

comitant protein synthesis has recently been demonstrated by Tissières and Watson (15).

It is not yet known whether poly U acts catalytically or stoichiometrically, that is, whether one molecule of poly U directs the synthesis of many or only one molecule of polyphenylalanine. Since more than 80 percent of the poly U added to reaction mixtures was degraded before significant polyphenylalanine synthesis had occurred, only a small fraction of the poly U added may actually function in directing protein synthesis. In making estimates of the number of phenylalanine residues directed into protein by each uridylic acid residue in poly U, the amount of poly U which had been degraded before significant protein synthesis occurred should therefore be taken into consideration. From the data of Fig. 3 it can be estimated that one uridylic acid residue in the poly U which survives rapid degradation acts to direct one phenylalanine into protein. If the suggestion that the coding ratio is three nucleotides per amino acid (16) can be applied to poly U, it might be concluded that one poly U molecule directs the synthesis of more than one polyphenylalanine molecule and thus behaves catalytically. Recent experiments with intact *E. coli* (17) have suggested that "messenger RNA" may act catalytically in vivo.

To help clarify the enzymic routes of  $H^3$ -poly U breakdown, the  $H^3$ -products formed during incubation were identified. The action of the two well-characterized ribonucleases from *E. coli*, polynucleotide phosphorylase (13) and ribosomal ribonuclease (18, 19), can be distinguished by the nature of the products formed. Polynucleotide phosphorylase catalyzes a reversible phosphorolysis of RNA in the presence of inorganic phosphate and produces nucleoside-5'-diphosphates. However, during incubation of our reaction mixture, UDP was partially converted to UTP presumably by the action of phosphoenolpyruvate kinase (20) and to 5'-UMP. Thus the formation of  $H^3$ -5'-UTP,  $H^3$ -5'-UDP, and  $H^3$ -5'-UMP may represent degradation of  $H^3$ -poly U by polynucleotide phosphorylase. In contrast, ribosomal ribonuclease catalyzes the formation of nucleoside-2',3'-cyclic phosphates which are more slowly converted by the same enzyme to nucleoside-3'-phosphates (19). This enzyme is further characterized by its existence in

a latent ribosome-bound form in the presence of 0.01M magnesium (18, 19), the concentration used in our investigation. Another ribosomal ribonuclease from *E. coli* has been reported by Wade and Lovett (21) but has not been purified or extensively characterized. It catalyzes the hydrolysis of RNA to nucleotide-5'-monophosphates and is active even in the presence of 0.01M magnesium.

Since most of the  $H^3$ -poly U which has been degraded in reaction mixtures can be recovered as 5'-mononucleotides, such degradation possibly may be ascribed to catalysis by polynucleotide phosphorylase or by the ribonuclease of Wade and Lovett, or by both. Relatively little degradation of poly U by ribosomal ribonuclease was found after either short or long incubations. Whether the enzymes involved in poly U degradation are also responsible for the in vivo degradation of "messenger RNA" remains to be determined.

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#### References and Notes

- Abbreviations used: GTP, adenosine and guanosine-5'-triphosphate, respectively; PEP, potassium phosphoenolpyruvate; PEP kinase, phosphoenolpyruvate kinase, crystalline; poly U, polyuridylic acid; (pU), uridylic acid residues in poly U; tris HCl, tris (hydroxymethyl) aminomethane hydrochloride; UTP, UDP, and 5' UMP, uridine-5'-tri-, di-, and monophosphate, respectively; 2'- and 3'-UMP, a mixture of uridine 2'-monophosphate and uridine 3'-monophosphate; 2', 3'-cyclic UMP, uridine-2', 3'-cyclic monophosphate.
- M. W. Nirenberg and J. H. Matthaei, *Proc. Natl. Acad. Sci. U.S.A.* **47**, 1588 (1961).
- S. H. Baronides and M. W. Nirenberg, *Science*, this issue.
- O. H. Lowry, N. J. Rosenbrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
- UDP was obtained from the Schwartz Biochemical Corp. The Wilzbach procedure was carried out by the New England Nuclear Corp.  $C^{14}$ -phenylalanine was obtained from the Nuclear-Chicago Corp.
- M. R. Singer and V. K. Guss, *J. Biol. Chem.* **237**, 1862 (1962).
- J. H. Matthaei, O. W. Jones, R. G. Martin, M. W. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 666 (1962).
- Dr. J. Fresco made available unpublished data concerning some physical characteristics of poly U.
- S. W. Luborsky and G. L. Cantoni, *Biochim. Biophys. Acta*, in press.
- G. A. Bray, *Anal. Biochem.* **1**, 279 (1960).
- M. Grunberg-Manago, in *The Enzymes*, P. B. Boyer, H. Lardy, K. Myrback, Eds. (Academic Press, New York, 1961), vol. 5, p. 257.
- L. Heppel, personal communication.
- Pabst Laboratories circular OR-17 (1961).
- U. Z. Littauer and A. Kornberg, *J. Biol. Chem.* **226**, 1077 (1957).
- F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 302 (1961); S. Brenner, F. Jacob, M. Meselson, *Nature* **190**, 576 (1961); E. Volkin and L. Astrachan, *Virology* **2**, 149 (1956).
- A. Tissières and J. D. Watson, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1061 (1962).
- F. H. C. Crick, L. Barnett, S. Brenner, R. J. Watts-Tobin, *Nature* **192**, 1227 (1961).
- C. Levinthal, A. Keynan, A. Higa, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1631 (1962).
- D. Elson, *Biochim. Biophys. Acta.* **207**, 216 (1958).
- P. F. Spahr and B. R. Hollingworth, *J. Biol. Chem.* **236**, 823 (1961).
- J. L. Strominger, *Biochim. Biophys. Acta.* **16**, 616 (1955).
- H. E. Wade and S. Lovett, *Biochem. J.* **81**, 319 (1961).
- V. N. Schumaker and H. K. Schachtman, *Biochim. Biophys. Acta.* **23**, 628 (1957).
- P. Siekevitz, *J. Biol. Chem.* **195**, 549 (1952).
- The counsel of Drs. Leon Heppel, Samuel Luborsky, and Maxine Singer is acknowledged.

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## Fate of a Synthetic Polynucleotide Directing Cell-Free Protein Synthesis II. Association with Ribosomes

**Abstract.** Tritiated-poly U, when added to cell-free extracts of *Escherichia coli*, became associated with ribosomes. This association occurred at 3°C and did not require high-energy phosphate compounds. Small amounts of tritiated polyuridylic acid produced polydisperse ribosomal aggregates with sedimentation constants of approximately 100 to 130.  $C^{14}$ -phenylalanine initially was incorporated into protein only on these particles, which suggests that they are the sites of polyphenylalanine synthesis.

Rapidly synthesized fractions of RNA have been demonstrated in *Escherichia coli* (1-3) and have been considered to function in protein synthesis as "messenger RNA." Recently, Risebrough, Tissières, and Watson have reported that such RNA fractions associate with ribosomal particles, particularly with 100 S ribosomes (2). Since poly U (4) functions in cell-free extracts of *E. coli* as template RNA, some characteristics of its association with ribosomes have been studied.

**Experimental procedure.** The same preparations of extracts of *E. coli* and  $H^3$ -poly U discussed in the accompanying report (5) were used in this study. The rate of polyphenylalanine synthesis and the relationship between the amount of poly U added to a reaction mixture and the amount of phenylalanine incorporated into protein remained within the ranges cited (5). The sedimentation constants of the components of the ribosomal solution are shown in Fig. 1. The sedimentation constant of the major ribosomal component was approximately 70 S. Few 100 S ribosomes were found. For some of the experiments a preparation of washed 70 S ribosomes was prepared by repeated centrifugation of ribosomes in 0.1M tris HCl, pH 7.8, 0.01M magnesium acetate, and 0.05M KCl (6). The final ribosomal pellet suspended in this medium contained 3 mg of ribosomal protein per milliliter; it was stored in aliquots at -20°C.

Sucrose density gradient centrifugation experiments were performed as described

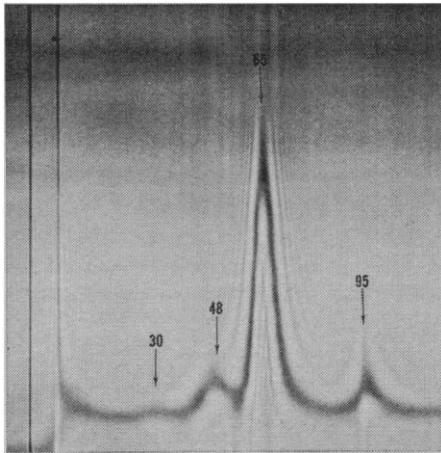


Fig. 1. Sedimentation of ribosomes. Ribosomes were suspended in 0.01M magnesium acetate, 0.05M KCl, 0.1M tris HCl, pH 7.8, at a concentration of 2.5 mg/ml and were centrifuged at 5°C in the Beckman model E analytical ultracentrifuge. The sedimentation constants,  $S_{20,w}$ , were not corrected for concentration effects.

by Britten and Roberts (7). Concentrated sucrose solutions were treated with bentonite to remove possible traces of ribonuclease (8). Sucrose solutions used in all density gradient centrifugation experiments contained 0.1M tris HCl, pH 7.8, and 0.01M magnesium acetate, 0.05M KCl; 2.4 ml each of 5 percent and 20 percent sucrose were used for the preparation of the gradients which were kept for several hours at 3°C, after which 0.2 ml samples were layered on top of each gradient. To remove large aggregates each thawed extract of *E. coli* was centrifuged at 10,000g for 10 minutes before addition to the other reagents. Gradient centrifugation was performed in a Spinco model L preparative ultracentrifuge with an SW-39 rotor. The tempera-

Table 1. Estimation of number of uridylic acid residues in  $H^3$ -poly U associated with each ribosome. The data in this calculation were derived from Fig. 2. The number of uridylic acid residues in each tube was calculated from the specific radioactivity of  $H^3$ -poly U corrected for quenching. The number of ribosomes in each tube was calculated from the O.D. at 260 m $\mu$  using the data of Tissières *et al.* (6) for the extinction coefficient and molecular weight of ribosomes. For the purposes of this calculation all of the ribosomes in tubes 7, 8, and 9 were assumed to have the molecular weight of 100 S ribosomes (6).

Sucrose gradient tube No.	Ribosomes ( $\times 10^{-13}$ )	(pU) ( $\times 10^{-13}$ )	Ratio (pU)/ribosome
<i>Not incubated</i>			
7	0.06	80	1300
8	0.08	100	1300
9	0.11	110	1000
<i>3-minute incubation</i>			
7	0.02	13	700
8	0.02	20	1000
9	0.03	20	700

ture setting of the chamber was -12°C, and the temperature of sucrose solutions at the completion of centrifugation was found to be approximately 3°C. Tubes were centrifuged, unless otherwise indicated, at 37,000 rev/min for 60 minutes. Fractions of 15 drops each were collected by puncturing the bottoms of the tubes. The number of drops in the final fraction is shown by the position of this fraction on the abscissa of each figure, and the contents of the final fraction plotted on each ordinate is corrected to 15 drops. After the fractions had been collected, the tubes were wiped to remove residual fluid and the bottom of each tube was washed with 1 ml of H<sub>2</sub>O which was collected and assayed. These data are presented in the figures above the arrow designating the bottom of the gradient.

Repeated calibration of the sucrose gradient with a standard was necessary, since short centrifugations, which did not clearly resolve 70 S from 50 S ribosomes, were found desirable in order to keep heavier material from moving to the bottom of the tube. The calibration standard permitted accurate interpretation of alterations in sedimentation constants of ribosomes produced by interaction with synthetic polynucleotides. Calibration was performed by including in most sucrose density gradient centrifugation experiments a separate tube containing purified 70 S ribosomes. The  $S_{20,w}$  of the major component of this preparation was 69.3 as determined with ultraviolet optics in the Beckman model E analytical ultracentrifuge at a concentration of 75  $\mu$ g/ml in 0.01M magnesium acetate, 0.05M KCl, and 0.1M tris HCl, pH 7.8. When this preparation was centrifuged in a sucrose gradient under the usual conditions, the peak of ultraviolet absorbing material was generally in the eighth tube from the top. The average position of this peak is indicated in the figures. The position of 100 S ribosomes was calculated by the method of Martin and Ames (9).

Randomly-ordered poly UG was synthesized by Oliver W. Jones (10); its nucleotide composition, determined by analysis (10), was uridylic acid, 1.7; guanylic acid, 1.12; the  $S_{20,w}$  was 6.5, determined at a concentration of 30  $\mu$ g/ml in 0.1M NaCl, 0.05M sodium cacodylate, pH 7.0, in the Beckman model E analytical ultracentrifuge equipped with ultraviolet optics. RNA from TMV containing approximately 10/ $\mu$ c of P<sup>32</sup> per milligram was the gift of H. Fraenkel-Conrat.

The fractions collected in liquid scintillation counting vials after sucrose density gradient centrifugation of  $H^3$ -poly U or P<sup>32</sup>-RNA from TMV were diluted to 1 ml with H<sub>2</sub>O, and their absorption at 260 m $\mu$  was determined in a Beckman model DU spectrophotometer. The fractions then were returned to their counting vials, 10 ml of Bray's solution (11) were added, and the samples were counted in a liquid scintillation counter for a minimum of 1000 counts. Addition of water and other materials produced approximately 40 percent quenching of tritium. The amounts of sucrose in both the top and bottom fractions of the sucrose gradient did not significantly alter the degree

of quenching. Since quenching was uniform, corrections were not made except where stated.

When incorporation of C<sup>14</sup>-phenylalanine into protein was determined, the fractions, after dilution and measurement of absorption at 260 m $\mu$ , were precipitated with 5 ml of 10 percent trichloroacetic acid; to insure an adequate precipitate 0.5 mg of crystalline bovine serum albumin was added to each fraction. The samples were heated at 90 to 95°C for 15 minutes to hydrolyze RNA; they were then cooled. Each sample was then filtered through a Millipore filter (pore size 0.45  $\mu$ ) and washed with 50 ml of 5 percent trichloroacetic acid. The filters were cemented on planchettes and, after drying, were counted in a low background gas flow counter with a thin Micromil window. Counting efficiency of C<sup>14</sup>-samples was approximately 30 percent. No counts attributable to H<sup>3</sup>-poly U were observed when this procedure was followed.

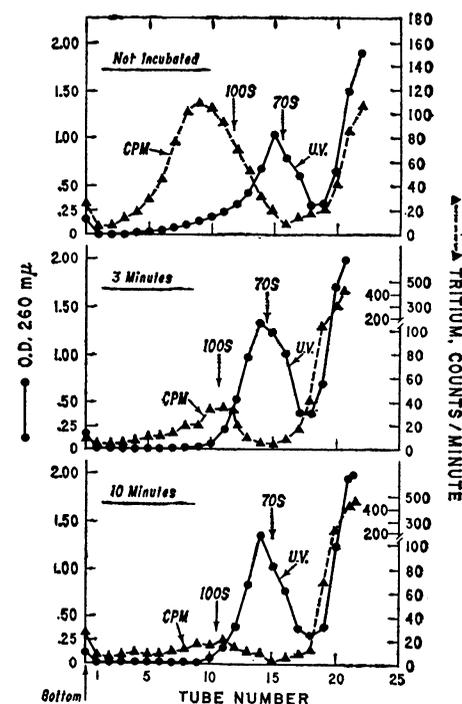


Fig. 2. Association of  $H^3$ -poly U with ribosomes. Each reaction mixture contained in a final volume of 0.25 ml: 25  $\mu$ mole tris HCl, pH 7.8; 2.5  $\mu$ mole magnesium acetate; 12.5  $\mu$ mole KCl; 1.5  $\mu$ mole  $\beta$ -mercaptoethanol; 0.25  $\mu$ mole ATP; 0.03  $\mu$ mole GTP; 1.25  $\mu$ mole potassium phosphoenolpyruvate; 5  $\mu$ g PEP kinase crystalline; 0.03  $\mu$ mole C<sup>14</sup>-phenylalanine; 105,000g supernatant solution of *E. coli* extract containing 0.45 mg protein; and ribosomes from *E. coli* extract containing 0.2 mg protein. Reactions were begun by addition of 20  $\mu$ mole (pU) in  $H^3$ -poly U to each 0.25 ml of reaction mixture. The samples were incubated at 35°C for periods indicated and chilled briefly in an ice bath, and 0.2-ml aliquots were layered on top of sucrose gradients at 3°C. Centrifugation and analysis of each fraction were performed as described in the text.

**Results.** The binding of  $H^3$ -poly U to ribosomes is demonstrated in Fig. 2. When  $80\text{ m}\mu\text{mole}$  of (pU) in  $H^3$ -poly U was added to a 1.0 ml reaction mixture at  $3^\circ\text{C}$ , most of the poly U was found associated with ribosomes which sedimented over a broad zone with a peak at approximately 130 S. Under these conditions little or no poly U was bound to 70 S ribosomes. The rapid degradation of poly U noted in the previous report (5) was reflected in a rapid decrease in the amount of tritiated material associated with ribosomes after incubation at  $35^\circ\text{C}$ . After incubation for 3 minutes at  $35^\circ\text{C}$  the amount of poly U on ribosomes was markedly diminished and the poly U that remained on ribosomes sedimented at approximately 100 S. After a 10 minute incubation only a small amount of poly U remained associated with ribosomes.

From the data of Table 1 it may be estimated that about 1000 (pU) in poly U were associated with each ribosome which sedimented in the range of 100 to 130 S. Since the weight-average molecular weight of the  $H^3$ -poly U used was shown to be approximately  $5 \times 10^5$  (5), the average  $H^3$ -poly U molecule would contain about 1700 nucleotide residues. These calculations suggest that no more than one poly U molecule was associated with each ribosome sedimenting in the range of 100 S.

The ribosomal fraction which supports  $C^{14}$ -phenylalanine incorporation into protein is shown in Fig. 3. After a 3 minute incubation at  $35^\circ\text{C}$ ,  $C^{14}$ -phenylalanine was incorporated into protein only on 100 S ribosomes. During this initial period of protein synthesis no incorporation of  $C^{14}$ -phenylalanine was observed on 70 S ribosomes. After incubation of reaction mixtures for 15 minutes at  $35^\circ\text{C}$ ,  $C^{14}$ -protein was found associated with 100 S and 70 S ribosomes. In addition, a substantial amount of rapidly sedimenting  $C^{14}$ -protein, which may represent insoluble  $C^{14}$ -polyphenylalanine, was found on the bottom of the tube.

The experiment described in Fig. 4 illustrates the characteristics of the association of different concentrations of  $H^3$ -poly U with washed ribosomes.  $C^{14}$ -phenylalanine incorporation into protein was proportional to the amount of poly U added in the range of approximately 0 to  $70\text{ m}\mu\text{mole}$  of (pU) in poly U per milliliter of reaction mixture;  $33\text{ m}\mu\text{mole}$  of (pU) per milliliter of reaction mixture therefore repre-

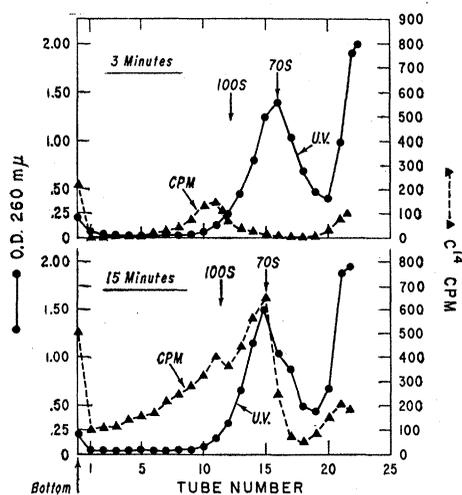


Fig. 3. Incorporation of  $C^{14}$ -phenylalanine into protein on ribosomes. A reaction mixture was prepared as described in the legend of Fig. 2, except that  $C^{14}$ -phenylalanine ( $7 \times 10^5$  counts/minute  $\mu\text{mole}$ ) was used.  $20\text{ m}\mu\text{mole}$  (pU) in  $H^3$ -poly U was added to  $0.25\text{ ml}$  reaction mixtures which were incubated at  $35^\circ\text{C}$  for the indicated periods;  $0.2\text{ ml}$  aliquots then were layered on sucrose gradients at  $3^\circ\text{C}$ .

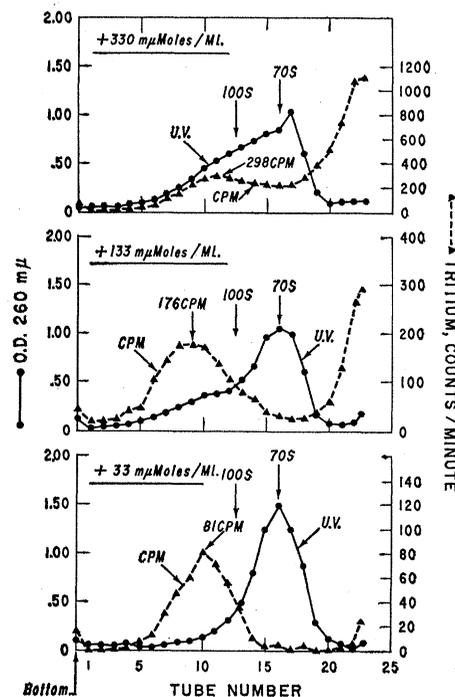


Fig. 4. Association of different concentrations of  $H^3$ -poly U with ribosomes. Note the difference in the  $H^3$  scale for the different diagrams. Ribosomes were washed three times by centrifugation at  $105,000g$  for 3 hours in  $0.1M$  magnesium acetate,  $0.05M$  KCl,  $0.1M$  tris HCl,  $pH$  7.8, and suspended in this solution at a concentration of  $0.8\text{ mg/ml}$ . The other components of the usual reaction mixture were omitted. The indicated amounts of (pU) in  $H^3$ -poly U were added per ml of ribosomes at  $3^\circ\text{C}$ , and  $0.2\text{ ml}$  aliquots were layered on sucrose gradients, centrifuged, and analyzed.

sents a "limiting concentration" of polymer, whereas  $330\text{ m}\mu\text{mole}$  of (pU) in poly U represents a large excess. When  $33\text{ m}\mu\text{mole}$  of (pU) in  $H^3$ -poly U was added, virtually all of the added polymer was associated with ribosomes whose sedimentation constants were greater than 100. Since the  $H^3$ -poly U was markedly heterogeneous, as shown in Fig. 1 of the preceding report (5), apparently both small and large poly U molecules can bind to ribosomes. No specific polynucleotide size seems to be required for binding. When large amounts of  $H^3$ -poly U were added, only part of the polymer associated with ribosomes and a significant fraction remained at the top of the gradient. Under such conditions, association with ribosomes whose sedimentation constants were less than 100 was also observed. It should be noted that even when an "excess" of poly U was added only a fraction of the ribosomes associated with the polymer to form heavy aggregates with sedimentation constants greater than 100.

The data of Fig. 4 also demonstrate that  $H^3$ -poly U can bind to washed ribosomes at  $3^\circ\text{C}$  in the absence of high energy phosphate compounds, phenylalanine, and  $105,000g$  supernatant solution.

Figure 4 demonstrates that the addition of  $H^3$ -poly U to a reaction mixture caused an aggregation of ribosomes. The aggregate sedimented faster than the 70 S ribosomal peak and was heterogeneous. The aggregation phenomenon may also be noted in the unincubated sample shown in Fig. 2. After incubation at  $35^\circ\text{C}$  for 3 minutes, most of the poly U could be recovered as mononucleotides (5) and the ribosomal aggregate largely disappeared (Fig. 2). In contrast to the evanescent aggregation induced by poly U, the addition of poly UG resulted in a more stable aggregate, which was found after even a 20 minute incubation at  $35^\circ\text{C}$ , (Fig. 5). The majority of  $C^{14}$ -phenylalanine directed into protein on ribosomes by poly UG was found on these aggregates.

To determine the origin of the aggregates,  $80\text{ m}\mu\text{mole}$  of nucleotide residues in poly U or poly UG was added to a 1.0 ml reaction mixture which then was centrifuged at  $3^\circ\text{C}$  in an analytical ultracentrifuge equipped with schlieren optics. A marked diminution in the size of the 70 S ribosome peak was observed

upon addition of polymer; whereas no significant alterations in the 30 S or 50 S peaks were found. Thus, the aggregates appear to be formed from 70 S ribosomes.

Since RNA from TMV stimulates  $C^{14}$ -amino acid incorporation into protein in the *E. coli* system (12), the association of  $P^{32}$ -RNA from TMV with ribosomes was studied (Fig. 6). When the viral RNA was added at 3°C, a peak was noted at approximately 60 S (Fig. 6). After centrifugation for 90 minutes, this peak was found to be the composite of a peak associated with 70 S ribosomes and another representing RNA from TMV unassociated with ribosomes. After incubating reaction mixtures at 35°C for 5 minutes a substantial portion of the RNA became associated with "heavy ribosomes" (see Fig. 6) and some RNA was degraded to small molecules which remained on top of the gradient. Unlike the synthetic polynucleotides, the association of RNA from TMV with "heavy ribosomes" occurred only after incubation at 35°C.

**Discussion.** "Messenger RNA" has been shown to associate with ribosomes (1, 2), particularly 100 S and 70 S ribosomes (2). Amino acids are then incorporated into protein on these particles (2, 13). We have now found that poly U both associates with ribosomes and directs  $C^{14}$ -phenylalanine into protein on these particles. These data further support the contention that poly U functions in the same

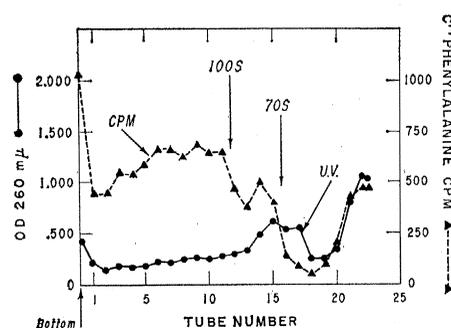


Fig. 5. Incorporation of  $C^{14}$ -phenylalanine into protein on aggregated ribosomes in the presence of poly UG. The reaction mixture contained the components described under Fig. 2, except that 0.05  $\mu$ mole of  $C^{14}$ -phenylalanine ( $4 \times 10^5$  count/min  $\mu$ mole) and 0.05  $\mu$ mole of 19 different  $C^{12}$ -L amino acids were added. After addition of 20  $\mu$ mole of nucleotide residues in poly UG, the reaction mixture was incubated for 20 minutes at 35°C and a 0.2 ml aliquot was layered on a sucrose gradient and centrifuged and analyzed as described in the text.

manner as natural "messenger RNA."

In 0.01M magnesium 70 S ribosomes reversibly aggregate to form 100 S ribosomes (6). Because of their interconvertibility it has not been possible to determine the extent of ribosomal aggregation necessary for protein synthesis. As shown in Fig. 1 very few 100 S ribosomes are found when these particles are suspended in the buffer and salt solution used in these experiments. This facilitated the demonstration that addition of poly U produces an aggregation of 70 S ribosomes into heavier particles. When a limiting amount of poly U is added to ribosomes all of the polymer is associated with these aggregates; and  $C^{14}$ -phenylalanine is initially incorporated exclusively on the small proportion of ribosomes which sediment at about 100 S. Thus, one way in which ribosomes with sedimentation constants greater than 70 may be formed is by the aggregation of 70 S ribosomes by "messenger RNA"; the aggregates formed in this manner may play an

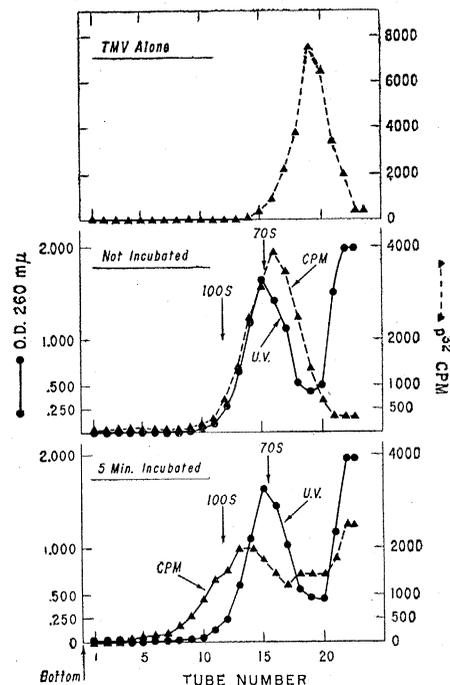


Fig. 6. Association of  $P^{32}$ -RNA of TMV with ribosomes. Reaction mixtures contained the components described under Fig. 2 except that they contained 0.05  $\mu$ mole each of 20  $C^{12}$ -L amino acids; 10  $\mu$ g  $P^{32}$ -RNA of TMV was added to each reaction mixture. Tubes were incubated at 35°C for the specified times and then were chilled in ice; 0.2 ml aliquots were layered on sucrose gradients at 3°C as was a sample of  $P^{32}$ -RNA of TMV suspended in 0.2 ml of 0.01M magnesium acetate, 0.05M KCl, 0.1M tris HCl, pH 7.8.

important role in protein synthesis. The appearance of  $C^{14}$ -protein on 70 S ribosomes after prolonged incubation with poly U may be due to disaggregation of the aggregates when the polymer has been degraded (5). It is interesting to note that in the experiments with poly UG more stable ribosomal aggregates are formed (Fig. 5), and that very little protein is found on 70 S ribosomes even after prolonged incubation.

Although ribosomal aggregates appear to be the site of protein synthesis, it is not clear whether aggregation of 70 S ribosomes is a necessary step in this process. Interpretation of the data should be made with caution since the sucrose density gradient experiments were performed at 3°C whereas protein synthesis was studied at 35°C, particularly since poly U acquires secondary structure below 5°C (14).

Since association of poly U with ribosomes occurs at 3°C in the absence of GTP, ATP, and supernatant solution obtained at 105,000g, it does not seem likely that this association is an energy requiring process. However, incubation of RNA from TMV in reaction mixtures was necessary before there was association with 100 S ribosomes. Similar results have been found with  $P^{32}$ -labeled turnip yellow mosaic virus (15). It therefore would appear premature to speculate about the possible requirement for energy in the attachment of "messenger RNA" to ribosomes.

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#### References and Notes

1. M. Nomura, B. D. Hall, S. S. Spiegelman, *J. Mol. Biol.* **2**, 306 (1960); S. Brenner, F. Jacob, M. Meselson, *Nature* **190**, 576 (1961); F. Gros, W. Gilbert, H. Hiatt, C. Kurland, R. W. Risebrough, J. D. Watson, *Nature* **190**, 581 (1961).
2. R. W. Risebrough, A. Tissières, J. D. Watson, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 430 (1962).
3. J. A. Boezi, E. T. Bolton, R. J. Britten, D. B. Cowie, B. J. McCarthy, J. E. Midgley, R. B. Roberts, *Carnegie Inst. Wash. Yearbook*, **60**, 263 (1961); E. Volkin, and L. Astrachan, *Virology* **2**, 433 (1956).
4. Abbreviations: ATP and GTP, adenosine and guanosine-5'-triphosphates respectively; Poly U, polyuridylic acid; (pU), uridylic acid residues in poly U; poly UG, polyuridylic-guanylic acid; TMV, tobacco mosaic virus; tris HCl, Tris (hydroxymethyl) amino-methane hydrochloride.
5. S. H. Baronides and M. W. Nirenberg, *Science*, this issue.
6. A. Tissières, J. D. Watson, D. Schlessinger, B. R. Hollingworth, *J. Mol. Biol.* **1**, 221 (1959).
7. R. J. Britten and R. B. Roberts, *Science* **131**, 32 (1960).

8. H. Fraenkel-Conrat, B. Singer, A. Tsugita, *Virology* 14, 54 (1961).
9. R. Martin and B. Ames, *J. Biol. Chem.* 236, 1372 (1961).
10. J. H. Matthaei, O. W. Jones, R. G. Martin, M. W. Nirenberg, *Proc. Natl. Acad. Sci. U.S.* 48, 666 (1962).
11. G. A. Bray, *Anal. Biochem.* 1, 279 (1960).
12. M. W. Nirenberg and J. H. Matthaei, *Proc. Natl. Acad. Sci. U.S.* 47, 1588 (1961); A. Tsugita, H. Fraenkel-Conrat, M. W. Nirenberg, J. H. Matthaei, *ibid.* 48, 840 (1962).
13. M. R. Lamborg and P. C. Zameznik, *Biochim. Biophys. Acta.* 42, 206 (1960); A. Tissières, D. Schlessinger, F. Gros, *Proc. Natl. Acad. Sci. U.S.* 46, 1450 (1960).
14. M. N. Lipsett, *Proc. Natl. Acad. Sci. U.S.* 46, 445 (1960).
15. R. Haselcorn, personal communication.

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## Application of an Additive Model to Impression Formation

**Abstract.** A simple mathematical model, based on the hypothesis that the psychological process underlying behavior is additive, was applied to the data of an experiment on the formation of personality impressions. Of 12 subjects, only three made responses which deviated by statistically significant amounts from responses predicted from the additive model, and these discrepancies were relatively small.

Recent work on the formation of impressions of personality, departing from an early gestalt orientation (1), has centered attention on the relation of the response to the individual stimuli from which the impression is formed. Predictive schemes involving correlation analysis, multiple regression, and weighted averages have yielded stimulus-response correlations ranging from medium to high (2). However, the associated problems of assessing the (statistical) significance and the meaning of the discrepancies from the predictive scheme have been given little or no attention.

This report describes the application of an additive model to the data obtained from an experimental design which permits joint evaluation of predicted response and of the discrepancy between observed and predicted values. Sets of three adjectives, describing hypothetical persons, were rated on a 20-point scale according to the "likeableness" of a person so described.

The basic stimuli presented to each test subject were nine common adjectives. These nine adjectives were split into three subgroups, each subgroup containing one adjective each of high, medium, and low "likeableness" value. For example, the three subgroups used for the first two subjects were as fol-

lows: (i) *good-natured, bold, humorless*; (ii) *level-headed, unsophisticated, ungrateful*; and (iii) *tactful, solemn, irresponsible*. These three subgroups were then used in a  $3^3$  factorial design yielding 27 possible combinations or sets of three adjectives such that each combination contained one adjective from each subgroup.

The adjectives were randomly selected from lists of 60 adjectives of each "likeableness" value, as determined from a separate normative study. Six groups of nine adjectives were used, and two subjects judged the combinations formed from each group for "likeableness" on the 20-point scale.

Twelve advanced-undergraduate males received \$5 each for serving five consecutive days. The eight initial (warm-up) sets of the day included two sets of very high value and two sets of very low value, presented to define the limits of the rating scale. An additional 27 sets were from the aforementioned six groups of adjectives; two subjects judged the combinations from each group. The eight initial sets and the 27 test sets were presented in a different random order each day, but each subject judged the same 35 sets each day.

The experimenter slowly read the adjectives of each set aloud. The card for the set was then handed to the subject, who read the adjectives aloud in reverse order and made his judgment. One set was presented each 20 seconds.

The basic purpose of the experiment was to study the degree to which the response to the sets of adjectives may be represented as the arithmetic mean of the psychological-scale values for the individual adjectives. Since three adjectives were used in each set, the following model is appropriate:

$$R_{ijk} = 1/3(a_i + b_j + c_k) + d_{ijk} + e,$$

$i, j, k, = 1, 2, 3$

Here  $R_{ijk}$  is the observed response to adjective set ( $i, j, k$ );  $a_i$ ,  $b_j$ , and  $c_k$  are the psychological values of these adjectives;  $d_{ijk}$  is the discrepancy from additivity; and  $e$  is the prevailing response variability (unreliability). If perfect additivity prevails, then the  $d_{ijk}$  terms will all be zero.

This model is standard in the analysis of variance (3), which yields significance tests of goodness of fit, and least-squares estimates of the psychological-scale values. Separate analyses were made for each test subject, since the values for the adjectives on the psycho-

Table 1. The summary statistical analysis (see text). The numerical suffixes to the initials of the subject designate the basic group of adjectives used for that subject. For 6 and 54 *df*, an *F* ratio of 2.28 is significant at the .05 level; for 20 and 54 *df*, an *F* ratio of 1.78 is significant at the .05 level.

Subject	Correlation: observed, predicted	<i>F</i> ratio		Error mean square, 54 <i>df</i>
		Additivity, 6 <i>df</i>	Nonadditivity, 20 <i>df</i>	
FF-1	.98	54.50	0.84	3.42
RH-1	.98	50.18	.48	1.67
AR-2	.99	223.94	1.65	0.57
AT-2	.97	52.33	0.94	1.75
JW-3	.99	126.87	.94	0.80
DB-3	.95	36.53	1.10	5.56
LL-4	.98	66.23	0.86	4.02
JZ-4	.96	54.75	1.37	2.02
BM-5	.97	31.41	0.67	6.11
FM-5	.95	74.65	2.53	1.47
MG-6	.94	105.97	2.84	2.90
NB-6	.95	74.88	2.45	2.79

logical scale vary with the individual. Only the data from tests given on the last three days were analyzed, tests on the first two days being considered practice.

Figure 1 is a plot of observed response versus response predicted on the basis of the additive model for three selected subjects, two of whom showed the largest and one of whom showed

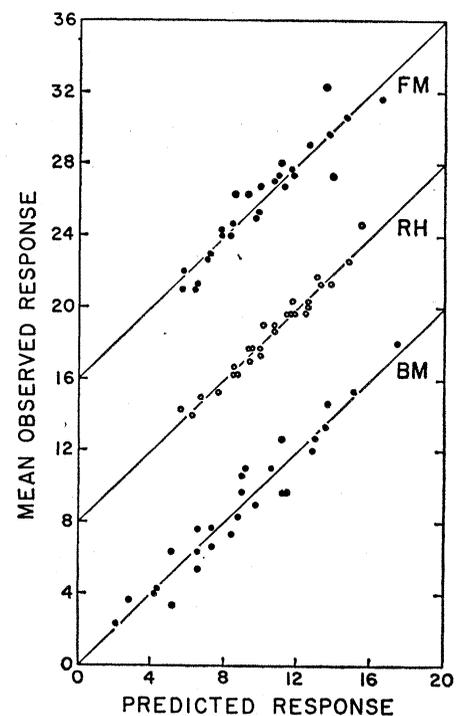


Fig. 1. Plots of observed response versus response predicted from the additive model for three selected subjects, two of whom showed the largest and one of whom showed the smallest deviations from the predicted values. The data points for subjects RH and FM are displaced upward by 8 and 16 units, respectively.