STUDIES OF *E. coli* RIBOSOMAL RNA AND ITS DEGRADATION PRODUCTS

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ABSTRACT The RNA of *E. coli* ribosomes has been extracted by the phenol method. The 70S ribosomes contain RNA molecules of 28 and 18S almost exclusively. When the 70S ribosomes are dissociated to 30 and 50S ribosomes the former contain only the 18S RNA and the latter a mixture of 28 and 18S RNA. There are also present, however, small quantities of ribosomal RNA having sedimentation coefficients of between 4 and 8S. These small molecules are particularly abundant in the smaller ribosomes present in the cell extract and account for most of the RNA of 20S ribosomes.

In addition it has proved possible to degrade the large molecules of RNA to a series of smaller molecules. Removal of magnesium ions from the growing cell, extensive dialysis of the RNA against a buffer of low ionic strength, and heating all resulted in such degradation. Three degradation products were observed having sedimentation coefficients of about 13.1S, 8.8S, and 4.4S. The integral sedimentation distributions of these preparations suggest a high degree of homogeneity among the molecules of each of the three classes. The three sizes seem to result from sequential breaks in the molecules since the proportion of smaller molecules increases with time of treatment.

The molecular weights of the 8.8S and 4.4S molecules have been estimated as 144,000 ± 4,900 and 29,200 ± 1,200 respectively by the Archibald method.

INTRODUCTION

The evidence that ribonucleoprotein particles have a major role in protein synthesis is now rather extensive. In growing *E. coli* it has been shown that most of the cell protein is transiently associated with the ribosomes before appearing free as soluble protein (McQuillen, Roberts, and Britten, 1959). In *E. coli* about 80 per cent of the RNA exists as part of the ribosomal particles. The particles themselves are distributed among five size classes having sedimentation constants of 20S, 30S, 50S, 70S, and 85 to 100S. In exponentially growing cells the fourth class is the most abundant and may account for as much as 80 per cent of the total ribosome material.

Phenol extraction of the ribosomes enables one to obtain the RNA essentially

free of protein (Schramm and Gierer, 1956). The RNA consists mainly of two components with sedimentation coefficients of 15 to 18S and 23 to 28S (Timasheff, Brown, Colter, and Davies, 1958, Littauer and Eisenberg, 1959, Hall and Doty, 1959). However, most of these preparations of ribosomal RNA used for physical studies were derived from a mixture of particles in which the 70S particles were predominant. In the present study the RNA of each of the five particle groups was examined so as to be able to detect which components were present in each particle group. This paper presents these results together with a study of the degradation of the ribosomal RNA to material of lower molecular weight. In a following paper the kinetic relationships among the different RNA fractions are discussed in conjunction with the known relationships among the ribosome groups.

MATERIALS AND METHODS

E. coli ML 30 was grown in C medium (Roberts *et al.*, 1955) with glucose or maltose. An overnight culture was diluted with fresh C medium and grown to about 10^9 bacteria/ml. Cells were aerated at 37°C until the late exponential phase had been reached. All cells were harvested, washed twice in the cold with 0.01 M tris, 0.004 M succinic acid, 0.01 M magnesium acetate, pH 7.4 (TSM 10^{-2}). The cells were resuspended in a small volume and an extract prepared by passing them through the orifice of a French pressure cell at 10,000 to 15,000 psi.

Ribosome Purification

Purified samples of 70S ribosomes and their constituent 50S and 30S parts, and of the small quantities of 50S, 30S, and 20S ribosomes "native" to the cell extract may be obtained by centrifugal techniques.

70S. The cell extract was spun in the model L Spinco ultracentrifuge No. 40 rotor at 40,000 R.P.M. for 5 minutes. The pellet (40K 5 P)[1] was discarded and the supernatant centrifuged at 40,000 R.P.M. for 45 minutes. The pellet (40K 45 P) was resuspended in TSM 10^{-3} buffer and following a short spin in the Servall SSl (5 minutes) to remove aggregates, was recentrifuged 40K 45 minutes. The final pellet was resuspended in TSM 10^{-3}, examined in the ultracentrifuge for purity, and stored at −20°C. Such a preparation consisted mostly of 70S ribosomes since they are the most abundant. Further purification steps yielded pure 30S and 50S derived from the 70S.

50S. For the preparation of a purified 50S fraction the first 40K 45 minute pellet was resuspended in tris, 0.01 M, succinic acid, 0.004 M, magnesium acetate, 10^{-4} M, pH 7.4 (TSM 10^{-4}) and centrifuged at 40,000 R.P.M. for 180 minutes. The 70S breaks down to 50S and 30S in the low magnesium concentration. This pellet was resuspended in the same buffer and contained only 50S and 30S ribosomes mostly derived from the 70S. The 50S particles were purified by four successive centrifugations of 40K 90 minutes (Tissières *et al.*, 1959), resuspending the pellet each time in TSM 10^{-4}.

30S. Either of two procedures was employed to prepare pure samples of 30S ribosomes. The first involved layering the 50S/30S mixture on a sucrose gradient (Britten and Roberts, 1959) and centrifuging in the swinging bucket rotor at 37,000 R.P.M. for 150 minutes (SWB 37K 150). Fractions of about 0.2 ml each were collected and

[1] K, 1000 R.P.M.; P, pellet.

the absorption at 260 mμ determined. The region containing the 30S fraction was then dialyzed briefly in the cold against TSM 10^{-4} and pelleted by centrifuging in the swinging bucket rotor at 37,000 R.P.M. for 6 hours.

Alternately, the 50S/30S mixture was centrifuged SWB 37K 90 minutes. The top third was removed with a syringe and the 30S particles pelleted by centrifugation SWB 37K 6 hours. By either of these procedures purities in the range 90 to 95 per cent 30S ribosomes were obtained as routine.

Native 50S, 30S, and 20S. The quantities of 50S, 30S, and 20S available in a cell extract are very limited. The 20S ribosomes are especially elusive due both to their small size and to the fact that less than 5 per cent of the ribosome material is present in this form. The first 40K 45 minute centrifugation removed the bulk of the 70S ribosomes. The pellet resulting from a long centrifugation (40K 300 minutes) of the supernatant contained the remainder of the 70S and most of the 50, 30, and 20S ribosomes. It was resuspended in TSM 10^{-3} and after a brief centrifugation to remove aggregates it was submitted to sucrose gradient centrifugation. A spin of 37K 150 minutes was sufficient to pellet most of the remaining 70S ribosomes and resolve the 50S, 30S, and 20S. The appropriate fractions were collected and the various ribosomes collected by centrifugation.

Preparation of RNA

The phenol used for RNA extraction was first purified by shaking at room temperature with an equal volume of 0.02 M phosphate buffer, pH 7.0. In special cases the buffer also contained 10^{-3} M versene. After removing the aqueous phase, the treatment was repeated four or five times. Such phenol is adequate for preparing biologically active poliomyelitis RNA (Hoyer, 1960). Phenol purified by steam distillation or by means of a column of aluminum oxide (Tissières, 1959) was also used and parallel preparations of RNA using the two phenol samples were indistinguishable in their stability and sedimentation properties. The purified phenol was stored in the dark at 2°C.

The RNA was extracted by raising the magnesium concentration of the particle preparation to 10^{-3} M and adding an equal volume of the purified phenol. The mixture was sucked up and down with a syringe several times at room temperature and the phases then separated by centrifugation. The aqueous layer was removed and treated with phenol twice again. The final preparation was shaken five or six times with ether saturated with phosphate buffer to remove phenol, and nitrogen was bubbled through to remove the ether. Any insoluble material remaining was then removed by low speed centrifugation. The recovery was usually 60 to 70 per cent and protein contamination as measured by an S^{35} label was less than 0.1 per cent. The RNA preparations are identified as RNA_{70}, RNA_{50}, RNA_{30}, and RNA_{20}, being derived from the 70, 50, 30, and 20S ribosomes respectively. All preparations were frozen to −20°C in the deep freeze and thawed immediately before use.

Dialysis. All dialysis was carried out at 2°C with constant stirring. Visking dialysis tubing was washed in versene (0.01 M) solution and boiled in $NaHCO_3$ before use.

Apparatus

Sedimentation coefficients were measured in a model E Spinco ultracentrifuge equipped with schlieren and ultraviolet absorption optics. A Spinco analytrol was used to read the films and the arithmetic mean sedimentation coefficient determined by measuring the rate at which the midpoint of the concentration distribution sedimented. Alternatively the in-

tegral sedimentation distribution of the preparation was obtained by measuring the rate of sedimentation of a range of different percentages of the molecules from zero to 100 per cent (Kurland, 1960). The sedimentation coefficients were corrected for density and viscosity of water to 20°C.

RESULTS

Since examination of preparations in the ultracentrifuge was to be the primary criterion of the size of the RNA molecules, an extensive study was made of the dependence of the sedimentation properties of RNA upon the concentration of RNA

TABLE I

THE EFFECT OF CONCENTRATION AND IONIC STRENGTH ON THE SEDIMENTATION COEFFICIENTS OF RIBOSOMAL RNA

RNA source	Salts added to standard TS buffer (0.01 M tris 0.004 M succinic pH 7.4)	Concentration *mg/ml*	$s_{20,w}$
70S	None	2.7	13.0
			17.3
70S	0.01 M KCl	2.5	12.8
			15.2
70S	0.05 M KCl	2.6	15.1
			18.1
70S	0.2 M KCl	2.5	14.9
			18.9
70S	0.4 M KCl	2.5	16.2
			22.3
70S	0.2 M KCl + 0.01 M magnesium acetate	2.5	19.7
			28.0
70S	0.4 M KCl + 0.01 M magnesium acetate	2.5	20.6
			28.1
70S	0.01 M magnesium acetate	2.7	20.6
			27.1
70S	0.01 M magnesium acetate	2.7	20.8
			27.1
70S	0.01 M magnesium acetate	0.056	19.8
			28.6
50S	0.01 M magnesium acetate	2.5	18.9
			26.4
50S	0.01 M magnesium acetate	0.060	20.0
			28.8
30S	0.01 M magnesium acetate	2.4	18.2
30S	0.01 M magnesium acetate	0.052	19.2
20S*	0.01 M magnesium acetate	1.8	4.50
			(17.6)
20S‡	0.1 M NaCl	0.060	4.40
			(19.7)
			(24.8)

* See Fig. 1. ‡ See Fig. 2.

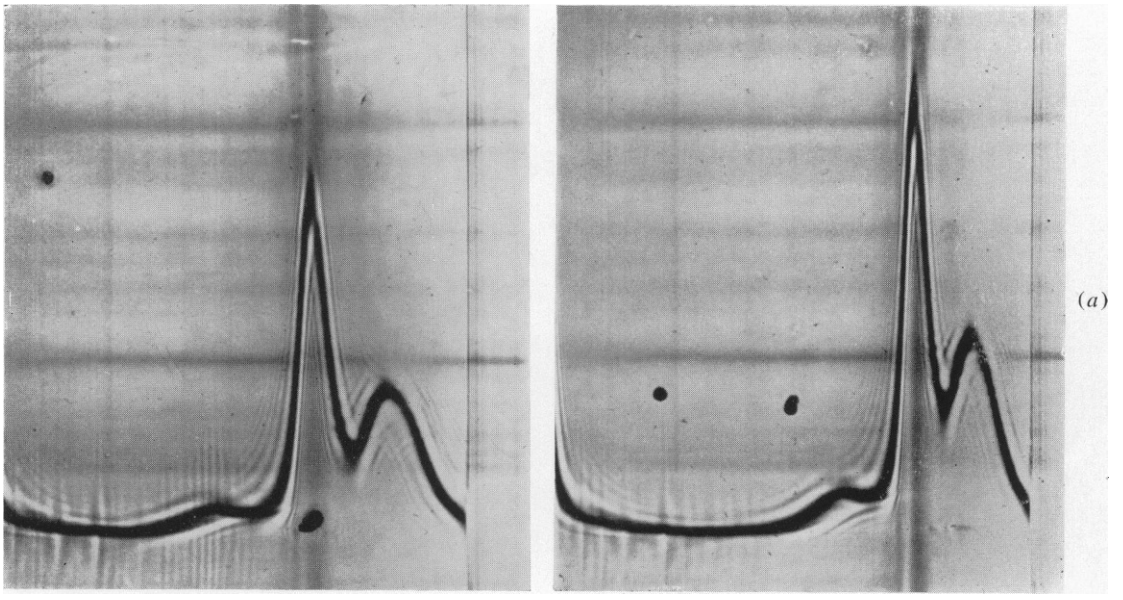


FIGURE 1 (*a*) Purified 20S ribosomes in TSM 10^{-2}. Pictures at 10 minutes (right) and 14 minutes (left) after reaching 50, 740 R.P.M. Concentration 2.1 mg/ml.

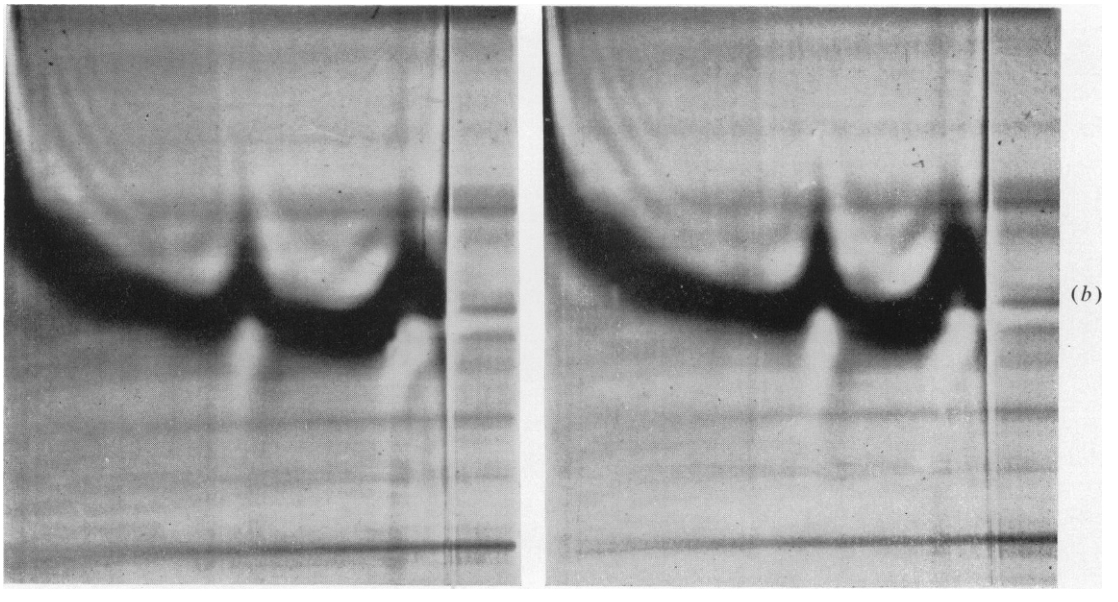


FIGURE 1 (*b*) RNA extracted with phenol from preparation shown in (*a*). Pictures at 19 minutes (right) and 27 minutes (left) after reaching 50, 740 R.P.M. Concentration 0.7 mg/ml. (The convection "spikes" in these figures are considered to be minor because of results of other centrifuge runs.)

and the composition of the solvent. At concentrations in the region of 2 mg/ml there was a considerable effect of ionic strength on the sedimentation coefficients of the RNA preparations. In 0.01 M tris buffer the sedimentation coefficients of the RNA from 70S particles were 13.0 and 17.3 (Table I). The addition of KCl from 0.01 M to 0.4 M increased these values progressively up to 16.2 and 22.3. The addition of 0.01 M magnesium acetate to the buffer in the presence or absence of a high concentration of KCl made a further increase in the sedimentation coefficients to about 20 and 27. There is a marked effect of ionic strength on the viscosity of RNA (Kawade, 1959, Cox and Littauer, 1960) and these changes may reflect an alteration in the shape of the molecules. It is apparent then that magnesium ions cause a tighter coiling of the RNA molecules than do potassium ions. The dependence of sedimentation coefficient on RNA concentration is not marked, particularly in the presence of magnesium ions (Table I).

The sedimentation analysis of RNA_{70} is summarized in Table I. For the RNA_{50} of 50S particles derived from the 70S, the two components have approximately the same $s_{20,w}$ values as those in RNA_{70} (Table I). The relative proportion of the two peaks did vary somewhat among different preparations. Thus the larger component could account for from about 25 to 75 per cent of the total. Changes in the magnesium or other cation concentration of the solution had no effect on the relative proportion of the two peaks. When a preparation was reextracted with phenol, however, a shift in the relative proportion of the two peaks was observed so that some of the larger component was apparently converted to the smaller. This shift could be due to removal of a protein component or to an alteration in hydrogen bonding.

There was only one major component in RNA_{30} of 30S particles derived from the 70S. Measured $s_{20,w}$ values are given in Table I. The small amount of faster moving material often observed is probably due to contaminating 50S particles in the original preparation. It did not prove possible to produce a second faster moving component by changing the cation concentrations. Fig. 1 *a* shows a preparation of 20S particles. The slower moving material of about 10S is evidently contaminating protein. Chromatography on a DEAE-cellulose column shows that about half of the material is protein and the other half nucleoprotein (Roberts *et al.*, 1960). These authors have analyzed pure 20S ribosomes and shown them to be of similar composition to the larger ribosomes; *i.e.*, 60 per cent RNA and 40 per cent protein. On the basis of this and a sedimentation coefficient very close to 20 the molecular weight of the smallest ribosome would be about half that of the 30S; *i.e.*, 5×10^5. The RNA of a 20S particle would therefore be expected to have a molecular weight of 3×10^5 and a sedimentation coefficient of about 13S. The faster moving of the two peaks has an $s_{20,w}$ of 17.6 (Table I). This large component could represent a dimer of a 13S molecule that is very readily formed upon extraction of the RNA in the presence of 10^{-2}M magnesium. Alternatively it could have resulted from contaminating larger particles but there seems to be more than could be attributed

to the small contamination visible in Fig. 1 *a*. The other component is 4.5S indicating another state of the RNA in these particles. Here again, the rather large amount found suggests that contamination is unlikely. An analysis of a second preparation of RNA from 20S particles at lower concentration using ultraviolet optics (Fig. 2) suggested that most of the RNA is about 4 to 5S, with a smaller quantity of larger molecules perhaps due to contamination of the preparation by traces of 30S and 50S ribosomes.

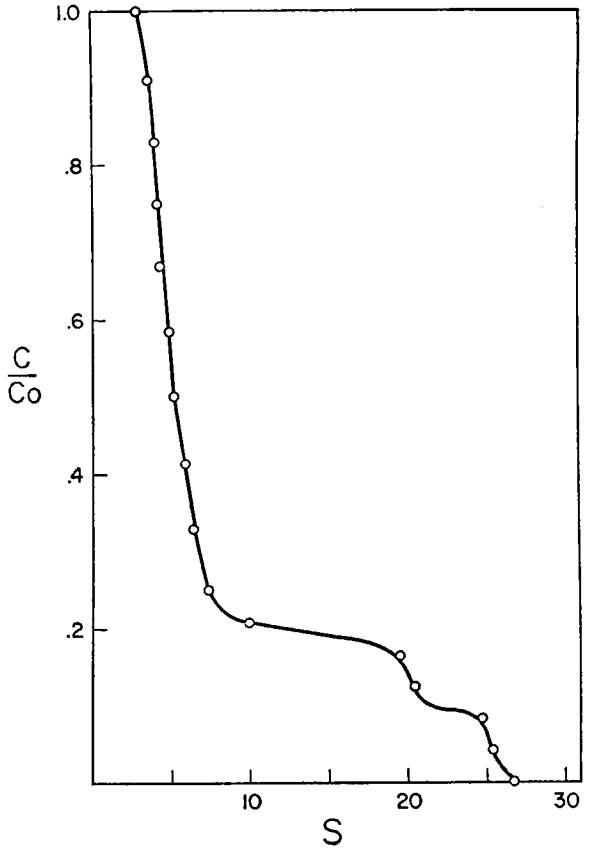


FIGURE 2 The integral sedimentation distribution of RNA from 20S ribosomes. Solvent 0.01 M tris, 0.004 M succinic acid, pH 7.4, 0.1 M NaCl. (Points are corrected for radial dilution.)

RNA Degradation

Instability of the RNA preparation with consequent breakdown into material of lower molecular weight is a characteristic feature of many of the studies made on both viral (Cheo, Briesen, and Sinsheimer, 1959, Boedtker, 1959) and ribosomal

(Hall and Doty, 1959, Kawade, 1959) RNA. Much of this instability has been previously ascribed to traces of nucleases in the preparation. This is, of course, a real possibility especially in the case of ribosomal RNA since the ribonucleoprotein itself contains a latent ribonuclease as an intimate part of the structure (Elson, 1958). In spite of this the RNA prepared as described showed no evidence of

TABLE II

THE DEGRADATION OF RIBOSOMAL RNA TO SMALLER MOLECULES

No.	RNA source	Treatment	Solvent	Concentration *mg/ml*	$s_{20,w}$
1	70S	20 hrs. dialysis against TS	TS 0.2 M KCl	2.3	5.23 10.8 16.0 21.7
2*	70S	24 hrs. dialysis against TS	TS 0.1 M NaCl	0.05	4.40 9.00 13.15
3‡	70S	32 hrs. dialysis against TS	TS 0.1 M NaCl	0.05	8.80
4	70S	70 hrs. dialysis against TS	TS 0.2 M KCl	2.1	4.28
5*	70S	72 hrs. dialysis against TS	TS 0.1 M KCl	0.05	4.15
6	50S + 30S	48 hrs. dialysis against 0.02 M PO_4, pH 7.2	TS 0.1 M NaCl	2.6	4.35
7	50S	68 hrs. dialysis against 0.01 M tris, 0.08 M KCl, pH 7.14	0.01 M tris, 0.08 M KCl, pH 7.4	1.9	4.68
8*	70S	Heated 90°C for 7 min in TS 0.2 M NaCl	TS, 0.2 M NaCl	0.05	4.40
9	Extract from cells Mg-starved 16 hrs	None	0.02 M PO_4, pH 7.4	2.9	4.14 10.9
10	Extract from cells Mg-starved 20 hrs	None	TS 10^{-3} M Mg acetate	2.4	4.24 11.1
11*	Extract from cells Mg-starved 28 hrs	None	TS, 0.1 M NaCl	0.05	4.35

TS = 0.01 M tris, 0.004 M succinic acid, pH 7.4.

* The integral sedimentation distributions of preparations 2, 5, 8, and 11 given in Figs. 4*e*, *f*, *c*, and *b* respectively.

‡ The molecular weight of this preparation was determined by the Archibald method (1947). See Table III.

ribonuclease activity as measured by the appearance of acid-soluble P^{32} from labeled RNA, even when treated with 4.5 M urea for 48 hours to release any latent enzyme. This is in agreement with the results of Littauer and Eisenberg (1959).

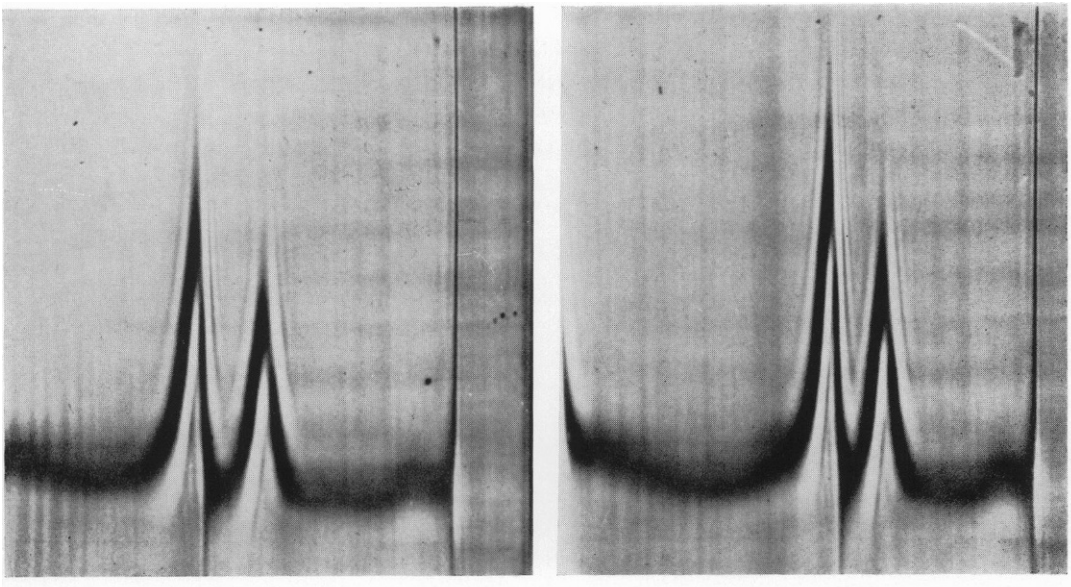
It became obvious that degradation to lower molecular weights could be brought about by three separate procedures. These are described in order followed by a discussion of the molecular weights of the fragments produced.

Magnesium Removal. Extraction of RNA from ribosomes by phenol in the presence of 0.002 M versene produced components of sedimentation constants lower than 28 and 18S. The sizes of these components and their relative quantities were somewhat variable but the preparation of Fig. 3 is a typical one. The drop in the baseline of Fig. 3 *b* is an artifact of the analysis. Such results suggested that the presence of magnesium ions during RNA extraction might have a considerable influence on the stability of the RNA. Removal of magnesium from both the intact growing cells and from the RNA preparation was studied.

An investigation was made of the RNA extracted from magnesium-starved cells. These cells deprived of magnesium for at least 16 hours in a medium complete except for magnesium suffer breakdown of their ribosomes into the constituent RNA and protein moieties without losing any of the RNA by degradation to nucleotides (McCarthy, 1959).

The starved cells were disrupted in the pressure cell and the resulting cell extract poured immediately into ice cold phenol. After extraction in the usual manner the RNA was examined in the ultracentrifuge. There was often a faster moving peak of about 11S which tended to flatten out on continued centrifugation suggesting heterogeneity (Table II). The slower peak of about 4.2S did, however, appear to be reasonably homogeneous. Longer starvation periods reduced the relative amount of the faster component until most of the material was converted to a single component of 4.35S (Fig. 4 *b*). The degradation process appeared to reach a limit with the conversion of all the RNA to this molecular size. Of course, soluble RNA is extracted under these conditions and accounts for some 20 per cent of the total but the presence of ribosomal RNA in this mixture of 4 to 5S components can be demonstrated by chromatography on DEAE-cellulose (McCarthy and Aronson, 1961).

Dialysis. Dialysis of the RNA preparation against the standard tris buffer with 0.01 M magnesium acetate (TSM 10^{-2}) had little effect on the sedimentation coefficients of RNA for periods up to several days (Figs. 4 *a*, 4 *d*). If, however, the dialyzing medium consisted of tris buffer pH 7.4 without any added salt, the RNA was progressively degraded to a number of discrete smaller components (Figs. 4 *e*, 4 *f*). The relative amounts of these smaller components varied among different preparations. The rate of breakdown was diminished when the ionic strength of the dialyzing buffer was increased by the addition of 0.1 M KCl. With more extensive dialysis (48 to 72 hours) there was degradation to a limiting component of



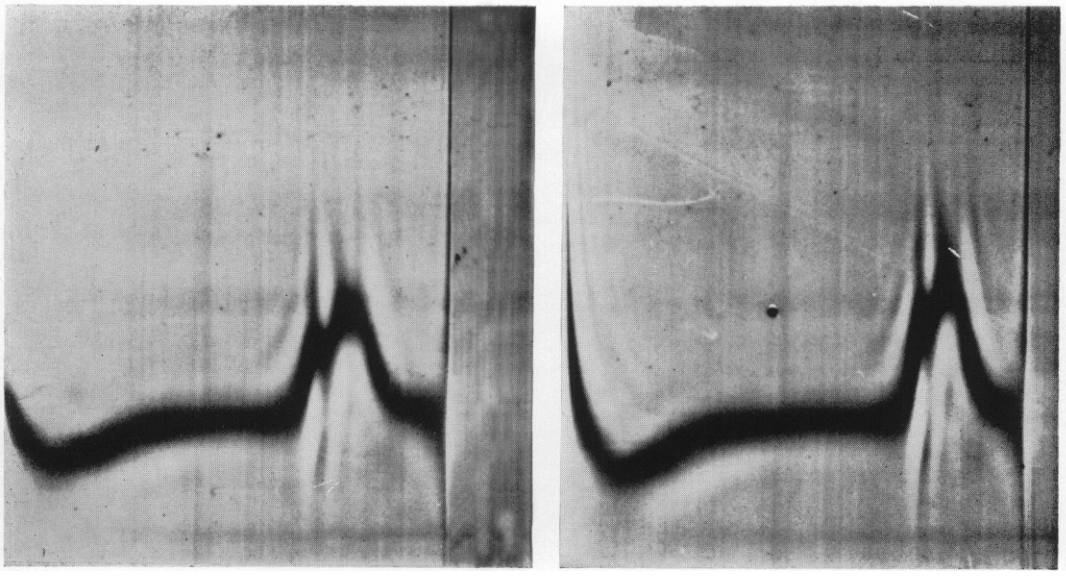


FIGURE 3 Two frames from schlieren pictures taken 14 minutes (right) and 18 minutes (left) after reaching 50, 740 R.P.M. Sedimentation from right to left. Concentration 20.0 mg/ml. (*a*) RNA extracted from 70S ribosomes suspended in TSM 10^{-2}. Phenol-saturated with 0.02 M phosphate buffer, pH 7.3. (*b*) As (*a*) but phenol-saturated with 0.02 M phosphate buffer containing 2×10^{-3} M versene.

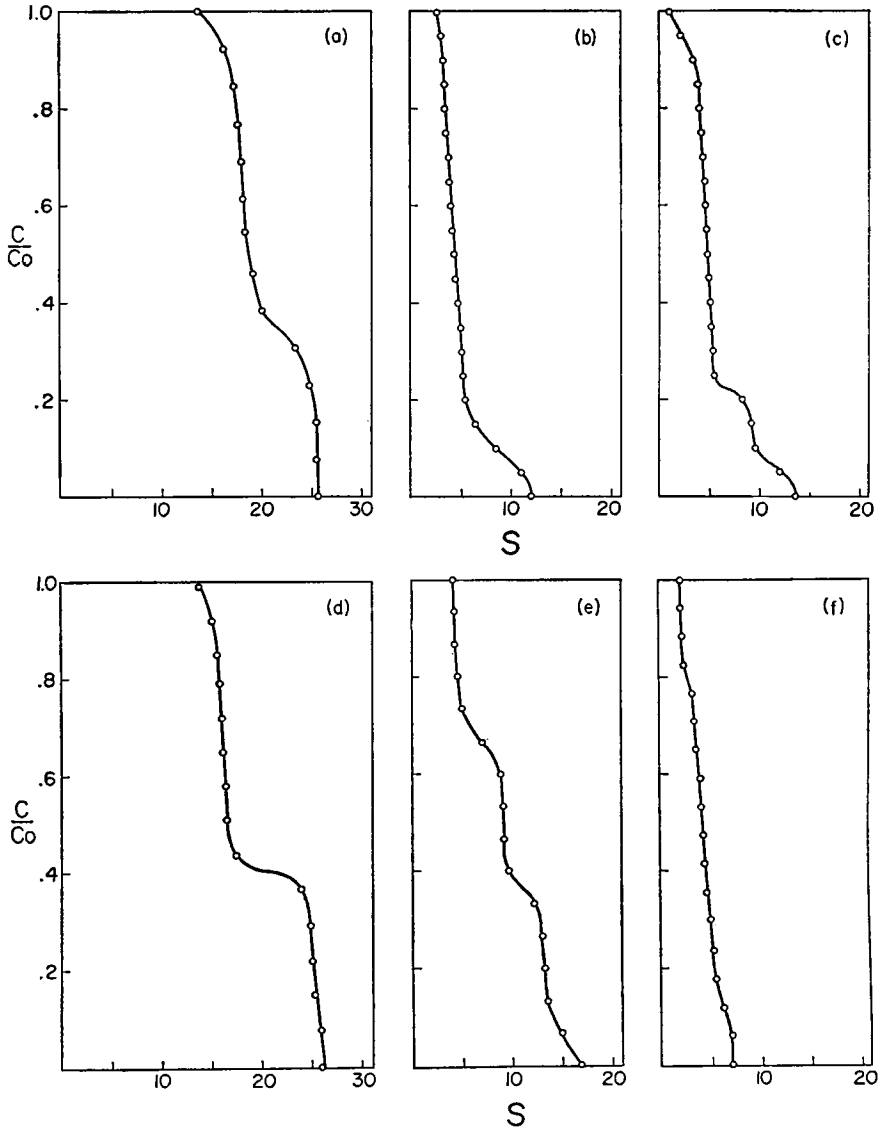


FIGURE 4 The integral sedimentation distributions of six RNA preparations. (*a*) RNA from 70S particles in TSM 10^{-3}. (*b*) RNA from magnesium-starved bacteria in TS, 0.1 M NaCl, pH 7.4. (*c*) RNA from 70S particles heated 90° 7 minutes in TS, 0.2 M NaCl, pH 7.4. (*d*) RNA of (*a*) dialyzed 60 hours against TSM 10^{-3}. (*e*) RNA of (*a*) dialyzed against TS 10^{-4} M, 32 hours, run in TS, 0.1 M NaCl, pH 7.4. (*f*) RNA of (*a*) dialyzed against TSM 10^{-4} 72 hours, run in TS, 0.1 M NaCl, pH 7.4. (Points are corrected for radial dilution.)

TABLE III

MOLECULAR WEIGHTS OF TWO RNA PREPARATIONS

	Concentration *mg/ml*	R.P.M.	Time	Molecular weight	Average
			min.		
1. 28 and 18S RNA from 70S particles dialyzed 2 days in TS, run in TS 0.1 M NaCl, $s_{20,w} = 8.80$	4.2	15,220	6	150,000	
			10	154,000	
			14	133,000	
			18	141,000	
			22	144,000	
			30	136,000	144,000±4,900
2. 28S and 18S RNA from 70S particles dialyzed 3 days in TS, run in TS 0.1 M NaCl, $s_{20,w} = 4.20$	2.9	24,630	6	29,700	
			10	27,000	
			14	28,600	
			18	29,900	
			22	30,100	
			26	29,100	
			30	28,800	29,200±1,200

4 to 5S (Fig. 4 *f*). Further dialysis did not produce smaller components, decrease the quantity of the 4 to 5S material, or cause any loss of dialyzable material to the outside buffer.

Heating. Periods of heating of a solution of RNA in tris buffer and 0.1 M KCl or 0.01 M magnesium acetate at 85°C for 10 or 15 minutes in the manner of Hall and Doty (1959) resulted in degradation to a mixture of smaller components between 4 and 18S. Heating at a higher temperature (90°C for 7 minutes) gave a limiting product of 4 to 5S (Fig. 4*c* and Table III). Degradation of RNA to a limiting size molecule of 4S by heating has also been shown by Takanami (1959) for rat liver and Osawa (1960) for yeast ribosomal RNA's.

Molecular Weights

The integral sedimentation plots of the degraded components examined by ultraviolet absorption optics suggest a non-random degradation. There appear to be two distinct molecular species between the original 28 and 18S and the limiting 4 to 5S components (Fig. 4 *e*). The sedimentation coefficients fall in the range 8 to 9S and 12 to 13S. An attempt was made to isolate each of the three components for a molecular weight measurement. The 4 to 5S species was prepared by extensive dialysis until all the material was in this form. By following the course of the degradation process it proved possible to obtain the intermediate size molecule as a reasonably homogeneous preparation of 8.8S (Table II). Attempts to prepare the largest degraded molecule were not successful; preparations in the early stages of

dialysis always contained at least two components and were not suitable for molecular weight studies.

The molecular weights were estimated by the method of Archibald (1947) using the modification of Ehrenberg (1957). Table III shows the molecular weight values obtained from the top meniscus after different times of centrifugation. A partial specific volume of 0.550 was used. There was no marked trend in the values as a function of time, indicating reasonable homogeneity. The average molecular weights obtained were 144,000 ± 4,900 for the 8.8S component and 29,200 ± 1,200 for the 4.2S component.

DISCUSSION

It has been established that most of the ribosomal RNA can be accounted for as two components here described as 18 and 28S with molecular weights of 5.5×10^5 and 1.1×10^6 respectively (Kurlund, 1960). In addition Fig. 2 shows that smaller molecules can be obtained from the 20S particles. Further evidence for the occurrence of small RNA molecules in ribosomes is given in the subsequent communication (McCarthy and Aronson, 1961).

Moreover, the larger molecules are susceptible to non-random degradation to smaller molecules of at least three different size ranges. The integral sedimentation analyses and the molecular weight determinations suggest quite a high degree of homogeneity among the molecules in these three groups. Accordingly we may consider each as a new molecular species derived from the original pair of molecules.

The 8.8 and 4.4S molecules for which the molecular weights have been measured obey the empirical expression $s^0 = kM^{0.5}$ relating sedimentation coefficient and molecular weight. The same relationship holds also for the two original molecules. By implication, therefore, the third components of 13.1S would have a molecular weight close to 300,000. There is thus an interesting quantization of molecular weights among the five components under discussion. Starting with the limiting size 4.4S molecule a fourfold increase in molecular weight gives the 8.8S component. Further increases by factors of two lead to the 13.1S component and the 18S and 28S molecules successively. All the RNA molecules observed during this study fall into one of these five classes.

The degradation of RNA molecules could be ascribed to at least three possible mechanisms; contaminating nucleases, breaks at weak points of the molecule, or a depolymerization into subunits. The first alternative would seem to be a rather unlikely one. Contaminating nuclease action would not be expected to lead to discrete components, quantized in terms of molecular weight or to an ultimate unit, although it is possible that the secondary structure is such as to expose regularly spaced covalent linkages susceptible to nuclease action. When bacterial RNAase

is intentionally added it does not produce a polynucleotide chain of about 100 units but the reaction goes to completion resulting largely in mononucleotides.

Alternatively the folding of the molecule could result in bonds, again regularly spaced, that are more strained than most of the phosphodiester linkages and thus more susceptible to heating, alteration in the ionic environment, or extent of hydrogen bonding. Such linkages may be of a different type from those commonly supposed to be universal in polynucleotide chains. On the basis of the present results it is not possible to distinguish between such a non-random degradation and the existence of real subunits. One possible way of making this distinction is to examine the kinetics of ribosomal RNA synthesis *in vivo*. These experiments are discussed in the subsequent paper (McCarthy and Aronson, 1961).

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