Proc. Chem. Soc., 310.
Dhar, M. L., Thaller, V., and Whiting, M. C. (1964), *J. Chem. Soc.*, 842.
Djerassi, C., Ishikawa, M., and Budzikiewics, H. (1961), *Tetrahedron Letters 12*, 383.
Golding, B. T., Rickards, R. W., and Barber, M. (1964), *Tetrahedron Letters 37*, 2615.
Whitfield, G. B., Brock, T. D., Ammann, A., Gottlieb, D., and Carter, H. E. (1955), *J. Am. Chem. Soc. 77*, 4799.

Analytical Studies on Nuclear Ribonucleic Acid Using Polyacrylamide Gel Electrophoresis*

C. Wesley Dingman and Andrew C. Peacock

ABSTRACT: Rat liver nuclear and cytoplasmic ribonucleic acids (RNAs) have been analyzed by electrophoresis on polyacrylamide and mixed polyacrylamide–agarose gels. Many species of nuclear RNA were found which are not detected in the cytoplasm. On the lower molecular weight side, the nucleus contained, in addition to a distinct 18S RNA species, several species with mobilities somewhat slower than 4S transfer ribonucleic acid (tRNA) and without detectable amino acid acceptor activity. On the high molecular weight side, there were several species of nuclear RNA, with mobilities slower than 30S RNA, which labeled rapidly, unlike cytoplasmic RNA. Newly synthesized 30S RNA and 18 S were found to appear in the cytoplasm at the same time, in contrast to results of studies by others. These results also suggested that "35S" and "45S" nuclear RNAs, as defined by sucrose gradient analysis, were not single species, but rather are composed of many components of different molecular weights. This highly complex nature of nuclear RNA makes it difficult to define precursor–product relationships between nuclear and cytoplasmic RNAs. However, these studies indicate that polyacrylamide gel electrophoresis offers a more discriminating technique for the study of nuclear RNA function and metabolism.

In a previous paper, we have described the usefulness of polyacrylamide gels for the analytical electrophoretic fractionation of cRNA from mammalian tissues (Peacock and Dingman, 1967). Recently, Loening (1967) and Loening and Ingle (1967) have also presented similar studies on the RNA from a variety of tissues. Because of the importance of the cell nucleus in the synthesis and maturation of cRNA (for reviews, see Harris, 1963a; Prescott, 1964; Perry, 1967) and because of the extensive evidence for the existence of species of RNA in the nucleus that are not found in the cytoplasm (Hiatt, 1962; Sporn and Dingman, 1963; Shearer and McCarthy, 1967), we have sought, in the present study, to use the high resolving power of gel electrophoresis to reexamine some of the physical and metabolic properties of nuclear RNA (nRNA).

Experimental Section

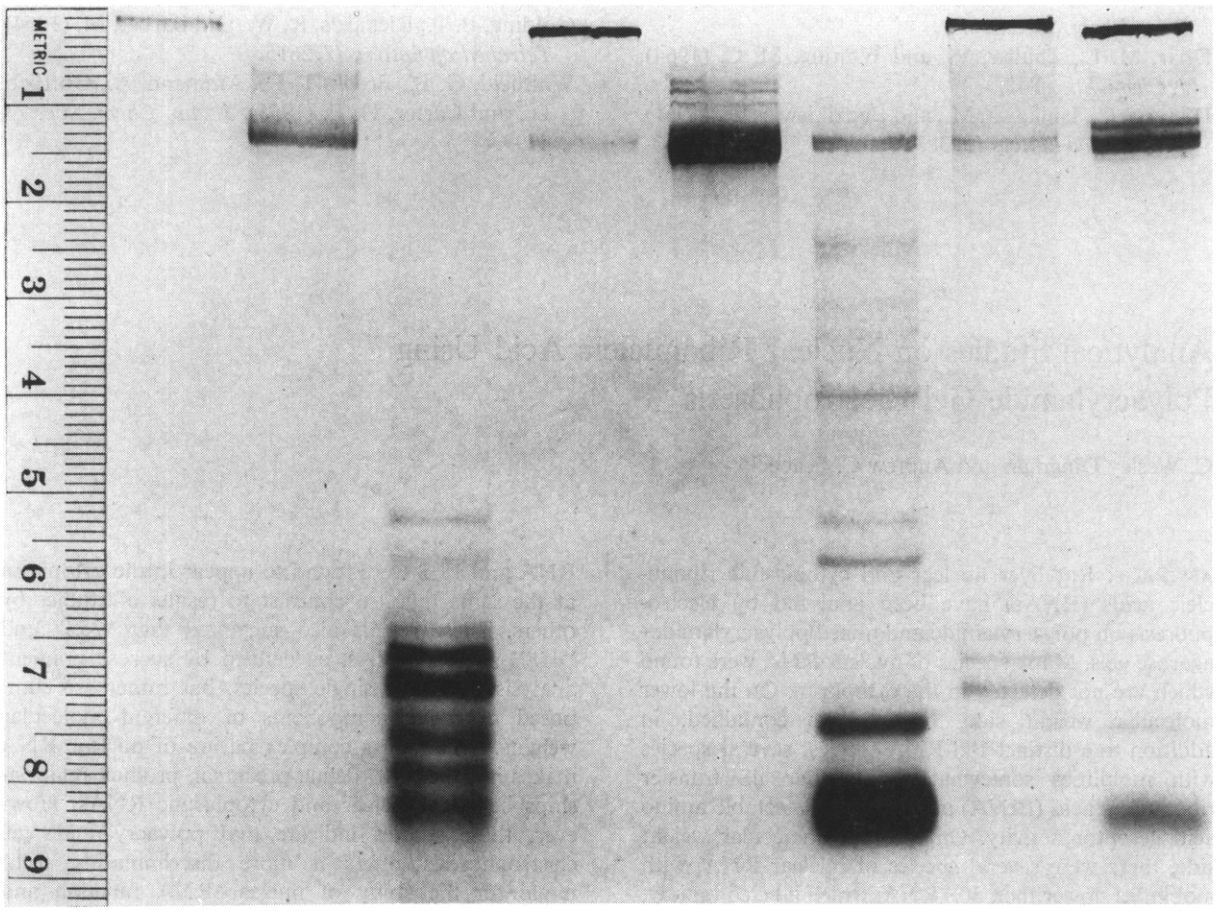
Materials. Pancreatic ribonuclease A (salt free, lyophilized) and pancreatic deoxyribonuclease I (electrophoretically purified, RNase free) were the products of Worthington Biochemical Corp. Sarkosyl NL-97 (sodium lauroyl sarcosinate) was a gift of the Geigy Chemical Corp. [6-^{14}C]Orotic acid hydrate (4–5 mc/mmole), [^{3}H]cytidine (5–6 c/mmole), and [^{14}C]amino acid mixture were purchased from New England Nuclear Corp.

Rats were Fischer males weighing 120–250 g. *In vivo* labeling of rat liver RNA was accomplished by intraperitoneal injection of aqueous solutions of the appropriate isotope at specified times prior to removing the tissue. All animals were fed *ad lib.*

Methods. ISOLATION OF SUBCELLULAR FRACTIONS. The methods used for the removal and homogenization of the tissue have been previously described (Peacock and Dingman, 1967). Purified liver nuclei were obtained from crude nuclear pellets that had been twice washed with solution A by methods described previously (Sporn and Dingman, 1966). In some cases, extraction of purified nuclei with Triton X-100 was performed by suspending the purified nuclei homogeneously in a solution which was 0.32 M sucrose, 0.001 M $MgCl_2$, 0.001 M potassium phosphate (pH 6.5), and contained 0.3% Triton X-100 (Lazarus and Sporn, 1967) followed by centrifugation. The extraction was repeated once more prior to isolation of nRNA.

ISOLATION OF RNA. cRNA was isolated as described

* From the Chemistry Branch, National Cancer Institute National Institutes of Health, Bethesda, Maryland 20014
Received September 25, 1967.



A

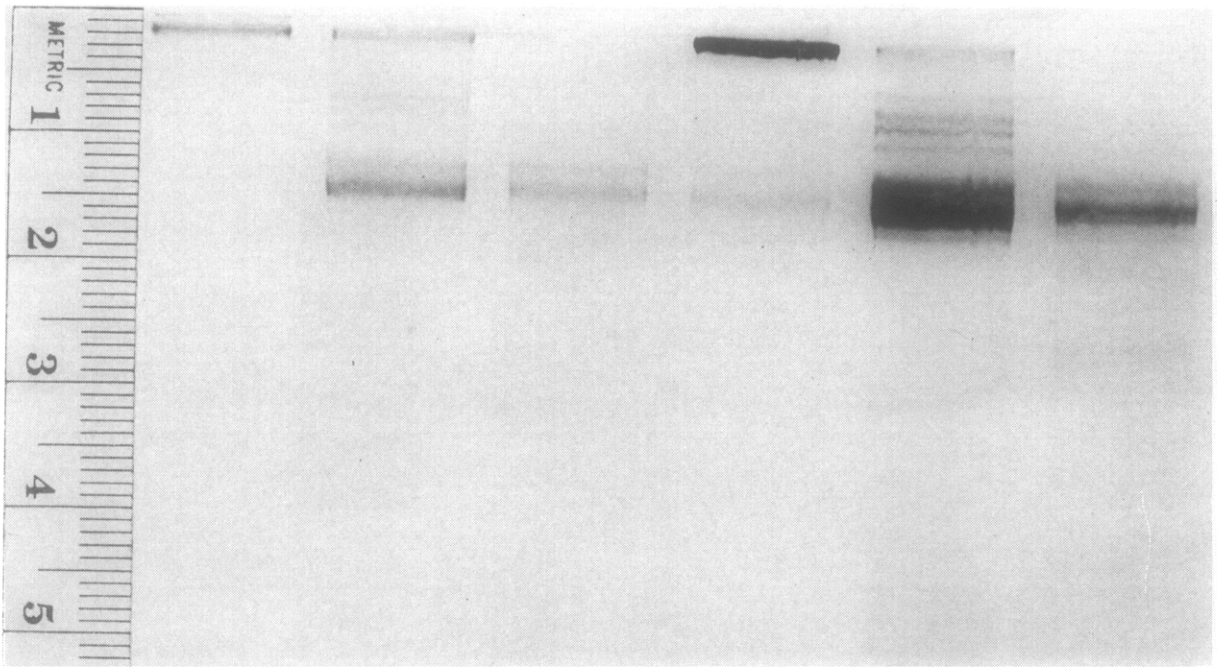
FIGURE 1: Electrophoretic analyses. (A) Electrophoregrams (in a 3.5% polyacrylamide gel run for 1.75 hr) of rat liver nRNA and cRNA fractions derived from zone sedimentation in sucrose gradients. From left to right, the samples applied were: slot 1, nRNA fraction 1, DNase treated; slot 2, nRNA fraction 2, DNase treated; slot 3, nRNA fraction

previously (Peacock and Dingman, 1967) except that Sarkosyl NL-97 was frequently substituted for SDS.[1] For the isolation of nRNA, homogeneously suspended nuclei in solution A were stirred at room temperature with seven-tenths volume of 0.5% SDS or Sarkosyl NL-97 until the solution was clear and then the usual phenol extraction was performed. To ensure an adequate yield of nRNA, the material in the phenol phase after the first phenol extraction was reextracted once more with 0.2% detergent in solution A containing either 0.2 M NaCl or 0.2 M potassium acetate, and the resulting aqueous phase was added to the first aqueous phase prior to the second phenol extraction. This procedure increased the amount of DNA contaminating the nRNA preparation, but the DNA was easily removed by either fractionally precipitating the DNA from the final aqueous phase with one volume of cold ethanol, or by incubating the nRNA with DNase just prior to electrophoresis (Peacock and Dingman, 1967).

FRACTIONATION OF RNA. Preparative zone sedimentation of RNA in sucrose gradients was performed as previously described (Peacock and Dingman, 1967). In most cases, the gradients were fractionated into only three fractions (with nominal range of S values indicated in parentheses): 1 (27–50), 2 (14–27), and 3 (2–14).

ASSAY OF AMINO ACID ACCEPTOR ACTIVITY. The amino acid acceptor activity was determined as follows. Aliquots of RNA (approximately 450–850 μg in 0.5 ml) were added to a 0.32-ml reaction mixture which contained 100 μc of [^{14}C]amino acids (67 μg), and which was 0.02 M Tris-HCl, 0.01 M $MgCl_2$, 0.01 M DTT, 0.004 M ATP, 0.00025 M CTP, 0.002 M phosphoenolpyruvate, and 0.01 M K_2HPO_4 (pH 7.0) and contained approximately 30 μg of pyruvate kinase (Calbiochem). The mixture was brought to 37°, and 0.2 ml of warmed, freshly prepared, 105,000g rat liver supernatant fraction (see below) was added. After 10-min incubation,

[1] Abbreviations used: DTT, dithiothreitol; PPO, 2,5-diphenyloxazole; POPOP, *p*-bis[2-(5-phenyloxazolyl)]benzene; SDS, sodium dodecyl sulfate; ATP and CTP, adenosine and cytidine triphosphates.



B

FIGURE 1 (Continued)

3, DNase treated; slot 4, cRNA fraction 1; slot 5, cRNA fraction 2; slot 6, cRNA fraction 3; slot 7, unfractionated nRNA, DNase treated; and slot 8, unfractionated cRNA, DNase treated. (B) Electrophoregrams (in a 3.5% gel run for 1.75 hr) of rat liver nRNA and cRNA fractions similar to those shown in part A. In this figure, only the RNAs with relatively slow mobilities are shown to present the various species in this region at greater enlargement (note scale at left). The RNA samples used in this case are derived from entirely different preparations than those used in part A. From left to right, the samples applied were: slot 1, nRNA fraction 1, DNase treated; slot 2, nRNA fraction 2, DNase treated; slot 3, nRNA fraction 3, DNase treated; slot 4, cRNA fraction 1, DNase treated; slot 5, cRNA fraction 2, DNase treated; and slot 6, cRNA fraction 3, DNase treated.

the reaction was stopped by the addition of 1 ml of 0.5% Sarkosyl NL-97. The RNA was then extracted with phenol as described previously (Peacock and Dingman, 1967) and, after ethanol precipitation, dialyzed for 3 days against solution B. This RNA was then concentrated by another ethanol precipitation and washed twice with a 2:1 (v/v) mixture of ethanol and solution B. Under the conditions used, there was no detectable degradation of either nRNA or cRNA as determined by gel electrophoresis. Rat liver supernatant fraction was prepared by homogenizing rat liver in three volumes of a solution which was 0.32 M sucrose, 0.01 M Tris-HCl, 0.01 M $MgCl_2$, and 5 mM DTT (pH 7.2–7.4) and centrifuging at 1500*g* (average) for 15 min. The supernatant was then centrifuged at 105,000*g* (average) for 2 hr.

For comparison incubations were performed with (1) unfractionated nRNA, (2) unfractionated cRNA, and (3) without added RNA, but to which nRNA was added after the reaction was stopped. The latter served as a control for the presence of tRNA in the rat liver supernatant fraction used as the source of amino acid activating enzymes.

ELECTROPHORESIS. The preparation of polyacrylamide gels of different strength and the electrophoretic techniques were as previously described (Peacock and Dingman, 1967). Because gels with a concentration of acrylamide less than 3.5% are weak and difficult to handle, a means was sought to strengthen such gels so that RNAs of higher molecular weight could be adequately analyzed (30S RNA scarcely enters the 3.5% gels). A solution to this problem was found by combining 0.5% agarose with the acrylamide (Peacock and Dingman, 1968) which enabled us to perform analyses in gels containing any desired concentration of acrylamide. The required amount of agarose was dissolved in water with heating, cooled to 40°, and mixed with a second solution, also at 40°, containing the acrylamide, *N,N'*-methylenebisacrylamide, dimethylaminopropionitrile, and buffer. Ammonium persulfate (not warmed) was added, and the solution was poured into an electrophoretic cell which had been previously equilibrated at 20°. The slot form was introduced without delay. The temperature was kept at 20° for 1 hr until the acrylamide had polymerized; then the temperature was set at the running temperature, usually 2°.

DETERMINATION OF RADIOACTIVITY IN GEL FRACTIONS.

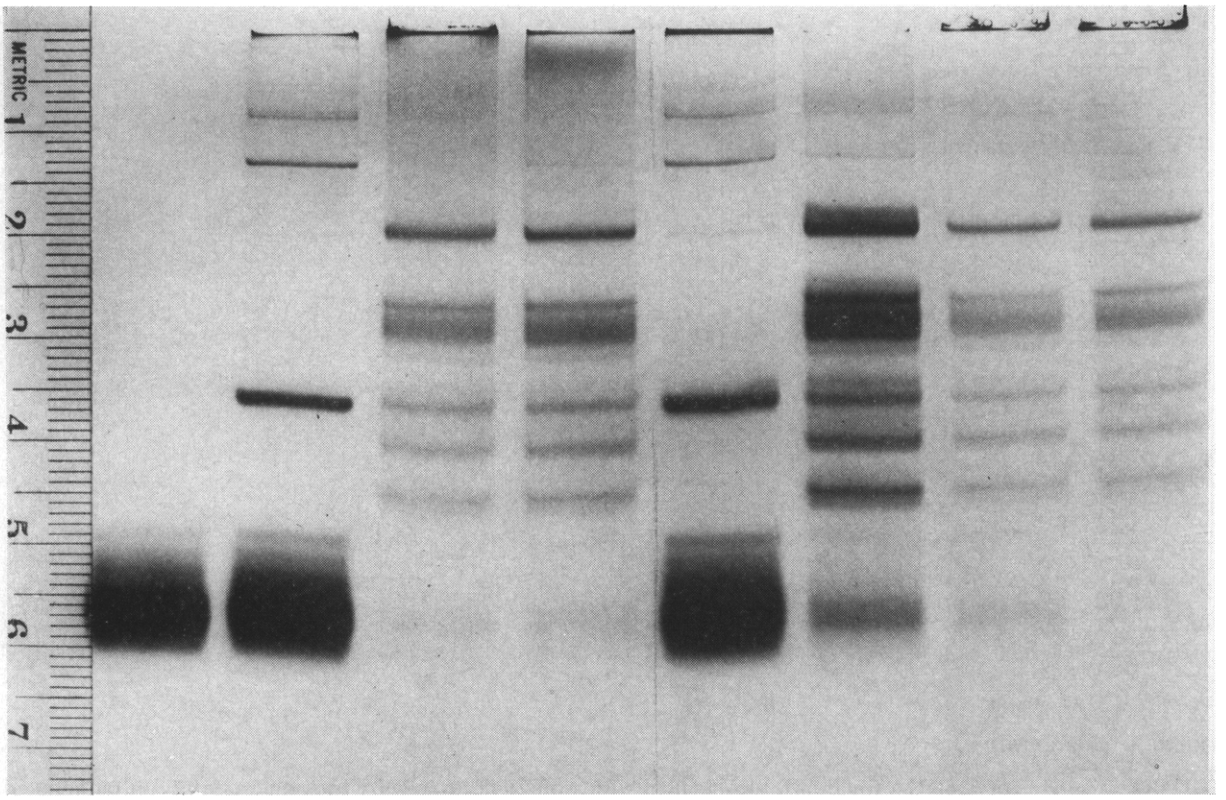


FIGURE 2: Electrophoregrams (in a 10% polyacrylamide gel run for 4 hr) of rat liver nRNA and cRNA fractions. From left to right, the samples applied were: slot 1, sRNA; slot 2, cRNA fraction 3; slot 3, unfractionated nRNA; slot 4, nRNA fraction 3; slot 5, cRNA fraction 3 (a different preparation than run in slot 2); slot 6, nRNA fraction 3 (a different preparation than run in slot 4); slot 7, unfractionated nRNA (a different preparation than run in slot 3); and slot 8, unfractionated nRNA from a preparation of nuclei extracted twice with Triton X-100 (see Experimental Section).

Gel strips (12 mm wide) were placed on Saran Wrap overlaying a paraffin-coated tray, and cut using a lucite block which contained 150 stainless-steel blades set 1 mm apart. The Saran Wrap enabled one to retain each section, in proper order within the cutting device until needed. We found that counting labeled RNA fractions did not require the use of ethylene diacrylate cross-linked gels (Choules and Zimm, 1965; Peacock and Dingman, 1967). Good recovery of isotope (usually better than 90% for both ^{3}H and ^{14}C) was obtained simply by first incubating the gel fractions in 0.2 ml of 1 M NaOH overnight at room temperature then adding 1 ml of NCS Reagent (Nuclear-Chicago Corp.), mixing, allowing the mixture to stand with occasional agitation for 1 hr at room temperature, and then adding 10 ml of toluene scintillator containing 5 g of PPO and 0.1 g POPOP/l. of toluene. Counting efficiencies for doubly labeled material (obtained in a Packard Tri-Carb scintillation spectrometer, Model 4312) averaged 31% for ^{3}H and 56% for ^{14}C. All counts reported have been corrected for background.

Results and Discussion

Analytical Studies. Figures 1 and 2 show the electrophoretic patterns obtained with n- and cRNAs in standard 3.5 and 10% polyacrylamide gels. The most striking difference between these RNAs is seen in the low molecular weight region where six major classes of nRNA are found compared with only two in the cytoplasm. This difference is brought out most impressively in 10% gels (Figure 2) where these six classes of nRNA are seen to be further resolved into both major and minor species most of which have no detectable counterpart in cRNA. The existence of a marked difference between the nucleus and cytoplasm with respect to the low molecular weight classes of RNA had been previously indicated both by physical techniques (Sporn and Dingman, 1963) and by electrophoresis in agarose gels (McIndoe and Munro, 1967).

Other differences between nRNA and cRNA are demonstrated by electrophoretic analysis in mixed acrylamide–agarose gels (Figure 3). Particularly prominent is the existence of multiple species with mobilities slower than 30S RNA. These latter species presumably include the 32S, 35S, and 45S nRNAs that have been described previously (*e.g.*, Muramatsu and Busch, 1965; Penman, 1966; Lamar *et al.*, 1967). We have tentatively identified (see Figure 3) the prominent band

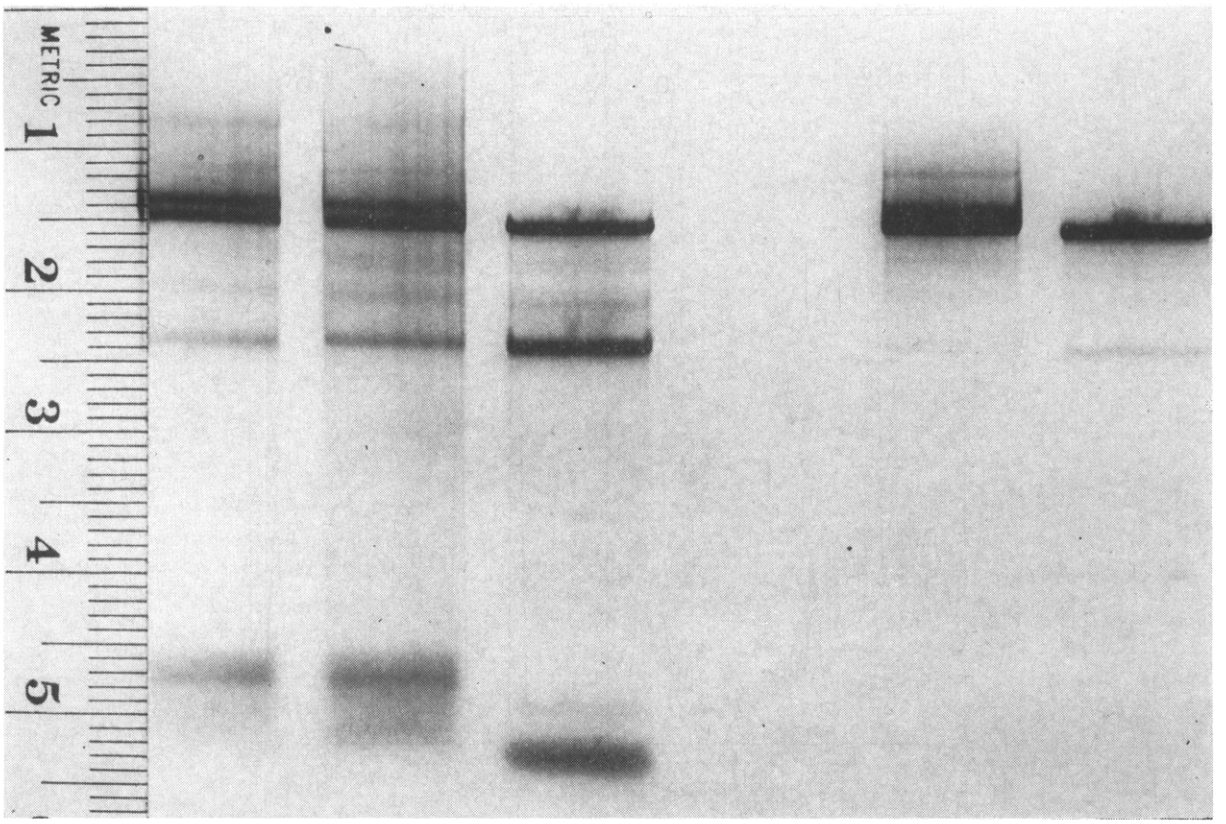


FIGURE 3: Electrophoregrams (in a 2.0% polyacrylamide plus 0.5% agarose gel run for 1 hr) of rat liver nRNA and cRNA fractions. From left to right, the samples applied were: slot 1, unfractionated nRNA from a preparation of nuclei extracted twice with Triton X-100, DNase treated; slot 2, unfractionated nRNA from a preparation of nuclei not extracted with Triton X-100, DNase treated; slot 3, unfractionated cRNA, DNase treated; (nothing was applied to slot 4); slot 5, nRNA fraction 1; and slot 6, cRNA fraction 1.

of nRNA migrating just behind the 30S RNA at 13.5 mm as "32S" RNA, the pair of nRNA bands at 11 and 12 mm as "35S" RNA, and the band at 8 mm as "45S" RNA. Labeling studies (see below) indicate, however, that multiple less prominent nRNA species are also present in this region; these species are not readily detected on the stained gels. Thus, the above nomenclature, based on sedimentation coefficients obtained by sucrose gradient analysis, may be grossly misleading with respect to the variety of nRNAs actually present. Also of note is the small amount of 18S nRNA relative to 30S nRNA, in contrast to the situation with cRNA. Penman (1966) found nearly complete absence of 18S RNA in HeLa cell nuclei purified with a combination of ionic (sodium desoxycholate) and nonionic (Tween 40) detergents. However, since sodium desoxycholate tends to disrupt rat liver nuclei, it is not a useful reagent for their purification. Extraction of previously purified nuclei with Triton X-100 (see Experimental Section) did not result in any detectable change in the nRNA pattern as observed on the electrophoregrams (Figures 2 and 3), a finding of interest in light of the evidence that such detergent treatment removes the outer nuclear membrane (Hubert *et al.*, 1962; Hymer and Kuff, 1964a). Our results indicate that 18S RNA is present in rat liver nuclei, although there is relatively less of it than in the cytoplasm. Furthermore, the staining pattern of the more slowly migrating (and presumably higher molecule weight) nRNA species (Figure 3) indicates that, underlying the more discrete bands in this region, there is a significant amount of relatively polydisperse material. Thus there exist many species of nRNA which are not represented in cRNA. A similar conclusion was reached by Shearer and McCarthy (1967) using RNA:DNA hybridization techniques.

Amino Acid Acceptor Activity of nRNA. In a typical experiment, the specific activity of unfractionated cRNA incubated with [^{14}C]amino acids (see Experimental Section) was 150 cpm/μg, while that of nRNA, incubated under similar conditions, was 70 cpm/μg. When nRNA was added after the incubation in the control reaction, the specific activity dropped to 56 cpm/μg. The difference, approximately 14 cpm/μg, indicated that the nRNA contained small, but measurable amounts of tRNA, in agreement with the results of McCarty *et al.* (1966). We thought it of interest to determine if any of the species of low molecular weight RNA peculiar to the nucleus had amino acid acceptor activity. The use of electrophoretic fractiona-

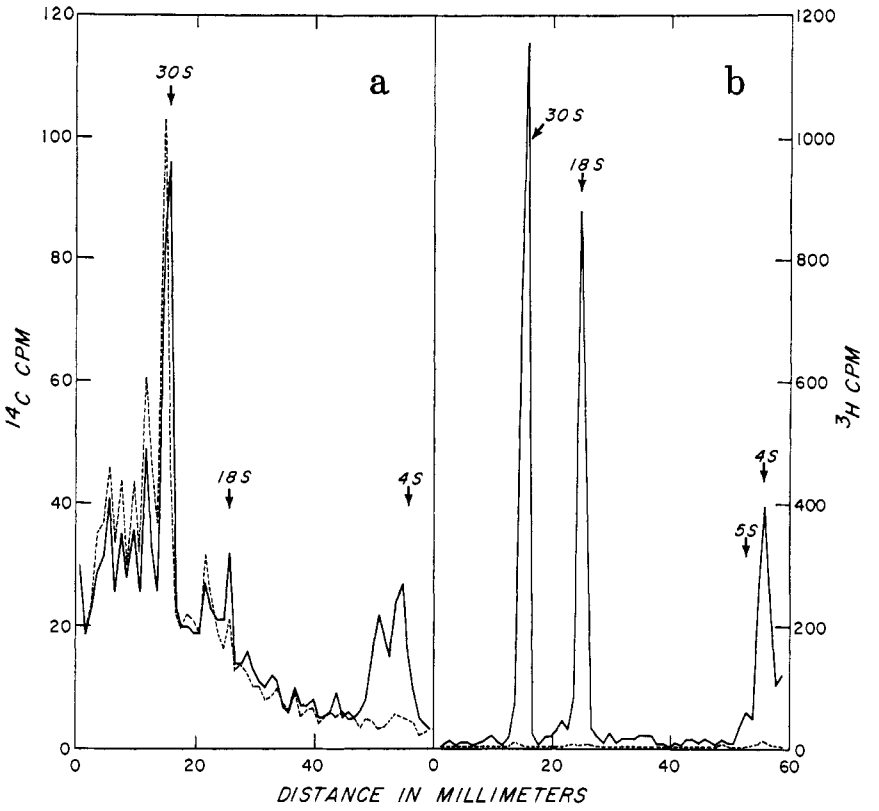


FIGURE 4: Labeling patterns obtained by electrophoresis for 1 hr (in 2% polyacrylamide, 0.5% agarose gels) of RNA isolated from rat liver 20 hr after injection of [^{14}C]orotic acid and 30 min after injection of [^{3}H]cytidine. (a) nRNA. (b) cRNA. ^{14}C (——) and ^{3}H (- - -). The nominal S values are given to facilitate comparison with similar studies employing zone sedimentation in sucrose gradients.

tion on polyacrylamide gels for this purpose, however, gave rise to some difficulties because of the lability of the tRNA-aminoacyl linkage. This lability led to loss of a portion of the amino acids from the tRNA during the electrophoretic run; these amino acids were subsequently eluted from the gel during the staining and destaining process. The per cent recovery of isotope from the gels, nonetheless, was always the same for both nRNA and cRNA (36–37% recovery in the usual pH 8.3, 3.5% gel run for 1.5 hr; 4–5% recovery in the usual pH 8.3, 10% gel run for 4 hr; and 27–28% recovery in a pH 5.2, 10% gel buffered with acetic acid and sodium acetate and run for 4 hr). In all cases, in both nRNA and cRNA, the counts recovered were localized strictly in the region corresponding to the 4S tRNA found in the cytoplasm (Figure 2). The cytoplasmic 5S RNA and the other RNA species showed no amino acid acceptor activity. The absence of amino acid acceptor activity associated with cytoplasmic 5S RNA confirms an earlier report by Bachvaroff and Tongur (1966).

Labeling Studies. Previous studies (Hiatt, 1962; DiGirolamo *et al.*, 1964; Peacock and Dingman, 1967) have indicated that 17–20 hr after a single injection of [^{14}C]orotic acid all classes of rat liver RNA separable by zone sedimentation in sucrose gradients are labeled uniformly. For these reasons, the amount of ^{14}C label associated with the RNA in any given experiment has been used as an approximate measure of the amount of RNA present. Figure 4, showing the labeling pattern found in nRNA and cRNA, 20 hr after [^{14}C]orotic acid and 30 min after [^{3}H]cytidine, demonstrates three points with respect to nRNA. (1) There exist several nRNAs of lower mobility (and therefore of higher molecular weight) than 30S nRNA (see also Figure 5); (2) except for the 30S band and the lower molecular weight RNAs, the ^{3}H-labeling pattern is very similar to that of the ^{14}C-labeling pattern; and (3) the low molecular weight (4–6 S) nRNAs are relatively slow to label. Figure 4b shows that in 30 min very little ^{3}H label has been incorporated into cRNA. Indeed, it is quite likely that the ^{3}H label that is present in cRNA at this time is derived from the few broken nuclei which are unavoidably included in the cytoplasmic fraction.

In order to examine the labeling pattern of the high molecular weight nRNAs more closely, preparations of nRNA, labeled with ^{3}H for different lengths of time, were electrophoresed in mixed polyacrylamide–agarose gels for 2 hr instead of the usual 1 hr. The results of these studies are shown in Figure 5a–c. Some variation in the observed ^{14}C-labeling pattern of different electrophoregrams of the same RNA preparation is to be

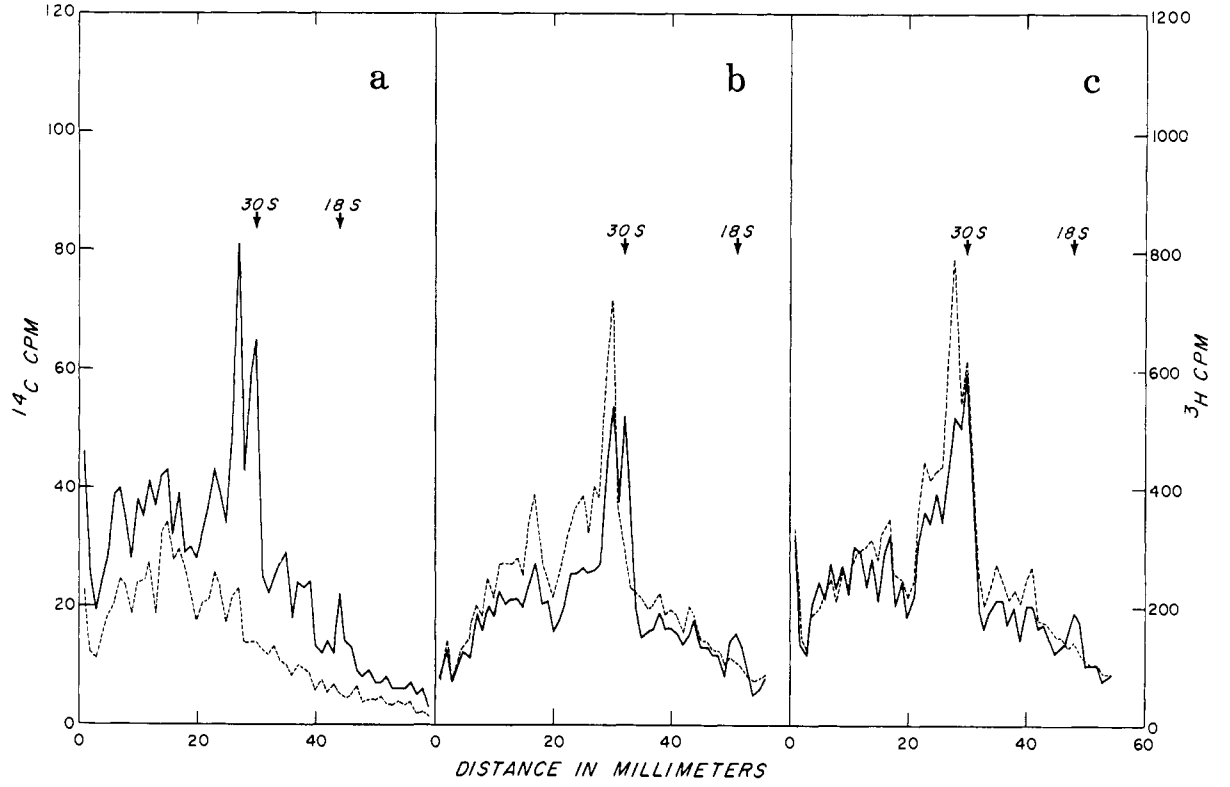


FIGURE 5: Labeling patterns obtained by electrophoresis for 2 hr (in 2% polyacrylamide, 0.5% agarose gels) of rat liver nRNA. In this instance, only the high molecular weight nRNAs have been examined. (a) nRNA isolated 20 hr after injection of [^{14}C]orotic acid and 10 min after injection of [^{3}H]cytidine. (b) As in part a except nRNA isolated 30 min after injection of [^{3}H]cytidine. (c) As in part a except nRNA isolated 90 min after injection of [^{3}H]cytidine. ^{14}C (——) and ^{3}H (- - -).

expected because the relative heights of two adjacent ^{14}C peaks will depend to some extent upon the position of the cutter blades relative to the RNA bands. In all cases, however, the position of the major ^{14}C peaks coincided with the position of the major methylene blue staining bands on the electrophoregrams. For comparison with previous fractionations of nRNA on sucrose gradients (*e.g.*, Muramatsu and Busch, 1965; Penman, 1966), we have tentatively identified the bands migrating between 10 and 18 mm (Figure 5) as "45S" RNA, those migrating between 22 and 26 mm as "35S" RNA, and that prominent band migrating just behind the 30S RNA as "32S" RNA (see also Figure 3). Examination of Figures 5a–c reveals that with increasing labeling time with [^{3}H]cytidine there is a gradual shift in the position of the maximally labeled region from the higher to the lower molecular weight regions in agreement with the results of Penman *et al.* (1966). Furthermore, distinct labeling of the 30S nRNA with ^{3}H did not become apparent until 90 min, while ^{3}H labeling of the 32S, 35S, and 45S RNAs was present as early as 10 min. Of particular interest was the finding of numerous high molecular weight species of nRNA not previously observed on sucrose gradients.

Meaningful interpretations of both these data and those resulting from the use of sucrose gradients to fractionate nRNA, in terms of nRNA metabolism, are hindered by two problems: (1) the likely existence of a significant amount of reutilization of labeled nucleotides during the normal turnover of nRNA (Thomson *et al.*, 1958; Watts and Harris, 1959), and (2) the presence of a significant amount of intranuclear degradation of RNA (*e.g.*, Harris, 1963b; Hymer and Kuff, 1964b; Shearer and McCarthy, 1967). This latter process would give rise to RNA fragments, polydisperse with respect to molecular weight, which would obscure the resolution of the more significant RNA species. In any event, the highly complex nature of newly synthesized liver nRNA (Figure 5a–c), with respect to the variety of species present, makes it difficult to fit these data into certain hypothetical schemes (*e.g.*, Perry, 1967) describing possible precursor–product relationships among nRNAs. It is apparent, nevertheless, that electrophoretic fractionation of these high molecular weight nRNAs in mixed polyacrylamide–agarose gels offers a potentially productive approach to studying their function and metabolism.

The results of our studies on the appearance of newly synthesized RNA in the cytoplasm (Figure 6, and Table I) indicated that 30S RNA appears simultaneously with 18S RNA. Analytical data on the labeling pattern of cRNA are presented in Table I. With respect to the

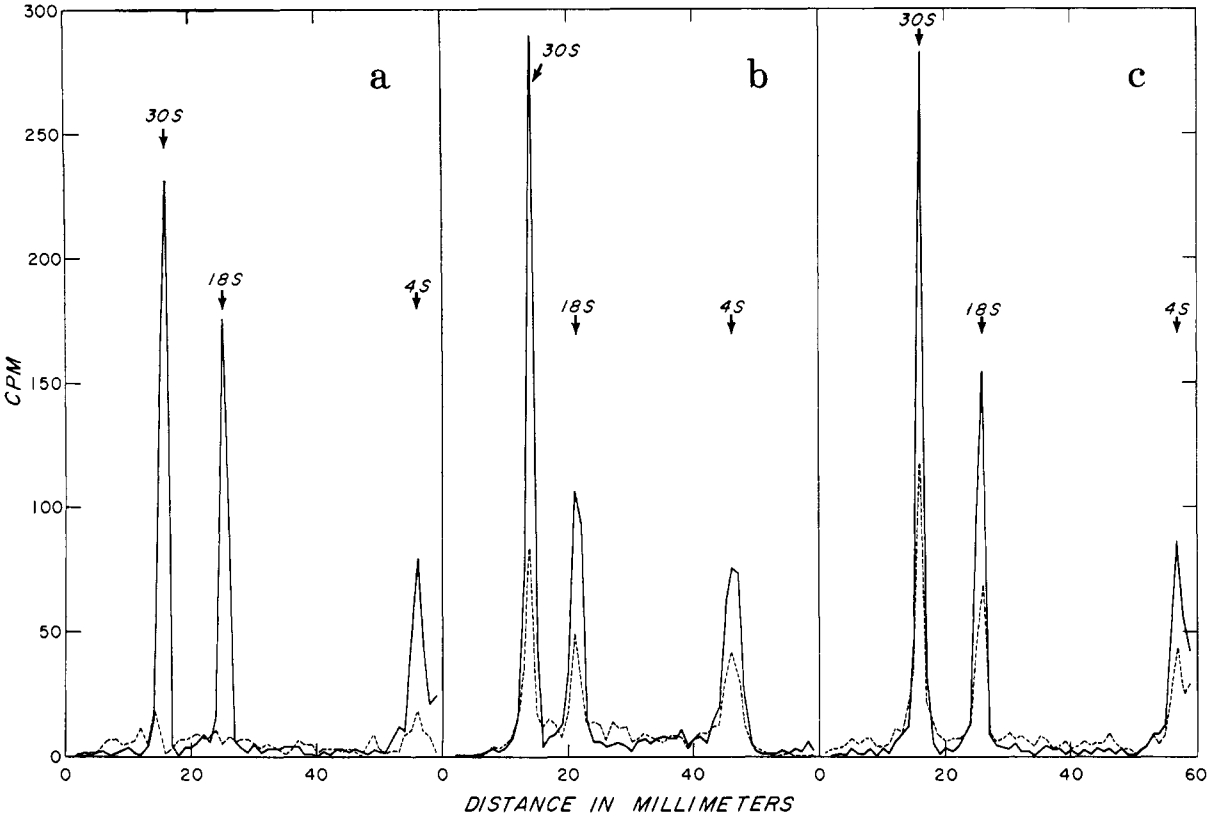


FIGURE 6: Labeling patterns obtained by electrophoresis for 1 hr (in 2% polyacrylamide, 0.5% agarose gels) of rat liver cRNA. (a) cRNA isolated 20 hr after injection of [^{14}C]orotic acid and 30 min after injection of [^{3}H]cytidine. (b) as in part a except cRNA isolated 60 min after injection of [^{3}H]cytidine. (c) As in part a except cRNA isolated 90 min after injection of [^{3}H]cytidine. ^{14}C (——) and ^{3}H (- - -).

major classes of RNA, the labeling pattern obtained only 60 min after [^{3}H]cytidine injection is nearly the same as that obtained 20 hr after [^{14}C]orotic acid (Figure 6b and Table I, expt B). Starving the rats for 24 hr prior to injection of the [^{3}H]cytidine did not alter significantly the pattern of newly synthesized cRNA. Furthermore, even when only 45 min was allowed for incorporation of [^{3}H]cytidine into cRNA approximately equal amounts of label were present in both the 30S and 18S regions on the electrophoregram. There is some indication that the 4S RNA does become labeled somewhat earlier than either 18S or 30S RNA (Figure 6a and Table I, expt B2), and this may result from intramolecular turnover of the –CCA terminus of the tRNA. When cRNA was fractionated on a sucrose gradient and the 30S, 18S, and 4S RNAs were analyzed separately by electrophoresis, the ^{14}C:^{3}H ratio in each major band remained unaltered. Microsomal RNA, isolated from animals injected 20 hr previously with [^{14}C]orotic acid and 60 min previously with [^{3}H]-cytidine, gave a labeling pattern essentially the same as that of the unfractionated cRNA, isolated from the same animals (Figure 6b), except for a much reduced amount of 4S RNA. With respect to the minor RNA bands lying between the 4S and 18S RNAs and between the 18S and 30S RNAs (Peacock and Dingman, 1967), the count levels obtained were too low to be analyzed with accuracy. The fact that at the time intervals studied, the ^{14}C:^{3}H ratio was frequently less than one in these regions (Figures 6a–c) suggested that these less prominent RNA species might have a faster rate of turnover; however, the distinct possibility that a small amount of nRNA was present in these preparations of cRNA (see above) precluded such an interpretation.

Thus these data indicate that newly synthesized 30S RNA appears in the cytoplasm at approximately the same rate (in terms of nucleotides per minute) as does 18S RNA. Moreover, comparison of Figure 5c with Figure 6b,c suggests the possibility that 30S and 18S RNAs make their appearance in the nucleus and cytoplasm at about the same time. This interpretation is inconsistent with the proposal that 18S RNA appears in the cytoplasm prior to 30S RNA (Girard *et al.*, 1965; Henshaw *et al.*, 1965; Penman, 1966; Perry, 1967). This latter theory has been based chiefly on evidence obtained from L cells and HeLa cells in tissue culture (*e.g.*, Fenwick, 1964; Rake and Graham, 1964; Joklik and Becker, 1965; Scharff and Robbins, 1965; Perry, 1965; Penman *et al.*, 1966) and it may be

TABLE I: Quantitative Evaluation of the Labeling Pattern of Liver Cytoplasmic RNA Obtained by Gel Electrophoresis (see Figure 6).

Expt	Isotope	Interval (hr)	30 S:18 S[a]	30 S:4 S[a]	$^{14}C:^{3}H$[b]		
					30 S	18 S	4 S
A							
1	[^{14}C]Orotic acid	20	1.5	2.1			
2	[^{3}H]Cytidine	0.5	...	...	...	...	...
B							
1	[^{14}C]Orotic acid	20	1.7	1.8			
					2.8	2.3	2.0
2	[^{3}H]Cytidine	1	1.4	1.3			
C							
1	[^{14}C]Orotic acid	20	1.5	2.0			
					1.9	2.0	1.8
2	[^{3}H]Cytidine	1.5	1.5	2.0			

[a] These figures indicate the amount of label in the 30S RNA relative to that in either the 18S or 4S RNAs. [b] These figures indicate the amount of ^{14}C label relative to ^{3}H label in each class of RNA.

that rat liver RNA metabolism is different in this respect. However, a problem that must be considered in this context is the possibility of selective degradation of newly synthesized RNA during its extraction from the tissue (Watts, 1964). Newly synthesized RNA, both cytoplasmic and nuclear, has been found by many workers (*e.g.*, Harris, 1963b; DiGirolamo *et al.*, 1964; Henshaw *et al.*, 1965; Lazarus and Sporn, 1967) to be particularly sensitive to the action of nucleases. This latter phenomenon might well be the cause for the frequent appearance of large amounts of labeled polynucleotide material in the low molecular weight regions of newly synthesized RNA fractionated on sucrose gradients (*e.g.*, Hiatt, 1962; Rake and Graham, 1964; DiGirolamo *et al.*, 1964; Girard *et al.*, 1965; Henshaw *et al.*, 1965; Penman *et al.*, 1966). Analytical studies on the kinetics of appearance of newly synthesized RNA in the cytoplasm using sucrose gradient fractionation (*e.g.*, Rake and Graham, 1964; Perry, 1964; Girard *et al.*, 1965; Penman, 1966) are particularly sensitive to any "noise" arising from the presence of polydisperse degradation products with relatively low sedimentation coefficients, since these labeled products may contribute to the total label present in the 18S and 4S regions. Fractionation by gel electrophoresis is less influenced by these factors. Thus, in Figure 6b, RNAs migrating between 25 and 45 mm do not contribute label to either the 18S and 4S species.

Acknowledgments

We thank Miss Sylvia L. Bunting for the excellent technical assistance she has provided in the electrophoretic analyses.

References

Bachvaroff, R., and Tongur, V. (1966), *Nature 211*, 248.

Choules, G. L., and Zimm, B. H. (1965), *Anal. Biochem. 13*, 336.

DiGirolamo, A., Henshaw, E. C., and Hiatt, H. H. (1964), *J. Mol. Biol. 8*, 479.

Fenwick, M. L. (1964), *Biochim. Biophys. Acta 87*, 388.

Girard, M., Latham, H., Penman, S., and Darnell, J. E. (1965), *J. Mol. Biol. 11*, 187.

Harris, H. (1963a), *Progr. Nucleic Acid Res. 2*, 20.

Harris, H. (1963b), *Proc. Roy. Soc. (London) B158*, 79.

Henshaw, E. C., Revel, M., and Hiatt, H. H. (1965), *J. Mol. Biol. 14*, 241.

Hiatt, H. H. (1962), *J. Mol. Biol. 5*, 217.

Hubert, M. T., Favard, P., Carasso, N., Rozencwajg, R., and Zalta, J. P. (1962), *J. Microsc. 1*, 435.

Hymer, W. C., and Kuff, E. L. (1964a), *J. Histochem. Cytochem. 12*, 359.

Hymer, W. C., and Kuff, E. L. (1964b), *Biochem. Biophys. Res. Commun. 15*, 506.

Joklik, W. K., and Becker, Y. (1965), *J. Mol. Biol. 13*, 496.

Lamar, C., Munger, W. L., and Pitot, H. C. (1967), *Arch. Biochem. Biophys. 119*, 98.

Lazarus, H. M., and Sporn, M. B. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1386.

Loening, U. E. (1967), *Biochem. J. 102*, 251.

Loening, U. E., and Ingle, J. (1967), *Nature 215*, 363.

McCarty, K. S., Parsons, J. T., Carter, W. A., and Laszlo, J. (1966), *J. Biol. Chem. 241*, 5489.

McIndoe, W., and Munro, H. N. (1967), *Biochim. Biophys. Acta 134*, 458.
Muramatsu, M., and Busch, H. (1965), *J. Biol. Chem. 240*, 3960.
Peacock, A. C., and Dingman, C. W. (1967), *Biochemistry 6*, 1818.
Peacock, A. C., and Dingman, C. W. (1968), *Biochemistry 7*, 668 (this issue; following paper).
Penman, S. (1966), *J. Mol. Biol. 17*, 117.
Penman, S., Smith, I., and Holtzman, E. (1966), *Science 154*, 786.
Perry, R. P. (1964), *Nat. Cancer Inst. Monogr. 14*, 73.
Perry, R. P. (1965), *Nat. Cancer Inst. Monogr. 18*, 325.
Perry, R. P. (1967), *Progr. Nucleic Acid Res. Mol. Biol. 6*, 219.
Prescott, D. M. (1964), *Progr. Nucleic Acid. Res. Mol. Biol. 3*, 33.
Rake, A. V., and Graham, A. F. (1964), *Biophys. J. 4*, 267.
Scharff, M. D., and Robbins, E. (1965), *Nature 208*, 464.
Shearer, R. W., and McCarthy, B. J. (1967), *Biochemistry 6*, 283.
Sporn, M. B., and Dingman, W. (1963), *Biochim. Biophys. Acta 68*, 389.
Sporn, M. B., and Dingman, C. W. (1966), *Cancer Res. 26*, 2488.
Thomson, R. Y., Paul, J., and Davidson, J. N. (1958), *Biochem. J. 69*, 553.
Watts, J. W. (1964), *Biochem. J. 93*, 297.
Watts, J. W., and Harris, H. (1959), *Biochem. J. 72*, 147.

Molecular Weight Estimation and Separation of Ribonucleic Acid by Electrophoresis in Agarose–Acrylamide Composite Gels*

Andrew C. Peacock and C. Wesley Dingman

ABSTRACT: An electrophoretic method has been developed for the analysis of ribonucleic acids (RNAs) ranging in size from 10^4 to 10^8 daltons. The method depends on the use of acrylamide gels strengthened with agarose for analysis of the larger RNAs. The resolving power of the method permitted individual characterization of RNAs in mixtures containing multiple species of RNA, without prior purification of each species; RNA molecules which differed in molecular weight by only a few per cent could be clearly distinguished, and the molecular weight of each estimated. This unusual application of electrophoretic methods for the determination of molecular weight is based on the observation that, for RNAs, smaller molecules migrate more rapidly than larger ones. The mobility and the logarithm of the molecular weight are inversely related and this relationship is approximately linear. The molecular weights estimated by this technique, although numerically dependent on values assigned to known RNA standards, are highly reproducible in gels of various composition, and are at present the best means of identification of species resolved by gel electrophoresis. By this means, liver 18S RNA is identified as a doublet of RNAs of 0.66 and 0.62 $\times$ 10^6 daltons and the analogous 16 S of *Escherichia coli* as a doublet of 0.58 and 0.54 $\times$ 10^6 daltons, while liver 5S RNA has a molecular weight of 38,000 daltons.

Several recent publications have described the usefulness of acrylamide gels in studying the electrophoretic behavior of ribonucleic acids of mol wt 10^4–10^6 daltons (Loening, 1967; Loening and Ingle, 1967; Mills *et al.*, 1967; Bishop *et al.*, 1967a,b; Peacock and Dingman, 1967). The resolution of RNA species in this molecular weight range appears much superior to that afforded by other techniques, such as centrifugation in density gradients or chromatography on methylated albumin kieselguhr.

We have been able, by incorporation of agarose in the gel mixtures, to prepare acrylamide gels of very low concentration, which still retain sufficient strength to permit easy handling. These new porous gels provide a medium suitable for the high-resolution analysis of RNAs up to 10^8 daltons, and are thus useful for the study of nuclear RNA (nRNA) (Dingman and Peacock, 1968). The addition of agarose also improves the handling characteristics of the 3.5% gels which we previously described as being useful for the study of cRNA (Peacock and Dingman, 1967).

Uriel (1966) and Uriel and Berges (1966) have described the preparation of composite agarose–acrylamide gels for protein electrophoresis, but they could

* From the Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.
Received October 19, 1967.