀-ribosomes were used in both experiments (see Table 1).

Expt. no.	¹⁴ C-labelled amino acid	RNA added (mg./mg. of ribosomes)		Source of s-RNA	Incorporation of amino acid into protein (μm-moles/mg. of ribosomes)	
		Reticulocyte RNA	s-RNA		Total	Increase due to RNA
14	Valine	None	None	—	0.358	—
		0.088	None	—	1.275	0.917
		None	0.078	Yeast	0.240	—
		0.088	0.078	Yeast	0.915	0.675
15	Leucine	None	None	—	0.0782	—
		None	0.05	Reticulocyte	0.0759	—
		None	0.10	cell sap	0.0809	0.003
		None	0.06	Reticulocyte	0.0969	0.019
		None	0.12	pH 5 enzymes	0.0964	0.018
		None	0.12	Liver	0.0734	—
		None	0.24	Liver	0.0702	—
		0.12	None	—	0.169	0.091
		0.24	None	—	0.184	0.106
		0.12	0.06	Reticulocyte	0.171	0.093
		0.24	0.06	pH 5 enzymes	0.167	0.089

Table 9. *Effect of high-molecular-weight reticulocyte ribonucleic acid on the incorporation of different amino acids into protein by L₆₀-ribosomes*

In Expt. 16 an unfractionated mixture of L₆₀-ribosomes and enzymes was used; in Expt. 17 L₆₀-ribosomes were isolated by the standard method. For experimental details see Table 1. The amino acid composition of haemoglobin in moles relative to valine (1.00) is as follows: leucine, 1.44; phenylalanine, 0.56; isoleucine, 0.16.

Expt. no.	¹⁴ C-labelled amino acid	RNA added (mg./mg. of ribosomes)	Incorporation of amino acid into protein	
			(μm-moles/mg. of ribosomes)	(relative to valine)
16	Valine	None	0.027	(1.00)
		0.11	0.478	(1.00)
	Leucine	None	0.048	1.77
		0.11	0.499	1.04
	Phenylalanine	None	0.020	0.74
		0.11	0.254	0.53
17	Valine	None	0.358	(1.00)
		0.09	1.275	(1.00)
	Isoleucine	None	0.100	0.28
		0.09	0.240	0.19

Table 10. *Release of soluble protein after the stimulation of protein synthesis with high-molecular-weight ribonucleic acid*

Experimental details are given in Table 1 (Expt. 18) and in the text. The L₆₀- and H₆₀-ribosomes were prepared by the standard method and the RNA was reticulocyte RNA prep. no. 80. The values in parentheses are percentages of total amino acid incorporation into soluble protein.

Ribosomes	RNA added (mg./mg. of ribosomes)	Incorporation of L-[¹⁴ C]valine into protein (μm-mole/mg. of ribosomes)	
		Ribosomes	Soluble protein
L ₆₀	None	0.070	0.194 (74%)
L ₆₀	0.24	0.153	0.549 (78%)
H ₆₀	None	0.179	0.667 (79%)
H ₆₀	0.25	0.112	0.390 (78%)

Table 11. *Recovery of radioactive soluble proteins from intact cells or cell-free incubations with [¹⁴C]valine after chromatography on carboxymethylcellulose*

Experimental details of the cell-free incubation mixture are given in Table 1 (Expt. 18). After incubation, carrier haemoglobin was added, the ribosomes were removed by centrifugation and the soluble protein was chromatographed on CM-cellulose, as described in the text. The peaks are numbered in order of elution from the columns (see Fig. 7). Intact reticulocytes (2 ml.) were labelled by incubation with L-[¹⁴C]valine (5 μ C) at 37° for 40 min. (Borsook *et al.* 1957). The cells were washed, lysed and the ribosomes were removed by centrifugation for 1 hr. at 105000g. Haemoglobin was prepared from the supernatant as described in the Experimental section.

Incubation system	Total radioactivity (counts/min.)				Recovery (%)
	In soluble protein before chromatography	After chromatography			
		Peak 1	Main peak	Peak 3	
L ₈₀ -ribosomes	Not determined	6860	20 500 (67 %)	3320	—
L ₈₀ -ribosomes + RNA	108 200	13 580	64 100 (73 %)	10 630	82
H ₈₀ -ribosomes	151 300	25 700	97 300 (79 %)	None	81
Intact cells	113 600	3 910	61 100 (86 %)	6 080	63

Table 12. *End-group analysis of purified haemoglobin synthesized from [¹⁴C]valine in the cell-free system*

Haemoglobin was isolated by chromatography (see Table 11 and Fig. 7) and the percentage of *N*-terminal valine was determined by preparing the DNP derivative.

Origin of labelled protein	Amount of DNP-protein hydrolysed (mg.)	Total radioactivity (counts/min.)		Radioactivity in end group (%)
		In non-terminal valine	In DNP-valine	
Cell-free system with L ₈₀ -ribosomes	16.8	3035	158.5	5.0
Cell-free system with L ₈₀ -ribosomes and RNA	11.5	6150	282.5	4.4
Cell-free system with H ₈₀ -ribosomes	9.3	4700	85.0	1.8
Intact cells	10.2	6050	358.5	5.6

incubation of L-ribosomes with RNA, compared with about 65 % from the system containing H-ribosomes. These values are also very similar to the percentage of radioactivity incorporated into haemoglobin by intact reticulocytes (Table 11).

The amount of [¹⁴C]valine incorporated into the *N*-terminal position (Table 12) shows that there is considerable synthesis of new peptide chains by the cell-free system containing L-ribosomes, as well as by intact cells, but rather less by H-ribosomes. The addition of RNA to the L-ribosome system appears to result in the initiation of additional new peptide chains, since the percentage of label in the *N*-terminal valine is about the same in the presence as in the absence of high-molecular-weight RNA, whereas there is a threefold increase in the total incorporation of valine into haemoglobin (Table 11).

DISCUSSION

In earlier work, a cell-free system from rabbit reticulocytes was found to respond to polyuridylic acid by a specific increase in the incorporation of phenylalanine into trichloroacetic acid-insoluble

product, presumably polyphenylalanine (Arnstein *et al.* 1962a; Weinstein & Schechter, 1962; Arlinghaus & Schweet, 1962). Fractionation of reticulocyte ribosomes (Gierer, 1963) indicated that the monomeric 80s ribosomes are comparatively inactive in protein synthesis, but are stimulated to a greater degree by polyuridylic acid than are the polyribosomes which are active in protein synthesis. The present experiments are in agreement with these observations and also show that the maximum amount (5 μ m-moles/mg. of ribosomes) of phenylalanine converted into polypeptide by our 'light'-ribosome fraction (i.e. largely 80s ribosomes) is similar to the best incorporation (approx. 8 μ m-moles/mg. of ribosomes) by the cell-free system from *E. coli* (Lengyel, Speyer & Ochoa, 1961). It is clear from these results, as well as from those reported by Arlinghaus & Schweet (1962), that mammalian systems can respond to a synthetic polynucleotide messenger such as polyuridylic acid, both qualitatively and quantitatively, in the same way as the bacterial system (Nirenberg & Matthaei, 1961; Lengyel *et al.* 1961).

High-molecular-weight RNA from reticulocytes,

Fig. 5. Kinetics of the stimulation of leucine incorporation into protein by high-molecular-weight reticulocyte RNA. Each tube contained either 0.76 mg. of L_{80} -ribosomes or 0.96 mg. of H_{80} -ribosomes, prepared by the standard method, and the usual components for protein synthesis by the cell-free system (see Expt. 19 in Table 1). The incorporation of L -[^{14}C]leucine into soluble protein (●, ■, ▲; solid lines) and protein attached to ribosomes (○, □, △; broken lines) was determined by resedimenting the ribosomes after incubation for various periods (see the Experimental section). The soluble and ribosome-bound protein was derived from H -ribosomes (○, ●), L -ribosomes (□, ■) or L -ribosomes in the presence of high-molecular-weight RNA (0.30 mg./mg. of ribosomes) (△, ▲).

which was obtained by extraction of the polyribosome-rich fraction with 5M-guanidinium chloride (Cox & Arnstein, 1963), has now been found to stimulate the incorporation of amino acids into protein. When the H -ribosome fraction, which is rich in polyribosomes, was used in the cell-free system, the stimulation was smaller than that obtained with the monomeric L -ribosomes. However, in the most favourable experiments, the addition of such RNA preparations restored the incorporation of amino acids by L -ribosomes to the same level as that by the H -ribosomes. The maximum increase in the incorporation of phenylalanine due to RNA was more than $0.5 \mu\text{m-mole/mg.}$ of ribosomes (Fig. 3 and Expt. 4a in Table 4). Since haemoglobin, which is one of the main products synthesized by the cell-free system, contains 5% of phenylalanine on a molar basis, this value corresponds to a stimulation in total amino acid incorporation of about $10 \mu\text{m-moles/mg.}$ of ribosomes, which compares favourably with the maximum effect of polyuridylic acid on the incorporation of phenylalanine.

Since various preparations of s-RNA from yeast, liver or reticulocytes did not stimulate the cell-free system to any significant extent, the effect of the RNA extracted from ribosomes on amino acid incorporation cannot be ascribed to traces of s-RNA

which might be present on the ribosomes. The pH 5-enzyme fraction, like s-RNA, could not replace high-molecular-weight RNA, and it may be concluded therefore that there is no significant amount of RNA other than s-RNA present in this fraction. The absence of m-RNA from the pH 5 fraction supports the conclusion that reticulocyte ribosomes contain sufficient information for haemoglobin biosynthesis (Bishop, Favelukes, Schweet & Russell, 1961; Arnstein *et al.* 1962a; Munro & Korner, 1963), although there is not complete agreement about the contribution of the pH 5-enzyme fractions and cell sap to the control of the specificity of haemoglobin synthesis (cf. Lamfrom, 1961; Kruh, Rosa, Dreyfus & Schapira, 1961).

Fig. 6. Fractionation of ribosomes activated for protein synthesis by high-molecular-weight RNA. Fractionated L_{80} - and H_{80} -ribosomes, prepared by the standard method, were incubated for 10 min. at 37° in the usual complete system with L -[^{14}C]valine (see Expt. 18 in Table 1). The incubation mixture was then fractionated by using a 15–30% sucrose density gradient as described in the Experimental section, and the distribution of radioactive protein was determined. ×, Radioactivity; ○, extinction at $260 \text{ m}\mu$. (a) H -ribosomes; (b) L -ribosomes; (c) L -ribosomes incubated with RNA.

Fig. 7. Chromatography of soluble protein synthesized in the cell-free system after the addition of high-molecular-weight RNA. The experimental conditions for incubation of the cell-free system are the same as those given in Fig. 6 and in Table 1 (Expt. 18), except that incubation was for 1 hr. The incubation mixture was fractionated into ribosomes and soluble protein as described in the Experimental section, the results being given in Table 10. Haemoglobin was converted into the cyanmet form and chromatographed as described in the text. The arrows indicate change of buffer. ○, Radioactivity of the protein; —, extinction of fractions at 415 mμ. The soluble protein was derived from a cell-free system containing (a) L-ribosomes, (b) L-ribosomes and high-molecular-weight reticulocyte RNA, or (c) H-ribosomes. Fig. 7(d) shows the chromatography of soluble protein synthesized by intact reticulocytes (for details see the Experimental section).

The kinetics of the effect of RNA on the activation of the L-ribosome fraction and analysis of the radioactive product by sucrose-density-gradient centrifugation (Figs. 5 and 6) suggest that an early result of the addition of the RNA to the cell-free system is the formation of active ribosomes. These ribosomes sediment faster than the 80s monomers and are probably at least dimers or trimers, i.e. aggregates of two or three 80s ribosomal particles presumably associated with m-RNA. Stimulation of the synthesis of soluble protein begins after a lag of 5–10 min. and reaches a maximum after about 1 hr. The observation of an early stimulation of synthesis of protein attached to ribosomes does not agree with some of the results of Kruh,

Dreyfus & Schapira (1962), who reported in a brief note that RNA prepared by phenol extraction of reticulocytes or liver depressed the incorporation of radioactive amino acids into ribosomes, whereas the biosynthesis of haemoglobin was stimulated.

Chromatography of the soluble protein synthesized by the cell-free system after the addition of high-molecular-weight RNA shows that the major product is identical with, or at least very similar to, haemoglobin. Moreover, determinations of labelled valine N-terminal groups support the view that in the presence of the RNA L-ribosomes can start additional peptide chains, most of the soluble protein being subsequently released as haemoglobin.

Although mammalian reticulocytes have no nucleus, contain no detectable amount of DNA (Holloway & Ripley, 1952; Burt, Murray & Rossiter, 1951) and do not synthesize any significant quantity of RNA (Marks, Willson, Kruh & Gros, 1962), there is good evidence that protein synthesis by isolated rabbit reticulocytes continues *in vitro* for several hours (Borsook *et al.* 1957). The m-RNA in these cells must therefore be relatively stable and, since most of the protein synthesized appears to be haemoglobin (Dintzis, 1961), reticulocytes should serve as a convenient source for the isolation of a m-RNA for a specific protein. The present experiments show that high-molecular-weight RNA extracted from ribosomes has messenger-like activity and seems to be able to induce the synthesis of haemoglobin. Since the RNA was tested in a homologous cell-free system, however, the possibility is not completely excluded that these results are due to activation of the L-ribosome system by a mechanism other than a m-RNA effect. Conclusive proof will therefore have to await experiments with RNA in a heterologous system.

SUMMARY

1. Ribosomes from rabbit reticulocytes were fractionated by differential ultracentrifugation into a 'heavy' fraction, consisting mainly of polysomes, and a 'light' fraction which contained mainly 80s monomers.

2. In a cell-free system, the incorporation of amino acids into protein by heavy ribosomes was greater than that by light ribosomes.

3. Amino acid incorporation into protein was stimulated by high-molecular-weight RNA isolated from reticulocyte ribosomes.

4. The increase in protein synthesis by light ribosomes when RNA was added, or in polyphenylalanine synthesis when polyuridylic acid was added, was greater than that by heavy ribosomes.

5. One of the products synthesized has been characterized as haemoglobin by chromatography on carboxymethylcellulose.

6. New active ribosomes (heavier than the 80S monomers) appeared to be formed and the synthesis of haemoglobin was increased when RNA was added to the cell-free system containing light ribosomes.

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Transamination of Peptides

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Despite the considerable literature on non-enzymic transamination of amino acids that has accumulated since the original observations (e.g. Nakada & Weinhouse, 1953; Metzler, Olivard & Snell, 1954a; Fleming & Crosbie, 1960), little work has been reported on the application of this reaction to peptides. Dixon & Weitkamp (1962) produced a

peptide (Gly¹-corticotrophin) from the corresponding α -oxo acyl compound; the reverse of this reaction, i.e. the conversion of the *N*-terminal residue of a peptide into an α -oxo acyl residue, is now reported. Cennamo, Carafoli & Bonetti (1956) described the same reaction with pyridoxal as amino acceptor and alum as a catalyst. It is