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Purification and Properties of Nuclear and Cytoplasmic "Deoxyribonucleic Acid like" Ribonucleic Acid from Ehrlich Ascites Cells*

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ABSTRACT: Newly synthesized ribonucleic acid was extracted from the nuclear and cytoplasmic fractions of Ehrlich ascites cells. These ribonucleic acids were fractionated further by chromatography on methylated albumin kieselguhr columns. Methods were developed whereby the ribonucleic acid rich in adenylic acid and uridylic acid ("deoxyribonucleic acid like" ribonucleic acid) from the nucleus and cytoplasm could be isolated from methylated albumin kieselguhr columns in a highly purified form. The cytoplasmic deoxyribonucleic acid like ribonucleic acid was found to differ significantly from the nuclear deoxyribonucleic acid like ribonucleic acid in both sedimentation properties and in base composition. This provides additional evidence that within animal cells there exists a species of ribonucleic acid which is restricted to the nucleus.

The nuclei of animal cells have been shown to contain species of RNA which are heterogeneous in size and which are characterized by having a base composition high in adenylic and uridylic acid. This ndRNA,[1] therefore, is distinct from the rRNA, rpRNA (Scherrer *et al.*, 1963), tRNA, and 5S RNA (Knight and Darnell, 1967) which are also found in the nucleus. The ndRNA represents a large fraction of the rapidly labeled RNA in the cell and can be isolated from nuclei in amounts which are readily detectable by optical density measurements (Muramatsu *et al.*, 1966).

Several types of experiments have indicated that a sizable fraction of this ndRNA cannot be a precursor to cdRNA (see Discussion). This means that in animal cells there exists a species of RNA which is restricted to the nucleus, which may be of considerable importance to the cells, but whose function is presently unknown. Many experiments designed to help elucidate the function of the ndRNA would become feasible if a simple method could be found for the quantitative isolation of ndRNA free from contamination. This would enable chemical and biological studies to be made on the isolated RNA, as well as permit investigations concerning the effects of hormones, antigens, and other stimuli and stresses on the ndRNA in the appropriate target cells.

Several procedures have been used for the isolation of ndRNA. These include nuclear fractionation (Soeiro *et al.*, 1966; Willems *et al.*, 1968) and nuclear fractionation in combination with methylated albumin kieselguhr chromatography (Muramatsu *et al.*, 1966). Of the various methods of RNA fractionation which are presently available, chromatography on methylated albumin kieselguhr columns is potentially one of the most useful, since the procedure is convenient, the recovery can be made quantitative, and large amounts of RNA can be fractionated.

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[1] Abbreviations used in this paper: rpRNA, ribosomal precursor RNA; cdRNA, cytoplasmic "DNA-like" RNA; ndRNA, nuclear "DNA-like" RNA.

Methylated albumin kieselguhr chromatography has been used to fractionate various species of high molecular weight animal cell RNA (Koch and Kubinski, 1964; Yoshikawa-Fukada *et al.*, 1965). Its use for the separation of dRNA from rRNA and rpRNA was suggested by the observation that RNA molecules rich in A and U are bound very firmly to methylated albumin kieselguhr columns (Ellem and Sheridan, 1964; Asano, 1965; Ellem, 1966). Ellem (1966) has used this procedure to partially purify the combined cdRNA and ndRNA from HeLa cells.

Fractionation of RNA on methylated albumin kieselguhr columns depends upon differences in size, conformation, or base composition of the RNA species, as well as selecting the appropriate conditions for elution. This paper describes conditions which permit the almost quantitative isolation of cdRNA and ndRNA from ascites cells by methylated albumin kieselguhr chromatography. The two species appear to be largely undegraded and free from contamination. Analysis of these RNAs provides additional evidence for a nonprecursor relationship between ndRNA and cdRNA.

Materials and Methods

Preparation of Radioactive RNA. Ehrlich ascites cells were harvested, washed, and suspended in modified Earle's saline as previously described (Roberts, 1965). To each of seven petri dishes, each containing 10 ml of the cell suspension, was added 0.2 ml of ^{32}P solution (carrier free, 0.15 M in NaCl, and containing approximately 2 mCi of $^{32}PO_4^{2-}$/ml). The cell suspensions were incubated at 37° for 45 min or for 3 hr. The 24-hr period of labeling was carried out by injecting 0.4 ml of ^{32}P solution directly into the interperitoneal cavity of a mouse bearing the tumor, and removing the ascites cells the next day. Using previously developed procedures (Roberts *et al.*, 1966), the cells were then washed, fractionated into nuclear and cytoplasmic components, and the RNA was isolated from each fraction by phenol extraction at pH 9.5. Nuclear RNA prepared in this manner is contaminated with large amounts of radioactive DNA which must be hydrolyzed with pancreatic DNase (Roberts *et al.*, 1965) before the RNA can be effectively analyzed. This degraded DNA is primarily responsible for the low molecular weight radioactive material observed in the sedimentation patterns and methylated albumin kieselguhr chromatography patterns of nuclear RNA.

Methylated Albumin Kieselguhr Column Chromatography. Column chromatography was carried out on methylated albumin kieselguhr columns (8 × 1.3 cm) which had been prepared according to the method of Mandell and Hershey (1960). Celite (Johns-Manville), previously washed by suspending the Celite to 5% in 1 M NaCl–0.1 M potassium phosphate–0.001 M EDTA (pH 6.7), boiling for 15 min, and centrifuging, was used as the source of kieselguhr for the preparation of these columns. The columns were stored at 4° for as long as 2 weeks before use.

The methylated albumin kieselguhr columns were washed for 24 hr immediately prior to use with 0.5 M NaCl–0.1 M potassium phosphate–0.001 M EDTA (pH 6.7). All column washings and elutions were carried out at 37° with a flow rate of approximately 0.25 ml/min.

Chromatography of cytoplasmic RNA was performed on columns which had been equilibrated with 0.2 M NaCl–0.1 M potassium phosphate–0.001 M EDTA (pH 6.7) by passing 50 ml of this solution through the column after the 24-hr wash. Approximately 1 mg of cytoplasmic RNA (about 20% of the RNA from a single preparation) was dissolved in 2 ml of the 0.2 M NaCl buffer solution and placed on the column. It was washed into the column with 1 ml of solution and the elution was carried out in four steps. (1) The 0.2 M NaCl buffer solution (40 ml) was passed into the column to wash through low molecular weight contaminants. (2) A linear gradient was then used for elution which consisted of 175 ml of 1.5 M NaCl–0.1 M potassium phosphate–0.001 M EDTA (pH 9.0) passing into 175 ml of the 0.2 M NaCl buffer solution. This gradient elution was not permitted to go to completion but was stopped when about 200 ml of solution has passed through the column (after complete elution of the rRNA). (3) About 40 ml of distilled water was added to wash potassium ions from the column and prevent the subsequent precipitation of potassium dodecyl sulfate. (4) A linear gradient of 100 ml of 1% sodium dodecyl sulfate passing into 100 ml of water was used for the final elution from the column. This gradient was stopped after about 60 ml had passed through the column, this being sufficient to elute the remainder of the RNA.

Nuclear RNA was prepared for chromatography by precipitating the nucleic acid from two-thirds of a DNase-treated nuclear sample with two volumes of cold ethanol and dissolving the precipitate in 2 ml of 0.5 M NaCl–0.1 M potassium phosphate–0.001 M EDTA (pH 6.7). This sample was placed upon a washed column and elution was again carried out in four steps. Step 1 was with 50 ml of the 0.5 M NaCl buffer solution. Steps 2, 3, and 4 were identical with those used for the elution of cytoplasmic RNA.

Fractions of 5 ml were collected and each fraction was analyzed for absorbancy at 260 mμ and for radioactivity by plating 0.5-ml aliquots on planchets and counting the ^{32}P in a Nuclear-Chicago gas-flow counter. The fractions from step 4 were turbid and were cleared by adding 0.5 ml of 1 M KCl to each fraction, chilling for 1 hr on ice, and removing the potassium dodecyl sulfate precipitate by centrifugation of the samples for 10 min at 10,000*g*. The clear supernatants from these fractions were used for optical density measurements, and they either showed no absorbancy or typical nucleic acid absorbancy spectra.

Sedimentation Analysis. Zonal centrifugation of RNA was carried out in 5–20% sucrose density gradients in the usual manner (Roberts, 1965). Fractions from methylated albumin kieselguhr columns were prepared for sedimentation analysis in several ways. If sufficient radioactivity was present in the fractions, 0.3-ml aliquots of the fractions were simply diluted to 1.0 ml with gradient buffer containing 0.5 mg of nonradioactive cytoplasmic RNA to serve as an optical density marker. If additional radioactivity was required, 2-ml aliquots of fractions from elution steps 1, 2, or 3 were combined

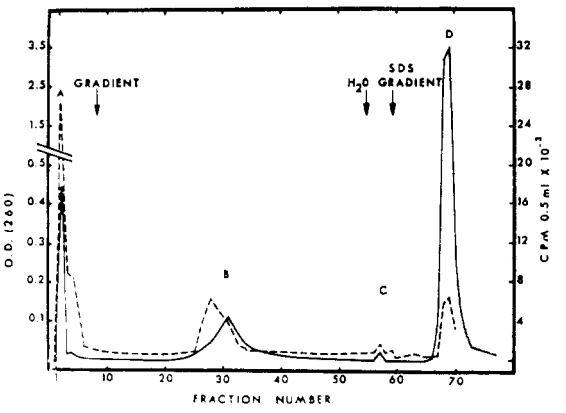


FIGURE 1: Chromatography of nuclear RNA on methylated albumin kieselguhr columns. The elution was carried out as described in the text using RNA from cells labeled for 45 min. Fractions of 5 ml were collected and 0.5 ml of each was plated and counted to determine the radioactiviy pattern shown. (——) Counts per minutes of ^{32}P; (- - -) optical density at 260 mμ.

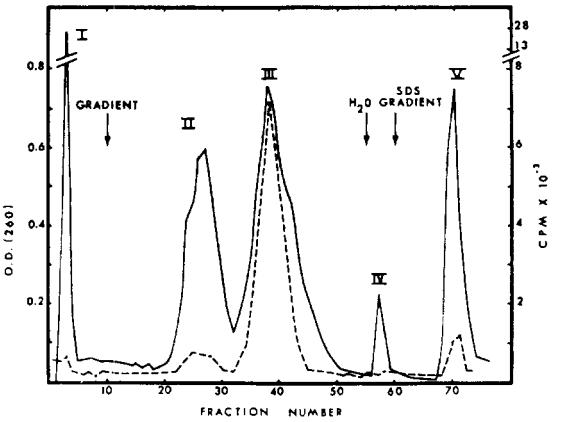


FIGURE 2: Chromatography of cytoplasmic RNA on MAK columns. The elution procedure is described in the text. RNA was isolated from cells labeled for 3 hr. (——) Counts per minute of ^{32}P; (- - -) optical density at 260 mμ.

with 0.5 mg of carrier RNA, the pH was adjusted to around 5 with several small drops of 3 M acetic acid, one volume of ethanol was added, and the samples were incubated at room temperature for at least 1 hr before centrifugation. This procedure eliminated the coprecipitation of potassium phosphate with the RNA. The RNA in fractions from step 4, where there is little if any potassium phosphate, could be precipitated by adding to 2-ml aliquots 0.5 mg of carrier RNA, 0.1 ml of 3 M sodium acetate buffer (pH 5.3), two volumes of ethanol, and placing the solution on ice for at least 1 hr. After centrifugation, each RNA pellet was dissolved in 1 ml of gradient buffer and layered onto a sucrose gradient. The sedimentation patterns of the radioactive RNA from different fractions was not found to vary depending upon the method of sample preparation used.

Base Compositions. Following elution from the methylated albumin kieselguhr column, aliquots of the fractions to be analyzed were combined with 1 mg of nonradioactive carrier RNA and the RNA precipitated by the addition of cold trichloroacetic acid to 5%. As previously described (Roberts, 1965), the precipitates were then washed, hydrolyzed with KOH, and the nucleotides were separated by electrophoresis and the radioactivity of each was determined.

Results

Fractionation of Nuclear and Cytoplasmic RNA by Chromatography on Methylated Albumin Kieselguhr Columns. Species of RNA which differ sufficiently in size, conformation, or base composition can be separated by chromatography on methylated albumin kieselguhr columns, providing the appropriate conditions for elution are selected. Our early attempts at RNA fractionation on methylated albumin kieselguhr columns, using a variety of salt and pH gradients, usually gave results in which the cdRNA and ndRNA were either contaminated with rRNA or rpRNA, partially degraded, or not eluted quantitatively from the columns. Eventually the conditions described in Materials and Methods were worked out which gave satisfactory separations of the relevant RNA species.

Typical elution patterns which were obtained for nuclear and cytoplasmic RNA using these chromatographic procedures are shown in Figures 1 and 2. Under these conditions over 97% of the added radioactivity was eluted from the columns, as judged by a final stripping of the columns with 0.1 M NaOH.

Peak A in Figure 1 would be expected to contain degraded DNA, tRNA, and 5S RNA from the nucleus. Region B of the elution patterns consists of rRNA and the larger rpRNA which elutes slightly later. The optical density peak at B is composed largely of the rRNA, whereas after relatively brief periods of labeling the radioactivity peak contains almost exclusively the more rapidly labeled rpRNA.

Following the elution of the rpRNA, the column is washed with distilled water to remove potassium ions and prevent the subsequent precipitation of sodium dodecyl sulfate as the potassium salt. This distilled water wash causes a rise in the pH of the solution which results in a small amount of RNA, primarily ndRNA, being eluted as peak C. Finally, the sodium dodecyl sulfate gradient causes the ndRNA to be eluted as peak D.

Most of the radioactivity in peak I of Figure 2 cannot be precipitated with acid, indicating that this peak is composed primarily of low molecular weight material which was contaminating the RNA. Peak II should contain most of the tRNA and 5S RNA of the cytoplasm. Peak III has been shown to contain the cytoplasmic rRNA. Peak IV is primarily cdRNA and peak V contains what we regard as "pure" cdRNA.

Base Compositions of Column Fractions. As an aid in identifying each of the RNA species eluted from the methylated albumin kieselguhr columns and in judging their purity, base ratios were determined on the RNA across each of the elution peaks shown in Figures 1 and 2. In addition, these base ratio determinations were repeated on the peaks which appeared at identical elution positions following the column chromatography of

TABLE I: Base Compositions of RNA Fractions from Methylated Albumin Kieselguhr Columns.[a]

Fraction[b]	Time of Labeling	Mole % C	A	G	U
Nucleus 31	45 min	28.2	15.7	33.8	22.3
Nucleus 31	3 hr	29.3	14.6	34.3	21.9
Nucleus 33	3 hr	28.9	16.3	33.4	21.5
Nucleus 69	45 min	23.2	22.7	25.9	28.1
Nucleus 69	3 hr	22.9	22.0	25.8	28.2
Nucleus 69	24 hr	23.7	22.8	27.3	25.7
Nucleus (actinomycin)[c]	4 hr	22.8	25.3	23.8	28.1
Cytoplasm 27	3 hr	28.2	19.4	32.0	20.6
Cytoplasm 38	3 hr	27.6	19.5	32.8	20.1
Cytoplasm 45	3 hr	23.4	26.6	25.3	24.7
Cytoplasm 70	45 min	22.6	29.5	23.5	24.4
Cytoplasm 70	3 hr	22.4	29.6	24.0	24.1
Cytoplasm 70	24 hr	22.6	29.1	26.1	22.4
Cytoplasm (actinomycin)[c]	4 hr	23.9	27.7	23.4	25.0

[a] The base compositions represent averages of at least three separate determinations. [b] Fraction refers to the fraction number in the elution pattern shown in Figures 1 and 2 from which the RNA was isolated. [c] Nuclear and cytoplasmic RNA from cells labeled for 4 hr in the presence of 0.04 μg/ml of actinomycin D; from Roberts and Newman (1966).

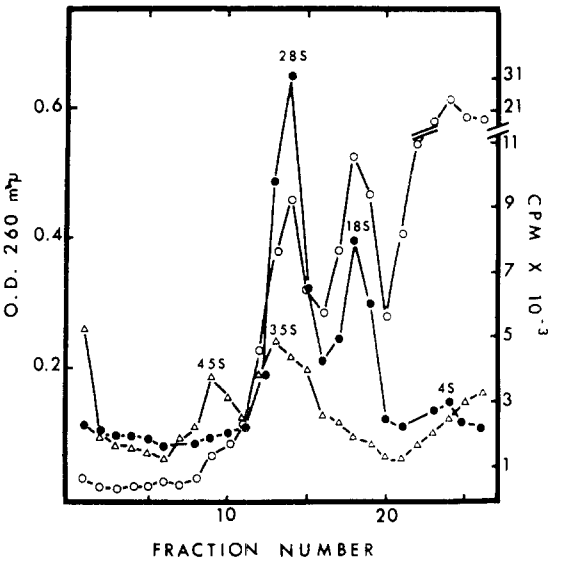


FIGURE 3: Sedimentation patterns of nuclear and cytoplasmic RNA. Superimposed profiles from the centrifugation of 2% of the total RNA in preparations of radioactive nuclear and cytoplasmic RNA from cells labeled for 3 hr. (△) Counts per minute of nuclear RNA; (○) counts per minute of cytoplasmic RNA; (●) optical density of cytoplasmic RNA.

RNAs which had been labeled for different periods of time. This was to assess the possible effects on the base compositions of any nonequilibration of RNA precursor pools as well as contamination of the peaks by other RNA species whose relative specific activities would be expected to change significantly with changes in the period of labeling. These results are summarized in Table I.

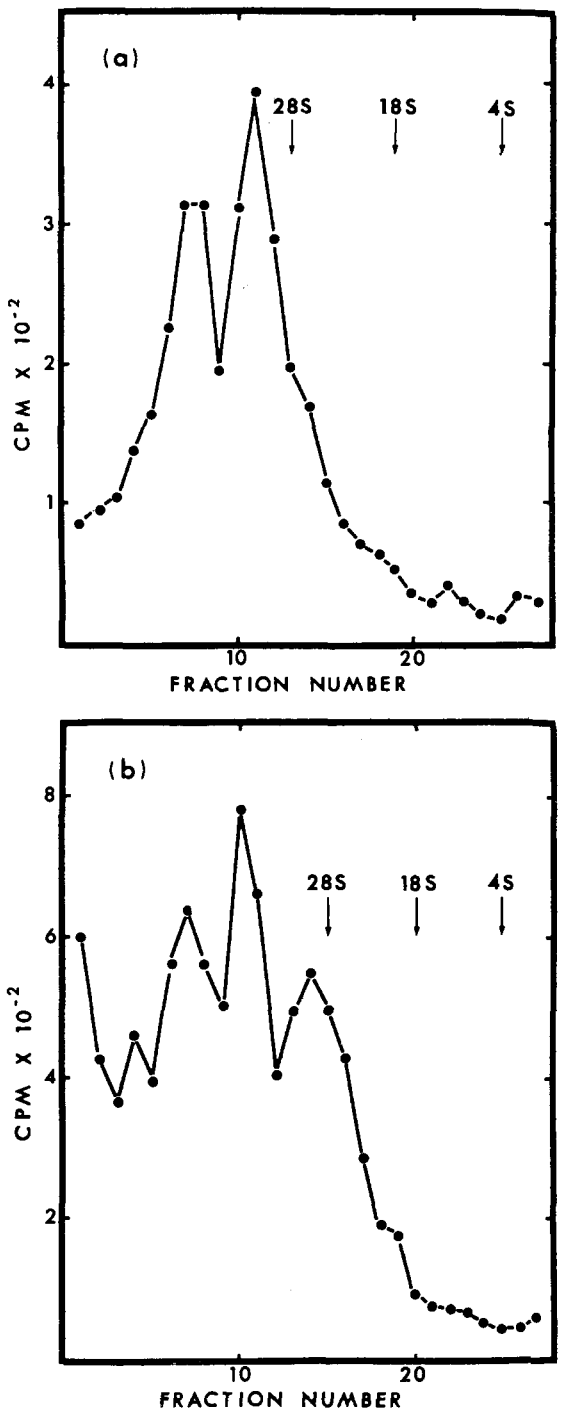
The base composition of the rpRNA in nuclear fraction 31 is in close agreement with the values obtained earlier for this RNA (Roberts and D'Ari, 1968). The base ratios of the ndRNA from fraction 69 can be seen to be very similar to, although not identical with, the base ratios obtained by surpressing the synthesis of rRNA and rpRNA with low concentrations of actinomycin D (Roberts and Newman, 1966). The similarities in base compositions of fractions from 45-min-labeled RNA compared with the same fractions from 3-hr-labeled RNA indicate that the ^{32}P base ratios are not being distorted by a nonrandom incorporation into RNA of nucleotides with different specific activities.

Only a rough estimate can be made concerning the purity of the ndRNA in fraction 69 because of the assumptions involved. It was found that between 45 min and 3 hr the amount of radioactive rpRNA, relative to radioactive ndRNA, increased two- to threefold. During this period analysis of the RNA in fraction 69 showed no detectable shift to more rpRNA-like base ratios. From this and the similarity between the base compositions of fraction 69 and actinomycin-treated nuclear RNA (assuming this to represent the "correct" values for ndRNA), we can estimate that the 3-hr-labeled ndRNA must be less than 20% contaminated with radioactive rpRNA.

The base composition of the ndRNA from cells labeled for 24 hr does differ significantly from the 45-min and 3-hr samples. This could be due to the enrichment of a more stable species of ndRNA whose base composition is different from the rapidly turning-over species. It might also be due to contamination from another species of nuclear RNA whose specific activity, relative to that of ndRNA, is greater after 24 hr of labeling than after a 3-hr period. This is true with the nuclear rRNA where small amounts of both 28S and 18S rRNA are found in nuclei prepared by our procedure (Roberts and Newman, 1966). Both the 28S and 18S species would be possible contaminants of the ndRNA; contamination by 18S RNA perhaps being more likely because of the higher A plus U content of this species.

Peak D from cells labeled for 3 hr can be spread out by using a more gradual sodium dodecyl sulfate gradient. Preliminary evidence indicates that if this is done and base compositions are determined across the entire peak, then one finds the early elution of an RNA high in both A and U (possibly containing newly synthesized cdRNA), followed by RNA with a typical ndRNA base composition, followed, in some cases, by RNA which is higher in G and C (perhaps contaminated ndRNA).

The base composition of fraction 38 from Figure 2 is typical for that of a mixture of 28S and 18S rRNA from the cytoplasm of ascites cells (Roberts and D'Ari, 1968). Fraction 70 gives base ratios similar to those ob-



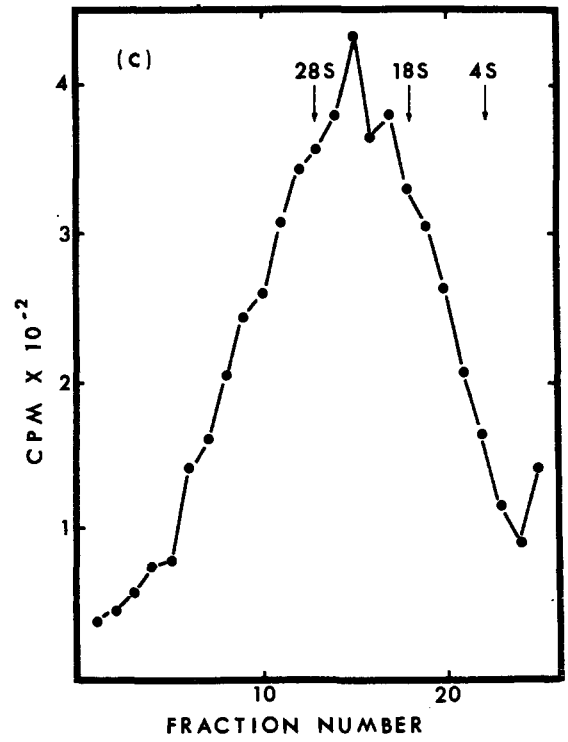


FIGURE 4: Sedimentation patterns of fractions of nuclear RNA from methylated albumin kieselguhr columns. Fractions were used from a methylated albumin kieselguhr chromatography run identical with that shown in Figure 1, except that the RNA was from cells labeled for 3 hr. Aliquots from the fractions were removed, mixed with marker cytoplasmic RNA, and centrifuged in sucrose gradients as described in the text. Pattern a was from fraction 31, pattern b was from fraction 33, and pattern c was from fraction 69.

tained for cdRNA by inhibiting rRNA synthesis with low concentrations of actinomycin D (Roberts and Newman, 1966). The trailing edge of peak III, as exemplified by fraction 45, exhibits base compositions consistent with it being composed of a mixture of cdRNA and rRNA.

The relative specific activities of the cytoplasmic cdRNA and rRNA were about equal following a 24-hr period of labeling, whereas after 3-hr labeling the specific activity of the cdRNA was roughly ten times that of the rRNA (this varied somewhat from one RNA preparation to another). Since the base compositions of the cdRNA from cells labeled for 3 or 24 hr were quite similar, we conclude that any contamination of the cdRNA with rRNA must be small, less than 20% of the total absorbance. Also, the base ratios from each fraction across peak V were fairly uniform, indicating again that the cdRNA is free from significant amounts of contamination.

Zonal Centrifugation of Column Fractions. Various RNA species which had been eluted from the methylated albumin kieselguhr columns were investigated further for possible contamination and degradation by zonal

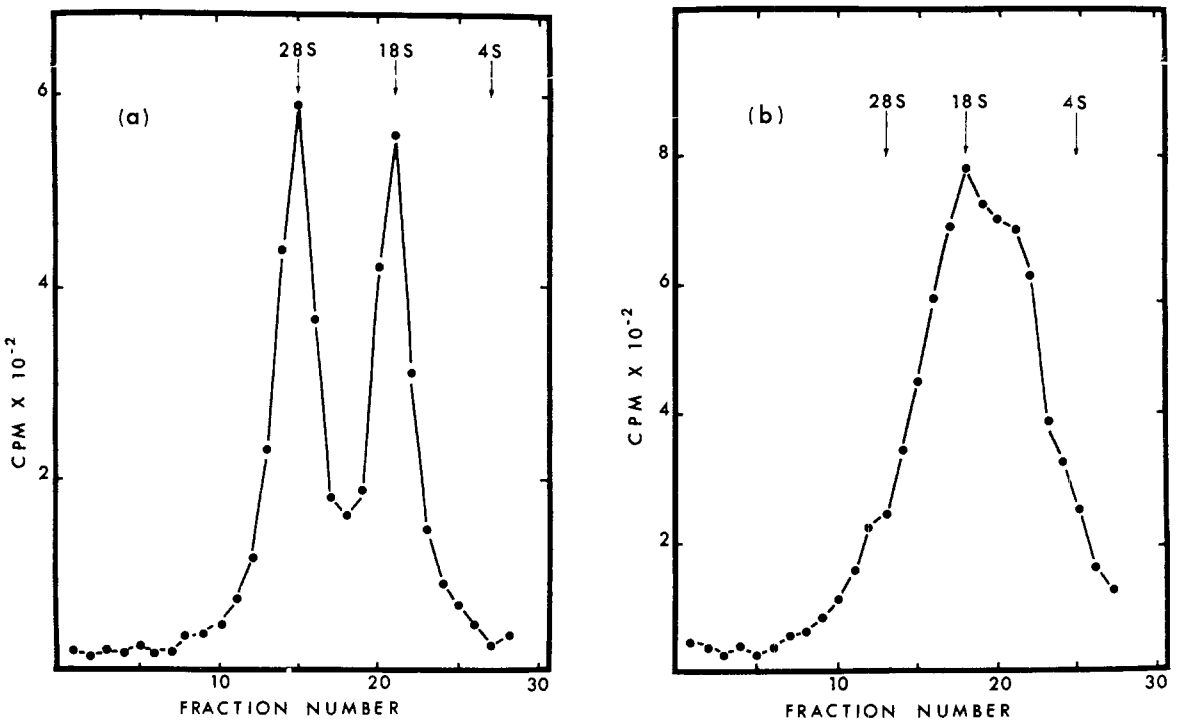


FIGURE 5: Sedimentation patterns of fractions of cytoplasmic RNA from methylated albumin kieselguhr columns. Same as Figure 3 except that a and b correspond to fractions 38 and 70, respectively, from Figure 2.

centrifugation on sucrose gradients. The radioactive samples were mixed with marker cytoplasmic RNA and either layered directly onto the gradients or else precipitated, redissolved, and layered. No difference in sedimentation profiles were observed when a given RNA sample was prepared using either method.

The sedimentation patterns of total radioactive nuclear and cytoplasmic RNA from ascites cells is shown in Figure 3. Aliquots of preparations of nuclear RNA and cytoplasmic RNA were each mixed with marker cytoplasmic RNA, centrifuged, and the sedimentation patterns were superimposed for the figure. The radioactivity profile of the nuclear RNA is characterized by peaks of 45S and 35S rpRNA (the S values in this figure have not been accurately determined but are approximations used for the purpose of reference). The cytoplasmic RNA is distinguished by the usual peaks of 28S and 18S rRNA and 4S tRNA.

Nuclear RNA from cells labeled for 3 hr was fractionated by methylated albumin kieselguhr chromatography and several fractions identical in position with those shown in Figure 1 were subjected to zonal centrifugation. The patterns from these sedimentation runs are shown in Figure 4.

The sedimentation profiles of fractions from the front side of peak B showed predominately 28S rRNA and 35S rpRNA. Profiles of fractions taken from the middle of the peak (Figure 4a) showed approximately equal amounts of 45S and 35S rpRNA. Fractions taken from the back side of peak B (Figure 4b) gave sedimentation patterns which revealed a considerable amount of RNA which sedimented more rapidly than 45 S. This type of pattern persisted after a gentle phenol extraction of fraction 33, but a vigorous phenol extraction of the fraction, carried out by agitating the phenol extraction mixture violently on a Vortex mixer for several minutes, resulted in the RNA having a sedimentation profile similar to Figure 4a, with little RNA now sedimenting faster than 45 S. This suggests that the rapidly sedimenting RNA in Figure 4b may be due to an aggregation phenomenon, perhaps resulting from a small amount of methylated albumin being eluted from the column at higher salt concentrations. RNA from the back side of peak B resembles rpRNA in its base composition but is sometimes slightly higher in A plus U, suggesting a small amount of ndRNA contamination.

Figure 4c shows that the ndRNA sediments as a broad band with a peak between 28 and 18 S.

The zonal centrifugation of fractions across peak III Figure 2 revealed a size distribution of rRNA as reported by Ellem (1966). Fractions from the front of the peak contained primarily 18S rRNA and those from the back almost exclusively 28S RNA. A fraction from the middle of the peak is shown in Figure 5a.

The cdRNA in peak V was found to sediment as heterogeneous RNA with a broad peak between 12 and 18 S. The exact location of the peak varied from one preparation to the next, perhaps due to the partial degradation of the cdRNA in some cases. The sedimentation profile of a preparation with a peak at 18 S is shown in Figure 5b.

The sedimentation profiles of ndRNA and cdRNA were routinely examined for possible peaks from contaminating rpRNA or rRNA. No significant peaks from these RNAs could be detected. However, due to the broad sedimentation patterns of ndRNA and cdRNA

these observations can only serve to rule out gross contaminations by these species.

Discussion

The general shape of the elution profiles shown in Figures 1 and 2 can be easily reproduced using methylated albumin kieselguhr chromatography with a variety of salt gradients for elution. In our experience, however, a number of factors must be carefully controlled if the RNA species eluted are to be free from detectable contamination. The most important of these factors are the extent to which the column has been washed and the initial pH and salt concentration used to put the sample onto the column.

Using the elution procedures given in Materials and Methods rRNA and rpRNA could routinely be isolated with the sedimentation properties and base compositions expected for the purified species. The cdRNA always exhibited a base composition high in A and had a sedimentation profile which peaked between 12 and 18 S. These properties are similar to what was observed for ascites cell cdRNA using actinomycin D to inhibit the synthesis of rRNA (Roberts and Newman, 1966). In contrast, the ndRNA showed the characteristic high U base composition and the broad pattern of more rapidly sedimenting RNA which have been found for the ndRNA from a number of different sources (Muramatsu *et al.*, 1966; Attardi *et al.*, 1966; Houssais and Attardi, 1966; Soeiro *et al.*, 1966; Roberts and Newman, 1966; Willems *et al.*, 1968).

Several types of evidence have indicated that there exists in the nuclei of animal cells a species of RNA whose function is restricted to the nucleus. These include DNA–RNA hybridization experiments (Shearer and McCarthy, 1967), base ratios of very short pulse-labeled RNA from the nucleus and cytoplasm of duck erythrocytes (Attardi *et al.*, 1966) and HeLa cells (Houssais and Attardi, 1966), the properties of nuclear and cytoplasmic RNA from cells labeled in the presence of low concentrations of actinomycin D (Roberts and Newman, 1966; Penman *et al.*, 1968) and the properties of ndRNA isolated by nuclear fractionation techniques (Muramatsu *et al.*, 1966; Soeiro *et al.*, 1966; Willems *et al.*, 1968). The argument could be made that the unique base composition of ndRNA was due, in the case of the pulse-labeled RNA experiments, to non-equilibration of ^{32}P pools and, in the case of the actinomycin experiments, to the "unnatural" influence of actinomycin on RNA synthesis within the nucleus. The results reported here make these possibilities seem unlikely and provide additional evidence that much of the ndRNA is indeed distinct from cdRNA.

The function of the ndRNA that is restricted to the nucleus is open to speculation. The fact that it has a "DNA-like" base composition, is heterogeneous in size and is rapidly turning over (although this may not be true in some cells which are not dividing (Kubinski and Koch, 1966)) suggests a messenger function. This could mean that it is nuclear mRNA (although the extent to which protein synthesis takes place in the nucleus of animal cells is a debatable subject) or else cytoplasmic mRNA whose function is controlled by degradation before entry into the cytoplasm (Scherrer *et al.*, 1966). Alternately, the ndRNA could be postulated to control transcription off DNA in the capacity of "repressor" or "derepressor" RNA (Holoubek and Crocker, 1968), or to have a function that is unsuspected at the present time. Discovery of the function (s) of ndRNA, as well as cdRNA (Penman *et al.*, 1968), should be possible once adequate techniques of cell fractionation and RNA fractionation and analysis become available.

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