Interaction of *Escherichia coli* Ribosomal Ribonucleic Acid with Synthetic Polynucleotides. Sedimentation Properties, and Thermal Stability as Measured by Fluroescence Polarization*

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ABSTRACT: The interaction of some synthetic polyribonucleotides with *Escherichia coli* ribosomal ribonucleic acid (RNA) has been found to be dependent upon the availability of free bases in the polynucleotide and the RNA. Thus a mixture of polyribouridylic acid (poly-U) and polyriboadenylic acid (poly-A) (U/A = 2:1) does not interact with ribosomal RNA, while poly-U alone does. RNA treated with formaldehyde does not react with poly-U. It has been found that both the 23 S and 17 S fractions of ribosomal RNA react with poly-U and that the reaction of ribosomal RNA is *p*H dependent. The thermal profile of polarization of fluorescence of an acriflavine conjugate of poly-U complexed with ribosomal RNA indicates that the bonds between the interacting nucleotide pairs are not as extensive as those in a poly-(U + A) 1:1 complex.

It has been known for some years now that the chief ribonucleic acid components of *Escherichia coli* ribosomes are high molecular weight species with sedimentation coefficients of approximately 16 and 23 S (Kurland, 1960), and that they possess a considerable degree of helical content which is due to intramolecular hydrogen bonds (Fresco *et al.*, 1960; Doty, 1961). The recent demonstrations (Yanofsky and Spiegelman, 1962; McCarthy and Bolton, 1964) that *E. coli* RNA hybridizes with homologous DNA clearly points out the ability of *E. coli* ribosomal RNA to form hydrogen-bonded structures with non-RNA nucleotide sequences of the appropriate complementarity. We should like to present experiments which show that *E. coli* RNA is able to form complexes with synthetic polynucleotides as well and that the bonds joining the RNA and homopolynucleotides are internucleotide bonds.

Materials and Methods

Extraction of Ribosomal RNA from E. coli Ribosomes. Ribosomal particles were isolated from *E. coli*, W3100, following the procedure of Tissières *et al.* (1959). The ribosomes were disrupted and the RNA was extracted by vigorous mechanical shaking at room temperature of a 1:1 (v/v) mixture of ribosomes and redistilled H_2O-saturated phenol (Merck, Silver Label). The aqueous supernatant solution was removed by aspiration and the phenol treatment was repeated five times. The aqueous supernatant from the last phenol treatment was adjusted to *p*H 4.6 and 0.1 M acetate with 1 M acetate buffer, and magnesium acetate to a final concentration of 0.01 M was added. Two volumes of ice-cold ethanol were then added; the suspension was allowed to stand 30 minutes in the cold. Finally the flocculent RNA precipitate was harvested by 20 minutes' centrifugation at 4° at a speed of 2400 rpm in an International centrifuge. This procedure was repeated three times. The RNA pellet was taken up in a minimum volume of 0.001 M KCl and adjusted to *p*H 4.6 with stock acetate as before. Aliquots of the RNA were used for the determination of residual protein and DNA by the procedures of Lowry *et al.* (1951) and Barton (1956), respectively. In the several preparations of RNA used in this investigation both DNA and protein were generally less than 0.3% (w/w).

Synthetic Polynucleotides. The preparation of the radioactive polynucleotides from their respective nucleoside diphosphates utilizing the enzyme polynucleotide phosphorylase has been previously described (Barondes and Nirenberg, 1962). Two preparations of [H^3]poly-U were employed in this study; [H^3]poly-U (no. 2) had a specific radioactivity of approximately 160 cpm/μg. Poly-U (no. 1) had approximately one-tenth as high a specific activity. Poly-C (C^{14}) had a specific activity of 870 cpm/μg.

Sucrose Gradient Centrifugation. Sucrose density-gradient centrifugations were performed either in the SW-39 swinging-bucket rotor or the SW-25 rotor fitted with adaptors to allow the use of No. 2232 celluloid tubes. Generally, 0.2 ml of ice-chilled reaction mixture

* *Received January 25, 1965.* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

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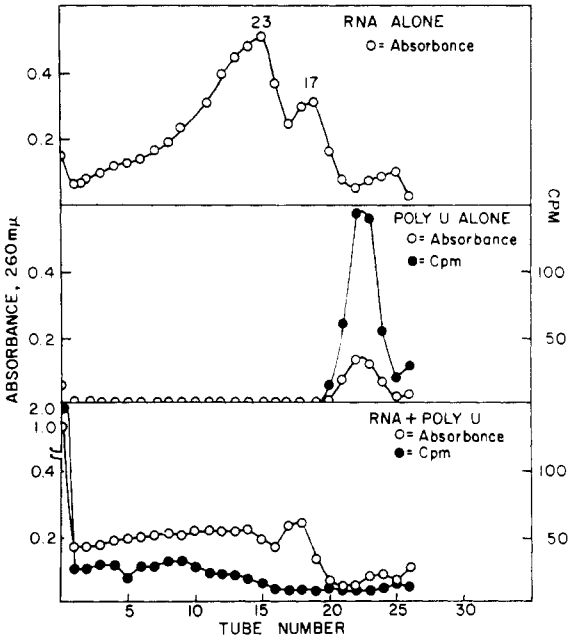

FIGURE 1: Interaction of unfractionated *E. coli* ribosomal RNA and [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/poly-U = 20 (w/w). Incubated 10 minutes at 37°.

removed from the buckets, the bottoms were pierced by means of a device similar to that described by Martin and Ames (1961), and approximately 12-drop fractions were collected. About 0.8 ml of Tris-Mg^{2+}-KCl buffer was added and the absorbance at 260 mμ was estimated in a Beckman Model DU spectrophotometer using 1.0-ml capacity matched cells with a 1-cm light path. Between readings the cuvets were rinsed three times with double-distilled water, then with distilled methanol, and then were air dried. After the absorbance had been determined, 15 ml of Bray's solution (Bray, 1960) was added and the radioactivity of each fraction was determined in a Nuclear-Chicago counter Series 720 or in a Packard liquid scintillation counter. It was found that identical samples gave different counting rates in the two counters; the difference was generally of the order of 20%. No correction has been made for this discrepancy since polynucleotide controls were always performed with each run. The sedimentation coefficients of the various RNA fractions were estimated by the technique of Martin and Ames (1961) using the value of the sedimentation coefficient of the [H^3]poly-U no. 1 which had been determined in the Spinco Model E analytical ultracentrifuge employing ultraviolet optics (Barondes and Nirenberg, 1962).

Polarization of Fluorescence. The fluorescent dye,

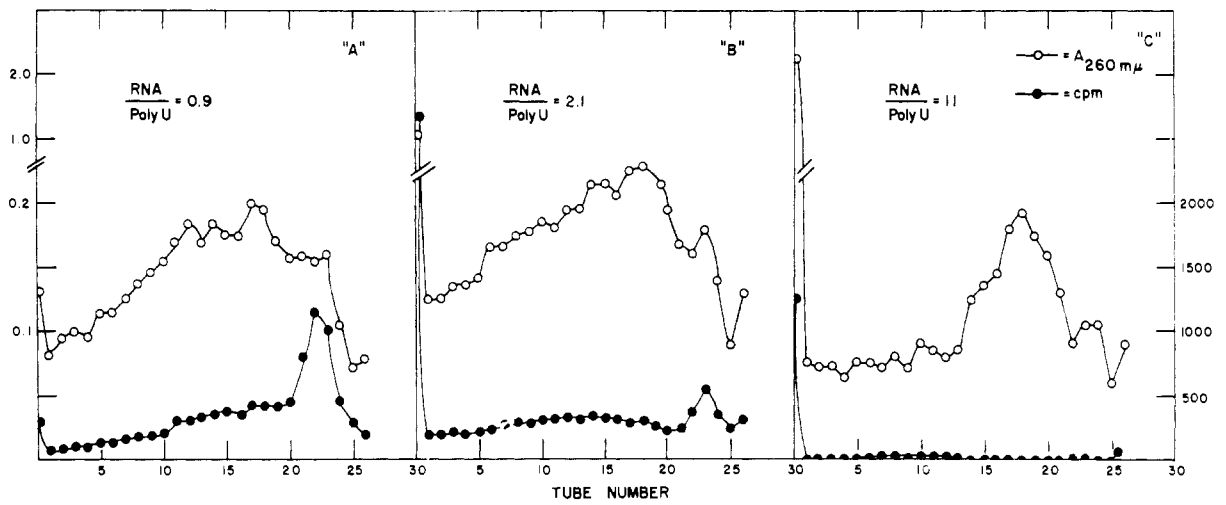

FIGURE 2: Interaction of unfractionated *E. coli* ribosomal RNA and [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/[^{3}H]poly-U as indicated. Incubated 2 minutes at 37°.

was applied to approximately 5 ml of a 5–20% sucrose (National Bureau of Standards Calorimetric Grade Sucrose) gradient, 0.014 M in Mg^{2+}, 0.05 M in KCl, and 0.1 M in Tris buffer, *p*H 7.8. Centrifugation took place in the Spinco Model L preparative centrifuge at an average temperature of 3° for periods of 13–19 hours at computed speeds of 17,000–21,000 rpm when using the SW-25 rotor and 3–5 hours at 37,000 rpm when using the SW-39 rotor. At the end of the run the tubes were

acriflavine (National Dye Div., Allied Chemical and Dye Corp.), was coupled to a nonradioactive sample of poly-U (Miles Chemical Co.) by the procedure described by Churchich (1963). Noncovalently bonded dye was removed from the poly-U by repeated ethanol precipitation, dialysis, and passage through a Sephadex G-25 column. The properties of the poly-U-acriflavine conjugate are the subject of a separate report (Millar and Steiner, 1965). Measurements of the polarization of

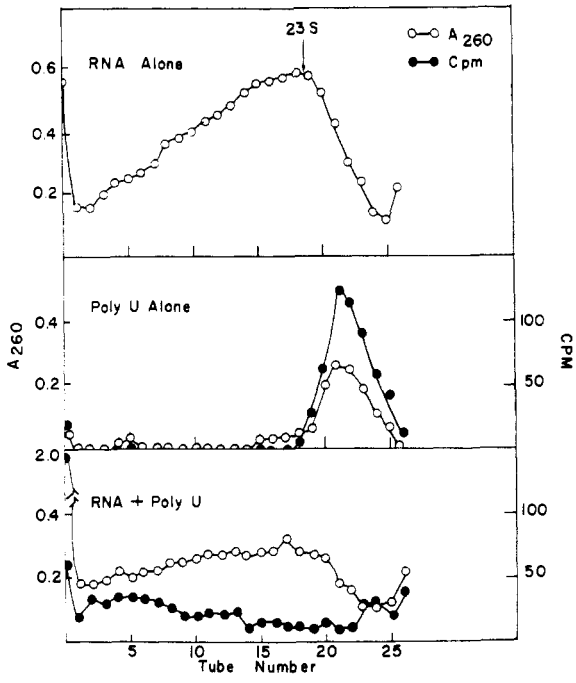

FIGURE 3: Interaction of 23 S *E. coli* ribosomal RNA and [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/[^{3}H]poly-U = 10 (w/w). Incubated 2 minutes at 36°.

fluorescence were performed in a cell compartment of a specially modified Brice-Phoenix universal light-scattering photometer (Steiner and Edelhoch, 1961). Unpolarized incident light of wavelength 436 mμ was employed and the fluorescent emission was passed through a No. 3334 Corning cutoff filter prior to impinging upon the polaroid analyzer which was inserted before the photomultiplier tube.

The polarization of fluorescence of a labeled polynucleotide may be described by equation (1)

$$\frac{(1/P + 1/3)}{(1/P_0 + 1/3)} = 1 + \frac{\tau}{\rho_h} \equiv R \qquad (1)$$

where P = polarization of fluorescence, P_0 = limiting value of P at high values of ρ_h, τ = excited lifetime of the fluorescent label, ρ_h = the harmonic mean of the characteristic relaxation times of the polynucleotide approximated as an ellipsoid:

$$\frac{1}{\rho_h} = \frac{1}{3}\left(\frac{1}{\rho_1} + \frac{1}{\rho_2} + \frac{1}{\rho_3}\right)$$

where ρ_1, ρ_2, and ρ_3 are the three relaxation times corresponding to the three axes of the ellipsoid. If the polynucleotide possesses spherical symmetry, then ρ_1 =

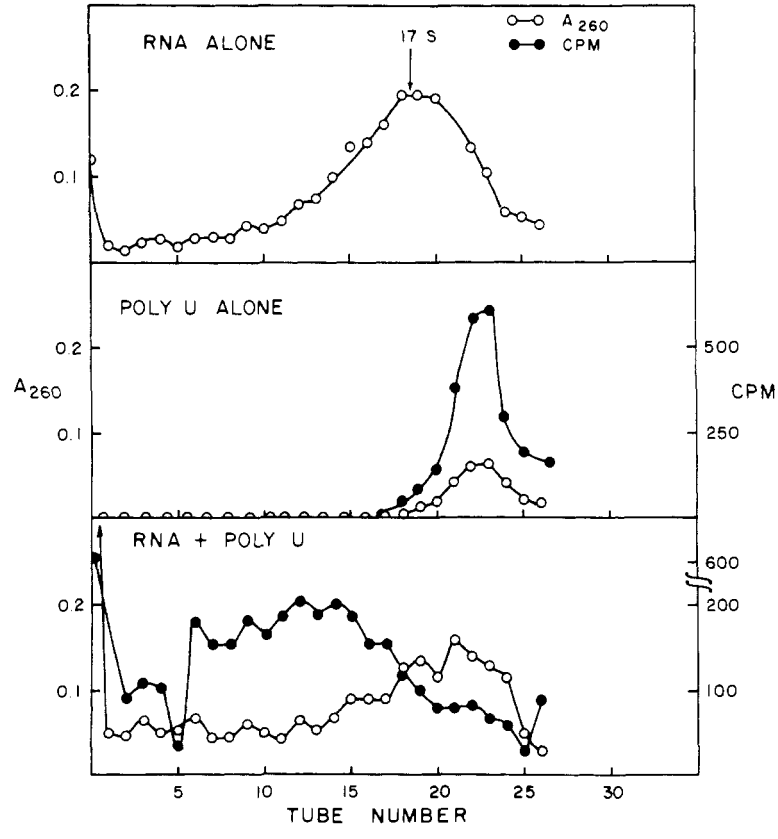

FIGURE 4: Interaction of 17 S *E. coli* ribosomal RNA and [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/[^{3}H]poly-U = 4 (w/w). Incubated 2 minutes at 36°.

$\rho_2 = \rho_3$ and equation (1) may be written:

$$R = 1 + \frac{R'T\tau}{\eta V} \qquad (2)$$

where R' is the gas constant, η is the solvent viscosity, V is the molar volume of the polynucleotide, and T is the temperature in degrees Kelvin.

While it is probably incorrect to assume that the polynucleotides under study here possess spherical symmetry, in practice (Steiner and Edelhoch, 1962) it has been found that equation (2) is valid for many nonspherical proteins and that deviations from linearity can be accounted for on the basis of changes in molecular configuration or association.

Results

Interaction of Unfractionated RNA with Poly-U and Poly-C. Figure 1 shows the results of a sucrose-gradient analysis of a mixture of RNA and the synthetic polynucleotide, poly-U. The mixture was incubated for 10 minutes at 37° prior to being chilled and layered on the gradient. Figure 1 shows that *E. coli* ribosomal RNA and poly-U react to form products of a polydisperse nature and that a significant quantity of radioactivity and absorbance has sedimented to the bottom of the tube, showing that the RNA-poly-U complex contains fractions of considerably higher sedimentation coefficients than the RNA itself.

It was found that under identical incubation conditions a mixture of RNA and poly-C (RNA/[C^{14}]poly-C = 2 [w/w]) also interacted but that the complex did not exhibit the very high sedimentation rates of the poly-U-RNA complex. In this case, the bulk of the reaction product was distributed over the region in which native ribosomal RNA sedimented.

We attempted to find an interaction between poly-A and *E. coli* ribosomal RNA but, confirming the prior observation of Bautz (1963), it was found that heating poly-A solutions in the presence of magnesium ion resulted in the formation of a visible turbidity. In general this turbidity disappeared upon chilling. Several experiments were performed and the results indicated a weak interaction between ribosomal RNA and poly-A. The possibility exists that the observed interaction might be due, in whole or part, to physical entanglement of the RNA by extended poly-A strands, i.e., a coprecipitation phenomenon. If the incubation is performed at 0°, there is no visible turbidity and no interaction. This experiment suggests that highly ordered poly-A will not react with RNA. Observations have been made (Okamoto and Takanami, 1963) concerning the lack of interaction between poly-A and *E. coli* ribosomes.

Effect of the Weight Ratio of Reactants on the Reaction. In an attempt to investigate the stoichiometry of the RNA-poly-U interaction, the following experiment was performed. To three test tubes containing [H^3]poly-U, unfractioned *E. coli* ribosomal RNA was added in amounts such that the weight ratio of RNA to poly-U was varied from approximately 0.9 to 11. The three mixtures were then incubated 2 minutes at 37°, chilled in ice, and centrifuged through a 5–20% sucrose gradient. The results are shown in Figure 2. In Figure 2A and B the amounts of [H^3]poly-U are the same. But B has two times as much RNA as does A. It is clearly shown that doubling the amount of RNA results in an approximately 9-fold increase in absorbance and radioactivity sedimenting to the botton of the tube. Figure 2C demonstrates the sedimentation profile of a mixture of RNA and [H^3]poly-U in the ratio of approximately 11:1. In this incubation mixture the RNA added is slightly less than in A and B and [H^3]poly-U added is one-seventh that of A and B. Under these conditions, 96% of the added radioactivity sediments to the bottom of the tube with a concomitant increase in material absorbing at 260 mμ.

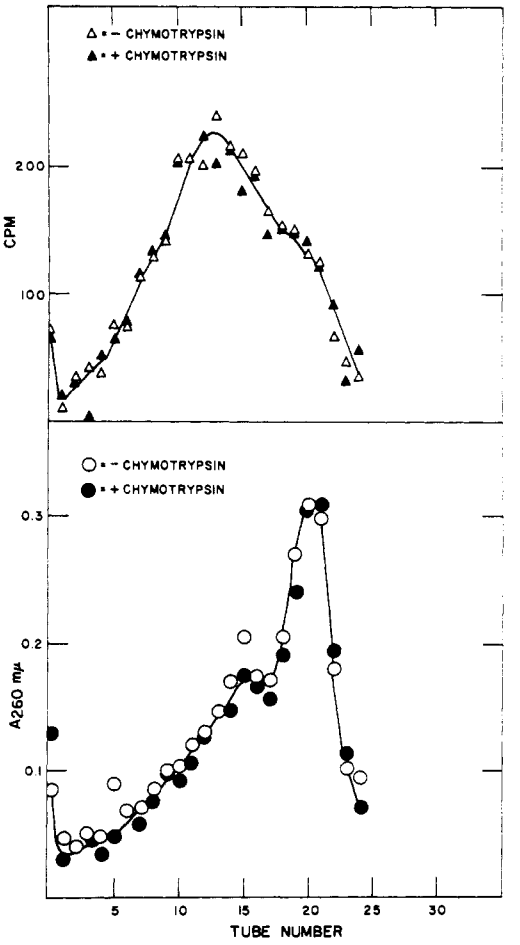

FIGURE 5: Effect of chymotryptic digestion on the interaction of 17 S *E. coli* ribosomal RNA with [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/[^{3}H]poly-U = 15 (w/w). Incubated 30 minutes at 36°.

The simplest explanation for these observations is that one molecule of poly-U can bind with two or more

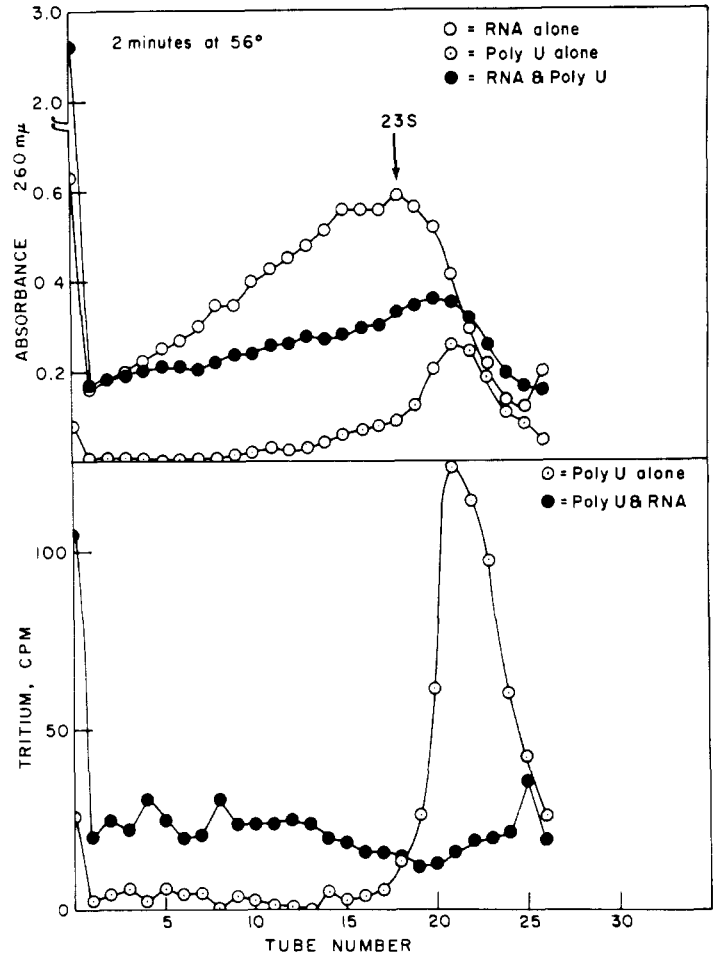

FIGURE 6: Effect of temperature on the interaction of 23 S *E. coli* ribosomal RNA with [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 Mg^{2+}. RNA/[^{3}H]poly-U = 10 (w/w). Incubated 2 minutes at 56°.

strands of RNA. At high RNA-to-poly-U ratios, then, multistranded complexes of poly-U and RNA with correspondingly high sedimentation constants are formed. When the ratio of RNA to poly-U approaches one, few multistranded complexes are formed and the resulting sedimentation profile is one in which low molecular weight complexes predominate.

Reactions of the 17 S and 23 S RNA Fractions with Poly-U. From the gradient results presented here it is difficult to determine whether the homopolymers are reacting with both the 23 and 17 S RNA fractions (Kurland, 1960; Littauer and Eisenberg, 1959) or with but one of these species. Since there is evidence that these two fractions are synthesized under the control of different cistrons (Yanofsky and Spiegelman, 1962) and that there are differences in both sequence and base composition (Aronson, 1962; Midgeley, 1962) between the fractions, the following operations were performed: Several 0.5-ml portions of unfractionated RNA (8 mg/ml) were layered onto 25 ml of a 5–20% sucrose gradient (*p*H 4.6, 0.1 M acetate) and centrifuged in the SW 25 swinging-bucket rotor for 17 hours at a computed speed of 18,100 rpm at an average temperature of 3°. The absorbance at 260 mμ of the fractions collected as previously described was determined and the tubes containing the bulk of the 23 and 17 S fractions were pooled. Overnight centrifugation at 40,000 rpm in the Spinco 40 head pelleted the fractions. The pellets were then taken up in a minimum volume of 0.001 M KCl, 0.01 M acetate buffer, *p*H 4.6, and dialyzed overnight against the same buffer.

As discussed above, the influence of the secondary structure of polynucleotides on both the rate and extent of polynucleotide interactions appears to be large (Ross and Sturtevant, 1962; Rich, 1958). Thus it was decided to employ poly-U, whose structure at room temperature and above is a random coil, in comparing the reactivity of the two chief fractions of *E. coli* RNA. Figures 3 and 4 show the results of incubating 23 and 17 S RNA with poly-U.

It can be seen that both fractions of RNA interact with poly-U and that the products of the reaction of 23 S RNA with poly-U possess higher sedimentation coefficients than are found for the reaction with 17 S RNA. This presumably reflects the difference in sedimentation coefficients of the RNA species themselves although we

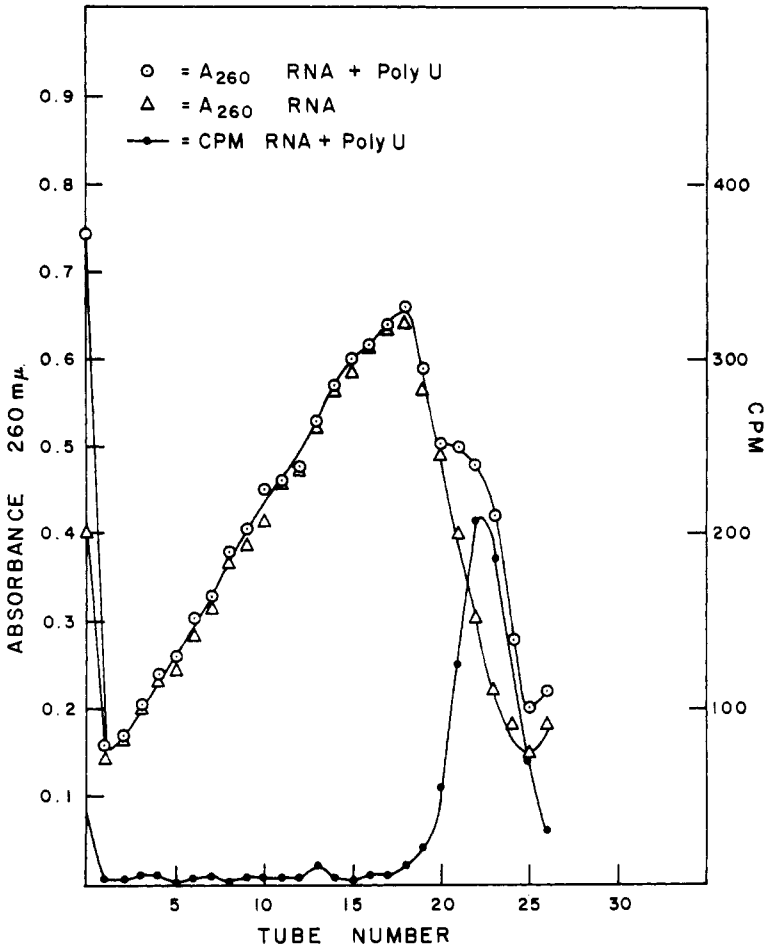

FIGURE 7: Effect of formaldehyde pretreatment on the interaction of 23 S *E. coli* ribosomal RNA with [^{3}H]poly-U. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/[^{3}H]poly-U = 10 (w/w). Incubated 2 minutes at 36°.

cannot rule out different degrees of affinity for poly-U by the two RNA's.

Effect of Chymotryptic Digestion upon the Interaction. It is conceivable that the small amount of ribosomal protein, perhaps tightly linked to the RNA fractions, resistant to the purification procedures and thus present during the incubation of the RNA with the synthetic polynucleotides, served as nuclei for an interaction formally similar to that observed for *E. coli* ribosomes and synthetic polynucleotides (Bautz, 1963). To eliminate this possibility, 17 S *E. coli* RNA was incubated with [H^3]poly-U in Tris-KCl-Mg^{2+} buffer in the presence and absence of 25 μg of chymotrypsin (Worthington Biochemical Corp.) for 30 minutes at 37°. The incubation mixtures were then chilled in ice and centrifuged in the usual sucrose gradients. Figure 5 shows that a good portion of the radioactivity migrates with the 17 S peak and a lesser fraction migrates ahead of the peak. The distribution of counts is probably due to the ratio of polynucleotide to RNA employed (see Figure 3). The fact that the absorbance and radioactivity profiles are superimposable in the presence and absence of chymotrypsin leads us to conclude that the complex formation between ribosomal RNA and synthetic polynucleotides is controlled by the polyribonucleotide structures themselves and not to traces of residual ribosomal protein.

Effect of Temperature upon the Reaction of 23 S and 17 S RNA with Poly-U. The partially helical character of ribosomal RNA has been amply documented (Fresco *et al.*, 1960; Doty, 1961). To determine the effect of "melting out" a portion of these helical regions upon the interaction of 23 S RNA with poly-U, mixtures of RNA and poly-U were incubated at 56° for 2 minutes and then quenched in an ice bath. As seen in Figure 6, there is no gross change in the extent of reaction. Mixing and incubating at 16° followed by an ice bath quench resulted in an only slightly diminished interaction. Exposing 17 S RNA to the same treatment produced no large change in the extent of interaction. Exposure to higher temperature, maximum attempted 90°, was tried with both RNA fractions but, in agreement with the results of Kurland (1960) and Petermann and Pavlovic (1963), some degradation of the RNA occurred

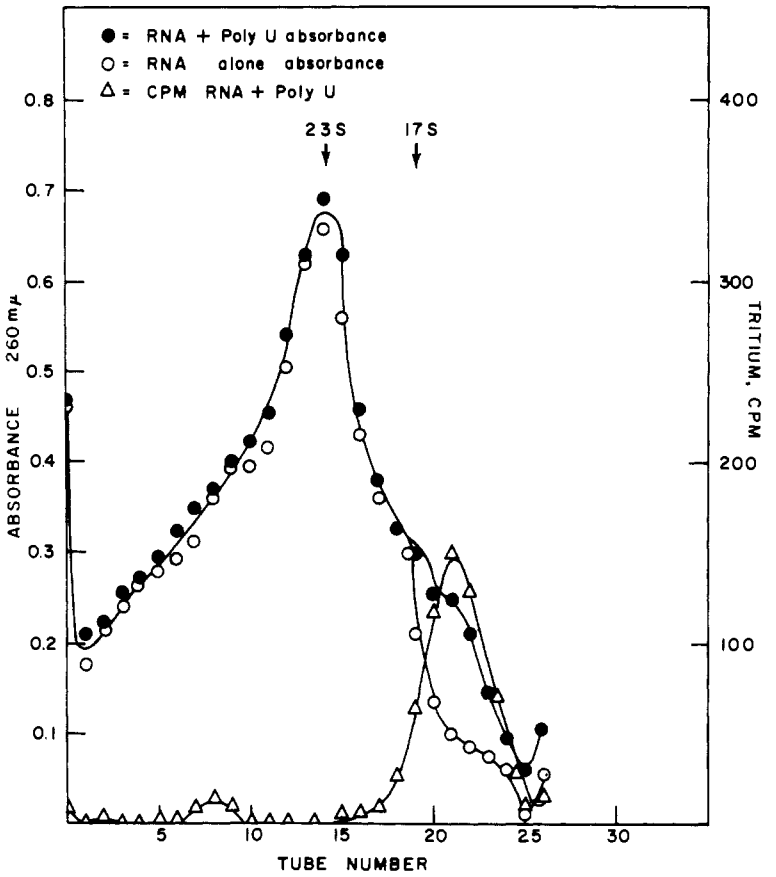

FIGURE 8: Effect of acid *p*H on the interaction of unfractionated *E. coli* ribosomal RNA with [^{3}H]poly-U. Medium: 0.1 M KAc, *p*H 4.08, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/[^{3}H]poly-U = 11. Incubated 2 minutes at 36°.

resulting in a broad distribution of sedimentation coefficients and an apparent enrichment of the 16 S peak. But in one case where extensive thermal fragmentation of the 23 S RNA had occurred, the reaction between poly-U and the fragments still proceeded to a measurable extent.

These results suggest that for complex formation to occur with poly-U, the RNA does not have to be in a fully extended, that is, random-coil, configuration, and that the sites of interaction available at 16° are the same as those available at 37°.

Effect of Formaldehyde Pretreatment on the Interaction. It has been demonstrated by Steiner and Beers (1959) that pretreatment of poly-A with formaldehyde prevents completely its interaction with poly-U. It is seen in Figure 7 that treatment of 23 S RNA with 2% formaldehyde for 16 hours at room temperature in a medium 0.014 M with respect to Mg^{2+}, 0.05 M with respect to KCl, and 0.1 M with respect to Tris, *p*H 7.8, also blocks the interaction of RNA with poly-U. The formaldehyde-treated RNA showed the expected increase in absorption at 258-260 mμ (Okamoto and Takanami, 1963) compared to untreated RNA at the same concentration.

*Effect of p*H *upon the Interaction.* It has been demonstrated by Steiner and Beers (1959) that the reaction between poly-U and poly-A diminishes rapidly with decreasing *p*H values and at *p*H values acid to 5.3 becomes immeasurably slow. As can be seen in Figure 8, incubation of unfractionated *E. coli* ribosomal RNA with poly-U at acid *p*H resulted in a sedimentation pattern essentially identical to the sum of the unmixed reactants with respect to absorbance and radioactivity profiles. Since the *pK* of the uracil residue in poly-U is approximately 9.5, this result suggests that the effect of acid *p*H upon the interaction is localized to a group or groups within the RNA molecule and that the presence of this charged group(s) renders interaction with poly-U impossible.

It is of interest to note the apparent diminution in the 17 S RNA peak at *p*H 4.08. Petermann and Pavlovic (1963), in an elegant study of the ribonucleic acid obtained from rat liver ribosomes, have reported a similar phenomenon which they demonstrate is due to the aggregation of 17 S RNA to faster-sedimenting species (28 S RNA). The reaction is favored by high hydrogen ion concentration, high ionic strength, and low temperature.

Polarization of Fluorescence of the Complexes. In general, an increase in the size or internal organization of the kinetic unit to which a fluorescent label is attached results in a change in polarization of fluorescence

(Steiner and Edelhoch, 1962). Thus it would be expected that combination of a fluorescently tagged poly-U molecule ($s_{20,w}$ approximately 5 S) with either the 17 or 23 S fractions of *E. coli* RNA would result in changes in the polarization of fluorescence of the poly-U-dye conjugate. Poly-U is particularly appropriate for this type of study since, as discussed previously, the structure of poly-U at room temperature is that of a random coil and measurements of the thermal dependence of the polarization of fluorescence of a poly-U-acriflavine conjugate show the polarization to be small at room temperature (Millar and Steiner, 1965). Accordingly, unfractionated *E. coli* ribosomal RNA and the isolated 17 and 23 S fractions were titrated at room temperature with poly-U–acriflavine dye conjugate. It was observed that with increasing concentration of the conjugate polarization of fluorescence appeared. This result can only be taken to mean interaction between the conjugate polynucleotide and the RNA molecule was occurring. When the value of polarization had reached an apparent maximum (it was difficult to estimate the quantity of polynucleotide required to saturate the RNA molecules, owing to the low fluorescent yield) the solutions were taken through a thermal cycle and the results are shown in Figure 9. The large drop in polarization which starts at values of τ/η of approximately 400 for the ribosomal RNA-poly-U complex is taken to reflect changes in the internal rigidity of the complexes and their eventual breakup at higher temperatures. However, it must be pointed out that a selective freeing of the labeled terminal residue from the complex and the consequent acquisition of a localized rotational freedom would heavily weight the apparent polarization in such a manner as to indicate wrongly dissolution of the bulk of the complex. But, as is shown in detail elsewhere (Millar and Steiner, 1965) for poly-U, poly-A, and their complex, the thermal profile of polarization of fluorescence faithfully reflects the breakup of organized helical structure.

Of particular interest is the shape of the $1/p + {}^1/_3$ versus T/η curve describing the transition from an ordered structure at low temperature to the point at which polarization of fluorescence is vanishingly small. Comparison of these results with those obtained for a 1:1 mixture of poly-U and poly-A, whose thermal breakup is highly cooperative (Millar and Steiner, 1965), suggests that the bonding sites in the RNA-poly-U complexes are not extensive in length and possibly that not all bond sites are of the same length. The polarization of fluorescence thermal profile for a complex formed between a copolymer of adenine and uracil residues (U/A $\sim$ 3:1)[1] is significantly displaced to lower transition temperatures. It is assumed that this reflects the low molecular weight of this copolymer ($s_{20,w} \sim 3$ S) and possibly the statistical distribution of adenine residues in the copolymer.

However, the outstanding fact derived from this experiment is the thermal stability of the complex. Thus at temperatures above 33° ($T/\eta \sim 400$) the complex begins sharply to gain rotational freedom. If this observation is interpreted as reflecting only complex breakup, it suggests that at physiological temperatures (37°, $T/\eta \sim 440$) the complex bonds approach minimum stability.

A mixture of poly-U-dye conjugate and unfractionated RNA which had been treated with formaldehyde as described above showed no polarization of fluorescence in agreement with the previously discussed sucrose-gradient analysis of a similar experiment.

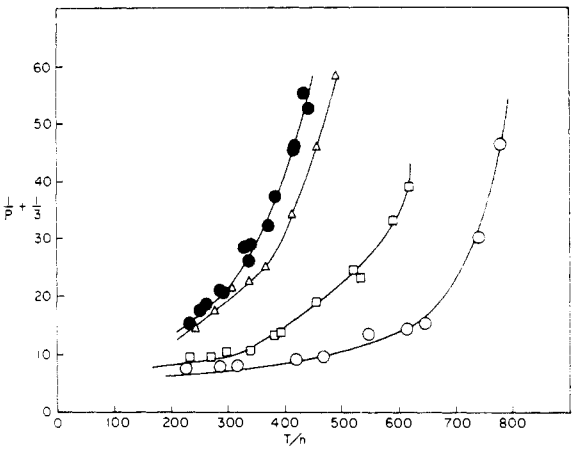

FIGURE 9: Thermal profile of the polarization of fluorescence of complexes. (●), poly-U-dye conjugate alone; (△), a 1:1 complex of poly-U-dye conjugate and a copolymer of A and U residues (A/U ⌢ 3:1); (○), 1:1 complex of poly-U-dye conjugate–poly-A; (□), ribosomal RNA + poly-U-dye conjugate, RNA/poly-U = 20 (w/w).

Influence of Secondary Structure of Polynucleotides upon the Reaction. Evidence that the secondary structure of polynucleotide influences the interaction is shown in Figure 10. Here unfractionated *E. coli* ribosomal RNA and a preformed triple-stranded structure of poly-A plus poly-U have been incubated at 36° for 10 minutes. It can be seen that the addition of this polynucleotide complex to RNA has no effect upon the sedimentation behavior of RNA. It is known (Steiner and Beers, 1959) that in both the doubly and triply stranded structures obtained from the interaction of poly-U and poly-A that a high degree of hydrogen bond formation occurs. The most probable explanation for the lack of interaction between the poly-U-poly-A complex and ribosomal RNA is that most if not all the hydrogen bonding sites in the complex are not available for reaction with potential sites in the RNA molecule.

It is noteworthy that Okamoto and Takanami (1963) have reported that a complex of poly-U and poly-A will not bind to 30 S ribosomes. We have also observed that such complexes do not bind to 70 S ribosomes.

[1] A gift from Dr. Charles O'Neal.

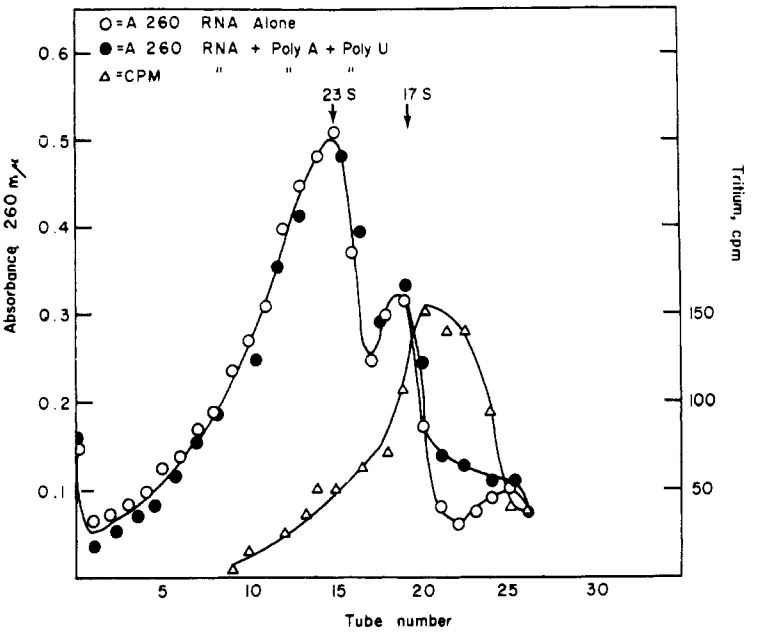

FIGURE 10: Effect of secondary structure on complex formation. Medium: 0.1 M Tris buffer, *p*H 7.8, 0.05 M KCl, 0.014 M Mg^{2+}. RNA/poly(H^3 U + A) = 12 (w/w). Incubated 10 minutes at 37°.

Discussion

The experiments presented in this paper clearly show that interaction between *E. coli* ribosomal RNA and synthetic polynucleotides occurs and suggest that forces responsible for the reaction are base pair interactions formed between the bases of the polynucleotide and those of the RNA. We are led to this point of view by the following considerations: (1) The fact that formaldehyde pretreatment of RNA completely blocks interaction with poly-U. Formaldehyde is known to react with the amino groups of nucleotide bases (Steiner and Beers, 1959). In the RNA molecule there are three residues which can react with formaldehyde, e.g., adenine, guanine, and cytosine. Uracil residues, possessing no free amino group, of course do not react with formaldehyde. A base so treated would not be expected to participate in hydrogen bonding. Indeed, formaldehyde pretreatment of poly-A completely blocks its interaction with poly-U. (2) The effect of acid *p*H values on the interaction of poly-U and ribosomal RNA: As mentioned, the *pK* of the uracil residue is in the region of 9.5, while the guanine, adenine, and cytosine bases are all titrated at *p*H values acid to *p*H 5. If the locus of the proton binding is, for example, at the N_1 position of the adenine residue, the latter would not be able to serve as an acceptor of a hydrogen bond. If the proton is bound by amino groups of the bases, the latter could serve as hydrogen bond donors only with distortion of bond angles (Steiner and Beers, 1961). (3) The failure of a preformed complex of poly-U and poly-A to react with RNA also indicates that, for complex formation to occur, the bases in the synthetic polynucleotide must not be extensively paired with other bases. In this connection it is interesting to note the recent report concerning the interaction of denatured DNA and synthetic polynucleotides (Ôpara-Kubinska *et al.*, 1964). It was observed that poly-A did not interact with denatured DNA but that poly-U did. It was also observed that simultaneous addition of poly-A with poly-U inhibited complex formation.

The variation of the polarization of fluorescence with temperature of the poly-U-dye conjugate–RNA complexes and the virtual absence of structure at moderate temperatures suggest that the bonds of the complex are limited in extent. In this connection it is noteworthy that the complex formed between pApApA and poly-U has a T_m of 17° and that formed between pApApApA and poly-U has a T_m which varies between 18 and 30° depending upon ionic strength (Lipsett *et al.*, 1961). This property of the RNA-poly-U complex is likely to be a consequence of the nucleotide sequence of RNA, since there is no reason to suppose that there is any distinction between residues in a structureless homopolymer such as poly-U.

The absence of observable structure in this complex at temperatures above 35–40° is consistent with the observation that there was little difference in reaction, as measured by sucrose-gradient centrifugation, when poly-U-RNA mixtures were incubated at either 36 or 56°. It is possible that in the 56° incubation mixture complex formation between RNA and poly-U took place as the reaction mixture cooled.

Thermal profiles of RNA obtained in this laboratory show a small increase in the absorption at 56° relative to the value at 20°. These results indicate that a fraction of the RNA secondary structure has been disrupted. Therefore, in the limited temperature range studied, it seems that no new binding sites with a ther-

mal stability different from those available at 36° had appeared. It is of course impossible to rule out the likelihood that a slow annealing process would have resulted in greater interaction. These results further raise the possibility that in the temperature range of 16–37° a large proportion of the bond sites in the RNA molecule are located in the nucleotide sequences which are uninvolved in intramolecular hydrogen bonding. Since the existence *in vivo* of some of the homopolymers studied here has been reported, it is possible that such interactions may be of importance in the synthesis of protein in the cell.

Finally, it should be pointed out that according to current usage the term *hybridization* should not be applied to the interactions described in this report. Hybridization involves the matching, in perfect register, of long sequences of differing nucleotide pairs, and since this cannot be so in the present case, the terms *interaction* or *complex formation* are more fitting.

Added in Proof

After this manuscript had been prepared we learned of investigations similar to those reported here (Watson, 1964).

References

Aronson, A. I. (1962), *J. Mol. Biol. 5*, 453.

Barondes, S. H., and Nirenberg, M. (1962), *Science 138*, 810.

Barton, K. (1956), *Biochem. J. 62*, 315.

Bautz, E. K. F. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28*, 205.

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Churchich, J. E. (1963), *Biochim. Biophys. Acta 75*, 275.

Doty, P. (1961), *Biochem. Soc. Symp. Cambridge, Engl. 21*, 8.

Fresco, J. R., Alberts, B. M., and Doty, P. (1960), *Nature 188*, 98.

Kurland, C. G. (1960), *J. Mol. Biol. 2*, 83.

Lipsett, M. N., Heppel, L. A., and Bradley, D. F. (1961), *J. Biol. Chem. 236*, 857.

Littauer, V. Z., and Eisenberg, H. (1959), *Biochim. Biophys. Acta 32*, 320.

Lowry, O. H., Rosebrough, N. J., Farr, L. A., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

McCarthy, B. J., and Bolton, E. T. (1964), *J. Mol. Biol. 8*, 184.

Martin, R., and Ames, B. (1961), *J. Biol. Chem. 236*, 1372.

Midgeley, J. E. M. (1962), *Biochim. Biophys. Acta 61*, 513.

Millar, D. B. S., and Steiner, R. F. (1965), *Biochim. Biophys. Acta* (in press).

Okamoto, T., and Takanami, M. (1963), *Biochim. Biophys. Acta 68*, 325.

Okamoto, T., and Takanami, M. (1963), *Biochim. Biophys. Acta 76*, 266.

Ôpara-Kubinzka, Z., Kubinzki, H., and Szybalski, W. (1964), *Proc. Natl. Acad. Sci. U.S. 50*, 923.

Petermann, M. L., and Pavlovic, A. (1963), *J. Biol. Chem. 238*, 318.

Rich, A. (1958), *Biochim. Biophys. Acta 29*, 502.

Ross, P. D., and Sturtevant, J. M. (1962), *J. Am. Chem. Soc. 84*, 4503.

Steiner, R. F., and Beers, R. F. (1959), *Biochim. Biophys. Acta 33*, 470.

Steiner, R. F., and Beers, R. F. (1961), Polynucleotides, Natural and Synthetic Nucleic Acids, Amsterdam, Elsevier.

Steiner, R. F., and Edelhoch, H. (1961), *J. Am. Chem. Soc. 83*, 1435.

Steiner, R. F., and Edelhoch, J. (1962), *Chem. Rev. 62*, 457.

Takanami, T., and Okamoto, T. (1963), *J. Mol. Biol. 7*, 323.

Tissières, A., Watson, J. D., Schlessinger, D., and Hollingsworth, B. (1959), *J. Mol. Biol. 1*, 221.

Watson, J. D. (1964), Lectures before the 50th Anniversary Meeting of the French Biochemical Society, Paris.

Yanofsky, S. A., and Spiegelman, S. (1962), *Proc. Natl. Acad. Sci. U.S. 48*, 1069.

Yanofsky, S. A., and Spiegelman, S. (1963), *Proc. Natl. Acad. Sci. U.S. 49*, 539.