Metal Ion–Nucleic Acid Interactions. I. A Method for the Fractionation of Rat Liver Ribonucleic Acids into Transfer Ribonucleic Acid and Ribosomal Ribonucleic Acids Using Zn^{II} as a Precipitant*

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ABSTRACT: A method is described for the fractionation of rat liver total ribonucleic acid, prepared by the hot phenol method, into transfer ribonucleic acid and ribosomal ribonucleic acids. In acetate buffer of pH 5.5, Zn^{II} at a concentration of 0.1 M quantitatively precipitates ribosomal ribonucleic acids. The zinc-insoluble fraction is soluble in neutral EDTA solution. The zinc-insoluble and zinc-soluble fractions have been characterized by chromatography on methylated albumin kieselguhr, sucrose density gradient centrifugation, gel filtration on Sephadex G-100, and by measurement of amino acid acceptor activity. The zinc-insoluble fraction consists of the two ribosomal ribonucleic acids and has no amino acid acceptor activity. The zinc-soluble fraction is transfer ribonucleic acid contaminated with a small proportion (10–15%) of a high molecular weight component. Sedimentation velocity measurement on this fraction gave 3.8 S for the major component and 8.3 S for the minor component. The molecular weight corresponding to the top meniscus, calculated by the Archibald method, decreased with the time of centrifugation reaching a limiting value of 30,000. The values for the optical rotatory dispersion constant, λ_c, and the melting temperature, T_m, of the zinc-soluble fraction were close to those of "standard transfer ribonucleic acid" obtained by Sephadex chromatography of total ribonucleic acid. This fraction has the ability to accept amino acids to form aminoacyl transfer ribonucleic acids; this ability is comparable with that of standard transfer ribonucleic acid. Evidence has been presented to show that the high molecular weight impurity in this fraction is not derived by degradation of ribosomal ribonucleic acids during precipitation with Zn^{II}.

The discovery that many metal ions are found in intimate association with nucleic acids in their natural environment (Loring and Waritz, 1957; Wacker and Vallee, 1959; Wacker *et al.*, 1963) and that these ions may stabilize the structure of nucleic acids (Wacker and Vallee, 1959; Fuwa *et al.*, 1960) has led in recent years to a number of studies on the interaction of metal ions with nucleic acids. The added metal ions seem to affect the biological activity (Huff *et al.*, 1964), resistance to the action of nucleases (Singer and Fraenkel-Conrat, 1962) and heat stability (Eichorn, 1962; Hai, 1965). Interaction of a nucleic acid or a polynucleotide with different metal ions may involve different binding sites on the macromolecule (Eichorn *et al.*, 1967). Differences in base composition may also affect the interaction of nucleic acids with a metal ion. Thus DNAs with different base composition interact differently with Ag^I or Hg^I and the resulting differences in the buoyant densities of metal–DNA complexes have been used to separate DNAs of different base composition (Davidson *et al.*, 1965).

Preparations of transfer ribonucleic acid are heterogeneous and contain a number of amino acid transfer ribonucleic acids. The molecular weight of unfractionated transfer ribonucleic acids from different biological sources have been found to be nearly the same, 25,000–27,000 (Tissiéres, 1959; Brown and Zubay, 1960; Otaka and Osawa, 1960; Lubrosky and Cantoni, 1962; Kay and Oikawa, 1966). The transfer ribonucleic acid preparations show a narrow distribution of molecular weights (Lubrosky and Cantoni, 1962; Lindahl *et al.*, 1965) suggesting that the molecular weights of individual transfer ribonucleic acids are similar. Although there is a similarity in molecular weight, nucleotide sequence studies have shown that the primary structure of individual transfer ribonucleic acids differ (Holley *et al.*, 1965; Madison *et al.*, 1966; Zachau *et al.*, 1966; Bayev *et al.*, 1967; Rajbhandary *et al.*, 1967). Further the melting curves of individual transfer ribonucleic acids are also different suggesting that the secondary structures also differ (Mahler *et al.*, 1963; Fresco, 1963; Nishimura *et al.*, 1967).

Because of the differences in primary, secondary, and possibly tertiary structures, we reasoned that the individual amino acid transfer ribonucleic acids may interact differently with a metal ion and that the metal–RNA interaction may provide a method for the separation of amino acid tRNAs. While this aspect of the problem is still the subject of our investigation,

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we have observed that Zn^{II} at a concentration of 0.1 M quantitatively precipitates rRNAs from RNA preparations which contain both rRNAs and transfer ribonucleic acid. The zinc-soluble RNA contains transfer ribonucleic acid, is virtually free of high molecular weight RNAs and has amino acid acceptor activity. The zinc-insoluble RNA fraction consists of the two rRNAs and is free of amino acid acceptor activity.

The use of NaCl (Crestfield *et al.*, 1955; Ofengand *et al.*, 1961; Zubay, 1962; Wicks *et al.*, 1965) and of $(NH_4)_2SO_4$ (Littauer and Eisenberg, 1959) to precipitate rRNAs have been reported. LiCl (2 M) has also been used for the separation of rRNA and transfer ribonucleic acid (Barlow *et al.*, 1963).

Materials and Methods

Preparation of RNA. Rats were fasted for 48 hr and killed. The livers were excised and homogenized in 0.01 M acetate buffer (pH 5.1) containing bentonite (5 mg/ml), sodium dodecyl sulfate (5 mg/ml), and polyvinyl sulfate (5 μg/ml). The homogenate was extracted with an equal volume of 90% phenol at 50° for 4 min. The aqueous layer was separated by centrifugation and the phenol layer was reextracted with the buffer. The combined aqueous layers were again extracted with phenol, separated from the phenol layer, and the RNA was precipitated with 2.5 volumes of cold ethanol. The precipitate was dissolved in a minimum volume of 0.1 M acetate buffer (pH 5.5) and dialyzed against the same buffer.

Zn^{II} Precipitation Curve. Unless otherwise stated these experiments were done in 0.1 M acetate buffer of pH 5.5. A stock solution of zinc acetate in acetate buffer was prepared and its Zn^{II} content was determined by titration with a standard EDTA solution. To 2-ml aliquots of cold RNA solution, 2-ml aliquots of cold zinc acetate solution of varying concentration were added. The mixtures were left in the cold for 4 hr and the resulting precipitates were removed by centrifugation. The absorbance of the supernatants, after dilution where necessary, was read at 260 mμ. From these values, the proportion of RNA precipitated as a function of Zn^{II} concentration was calculated. For this and other calculations, an optical density of 1.0 at 260 mμ in a 1-cm cell was taken as equivalent to 40 μg of RNA/ml.

Preparation of Large Amounts of Zn^{II}-Soluble and Zn^{II}-Insoluble RNA Fractions. RNA solution (20 ml) containing 40 mg of RNA was mixed with an equal volume of 0.2 M zinc acetate solution. After standing in the cold for 4 hr the precipitate was removed by centrifugation. The supernatant was dialyzed in the cold against distilled water for 12 hr and then passed through a column of Dowex 50W-X8 (in potassium form) to remove Zn^{II} as completely as possible. RNA was then precipitated with 2.5 volumes of ethanol. The zinc-insoluble RNA was dissolved in a minimal volume of 0.1 M EDTA (pH 7.0). The solution was then dialyzed against distilled water and RNA was precipitated with 2.5 volumes of ethanol.

Effect of pH on Precipitation. To a series of tubes containing 2-ml aliquots of RNA solution (1 mg/ml) in acetate buffers of pH 3.5–6.2, 2-ml aliquots of 0.4 M zinc acetate solutions, in corresponding buffers, were added. After standing in the cold for 4 hr, the precipitates were removed by centrifugation. From the absorbance readings of the supernatants at 260 mμ, the proportion of RNA precipitated at various pH values was calculated.

Chromatography on Methylated Albumin Kieselguhr. The method of Mandell and Hershey (1960) with the modification of Monier *et al.* (1962) was used. For elution of RNA, a linear gradient of 0.2–1.2 M NaCl in phosphate buffer of pH 6.8 was used. Fractions of 3 ml were collected.

Sucrose Density Gradient Centrifugation. A linear gradient of 5–20% sucrose in acetate buffer of pH 5.1, 0.01 M containing 0.1 M NaCl was used. After centrifugation for 16 hr at 24,000 rpm in a SW25.1 rotor in a Spinco Model L ultracentrifuge, approximately 1-ml fractions were collected by puncturing the tube bottom, and their optical density at 260 mμ was read after dilution to 2 ml.

Gel Filtration on Sephadex G-100. A column of 1.6 × 150 cm of Sephadex G-100 equilibrated with 0.1 M NaCl was used, elution being performed with the same solvent. The experiments were done at room temperature (25°). Fractions of 2 ml were collected.

Sedimentation Velocity. A 0.6% solution of zinc-soluble RNA in 0.1 M acetate buffer (pH 5.5) was sedimented at 59,780 rpm at room temperature in a Spinco Model E ultracentrifuge fitted with phase-plate schlieren optics and RTIC unit. From the pictures taken at 20-min intervals, the sedimentation coefficient was calculated and reduced to the standard state of water at 20° (Schachman, 1959).

Molecular Weight. The Archibald method of molecular weight determination was used (Klainer and Kegeles, 1955). In a standard 12-mm cell, a 0.6% solution of zinc-soluble RNA was centrifuged at 6000 rpm. Pictures were taken at 30, 45, 60, 90, and 120 min after the attainment of the operating speed. To obtain the concentration of the solution in refractive index units, a separate experiment using a Kegeles-type synthetic boundary cell was done. Molecular weight corresponding to the top meniscus only was calculated. A value of 0.5 has been assumed for the partial specific volume of RNA.

Melting Curve. The melting curve was determined by measuring the absorption at various temperatures of RNA solutions (in 0.15 M NaCl) at 260 mμ using a Unicam recording spectrophotometer (SP 700) fitted with an electrically controlled, constant-temperature cell holder and a thermocouple temperature-measuring device.

Optical Rotation. The optical rotation of RNA solutions (1–2 mg/ml) in 0.15 M NaCl was measured at four wavelengths (405–578 mμ) with a Carl-Zeiss photoelectric polarimeter 370265. The measurements were made at room temperature (25°).

Assay for Amino Acid Acceptor Activity. The incubation mixture contained, in a total volume of 0.5

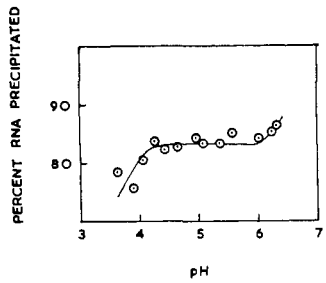

FIGURE 1: The effect of pH on the precipitation of RNA with 0.2 M Zn^{II}.

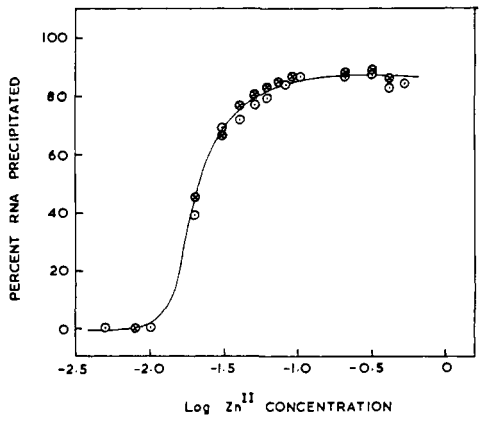

FIGURE 2: The effect of Zn^{II} concentration on the precipitation of RNA at pH 5.5. (—⊙—) RNA concentration, 1 mg/ml; (—⊗—) RNA concentration, 2 mg/ml.

ml, RNA varying from 50 to 500 μg, 50 μmoles of Tris, 5 μmoles of KCl, 5 μmoles of $MgCl_2$, 5 μmoles of ATP, 2.5 μmoles of phosphoenolpyruvate, 40 μg of pyruvate kinase, and 3 μCi of [^{14}C]chlorella protein hydrolysate (specific activity 54 mCi/matom of carbon). To this mixture, equilibrated at 36° for 5 min, 0.1 ml of "enzyme extract" (2 optical density units ml at 280 mμ) was added. The reaction was stopped after 20-min incubation by the addition of an equal volume of 10% trichloroacetic acid; 1 mg of bovine serum albumin was added as a carrier for the precipitation of RNA. The precipitate was washed four times with 5% trichloroacetic acid containing unlabeled amino acids. The precipitate was dissolved in 2 ml of 2 N NH_4OH, a small aliquot was plated on an aluminium planchet, dried, and radioactivity was measured in a Tracerlab counter with a counting efficiency of 6% for ^{14}C. The "enzyme extract" was obtained from the 105,000-*g* supernatant of lysed *Escherichia coli* cells. It was purified by adsorption on a DEAE-cellulose column (equilibrated with phosphate buffer of 0.02 M and pH 7.5) and by elution with the same buffer containing 0.25 M KCl.

Results and Discussion

In preliminary experiments it was observed that at Zn^{II} concentrations of 0.1 M or higher about 85% of RNA was precipitated. The RNA preparations used were found by methylated albumin kieselguhr chromatography and sucrose density gradient centrifugation to contain about 85% of rRNAs. The above observation, therefore, led us to infer that Zn^{II} may quantitatively precipitate rRNAs, leaving transfer ribonucleic acid in the supernatant. Before characterizing the zinc-soluble and zinc-insoluble RNA fractions, the effect of pH on precipitation was studied to determine the pH for optimum precipitation.

At a constant Zn^{II} concentration of 0.2 M, precipitation experiments were done in the pH range 3.5–6.2. Figure 1 indicates that about 85% of the total RNA was precipitated between pH 4.5 and 5.5. All the subsequent precipitation experiments were therefore done at pH 5.5.

In Figure 2, the amount of RNA precipitated at various Zn^{II} concentrations (0.005–0.500 M) is given. It is seen that (a) up to a Zn^{II} concentration of 0.01 M there is no precipitation of RNA; (b) between 0.01 and 0.10 M there is a steep rise in the proportion of RNA precipitated and this reaches a value of 85% at 0.10 M; and (c) there is no increase in precipitation from 0.1 to 0.5 M. The precipitation data were obtained at two different RNA concentrations, 1 and 2 mg per ml. Both the sets of data fit the same curve; thus at least a twofold increase in the RNA to Zn^{II} ratio does not seem to have any effect on the precipitation of RNA. It is, however, possible that if a much wider range of RNA concentration had been used, differences might have been observed.

The methylated albumin kieselguhr chromatograms (Figure 3) show the following: (a) the total RNA preparation gives two peaks, one eluting at 0.3 M NaCl and the other at 0.7 M NaCl; (b) the zinc-insoluble RNA fraction gives a single peak eluting at 0.7 M NaCl; and (c) the zinc-soluble RNA fraction gives a single peak eluting at 0.3 M NaCl. In methylated albumin kieselguhr chromatography (Mandell and Hershey, 1960; Sueoka and Cheng, 1962; Monier *et al.*, 1962), transfer ribonucleic acid elutes at 0.3–0.5 M NaCl and rRNAs at 0.7–0.9 M NaCl; DNA elutes between transfer ribonucleic acid and rRNAs. The total RNA preparation did not show any peak between transfer ribonucleic acid and rRNA indicating that it was free of DNA; this was also confirmed by testing for deoxyribose in the RNA preparation.[1]

In sucrose density gradient centrifugation experiments (Figure 4), the total RNA preparation gave two peaks due to rRNAs, and one due to transfer ribonucleic acid. The zinc-insoluble RNA fraction gave only the rRNA peaks and was free of transfer ribonucleic acid. The zinc-soluble RNA contained one major peak corresponding to transfer ribonucleic acid; in addition it contained a minor peak with sedimentation characteristics intermediate between those of

[1] It was observed that the pH of the medium employed for extraction of RNA had an effect on DNA contamination. If the pH was maintained around 5.0, there was no DNA contamination, as judged by deoxyribose test. If the pH was above 5.5 considerable amounts of DNA were also extracted along with RNA.

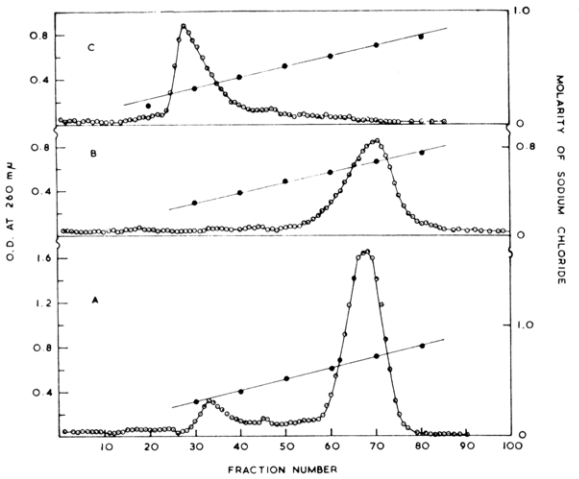

FIGURE 3: Chromatography of RNAs on methylated albumin kieselguhr. (A) Total RNA, (B) zinc-insoluble RNA, and (C) zinc-soluble RNA.

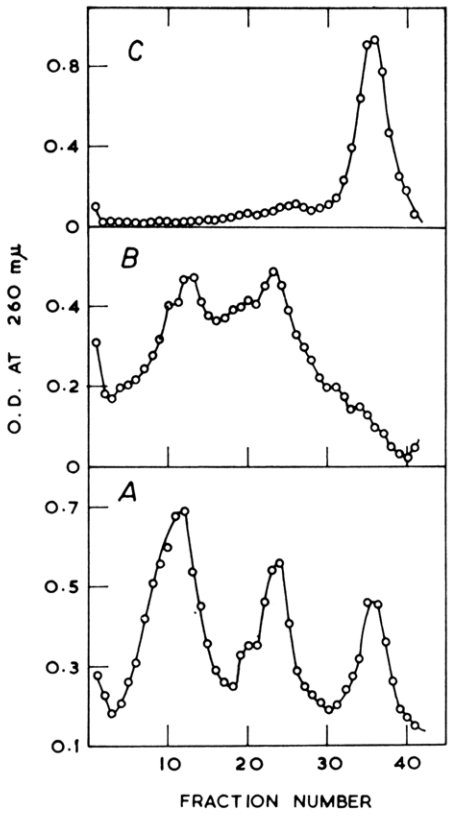

FIGURE 4: Sucrose density gradient centrifugation patterns. (A) Total RNA, (B) zinc-insoluble RNA, and (C) zinc-soluble RNA.

rRNAs and transfer ribonucleic acid. The presence of this impurity was also seen in the sedimentation velocity pattern of zinc-soluble RNA (Figure 5). The major component had a value of 3.8 S which is in good agreement with the values reported for transfer ribonucleic acid preparations (Tissiéres, 1959; Brown and Zubay, 1960; Otaka and Osawa, 1960; Lubrosky and Cantoni, 1962; Ofengand *et al.*, 1961; Kay and Oikawa, 1966). The minor component had a value of 8.3 S.

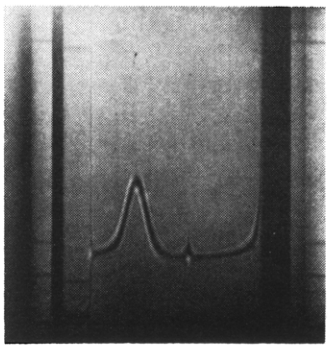

FIGURE 5: Sedimentation velocity pattern of zinc-soluble RNA. Centrifugation for 40 min at 59,780 rpm; bar angle 55°; sedimentation proceeds from left to right.

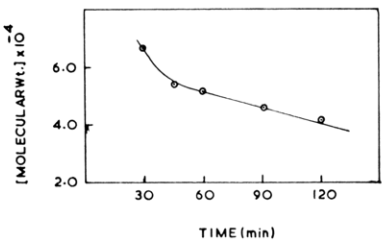

FIGURE 6: Variation of molecular weight of zinc-soluble RNA with time of centrifugation.

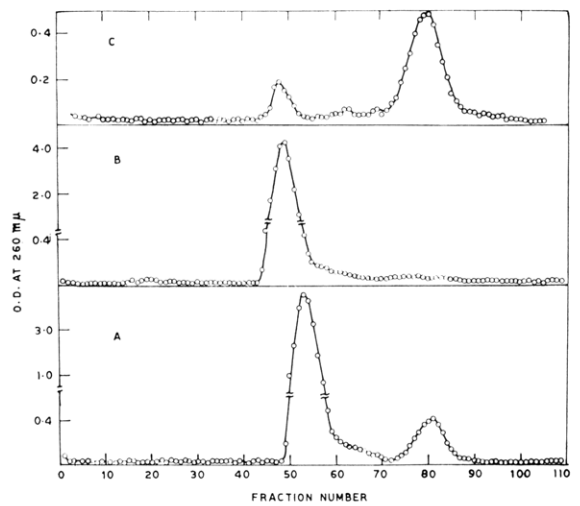

FIGURE 7: Gel filtration patterns. (A) Total RNA, (B) zinc-insoluble RNA, and (C) zinc-soluble RNA.

The molecular weight of the zinc-soluble RNA fraction measured at the top meniscus decreased with the time of centrifugation (Figure 6): the value at 30 min was 66,000 and this decreased gradually, reaching a limiting value of about 30,000, which falls within the range of molecular weights reported for transfer ribonucleic acid preparations.

The gel filtration patterns of the total RNA and the zinc-soluble and zinc-insoluble fraction are given in Figure 7. The total RNA preparations gave two peaks;

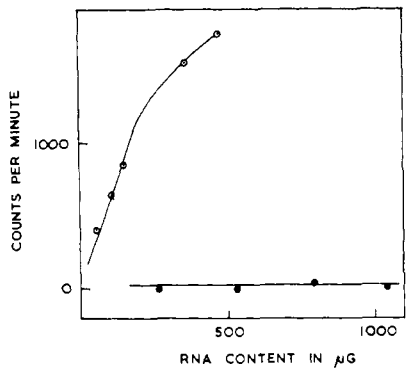

FIGURE 8: Variation of the amount of amino acids accepted with RNA concentration. (—⊙—) Zinc-soluble RNA; (—●—) zinc-insoluble RNA.

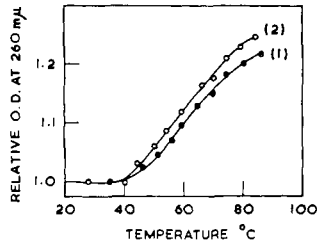

FIGURE 9: Melting curves of RNA. (1) Zinc-soluble RNA; (2) standard tRNA.

an rRNA peak emerging immediately after the void volume of the column and a transfer ribonucleic acid peak emerging later. Whereas the zinc-insoluble fraction gave a single peak at the position of rRNA, the zinc-soluble RNA gave two peaks, one where rRNA was eluted and the second where transfer ribonucleic acid was eluted. The former accounts for 10–15% of the zinc-soluble RNA fraction. This proportion appears to be higher than what can be estimated by visual examination of either Figure 4C or 5. The reason for the discrepancy is not clear. Gel filtration technique has been used for the separation of minor RNA components such as 5S and 7S RNA (Galibert *et al.*, 1965; Watson and Ralph, 1966). Since the gel filtration patterns obtained by us do not reveal any RNA fractions other than rRNA and transfer ribonucleic acid, it is difficult to estimate the contamination, if any, of the zinc-insoluble and zinc-soluble fractions with any of these minor RNA components.

The above-mentioned results indicated that the zinc-soluble RNA fraction consisted mainly of transfer ribonucleic acid. Therefore, the amino acid acceptor activity of the zinc-soluble RNA fraction was determined. It is seen from Figure 8 that this fraction had the ability to accept amino acids; the extent of this acceptance increased with increase in RNA concentration. The zinc-insoluble RNA fraction was free of amino acid acceptor activity; this observation, in conjunction with the other results described above, suggests that this fraction consists of the two rRNAs only.

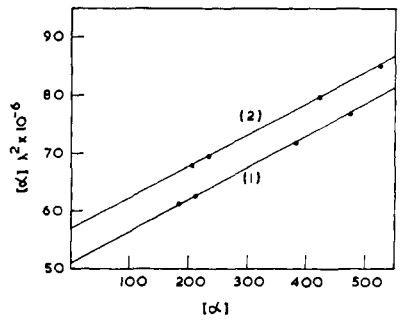

FIGURE 10: Drude plot of optical rotation data. (1) Zinc-soluble RNA; (2) standard tRNA; the lines drawn are least-square lines.

For comparison a "standard transfer ribonucleic acid" was obtained by gel filtration of total RNA on Sephadex G-150 column. It will be seen from Table I that the activity of zinc-soluble RNA fraction was close to that of the standard transfer ribonucleic acid suggesting that the biological activity of transfer ribonucleic acid was not impaired during fractionation with Zn^{II}.

Lindahl *et al.* (1966) have reported that transfer ribonucleic acid prepared by the usual methods is denatured and that it can be renatured by incubation at 60° for 5 min in the presence of 0.02 M Mg^{II}; the amino acid acceptor activity increases on renaturation. We have measured the amino acid acceptor activity of the zinc-soluble RNA fraction as well as that of standard transfer ribonucleic acid, before and after renaturation. Both the RNAs show enhanced activity after renaturation (Table I). It may be concluded from those results that fractionation of transfer ribonucleic acid with Zn^{II} does not cause any more denaturation than caused perhaps by any of the previously described methods for its isolation.

Metal ions have been reported to have both stabilizing and destabilizing effects on the secondary structure of nucleic acids (Huff *et al.*, 1964; Nishimura *et al.*, 1967; Comb and Zehavi-Willner, 1967; Eichorn, 1962; Hai, 1965). The melting curve and optical rotatory dispersion of zinc-soluble RNA fraction were therefore determined and compared with those of standard transfer ribonucleic acid to investigate whether fractionation with Zn^{II} had caused any changes in its secondary structure. The melting curves (Figure 9) show that the optical absorption at 260 mμ of both the RNAs increases on heating, the maximum increase being 24% for standard transfer ribonucleic acid and 22% for zinc-soluble RNA. From the midpoint of the curves, T_m may be estimated at 60° for standard transfer

TABLE I: Amino Acid Acceptor Activity Expressed as Counts per Minute per Milligram of RNA.

Sample	Before Renaturation	After Renaturation
Zinc-soluble RNA	5,920	19,640
Standard tRNA	7,200	25,940

ribonucleic acid and 64° for zinc-soluble RNA. The addition of metal ions has in some cases been observed to increase T_M by 15–20° (Huff *et al.*, 1964; Nishimura *et al.*, 1967; Comb and Zehavi-Willner, 1967). The small increase in the T_m of zinc-soluble RNA over that of standard transfer ribonucleic acid may be due to the reason that the procedure used by us to remove Zn^{II} may not remove Zn^{II} completely and that a few tightly bound Zn^{II} ions may stabilize the secondary structure. Bachvaroff and Tongur (1966) have reported a value of 49° for the T_m of rat liver 4S RNA which is considerably lower than the value obtained by us. Our value is, however, close to the values reported for the T_m of 4S RNA from *E. coli* (Tissiéres, 1959; Brown and Zubay, 1960; Ofengand *et al.*, 1960) and from yeast (Mahler *et al.*, 1963; Nishimura *et al.*, 1967).

Optical rotation measurements indicated that the degree of secondary structure in zinc-soluble RNA and standard transfer ribonucleic acid were the same. The specific rotations measured at 405, 436, 546, and 578 mμ were analyzed in terms of one-term Drude equation (Djerassi, 1960). The data gave a linear plot (Figure 10); from the slope the rotatory dispersion constant, λ_c, was estimated to be 237 mμ for zinc-soluble RNA and 234 mμ for standard transfer ribonucleic acid. It has been reported that RNA when it exists in a partially helical form has a λ_c value of 240–250 mμ and that this increases to 280–310 mμ when the conformation changes to a random coil (Haschemeyer *et al.*, 1959; Fresco, 1961; Boedtker, 1961; Kay and Oikawa, 1966). Our values are close to those reported for the partial helical conformation of RNA.

The foregoing results show that fractionation with Zn^{II} gives two well-separated fractions, one of rRNAs and another of transfer ribonucleic acid. In our experience two factors are important for achieving good separation: (1) the pH at which precipitation is carried out should be between 4.5 and 5.5; above pH 6.0 part of transfer ribonucleic acid is also precipitated, and (2) degraded materials in RNA preparations give a false estimate of the proportion of RNA precipitated and should therefore be removed by prior dialysis.

The zinc-soluble RNA fraction, though it is mainly transfer ribonucleic acid, contains a small proportion of a high molecular weight component whose sedimentation coefficient, 8.3 S, is lower than those of the two rRNAs, 30 and 18 S (Spirin, 1963). It could be either derived by degradation of rRNAs during precipitation with Zn^{II} or a species of RNA present in the total RNA preparation which is not precipitated by 0.1 M Zn^{II}. We did the following experiment to investigate whether precipitation of rRNA with Zn^{II} produces this component.

Rats were injected with ^{32}P and killed after 24 hr. The RNA from the liver of the animals was prepared by the method described earlier and was freed of unincorporated ^{32}P by passage over an ion-exchange column (Petrovic and Petrovic, 1966). It was found by sucrose density gradient centrifugation to be uniformly labeled. The tubes from the sucrose gradient run containing the two rRNA fractions were pooled, 20 mg of unlabeled *total* RNA was added as a carrier, and RNA was precipitated with ethanol. The precipitate, having a total count of 340,800/min, was dissolved in acetate buffer (pH 5.5) and precipitated with 0.1 M Zn^{II}. The zinc-insoluble and zinc-soluble fractions were freed of

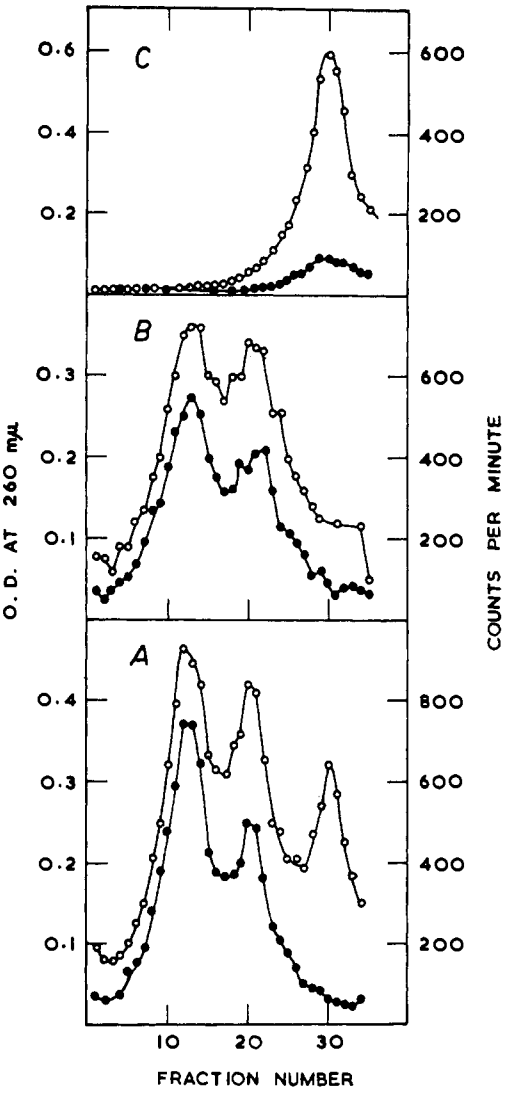

FIGURE 11: Sucrose density gradient centrifugation patterns of RNA mixed with ^{32}P-labeled rRNAs. (A) Total RNA, (B) zinc-insoluble RNA, (C) zinc-soluble RNA, (—O—) optical density, and (—●—) radioactivity.

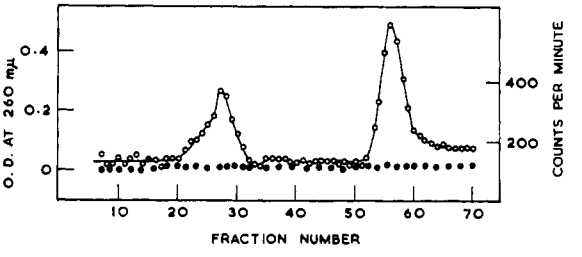

FIGURE 12: Gel filtration pattern of zinc-soluble RNA fraction obtained after the precipitation of total, unlabeled RNA mixed with ^{32}P-labeled rRNAs. Column, 1.2 × 150 cm; fraction, 3 ml/fraction; (—O—); optical density; (—●—) radioactivity.

Zn^{II} and run on sucrose density gradient; the zinc-soluble fraction was chromatographed on a Sephadex G-150 column also. Both optical density and radioactivity of the fractions were measured.

It was observed that only 1% of the total counts remained in the zinc-soluble fraction and the rest was precipitated by 0.1 M Zn^{II}. There was correspondence between the optical density and radioactivity peaks of the zinc-insoluble fraction (Figure 11). The zinc-soluble fraction gave a minor radioactivity peak in the 4S RNA region. There was no peak in the position of 8.3S RNA whose optical density peak could also not be clearly seen. However, the gel filtration pattern (Figure 12) clearly revealed the presence of the high molecular weight component in the zinc-soluble fraction. There was no radioactivity peak corresponding to its optical density peak suggesting that this fraction was not derived by degradation of rRNAs during precipitation with Zn^{II}. This fraction must therefore have been present in the total RNA preparation. The possibility that this fraction could be degraded DNA was ruled out by the observation that the deoxyribose test with zinc-soluble RNA was negative. ^{32}P-Labeled transfer ribonucleic acid, obtained by gel filtration, was mixed with Zn^{II} and rechromatographed on Sephadex after removal of Zn^{II}. It did not give the peak due to the heavier component suggesting that the 8.3S material is not an aggregate of 4S RNA formed during treatment with Zn^{II}. These results do not reveal whether this is a new species of RNA or an artifact of the method of preparation of total RNA.

Butzow and Eichorn (1965) have reported that when RNA is heated at 64° in the presence of Zn^{II}, it is degraded. Probably because of the low temperature employed in our fractionation procedure, the RNAs do not seem to undergo degradation. In sucrose density gradient centrifugation the zinc-insoluble RNA fraction gives the two peaks at the same positions as the two rRNAs of the total RNA preparation indicating that there was no gross degradation of the RNA fractions. The results of sedimentation velocity, melting curve, and amino acid acceptor activity experiments with the zinc-soluble RNA rule out any degradation of this fraction.

Acknowledgments

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Biosynthesis of Ubiquinones by Malarial Parasites. I. Isolation of [^{14}C]Ubiquinones from Cultures of Rhesus Monkey Blood Infected with *Plasmodium knowlesi**

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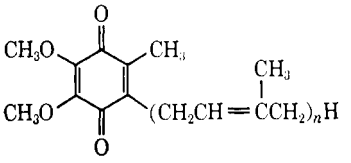
ABSTRACT: It has been demonstrated that *Plasmodium knowlesi* biosynthesizes [^{14}C]ubiquinones-8, -9, and perhaps -7 from [^{14}C]*p*-hydroxybenzoic acid by the isolation of these ubiquinones from parasitized (*P. knowlesi*) rhesus monkey blood cells cultured in synthetic medium containing [^{14}C]*p*-hydroxybenzoic acid. Only [^{14}C]ubiquinone-10 was isolated from control cultures of unparasitized rhesus monkey blood cells and was shown to be associated with the leucocytes.

The occurrence of ubiquinones-8 and -9 (I, n = 8 and 9, respectively) in duck blood infected with *Plasmodium lophurae*, in contrast to only ubiquinone-10 (I, n = 10) in normal duck blood, has been reported (Rietz *et al.*, 1967). A similar analysis (F. S. Skelton, P. J. Rietz, and K. Folkers, 1968, unpublished data) performed on rhesus monkey blood infected with either *Plasmodium knowlesi* or *Plasmodium cynomolgi* also showed only ubiquinone-10 in the normal blood and ubiquinones-8 and -9 in the infected blood.



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p-Hydroxybenzoic acid has been found to be a biosynthetic precursor of ubiquinone in a variety of microorganisms and animals (Rudney and Parson, 1963; Parson and Rudney, 1964; Olson *et al.*, 1963; Aiyar and Olson, 1964). We now report the incorporation of [^{14}C]*p*-hydroxybenzoic acid (uniformally labeled) into ubiquinones-8 and -9 by parasitized (*P. knowlesi*) rhesus monkey erythrocytes cultured *in vitro* using the rocker dilution technique (Geiman *et al.*, 1946). Only [^{14}C]ubiquinone-10 was identified in the normal blood cells, and is believed to be associated with the leucocytes.

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