THE KINETICS OF THE SYNTHESIS OF RIBOSOMAL RNA IN *E. coli*

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ABSTRACT The kinetics of the synthesis of ribosomal RNA in *E. coli* has been studied using C^{14}-uracil as tracer. Two fractions of RNA having sedimentation constants between 4 and 8S have kinetic behavior consistent with roles of precursors. The first consists of a very small proportion of the RNA found in the 100,000 *g* supernatant after ribosomes have been removed. It has been separated from the soluble RNA present in much larger quantities by chromatography on DEAE-cellulose columns. The size and magnitude of flow through this fraction are consistent with it being precursor to a large part of the ribosomal RNA.

A fraction of ribosomal RNA of similar size is also found in the ribosomes. This fraction is 5 to 10 per cent of the total ribosomal RNA and a much higher proportion of the RNA of the 20S and 30S ribosomes present in the cell extract. The rate of incorporation of label into this fraction and into the main fractions of ribosomal RNA of 18S and 28S suggests that the small molecules are the precursors of the large molecules. Measurements of the rate of labeling of the 20, 30, and 50S ribosomes made at corresponding times indicate that ribosome synthesis occurs by concurrent conversion of small to large molecules of RNA and small to large ribosomes.

INTRODUCTION

It has been demonstrated (Aronson and McCarthy, 1961) that although most ribosomal RNA occurs as large molecules having molecular weights of approximately 1.2×10^6 and 5.5×10^5, there are also present in ribosomes small quantities of RNA of lower molecular weight. In addition it has proved possible to degrade all the RNA by gentle procedures to discrete molecules of about 30,000 molecular weight. Together these observations could suggest an important role of small molecules of RNA as precursors to the large molecules. In this paper the presence of the small RNA molecules in the ribosomes of various sizes is studied together with their kinetic behavior. The small molecules of RNA are shown to be more rapidly labeled than the bulk of large molecules.

MATERIALS AND METHODS

The methods of growing bacteria have already been detailed (Aronson and McCarthy, 1961). The strains of *E. coli* used were ML 30 and 15 $T^-A^-U^-$ which requires thymine, arginine, and uracil for growth. The latter strain was always grown in the presence of thymine and arginine. The C^{14}-uracil used to observe RNA synthesis was obtained from the California Corporation for Biochemical Research and had specific activities in the range 2 to 4 mc/mM. Cells uniformly labeled with P^{32} were prepared by growing overnight in a glucose-salts medium, buffered with tris, containing 1 gm/liter nutrient broth to supply carrier phosphate and 1 to 2 mc P^{32} as orthophosphate.

Ribosomes and RNA were fractionated according to size by means of sucrose gradient centrifugation (Britten and Roberts, 1960).

Radioactivity present in macromolecules was estimated after adding trichloroacetic acid (TCA) (to 5 per cent) to the sample and passing through a millipore filter. This technique also served to follow rates of incorporation of tracers (Britten, Roberts, and French, 1955; McQuillen, Roberts, and Britten, 1959).

RESULTS

1. *Kinetics of a Free Ribosomal RNA Fraction*

(*a*) *Separation of Ribosomal RNA and S-RNA.* If ribosomal particles are built up by sequential additions of protein and RNA so that the smaller particles are precursors to the larger, it might be possible to detect ribosomal RNA before it becomes a part of a nucleoprotein. The proof of its existence would depend on an adequate method of differentiating it from the soluble RNA (S-RNA) which is present in much larger quantities. Sedimentation analysis would not be suitable if such a precursor was of a low molecular weight close to that of S-RNA. There-

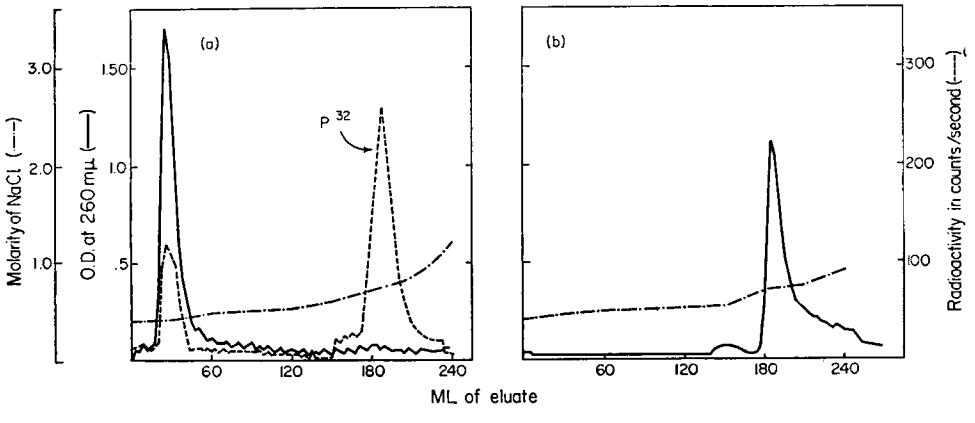

FIGURE 1 Chromatography on a DEAE-cellulose column. Non-linear sodium chloride gradient from 0.4 to 2.0 M in buffer (tris 0.01 M, succinic acid 0.004 M, magnesium acetate 0.01 M, pH 7.4). (*a*) A mixture of unlabeled S-RNA and P^{32} total RNA, phenol-extracted from magnesium-starved cells. (*b*) 28S and 18S RNA, phenol-extracted from 70S particles.

fore a chromatographic method of separation was tried to differentiate ribosomal RNA from S-RNA. It proved possible to obtain complete resolution between S-RNA and ribosomal RNA by chromatography on a DEAE-cellulose column. Fig. 1 *a* shows the result of elution of a mixture of a P^{32}-labeled total of RNA fraction and a large excess of unlabeled soluble RNA. The labeled cells had been exhaustively starved of magnesium. This procedure degrades the ribosomes to their constituent protein and RNA moieties (McCarthy, 1959) and the RNA to molecules of 4 to 5S (Aronson and McCarthy, 1961). The labeled RNA therefore consisted of a mixture of ribosomal RNA and S-RNA of closely similar sedimentation constants. Phenol-extracted unlabeled S-RNA was added in excess as carrier. The eluting buffer contained tris 0.01 M, succinic acid 0.004 M, magnesium acetate 0.01 M, pH 7.4 with a non-linear gradient of sodium chloride from 0.4 to 2.0 M. The salt gradient was made with the aid of a seven chamber device of the type used by Peterson and Sober (1959). Virtually all the ultraviolet absorbing material was eluted at the front together with the small fraction of P^{32} corresponding to S-RNA originally in the labeled cells. Most of the radioactivity associated with ribosomal RNA was eluted near 0.8 M.

The salt concentration at which ribosomal RNA is eluted does not seem to

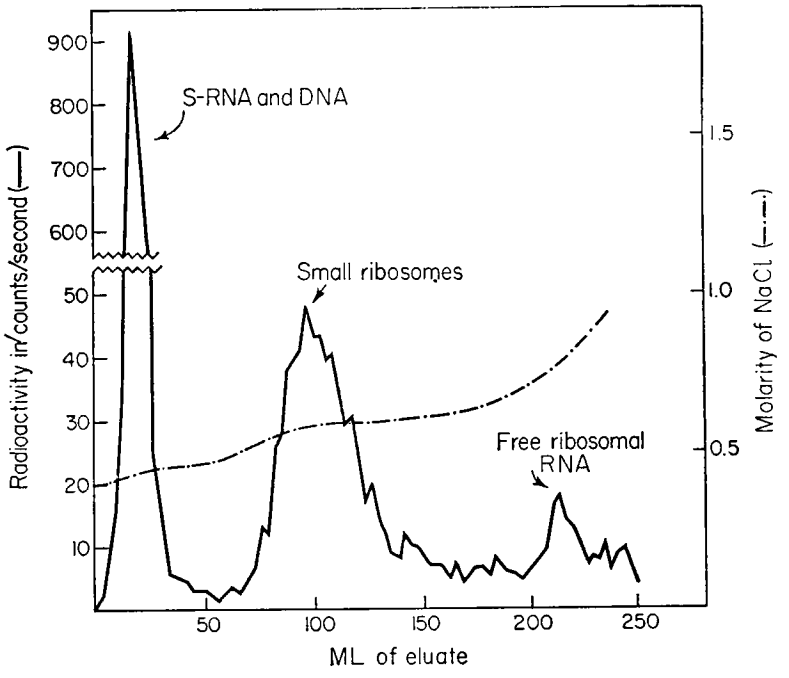

FIGURE 2 Chromatography on a DEAE-cellulose column. Non-linear sodium chloride gradient from 0.4 to 2.0 M in buffer (tris 0.01 M, succinic acid 0.004 M, magnesium acetate 0.01 M, pH 7.4). 40K 90 minutes SN of P^{32} randomly labeled cell juice.

depend very strongly on its molecular weight. Fig. 1*b* shows the elution pattern for a mixture of 28S and 18S RNA prepared by the phenol method from 70S particles. Again the RNA is eluted in the region of 0.8 M salt.

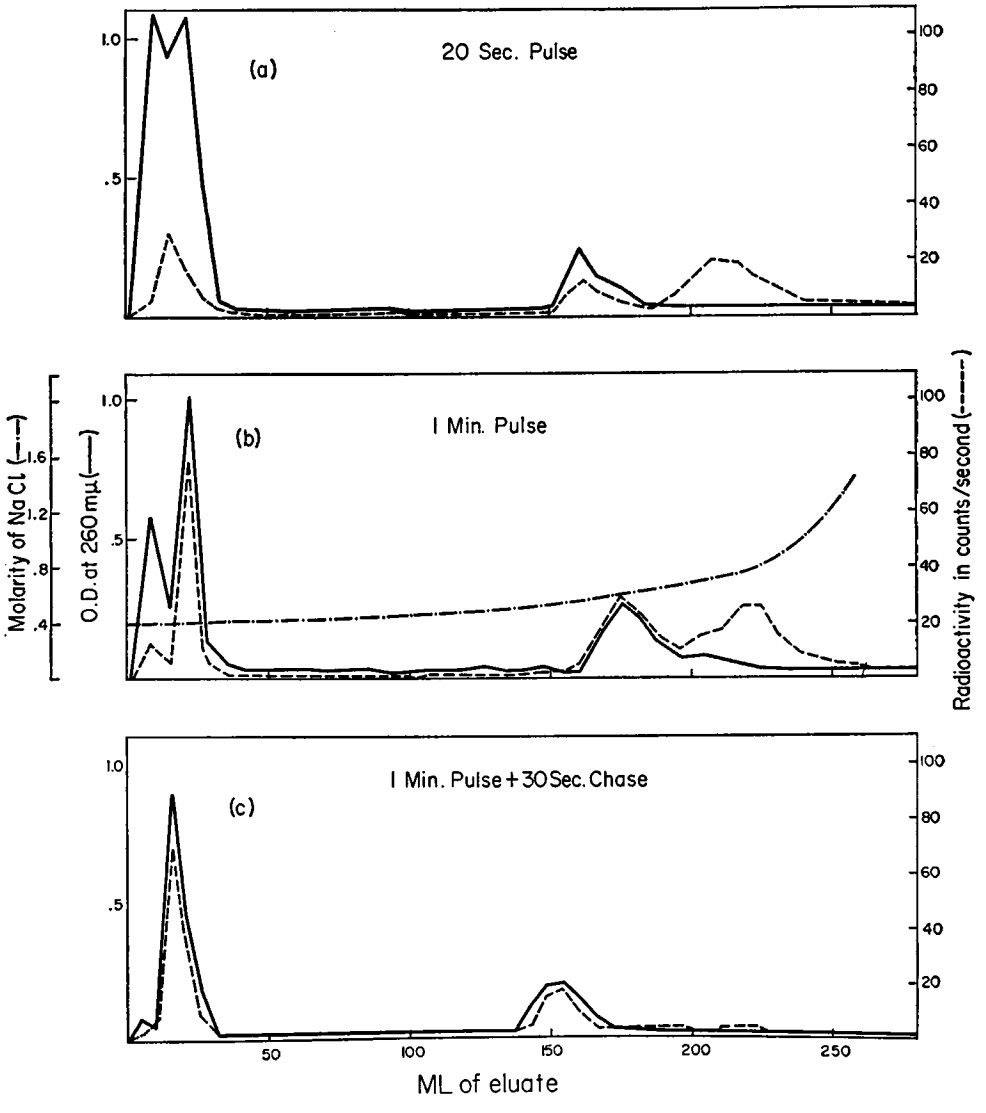

FIGURE 3 Chromatography on a DEAE-cellulose column of a 37K 180 minute SWB SN of three cell juices. Non-linear sodium chloride gradient from 0.4 to 2.0 M in buffer (tris 0.01 M, succinic acid 0.004 M, magnesium acetate 0.01 M, pH 7.4). (*a*) Cells given a 20 second exposure to C^{14}-uracil (0.5 μg/ml). (*b*) Cells given a 1 minute exposure to C^{14}-uracil. (*c*) Cells given a 1 minute exposure to C^{14}-uracil (0.5 μg/ml) followed by a 30 second chase in C^{12}-uridine and cytidine in excess (100 μg/ml of each).

Since soluble and ribosomal RNA's can be readily separated by this technique, it is possible to chromatograph cell extracts to determine whether any free ribosomal RNA is present. An extract from cells uniformly labeled with P^{32} was first centrifuged 40K[1] for 180 minutes (40K 180) to remove the bulk of the ribosomes. A sample of the supernatant was then choromatographed on a DEAE-cellulose column employing the same non-linear salt gradient (Fig. 2). The first peak contains a mixture of S-RNA and DNA, the second elutes at a salt concentration characteristic of small ribosomes and represents the few left in the supernatant after centrifugation, and the third small peak elutes in the region of free ribosomal RNA. Treatment of the first peak with DNAase showed 40 per cent of the P^{32} to be in DNA, so that the remaining 60 per cent would be in S-RNA.

As the sedimentation constants of S-RNA and the free ribosomal RNA are similar, as will be shown later, the same proportion of each should remain in solution after centrifugation. It is therefore justifiable to estimate the quantity of ribosomal RNA by comparison with the known quantity of S-RNA. Summing the radioactivity under the first and third peaks shows that the ribosomal RNA is approximately 1.5 per cent of the S-RNA. Accordingly, the quantity of free ribosomal RNA would be about 0.4 mg/gm dry weight of cells. If this were precursor material to all the particle-bound RNA, it would provide material for about 15 seconds' growth for cells growing with a generation time of about 1 hour. Consequently, studies with labeled uracil should reveal whether this material has the kinetic characteristics of a precursor or whether this small quantity of free ribosomal RNA arises from degradation of particles.

(*b*) *Pulse Studies with* C^{14}*-Uracil.* The uracil-requiring mutant (15 $T^-A^-U^-$) was allowed to incorporate C^{14}-uracil for periods of 20 seconds or 1 minute. The majority of the ribosomes were removed from the two cell juices obtained by means of a 37K 180 minute spin in the swinging bucket rotor. The resulting supernatants were analyzed on DEAE-cellulose columns (Figs. 3 *a*, 3 *b*). Radioactive uracil appears in three distinct regions. The first and third have been identified as S-RNA and ribosomal RNA, respectively, by the salt concentrations at the peaks. The second component is due to residual small ribosomes.

Sedimentation analysis of the same 1 minute pulse supernatant showed that most of the radioactivity appeared in a single peak corresponding to about 4 to 8S (Fig. 4). The 25 per cent of the radioactivity found in the pellet at the bottom of the tube can be attributed to the residual small particles in the preparation which must have sedimented since they have sedimentation constants of at least 20S. The other 75 per cent of the radioactivity must include both S-RNA and ribosomal RNA so that both must be in the range of 4 to 8S.

In Figs. 3 *a* and 3 *b*, it can be seen that the total label in the first peak increased with time, showing continued synthesis of S-RNA. The specific radioactivity of

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

the middle component, representing a mixture of residual small ribosomes, rises only slightly when compared to the S-RNA. The third component, the presumed precursor, has a constant quantity of label after 20 seconds. Such data are consistent with the small precursor pool. If it can be shown that this radioactivity is lost from the pool as quickly as it enters (20 seconds) then this fraction can be assumed to be either an intermediate in a kinetic sequence or rapidly degraded back to nucleotides. To determine whether label is rapidly lost from this fraction, extracts from cells exposed to radioactive uracil for a very short time followed by dilution with a large excess of unlabeled uridine and cytidine were analyzed.

Unfortunately, the mononucleotide pool which cells rapidly accumulate after

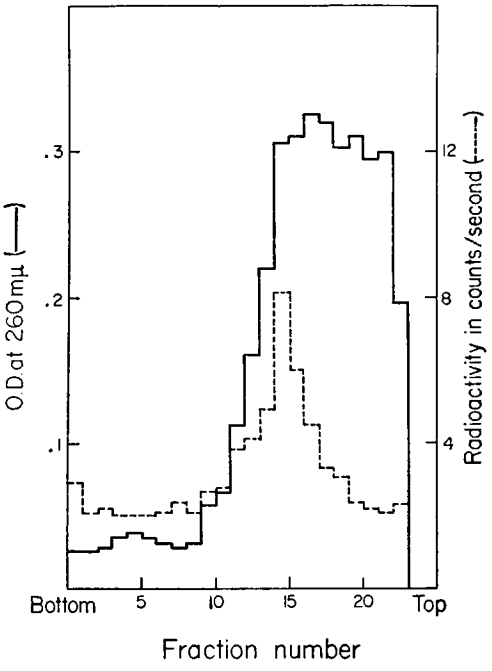

FIGURE 4 Sedimentation analysis of a 37K 180 minute SN of an extract from cells given a 1 minute pulse label with C^{14}-uracil. Most of the ultraviolet-absorbing material between fractions 17 and 24 is protein. Centrifugation 30K 16 hours. An object of about 6S would be expected to peak in the middle of the tube.

a very brief exposure to exogenous C^{14}-uracil is sufficient for some 5 minutes' growth. The addition of a gross excess of non-radioactive uracil and cytosine fails to dilute out the pool. Thus, under normal conditions of growth, a 5 to 10 minute dilution period ("chase") of C^{14}-uracil is the minimum possible. It is, however, possible to surmount this difficulty and to obtain rapid cessation of further incorporation of label into polynucleotide. Bacterial cells will grow in a medium of high osmotic strength without any appreciable loss of metabolic ability or diminution of

growth rate. If such a culture is quickly added to an equal volume of distilled water, the resulting osmotic shock is sufficient to cause the cells to lose their pools of amino acids and nucleotides almost completely. This technique makes possible the rapid cessation of further incorporation of label.

Uracil-requiring cells were grown in C^{14}-uracil (0.5 μg/ml) for 1 minute in C medium containing 0.25 M sucrose, and then quickly poured into an equal volume of distilled water. After a few seconds, a two hundred-fold excess of unlabeled uridine and cytidine was added and quickly followed by the salts necessary to bring the medium back to the original composition. Prior experiments had shown that such manipulations did not cause a delay in the resumption of protein or RNA synthesis. Measurement of the uptake of C^{14}-uracil showed a rapid decrease in the

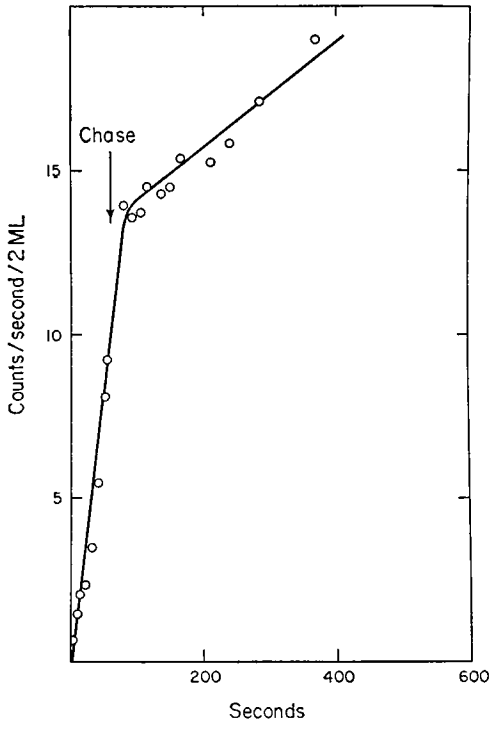

FIGURE 5 The incorporation of C^{14}-uracil (0.5 µg/ml) by *E. coli* $T^-A^-U^-$ into a TCA–precipitable fraction. Cells growing at 37° C in a maltose-salts medium containing 0.25 M sucrose. At 1 minute the cells were diluted by pouring into an equal volume of distilled water. This was followed by an addition of 100 µg/ml each of C^{12}-uridine and cytidine and the salts necessary to bring the medium back to the normal composition.

rate of incorporation by a factor of 12 (Fig. 5). Samples were harvested after chasing for various times, chilled, and processed in the usual manner.

Fractionation of the juice from cells given a 30 second chase by this technique

after a 1 minute uracil pulse, showed very little label left in the ribosomal RNA region of the elution diagram (Fig. 3*c*), Thus, this pool does have the properties of an intermediate, being rapidly labeled and just as rapidly emptied. Although neither the estimate of the steady state pool size nor the turnover time are accurate to better than a factor of two, the existence of such a pool does suggest that at least a large percentage of the particle-bound RNA could pass through a pool of free ribosomal RNA before it is assembled into nucleoprotein.

2. *Newly Synthesized RNA in Ribosomes*

The state of the newly synthesized RNA actually within nucleoprotein particles was then investigated. Total particle fractions were prepared by centrifuging a cell extract in the swinging bucket rotor at 37K for 3 hours. At least 90 per cent of the particle RNA was collected by this means. The pellets were then extracted with phenol to obtain the RNA and fractionated into the various sized components on the sucrose gradient. Fig. 6 compares the distribution of radioactivity in the ribo-

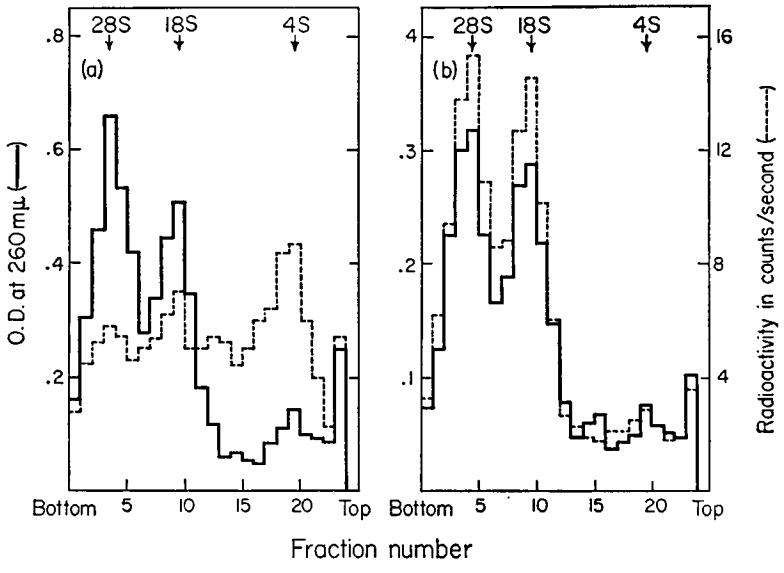

FIGURE 6 Sedimentation analysis of RNA extracted from ribosomes. (*a*) From cells given a 30 second exposure to C^{14}-uracil (0.5 μg/ml). (*b*) From cells given a 30 second exposure to C^{14}-uracil (0.5 μg/ml) followed by 20 minutes' growth in C^{12}-uracil (100 μg/ml). Centrifugation 37K 280 minutes.

somal RNA from cells given a 30 second C^{14}-uracil pulse with that of the same cells given a subsequent 20 minute chase with C^{12}-uracil. It is immediately apparent that low molecular weight RNA of between 4 and 8S is most highly labeled at early times and that at the subsequent time point virtually all of the radioactivity is found

in the 18 and 28S fractions. There can be little contamination of the particles by soluble RNA, as much of its radioactivity would persist and be present in the 4S region after the 20 minute chase. Therefore, it may be concluded that small molecules of RNA enter nucleoprotein in that form and that there seems to be a second process by which labeled material of this component passes on into the larger molecules.

Since small RNA components are prominent in short term labeling experiments, the distribution of the small RNA components among the various sized ribosomes was determined. Each of the four ribosome classes (70 to 100S, 50S, 30S, 20S) was isolated from two extracts, one from cells which had been exposed to C^{14}-uracil for 1 minute and the other from uniformly labeled cells. A 40K 180 minute pellet was fractionated by sedimentation to separate the 70, 50, 30, and 20S ribosomes (Fig. 7). Characterization of the RNA from each of these fractions was then made

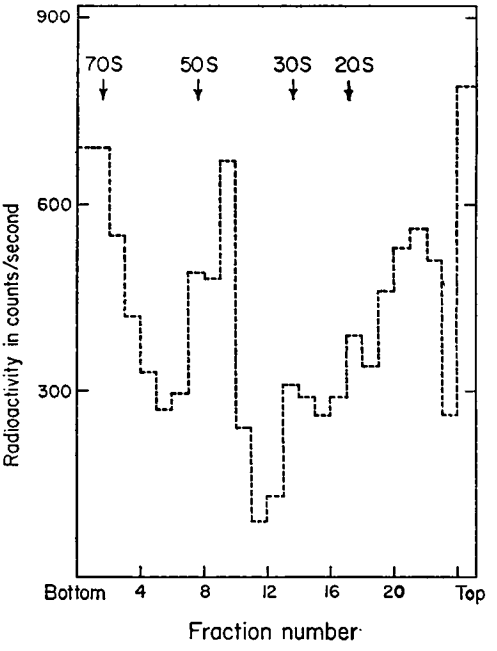

FIGURE 7 Sedimentation analysis of C^{14}-uracil uniformly labeled ribosomes (40K 180 P). The peaks correspond to 70S, 50S, 30S, and 20S ribosomes. The fractions from which RNA was extracted were 70S 2-5, 50S 8-10, 30S 14 and 15 and 20S 17 and 18. Centrifugation 37K 120 minutes.

by means of a second sucrose gradient separation (Fig. 8). This analysis of the uniformly labeled RNA's of the different particles is analogous to the schlieren studies already described (Aronson and McCarthy, 1961) but it is sufficiently more sensitive for it to permit the study of the RNA of the less abundant "native"

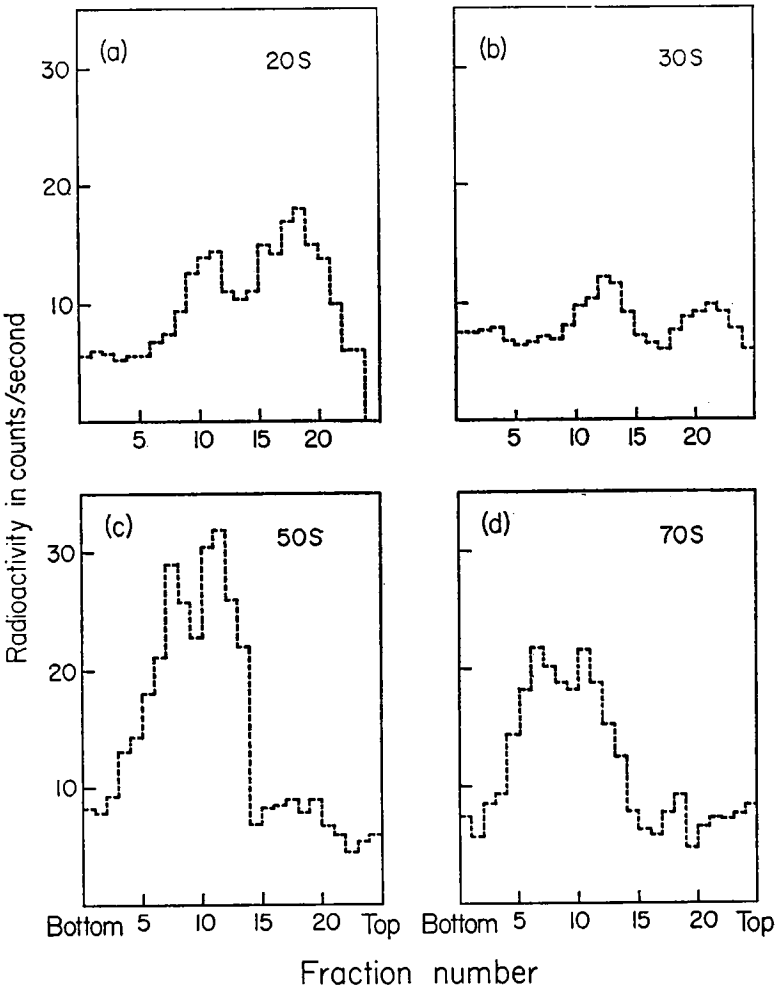

FIGURE 8 Sedimentation analysis of RNA extracted from C^{14}-uracil randomly labeled ribosomes. (*a*) 20S. (*b*) 30S. (*c*) 50S. (*d*) 70S. Ribosome fractions obtained by the separation shown in Fig. 7. Centrifugation 37K 240 minutes. An object of 18S would be expected to peak in the middle of the tube.

50, 30, and 20S particles. The native 50S particles are made up mostly of 28 and 18S RNA, but they also contain some RNA of 4 to 8S. Fig. 8 *a* showing the RNA extracted from 20S particles, demonstrates RNA of both 18S and 4 to 8S. As previously discussed (Aronson and McCarthy, 1961), the larger RNA component cannot be part of a single 20S particle. The fact that the 20S particles contain the highest proportion of low molecular weight RNA would be in close accord with a role as precursor of some, at least, of the larger particles.

The analogous experiments with 1 minute pulse-labeled ribosomes showed the distribution of newly formed RNA. Even in the 70S particles about one-third of the

radioactivity appeared as material of low sedimentation constant (4 to 8S) (Fig. 9). The 50S particles contained only traces of radioactive 18 and 28S RNA, the radioactivity appearing mainly in 4S and 12S RNA. The labeled RNA of both the 20 and 30S particles was mainly 4 to 8S. It is evident that the 4 to 8S material is not confined to any one class of ribosome.

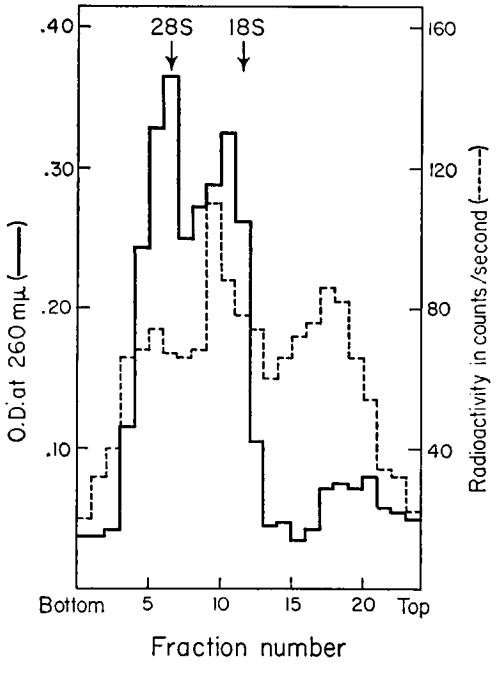

FIGURE 9 Sedimentation analysis of the RNA extracted from purified 70S ribosomes of cells given a 1 minute exposure to C^{14}-uracil. Centrifugation 37K 240 minutes.

To examine in more detail the fate of this low molecular weight fraction of the ribosomal RNA, a series of different length uracil pulses and chases were studied. Fig. 10 shows the analysis of RNA derived from particles of cells given 20, 40, and 60 second pulses of C^{14}-uracil and a 60 second pulse followed by 5 minute and 10 minute chases. The ribosomes were prepared by a 40K 180 minute centrifugation so that the yield of the smaller ribosomes was not complete. The small 4 to 8S RNA becomes labeled very quickly, and the specific radioactivity continues to increase linearly for at least 1 minute.

In Fig. 11 the total label in the three main classes of RNA 28S, 18S, and 4 to 8S is plotted as a function of time. The label in the smallest components has not reached a saturation value by 1 minute and, therefore, the pool of such material must be greater than a minute's supply if it is an intermediate. On the other hand, there is evidence of a lag in the flow of label into the 18S and 28S regions suggesting the existence of a precursor. On chasing the label is lost from the small com-

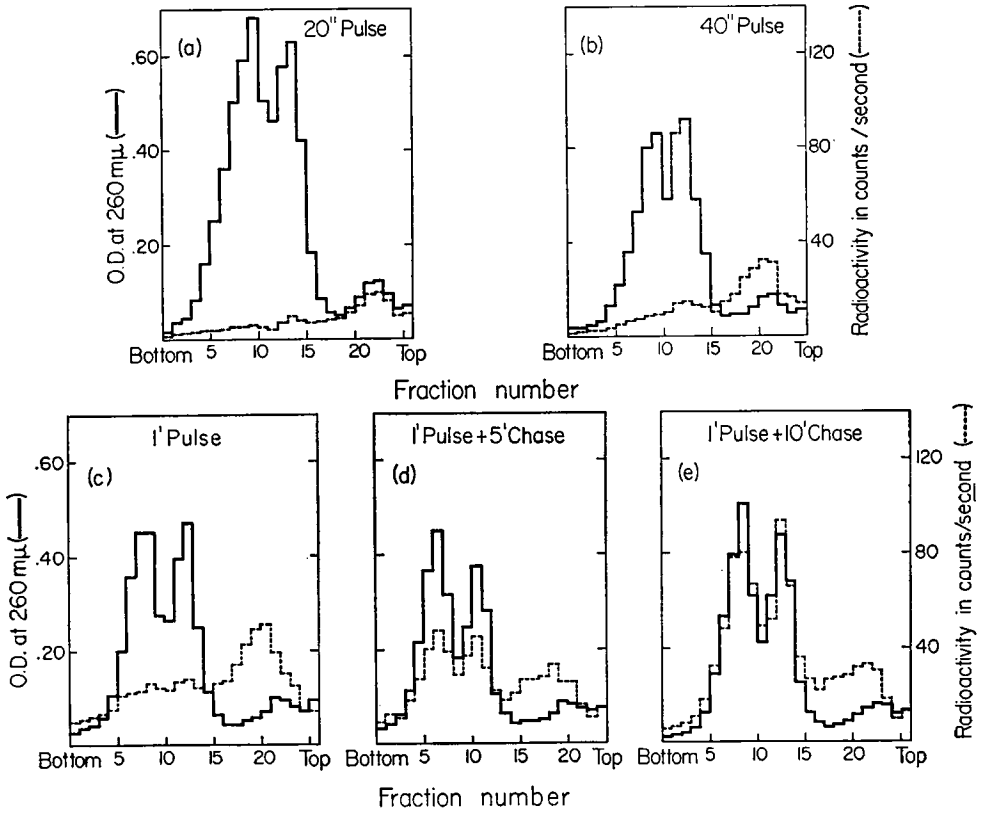

FIGURE 10 Sedimentation analysis of the RNA extracted from ribosomes. (*a*) From cells given a 20 second exposure to C^{14}-uracil (0.5 μg/ml). (*b*) 40 second exposure. (*c*) 1 minute exposure. (*d*) 1 minute exposure to C^{14}-uracil (0.5 μg/ml) followed by a 5 minute period in C^{12}-uridine and cytidine in excess (100 μg/ml of each). (*e*) 1 minute exposure to C^{14}-uracil followed by a 10 minute period in C^{12}-uridine and cytidine. Centrifugation 37K 240 minutes. An object of 18S would be expected to peak in the middle of the tube.

ponents to reach a constant value by about 5 minutes. At the same time, more label flows into the large components.

The distribution of ultraviolet-absorbing material in Figs. 6 and 10 shows that about 5 per cent of the ribosomal RNA is in the form of 4 to 8S material. With a generation time of 50 to 60 minutes the RNA increases at about 2 per cent per 100 seconds. Thus if the 4 to 8S RNA were entirely precursor to the 18S and 28S components, its quantity would be sufficient to provide material for about 4 minutes' growth. Accordingly it is possible to consider how well the observed data of Fig. 11 fit with such a precursor-product relationship, as opposed to complete independence of the synthesis of 4 to 8S RNA and the larger 18S and 28S.

The fact that the label in the 4 to 8S fraction decreases by only a factor of three

on chasing could be a result of the inefficient chase. A 4 minute pool of 4 to 8S RNA would have reached only one-quarter of its eventual specific radioactivity after a 1 minute labeling. But the chase itself decreased the rate of uptake of label by only a factor of twelve. Therefore the chase would reduce the specific radioactivity or the total label in the 4 to 8S pool by a factor of three from one-quarter to one-twelfth.

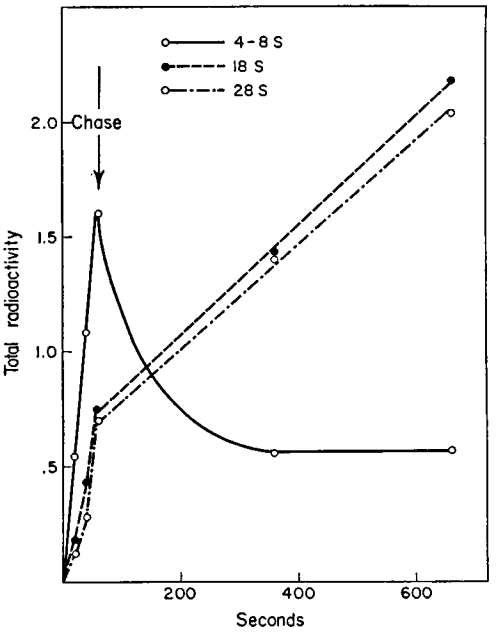

FIGURE 11 The total radioactivity in the three main fractions of ribosomal RNA of cells given a 1 minute exposure to C^{14}-uracil followed by a 10 minute period in C^{12}-uridine and cytidine. Data from Fig. 10.

3. *Kinetics of Ribosome Synthesis*

To correlate these observations on the synthesis of RNA with a scheme of particle synthesis, additional kinetic studies were made of the incorporation of C^{14}-uracil into the ribosomes. Such experiments represented a continuation of a series of pulse labeling and chasing studies performed in this laboratory over the past 3 years (Roberts *et al.*, 1958, 1959, 1960). For the most part, either S^{35} or P^{32} had been previously employed as a tracer. C^{14}-uracil was used in this series to avoid the kinetic delays introduced by the large pool of low molecular weight phosphorus compounds. Some effort was made to obtain results using pulses and chases of the same times as had been employed in the RNA studies so that a correlation could be made between particle kinetics and RNA kinetics. In many

cases, part of the sample was fractionated in the ribosome form and another as free RNA.

Sedimentation analyses of a total ribosome pellet (37K 180 P) in the region of the 50S, 30S, and 20S ribosomes do not show well resolved peaks corresponding to these objects. The interpretation of such analyses in terms of the labeling of these specific objects therefore requires some justification. This difficulty arises because of the presence of a large quantity of 70S ribosomes at the bottom of the tube. It is possible to remove most of these by suitable centrifugation and then to concentrate the remaining 50S, 30S, and 20S ribosomes by a further centrifugation. Subsequent sedimentation analyses then show much clearer indications of three peaks. In the present case, however, it seemed that the more prolonged manipulation of the preparation and the consequent increased possibility of degradation would more than nullify the advantages of a cleaner separation.

On the other hand, there is no doubt that the small ribosomes are present and separated by this technique. The relative proportions of the 50S, 30S, and 20S ribosomes indicated by the ultraviolet absorption agree with those determined in the model E analytical ultracentrifuge. These ribosomes represent about 10, 5, and 5 per cent respectively of the total ribosomal material of the cell juice. Fractions taken from the peaks of the swinging bucket analyses or from where the ribosome peak is expected and analyzed in the model E analytical ultracentrifuge show not only the presence of the particular ribosome expected for that fraction, but also that these ribosomes account for most of the ultraviolet absorption. In view of this it seems justifiable to interpret the results of these sedimentation analyses in terms of the three classes of ribosomes, rather than in terms of a heterogeneous mixture.

The results to be presented have been analyzed mainly on the basis of the distribution of radioactivity. Fig. 12 represents sedimentation analyses of five C^{14}-uracil pulse–labeled total ribosome pellets collected by a 37K 180 minute centrifugation. The five samples were taken after a 20 second, a 40 second, and a 1 minute pulse, and a 1 minute pulse followed by 1 and 2 minute chases. Specific radioactivities were calculated to normalize the data for differences in the quantity of material analyzed, and to summarize the results (Fig. 13).

These early pulse-labeling studies fail to elucidate the exact relationship among the 20, 30, and 50S ribosomes for there are no striking differences among the rates of labeling of the three regions corresponding to the three ribosome groups. At the earliest time, 20 seconds, the radioactivity is more or less equally distributed among the three regions. Although the specific radioactivities of the 20S and 30S seem to be very close throughout the three pulse points, that of the 50S lags behind during the 1st minute. The difference in specific radioactivity obtained from the data of Fig. 12 is consistent with the equal distribution of total radoactivity among the three regions, and with the greater quantity of 50S ribosomes present in a cell extract.

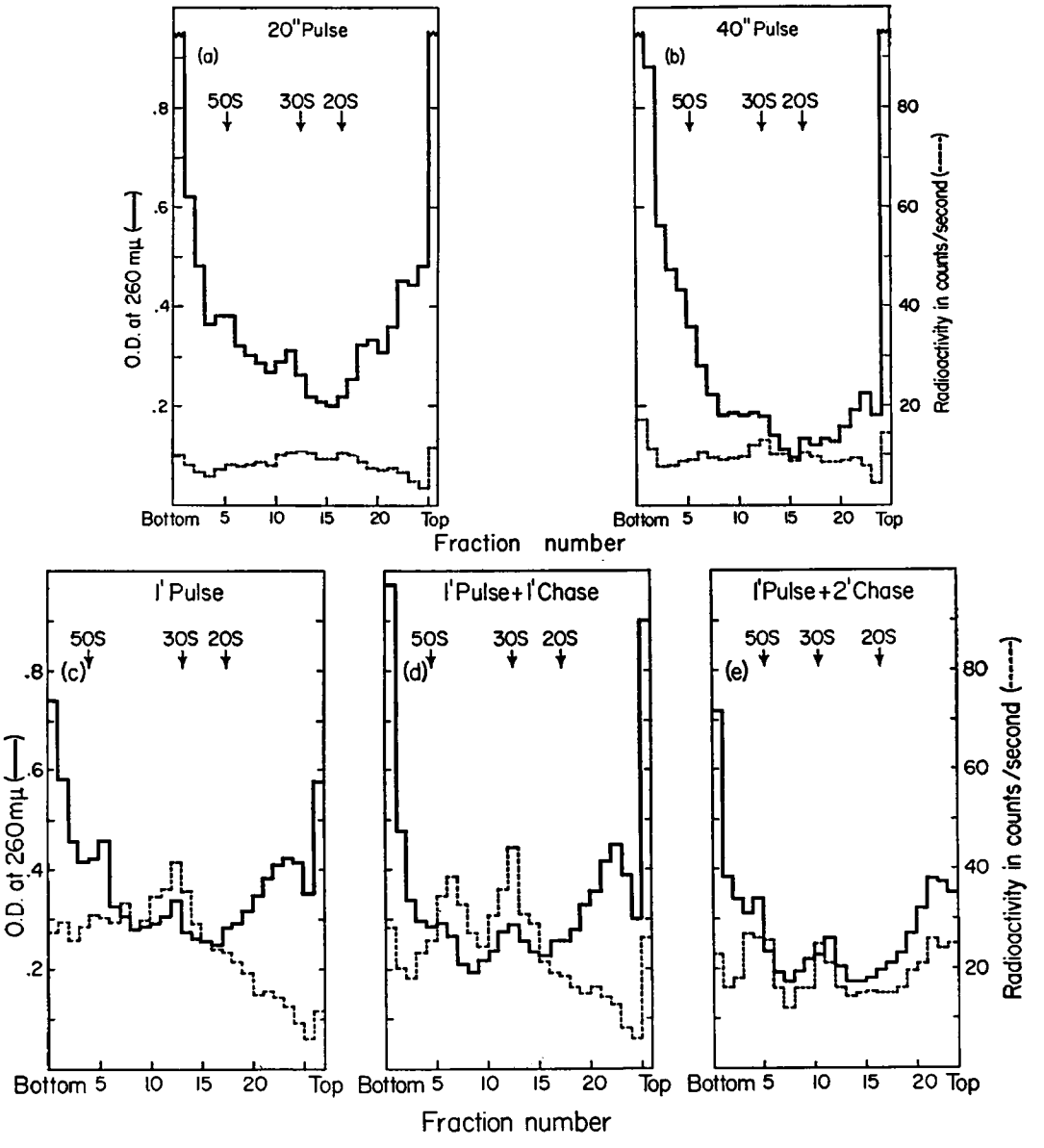

FIGURE 12 Sedimentation analysis of five total ribosome pellets (37K 180 P). (*a*) From cells given a 20 second exposure to C^{14}-uracil (0.5 μg/ml). (*b*) 40 second exposure. (*c*) 1 minute exposure. (*d*) 1 minute exposure to C^{14}-uracil (0.5 μg/ml) followed by a 1 minute period in excess C^{12}-uridine and cytidine (100 μg/ml of each). (*e*) 1 minute exposure to C^{14}-uracil followed by a 2 minute period in C^{12}-uridine and cytidine. Centrifugation 37K 150 minutes.

The distribution of label among the three ribosomes at the earliest times indicates three separate points of entry of newly synthesized RNA. An independent synthesis of each of the three small ribosomes would require a distribution of early radioactivity in proportion to the quantity of the three ribosomes. The radioactivity associated with the 50S at early times would then be twice that observed. It seems more reasonable, therefore, to suggest that the ribosomes are built by addition of RNA and protein to a preexistent core so that the 20S becomes a 30S and 30S becomes a 50S. This has already been proposed by Roberts *et al.* (1959) on the basis of early labeling studies using P^{32} and S^{35} as tracer, the results of which bear much similarity to those discussed here.

Of further interest is the proportional and rapid loss of specific radioactivity of the 50 and 30S components during the 2nd minute of chasing (Figs. 12 and 13). If the synthesis of 70S particles from 30S and 50S were a one way process, then the pools of these smaller particles based on the steady state quantities relative to 70S should empty only about five or six times per generation. The higher rate

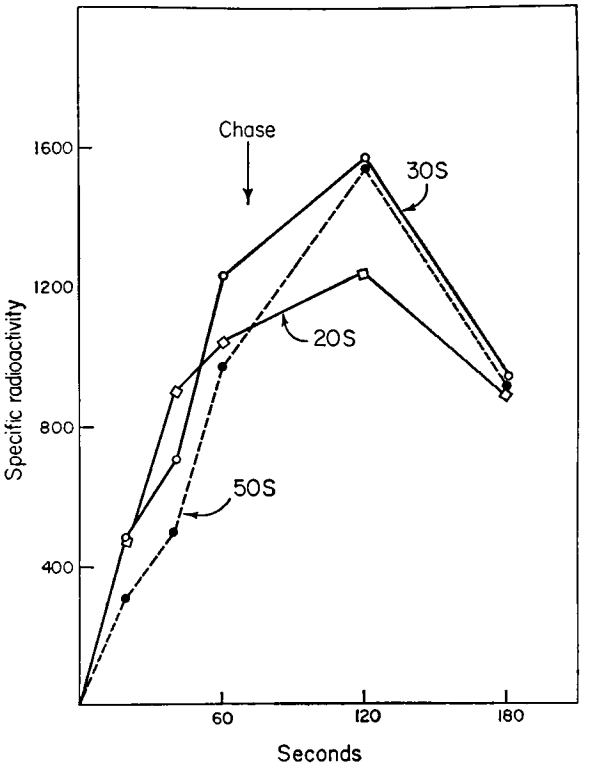

FIGURE 13 The specific radioactivities of the 20S, 30S, and 50S ribosomes of cells given a 1 minute exposure to C^{14}-uracil followed by a 2 minute period in excess C^{12}-uridine and cytidine. Data from Fig. 12.

of loss of specific radioactivity observed suggests that the new particles are diluted by an equal number from the 70S fraction about every 1½ to 2 minutes so that only about one-fifth of the 30S and 50S pools are really precursors. Since roughly 80 per cent of the ribosomal material is in the form of 70S and 100S particles, we can make an estimate of about 4 to 6 minutes as the average lifetime of a large particle before it breaks down to its component parts. These calculations are approximate and further experiments are needed to clarify this process.

DISCUSSION

The present studies on the kinetics of RNA synthesis have clearly shown that there are present minor components of between 4 and 8S which are very rapidly labeled. Such material can be demonstrated as free RNA in the 100,000 *g* supernatant and also as part of the ribonucleoprotein fraction. The former fraction has kinetic characteristics (magnitude of flow and time constant) which are consistent with its being a precursor to the main bulk of ribosomal RNA. The available data are, however, equally consistent with the behavior of an independent RNA fraction unrelated to the synthesis of ribosomal RNA which is rapidly synthesized and subsequently degraded to nucleotides or lost from the cell. At the moment, a choice cannot be made between these two possibilities.

The second component of similar molecular size found in the ribosome fraction is also rapidly labeled although it has a considerably longer turnover time. This extended time is not unreasonable since it is a much more abundant component; *i.e.,* about 5 per cent of the total ribosomal RNA. As with the soluble component it appears that this lower molecular weight RNA is a precursor to the 28S and 18S RNA. However, it is not clear from the crude separation techniques employed how many components are mixed in the 4 to 8S region of the swinging bucket separation. There could be a series of homologous polymers or a mixture of components of each of the two types.

The existence of any appreciable fraction of the pulse uracil label in an RNA fraction which is rapidly exchanging with the nucleotide pool is not supported by the incorporation data of Fig. 5. The sedimentation analysis of ribosomal RNA following a 1 minute uracil pulse shows about one-third of the label in the form of 4 to 8S material. If this were all rapidly turning over by degradation to nucleotide (TCA-soluble) material, the TCA-precipitable counts should fall by 30 per cent very rapidly after the chase was made. This is evidently not the case, so that although label leaves the 4 to 8S material on chasing, it cannot be feeding back into the uracil pool of TCA-soluble compounds. Counts leaving the 4 to 8S component find their way into the 18S and 28S components during the 5 minute chase with non-radioactive uracil. Such a quantitative reutilization of uracil counts would not be expected if there were an intermediate stage during which the label became TCA-soluble again and equilibrated with the uracil pool. In addition, the kinetic delay in

incorporation into 18 and 28S components does suggest the existence of a precursor. On the basis of these two points, then, we are inclined to believe that the 4 to 8S RNA is an intermediate.

The above experiments extentd the over-all view of the process of ribosome synthesis about which much information has ben collected (Roberts *et al.*, 1958, 1959, 1960). It is already clear that the small quantity of 20, 30, and 50S ribosomes present in a cell juice prepared in the presence of 0.01 M magnesium ions is not the result of breakdown of the more abundant 70S ribosomes but is their precursor. Furthermore, as we have seen in these studies there is newly formed RNA in each of these three particle groups at early times. At 20 seconds the uracil label is roughly equally distributed among these three groups. Thus there seem to be three points of entry of new RNA into the ribosome system before the formation of the 70S.

One other general feature of ribosome synthesis is important to the present discussion. Although the small particles are precursors to the large, the process is evidently not unidirectional. A long chase period following a short pulse results in the distribution of the tracers almost equally among all the particle groups (Roberts *et al.*, 1959). Evidently the 70S ribosomes, once formed, are not stable end products but rather break down into their constituent parts again. The short chase periods examined here have shown that the 30 and 50S ribosome pools lose their label very rapidly. This process takes place about ten times as rapidly as is necessary to account for the net synthesis alone. A breakdown process 70S $\rightarrow$ 50S + 30S occurring about ten times per generation, with a 70S particle having a lifetime of 5 minutes would fit the observed results.

How may we fit the observations of RNA synthesis with this scheme? It is immediately obvious that if the 20S particle is the ultimate 70S ribosome precursor, ribosomal RNA must exist at early times as molecules of not more than 300,000 molecular weight (the RNA content of a 20S ribosome). In fact, an examination of the RNA of the different ribosome groups has shown that the 20S and 30S particles have a high proportion of low molecular weight RNA. Most of the 5 per cent or so of the postulated ribosomal RNA precursor must therefore be located in these precursor ribosomes. Such a distribution would be expected if the conversion of small to large ribosomes and of small to large RNA molecules are concurrent processes. In addition, the rapid circulation betwen 30 and 50S, and 70S ribosomes could explain how some of the 4 to 8S pulse–labeled RNA is found in the 70S ribosomes when the pool of such material is about a 5 minutes' supply. A flow of ten times the net increase of material through the 30 and 50S ribosome pools would sweep some low molecular weight RNA on into 70S ribosomes. Therefore, the polymerization of precursor RNA cannot be mandatory for the conversion of 30 and 50S ribosomes to 70S.

In summary, the following scheme for the synthesis of ribosomal RNA may be

presented. Newly polymerized nucleotides appear first in 4 to 8S RNA which can be isolated from the 100,000 *g* supernatant of disrupted cells. Later these small molecules are found in the 20, 30, and 50S particles. Evidently the ribosomes grow by the aggregation of RNA and protein molecules, perhaps to a preexistent core (Roberts *et al.*, 1959). As the process continues, the RNA units are joined to form larger entities of about a half and one million molecular weight. Concurrently, the 30S and 50S particles join to form the 70S particles which have been shown to be active in protein synthesis.

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