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Resolution of Multiple Ribonucleic Acid Species by Polyacrylamide Gel Electrophoresis*

Andrew C. Peacock and C. Wesley Dingman

ABSTRACT: High molecular weight ribonucleic acid (RNA) from rat liver, kidney, and brain has been fractionated by polyacrylamide gel electrophoresis. A large number of new species of RNA have been resolved with electrophoretic mobilities and sedimentation coefficients intermediate between 30S and 18S RNA and intermediate between 18S and 4S RNA. The number and relative amount of each of these species were constant from preparation to preparation from the same tissue as well as from different tissues. Evidence is presented that these RNA components are present *in vivo* and are not the result of *in vitro* artifacts. The results suggest that the current classification of RNA into three major types (30, 18, and 4 S) is inadequate to describe the true heterogeneity of cytoplasmic RNA, and therefore hinders studies of the functional role played by different RNA species.

Earlier reports on the successful electrophoretic fractionation of RNA in supporting gels (Bachvaroff and McMaster, 1964; Richards and Gratzer, 1964; Tsanev, 1965; Beney and Székely, 1966; McPhie *et al.*, 1966; Bachvaroff and Tongur, 1966) had indicated that such methods might be capable of higher resolution than the more commonly used techniques of zone sedimentation and column chromatography. The first descriptions of this technique involved the use of agar gels (Bachvaroff and McMaster, 1964; Tsanev, 1965). These studies were significant in showing that high molecular weight RNAs (*e.g.*, rRNA) could be resolved and also suggested that rRNA might be composed of more than the two species resolved on sucrose gradients, *i.e.*, 18S and 30S RNA. Later, workers turned to polyacrylamide gels (Richards and Gratzer, 1964; McPhie *et al.*, 1966) or starch gels (Beney and Székely, 1966) in which even greater resolving power was observed; however, these studies were largely confined to fractionation of low molecular weight RNA (*e.g.*, sRNA (Richards and Gratzer, 1964), and nuclease digestion products of rRNA (McPhie *et al.*, 1966)).

In the present study we have used the very high resolving power of electrophoretic separations on polyacrylamide gels to examine the heterogeneity of RNA. It appears that cytoplasmic ribonucleic acid (cRNA) is composed of multiple species, and that these same species are present in rat liver, kidney, and brain in similar proportions.

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Experimental Section

Materials. Pancreatic ribonuclease A (salt free, lyophilized) and pancreatic deoxyribonuclease I (electrophoretically purified, RNase free) were the products of the Worthington Biochemical Corp. Sarkosyl NL-97 (sodium lauroyl sarcosinate) was a gift of the Geigy Chemical Corp. [6-^{14}C]Orotic acid hydrate (4.9 mc/mmole) and [^{3}H]uridine (7.2 c/mmole, generally labeled) were purchased from New England Nuclear Corp. Acrylamide, *N,N'*-methylenebisacrylamide, and DMAPN[1] were obtained from Eastman Kodak, Distillation Products Industries, Rochester, N. Y. Ethylene diacrylate was obtained from Monomer-Polymer Laboratories, The Borden Chemical Co., Philadelphia, Pa. 19124.

Rats were Fischer 344 males, weighing 200–300 g, and mice were adult C57 males. *In vivo* labeling of mouse liver RNA was accomplished by intraperitoneal injection of 0.2 ml of a solution containing about 30 μc of [^{14}C]orotic acid 18–20 hr prior to removal of the tissue, followed by intraperitoneal injection (in the same animal) of 0.2 ml of a solution containing about 200 μc of [^{3}H]uridine 20 min before removing the tissue.

Methods. ISOLATION OF SUBCELLULAR FRACTIONS. In all cases, the tissue was rapidly removed from decapitated animals, chilled, and minced in a small volume of solution A (0.32 M sucrose, 0.002 M $MgCl_2$, and 0.001 M potassium phosphate, pH 6.8). Homogenization was carried out with a Dounce homogenizer in five to ten volumes of solution A (Sporn *et al.*, 1962) and the homogenate was filtered through 230 mesh nylon monofilament stencil fabric and centrifuged at 1500*g* (average) for 10 min (Dingman and Sporn, 1964). The resulting pellet was washed once with five to ten volumes of solution A and again centrifuged at 1500*g* (average) for 10 min. The pooled supernatants from these two centrifugations were used for the isolation of total cRNA and the pellet was used for the isolation of crude nuclear ribonucleic acid (nRNA). Isolation of microsomal and sRNA was accomplished by first centrifuging the total cytoplasmic fraction 20,000*g* (average) for 15 min to remove mitochondria, and then centrifuging the supernatant from this step 105,000*g* (average) for 2 hr. The supernatant from this last step was used for the isolation of sRNA and the pellet was used for the isolation of microsomal RNA.

ISOLATION OF RNA. RNA was isolated essentially by methods previously described (Dingman and Sporn, 1962). Subcellular fractions were treated with 0.1% sodium dodecyl sulfate for 2–5 min at room temperature and then stirred with an equal volume of water-saturated phenol for 30 min at room temperature. Either potassium acetate or sodium chloride was added to a final concentration of 0.2 M and the mixture was chilled to 0° and centrifuged. The separated aqueous phase was then reextracted once more with a one-half volume of phenol for 10 min at room temperature. The mixture was again chilled to 0° and centrifuged. If the RNA was isotopically labeled and from the crude nuclear fraction, the aqueous phase was dialyzed against solution B (0.05 M NaCl–1 mM EDTA, pH 6.2), and the RNA was precipitated with two volumes of ethanol at −15° and redissolved in solution B as described below. RNA from other fractions was obtained by adding two volumes of cold ethanol to the aqueous phase and precipitating overnight at −15°. (In the case of RNA from fractions rich in glycogen, *e.g.*, liver cytoplasm, the aqueous phase was first centrifuged at 105,000*g* (average) for 1 hr to remove the glycogen; Dingman and Sporn, 1962.) The precipitated RNA was centrifuged and the pellet either (a) was washed twice at 0° with a 2:1 (v/v) mixture of ethanol and solution B and then dissolved in solution B for electrophoretic analysis or (b) dissolved in 0.1 M NaCl–1 mM EDTA (pH 6.2) and dialyzed against the same solution in preparation for fractionation by zone sedimentation in a sucrose gradient. RNA concentrations were estimated from the absorbance of the solution at 260 mμ (determined with a Beckman Model DU spectrophotometer); a solution containing 10 μg/ml was assumed to have an absorbance of 0.24 at 260 mμ. The A_{260}:A_{280} ratio of all RNA preparations was 2.1–2.2.

FRACTIONATION OF RNA. Zone sedimentation was

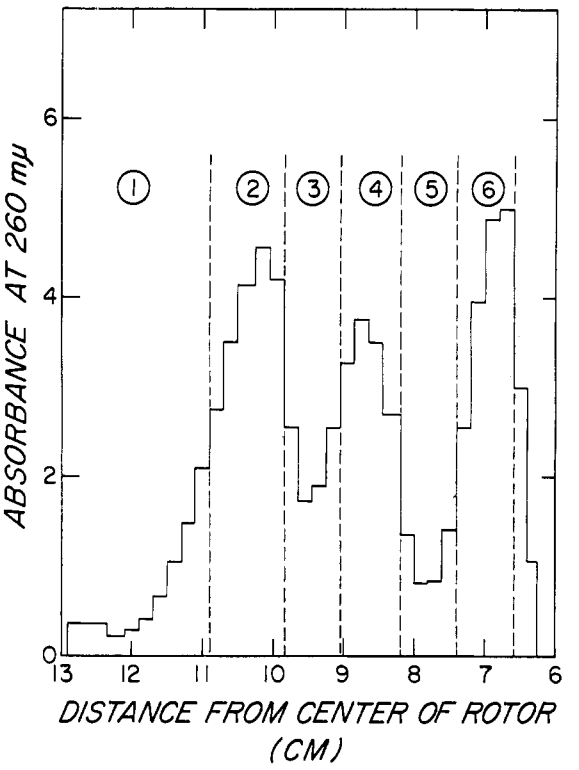

FIGURE 1: Preparative zone sedimentation of rat liver cRNA. Recovery, after sedimentation, of the 3.2 mg of RNA placed on gradient was 92%. Numbers in circles refer to zones in the gradient from which the RNA was isolated for electrophoretic analysis (see text).

[1] Abbreviations used: PPO, 2,5-diphenyloxazole; POPOP, *p*-bis[2-(5-phenyloxazolyl)]benzene; DMAPN, dimethylaminopropionitrile.

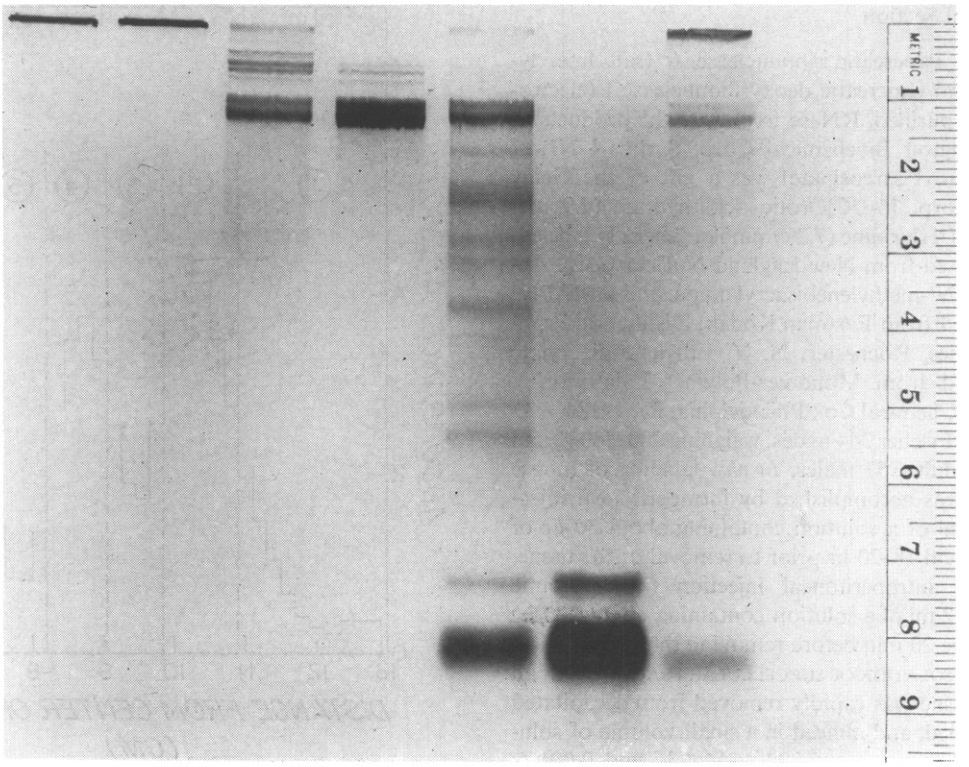

PLATE 1: Polyacrylamide gel electrophoregrams in 3.5% gel of rat liver cRNA fractions. From left to right, the samples applied were: fraction 1–6 from zone sedimentation (see Figure 1 above) and unfractionated cRNA.

performed using a linear, 4–20%, sucrose gradient of 30 ml containing 0.1 M NaCl and 1 mM EDTA (pH 6.2). Centrifugation was carried out in the Spinco SW 25.1 rotor at approximately 4°; about 1 ml of RNA containing solution was layered on the gradient and centrifuged at 23,000 rpm for 17 hr. The gradient was fractionated by collecting measured volumes from a needle hole made in the bottom of the tube.

An example of the sedimentation pattern achieved with preparative zone sedimentation of liver cRNA is shown in Figure 1. The RNA from such gradients was divided into seven fractions (with nominal range of S values indicated in parentheses): 1 (36–50), 2 (27–36), 3 (21–27), 4 (14–21), 5 (7–14), 6 (2–7), and 7 (<2). Fraction 7 was discarded and the RNA in the other fractions was precipitated by first adding $MgCl_2$ to a final concentration of 0.02 M and then adding two volumes of cold ethanol and precipitating overnight at −15°. The precipitated RNA was washed and dissolved in solution B (as described above) for electrophoretic analysis.

TREATMENT OF RNA WITH ENZYMES. Digestions with RNase were accomplished by incubating solutions of RNA (containing 0.5–3.0 mg/ml) with an equal volume of RNase solution (containing 100 μg of enzyme/ml of 0.01 M potassium phosphate, pH 7.0) for 30 min at 37°. Digestions with DNase were accomplished by incubating similar RNA solutions with an equal volume of DNase solution (containing 200 μg of enzyme/ml of 0.02 M NaCl, 0.001 M potassium phosphate, and 0.001 M $MgCl_2$, pH 7.0) for 30 min at 37°. Digestions with both RNase and DNase were performed by mixing equal volumes of all three of the solutions described above and incubating for 30 min at 37°. When RNA samples were preincubated with RNase or DNase or both prior to electrophoretic analysis, loss of methylene blue stained bands on the resulting electrophoregrams was taken as direct evidence regarding the chemical nature of the material within the band. Preincubating of RNA samples containing no DNA with DNase did not alter the resulting electrophoregram detectably, whereas preincubation of these samples with RNase completely degraded the specimen into products migrating faster than 4S RNA.

ELECTROPHORESIS. A stock solution of pH 8.3 buffer contained 108 g of Tris, 9.3 g of disodium-EDTA, and 55.0 g of boric acid, in 1 l. This buffer was used undiluted in the preparation of the gel and was diluted 1:10 to fill the buffer reservoirs in the electrophoresis cell. The standard gel mixture consisted of acrylamide and *N,N'*-methylenebisacrylamide in a weight ratio of 19:1. Gel compositions in per cent refer to total concentration of monomer. Four solutions were used in the preparation of gels: (1) 20% acrylamide (19 g of acrylamide and 1 g of bisacrylamide in 100 ml of water); (2) DMAPN, 6.4% in water; (3) ammonium persulfate, 1.6% in water; and (4) buffer, as above.

Gels were prepared by mixing in an erlenmeyer flask: acrylamide, 28 ml for 3.5% gel (or 80 ml for 10% gel);

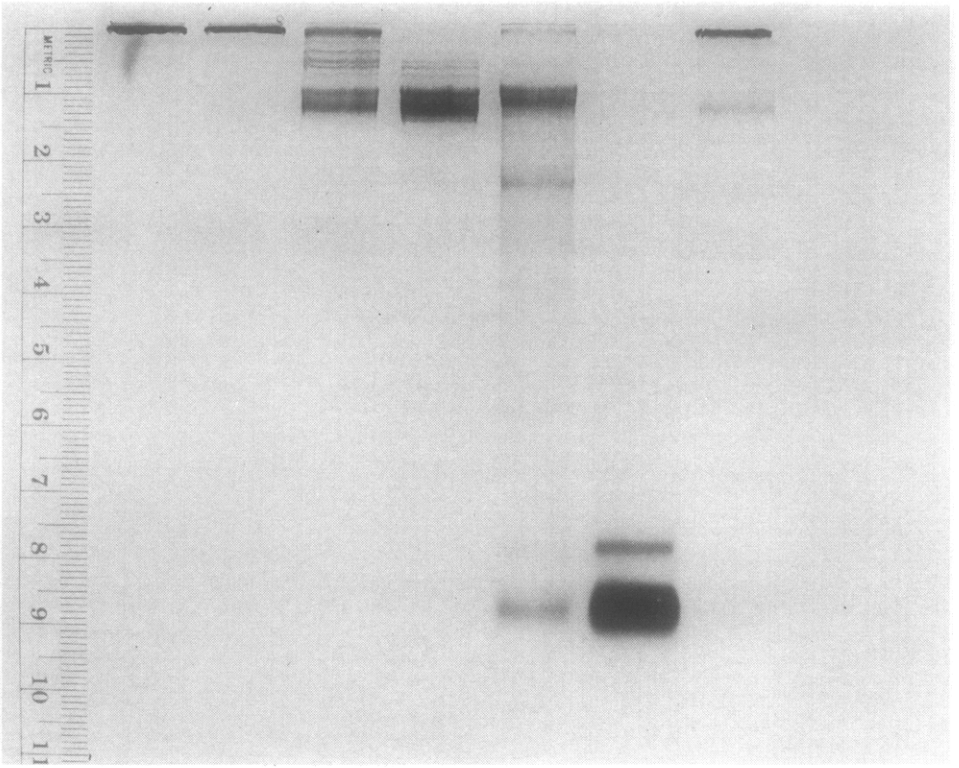

PLATE 2: Electrophoregrams in 3.5% gel of rat kidney cRNA fractions. Samples, similarly derived, were applied as in Plate 1.

DMAPN, 10 ml; buffer, 16 ml; and water to 150 ml. The gel solution was warmed to 25°, ammonium persulfate (10 ml) was added, and the solution was poured into the electrophoretic cell. The eight-place slot former was put in place and gelation allowed to proceed for 20 min before removing the excess gel. The cell was placed vertically, diluted buffer was added to top and bottom buffer reservoirs, and the slot former was removed.

Gels (3.5%) used for study of the distribution of radioactivity in RNA were prepared as above except that solution 1 (acrylamide) contained 19 g of acrylamide and 3 g of ethylene diacrylate in 100 ml (Choules and Zimm, 1965). Without an adequate prerun to establish equilibrium, the highly mobile, low molecular weight RNA molecules became trapped in boundaries formed by the discontinuities between the reservoir buffer solution and the gel, since the latter contained additional ions (persulfate). This difficulty was avoided entirely by a 45-min prerun at 200 v prior to the addition of the sample.

Samples of RNA dissolved in solution B were diluted 4:5 with 40% sucrose containing bromophenol blue. The sucrose was added to raise the density of the sample above that of the buffer, and bromophenol blue was added to make the sample clearly visible during its application to the slot. The subsequent migration of the bromophenol blue toward the anode afforded some indication that the electrophoretic process was proceeding as expected. The bromophenol blue migrated more rapidly than the 4S RNA in the 10% gel but somewhat slower than the 4S RNA in the 3.5% gel. Because of reduced rigidity in the slot walls, it was generally preferable to apply only 10–20 μl to the 3.5% gel, although substantially greater volumes could be applied to the 10% gel.

Specimens of RNA containing an appreciable percentage of 18S material could be applied to the gel at a concentration as great as 500–700 μg/ml. If solutions containing 18S RNA were applied at substantially greater concentrations, aggregation of 18S material appeared to result (*cf.* Plate 3); such aggregates resulted not only in the failure of 18S material to be resolved properly but also (probably by physical obstruction of the channels in the gel) in very significantly diminished resolution of those RNA molecules migrating between the origin and the normal position of the 18S RNA. RNAs sedimenting slower than 18S could be applied at substantially greater concentrations without loss of resolution. In the discontinuous system employed by Richards and Gratzer (1964) and by McPhie *et al.* (1966) very high concentrations of migrating substances may occur during the "stacking" phase (Ornstein, 1964). Such concentrations may be incompatible with the avoidance of these concentration artifacts and may account for the polydisperse smear reported by McPhie *et al.* (1966).

Electrophoretic conditions were as described previously (Peacock *et al.*, 1965). The vertical electrophoresis cell of Raymond (1962) with a 3-mm thick gel slab

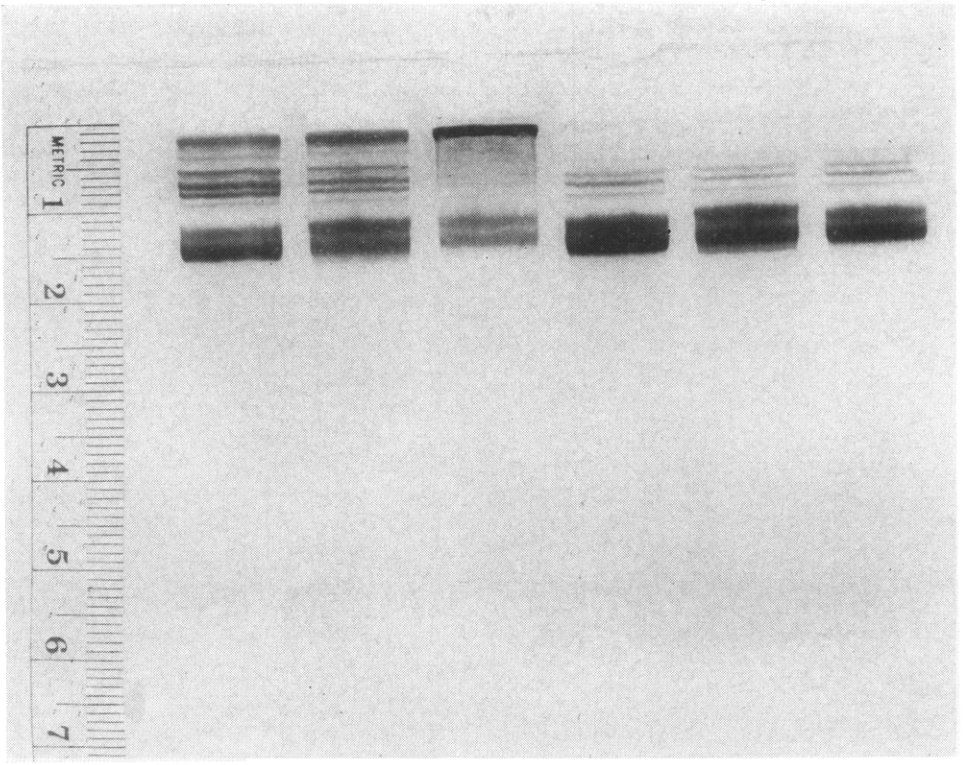

PLATE 3: Electrophoregrams in 3.5% gel of RNA sedimentation fractions 3 and 4 from rat liver, kidney, and brain. From left to right, the samples applied were: fraction 3, liver; fraction 3, kidney; fraction 3, brain; fraction 4, liver; fraction 4, kidney; and fraction 4, brain. The RNA in fraction 3 from brain apparently underwent some aggregation which led to poor resolution of the less prominent bands between 30S and 18S RNA; these bands are, however, well resolved in the electrophoregram of fraction 4 from brain; subsequent analysis of brain fraction 3 at 1:3 dilution provided a clear analysis.

was used; 200 v was applied, approximately 10 v/cm, resulting in a current of approximately 50 ma. For a gel concentration of 3.5%, the run was 1.5 hr, for a 10% gel concentration, the run was 4 hr. Cooling at 0° was employed throughout, but the buffer was not recirculated.

At the end of the run, the gel slab was removed from the electrophoresis assembly and rinsed for 10–15 min in 1 M acetic acid to drop the pH of the gel for staining. The buffer used in staining was a mixture of equal volumes of 0.4 M sodium acetate and 0.4 M acetic acid (pH 4.7). We have adopted a standard concentration for stain of 0.2% dissolved in the acetate buffer (Uriel and Avrameas, 1961). This stain may be reused several times and its concentration is apparently not critical. Preliminary results indicated that many basic dyes could be used. Methylene blue, toluidine blue O, thionin, and azure A were found to be nearly equivalent but methylene blue was used most extensively because of high solubility and easy availability. Less satisfactory were pyronin Y, acridine orange, alcian blue 8GX, methyl green, and gallocyanin. "Fixation" suggested by Richards *et al.* (1965) was found less useful than the procedure described above. Time for staining was arbitrarily chosen at approximately 1 hr but longer periods up to 16 hr have proved satisfactory. The excess stain was removed from the gel either by rinsing the gel in many successive changes of water or by a continuous flow of fresh water. Under the conditions described, the RNA bound the dye sufficiently strongly that 8 hr of washing removed dye from all areas of the gel not containing RNA, but left the RNA zones still darkly stained. More prolonged washing removed the stain from the RNA regions as well. Gels which have been over destained may be restained and subsequently destained to provide a display nearly equal to that observed with only one such cycle.

DETERMINATION OF RADIOACTIVITY IN GEL FRACTIONS. Polyacrylamide gels cross-linked with ethylene diacrylate (Choules and Zimm, 1965) were sectioned (after staining) into strips 12 mm wide and 1–4 mm long (in the direction of electrophoresis). The strips were then dissolved in 0.1 ml of 1 M NaOH; to this was added 2 ml of NCS Reagent (Nuclear-Chicago Corp.) followed by 15 ml of toluene scintillator containing 5 g of PPO and 0.1 g of POPOP/l. of toluene. Samples were counted in a Packard Tri-Carb scintillation spectrometer, Model 4312; counting efficiencies were 27% for ^{3}H and 39% for ^{14}C. Recovery of both ^{3}H and ^{14}C counts was always greater than 90%.

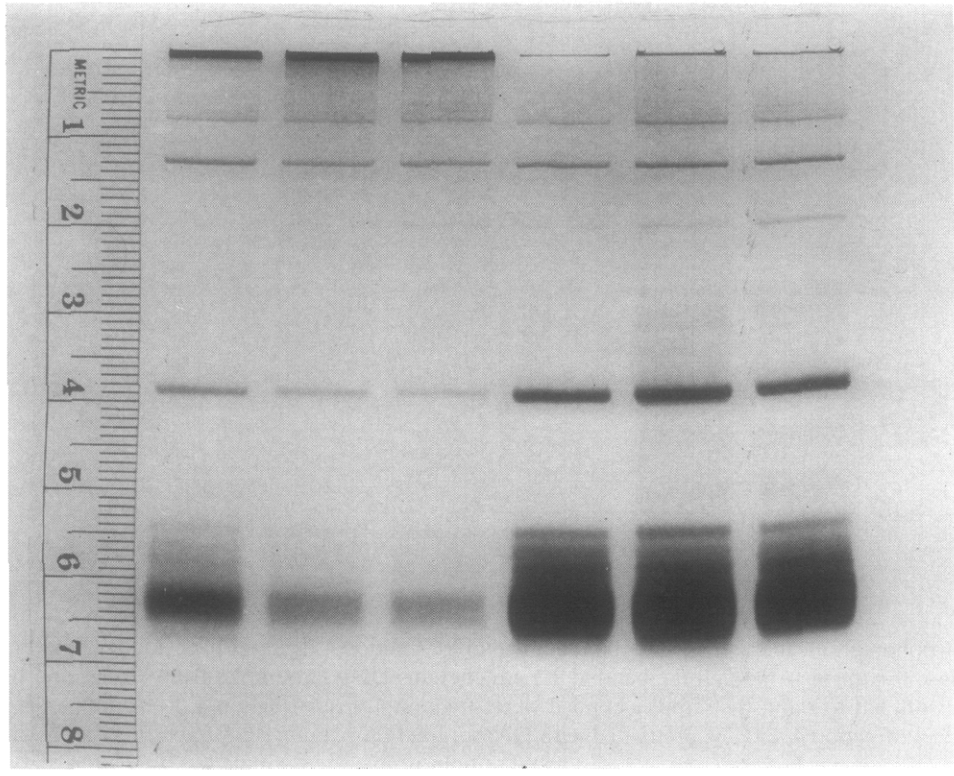

PLATE 4: Electrophoregrams in 10% gel of RNA sedimentation fractions 5 and 6 from rat liver, kidney, and brain. From left to right, the samples applied were: fraction 5, liver; fraction 5, kidney; fraction 5, brain; fraction 6, liver; fraction 6, kidney; and fraction 6, brain. The relationship of the lines on this plate to those on Plate 1 are as follows The bands at 5.4–6.7 cm on this plate correspond to the band at 8.2 cm on Plate 1; the band at 3.8 cm on this plate corresponds to the band at 7.2 cm on Plate 1; the band at 1.3 cm on this plate corresponds to the one at 5.4 cm on Plate 1 and the band at 0.8 cm on this plate corresponds to the band at 5.0 cm in Plate 1. These relationships were established by the use of a two-dimensional gel electrophoresis technique (Raymond and Aurell, 1962), which also verified that RNAs electrophoretically removed from the 3.5% gel, migrate subsequently in a 10% gel in the same order.

Results

Plate 1 shows electrophoretic patterns obtained from each of the six rat liver cRNA fractions derived from zone sedimentation. Comparison of Figure 1 and Plate 1 indicates that the material scarcely entering the gel is 30S RNA, the prominent pair of bands at about 1.2 cm, 18S RNA, and the leading band at approximately 8.2 cm, 4S RNA. Equally striking, however, are the multiple classes of RNA which exist with electrophoretic mobilities (and sedimentation coefficients) intermediate between the 30S and 18S RNA and between the 18S and 4S RNA.[2] The prominent band at approximately 7.2 cm, derived largely from fraction 6, was identified as microsomal 5S RNA, as it was present in samples of RNA isolated from microsomes but absent from preparations of sRNA, in agreement with the results of Beney and Székely (1966). Although some of these less prevalent classes of RNA could be detected by electrophoretic analysis of unfractionated cRNA, it was observed that resolution of all components was improved considerably by prior fractionation of RNA by zone sedimentation in sucrose gradients. Since each of the RNA fractions obtained by zone sedimentation have been applied to the gel at approximately the same concentration, the amount of RNA in the various classes isolated from sedimentation fractions 1, 3, and 5 relative to the total composition has been exaggerated in the electrophoregrams.

Results identical with those obtained with assay of fresh material were obtained on repetitive analysis of RNA preparations stored for up to 2 weeks at −70°. Identical electrophoretic patterns were observed with samples of RNA prepared on different occasions from different animals. This interesting result with liver cRNA led us to examine and compare other tissues as

[2] It would be possible to identify these RNAs by a term similar to relative mobility, a device found useful in the study of the electrophoretic properties of the serum proteins (Peacock *et al.*, 1965). Presumably, the 5S or the 4S RNA would be suitable as such a reference. However, it appears to us premature to make such an arbitrary identification, since the relative mobilities are highly dependent on gel concentration.

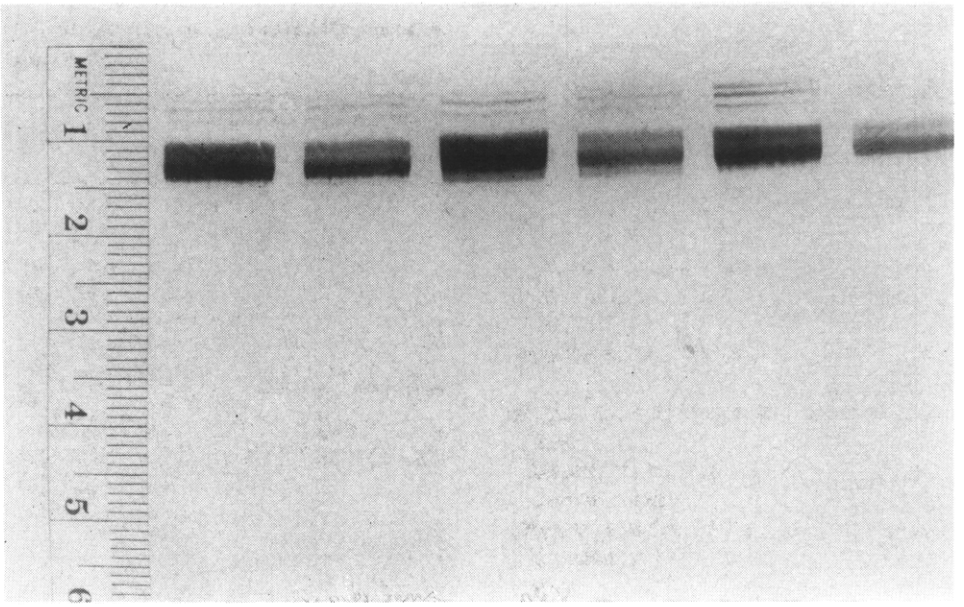

PLATE 5: Electrophoregrams in 3.5% gel of RNA sedimentation fraction 4 from rat liver, kidney, and brain untreated and after DNase treatment. Although the band at 1.1 cm contains DNA in samples from kidney and brain, RNA is also present. From left to right, the samples applied were: fraction 4, liver; fraction 4, liver, pretreated with DNase; fraction 4, kidney; fraction 4, kidney, pretreated with DNase; fraction 4, brain; and fraction 4, brain, pretreated with DNase. Approximately one-half as much DNase-treated sample as untreated sample was applied in each case.

well by this technique. The sedimentation patterns of cRNA were reproducible enough to allow us to fractionate the sucrose gradients directly into seven fractions and, following the procedure outlined above, six classes of RNA were isolated from rat liver, kidney, and brain. The electrophoretic distribution of cRNA from various sedimentation fractions of kidney (Plate 2) and brain (not shown) was very similar to that from liver (Plate 1). A more direct comparison was made by placing corresponding sedimentation fractions of each of the three tissues side by side on the electrophoretic gel (Plates 3 and 4). As shown in the photograph, identical patterns were obtained from all three tissues, and the relative intensities of the bands were similar from tissue to tissue.

DNA, although a negligible contaminant in RNA preparations from liver cytoplasm, was present to a more significant extent in similar preparations from kidney and brain, and could be distinguished visually in the electrophoregrams by its more pinkish staining characteristics. Although the DNA was present in RNA sedimentation fractions 1–5, it consistently migrated electrophoretically in 3.5% gels at the position occupied by the slower of the two major 18S RNA bands. Nonetheless, removal of the DNA by preincubation with DNase confirmed that the principal component of this band was RNA (Plate 5).

The high resolution obtained by polyacrylamide gel electrophoresis of RNA opened the possibility of studying some of the functional and metabolic properties of the various RNA fractions obtained. Crude nRNA was chosen because it would contain newly synthesized RNA from the nucleus as well as representative quantities of all cytoplasmic classes of RNA with the exception of sRNA. As described in the Experimental Section, the RNA was isolated 18–20 hr after [^{14}C]orotic acid administration and 20 min after [^{3}H]uridine administration. It was found that 18–20 hr after [^{14}C]orotic acid administration, the labeling pattern and absorbance pattern obtained by zone sedimentation in sucrose gradients coincided well (Figure 2), in agreement with the results of Hiatt (1962). Thus, the ^{14}C radioactivity could be used as an approximate measure of the amount of RNA in each of the gel fractions. The pattern of radioactivity obtained after sectioning and counting such gels is shown in Figure 3A. As in the studies using unlabeled RNA, it was found that preincubation with RNase completely displaced both ^{14}C and ^{3}H counts to the position occupied by RNase digestion products (*i.e.*, in front of sRNA, Figure 3B), whereas preincubation with DNase did not significantly alter the labeling pattern (Figure 3C). The extensive electrophoretic polydispersity of the ^{3}H-labeled RNA as seen in Figures 3A,C is consistent with the known molecular weight polydispersity of newly synthesized RNA as shown by sucrose gradient zone sedimentation (*cf.* Hiatt, 1962; Perry *et al.*, 1964; Scharff and Robbins, 1965). Studies are currently underway to determine if the minor RNA fractions show labeling characteristics distinct from those of the more prominent fractions.

Discussion

Electrophoretic Properties of RNA. In free solution, RNA has an electrophoretic mobility of 15–20 $\times$ 10^{-5} cm^2 v^{-1} sec^{-1} (Olivera *et al.*, 1964). Thus, the distribution of electrophoretic mobilities in free solution is very narrow in contrast to the wide distribution of electrophoretic mobilities in gels where there exists a strong inverse correlation between the electrophoretic mobility and the sedimentation coefficient (Bachvaroff and McMaster, 1964; McPhie *et al.*, 1966).

The Multiplicity of RNA Species. Although there is some precedence for believing that there exist classes of RNA not resolvable by the usual zone sedimentation in sucrose gradients (Rosset and Monier, 1963; Bachvaroff and McMaster, 1964; Perry *et al.*, 1964; Tsanev, 1965; Drach and Lingrel, 1966), the large number of distinct, electrophoretically resolvable, RNA species from cytoplasm found in this work clearly was unexpected. One is led immediately, therefore, to ask whether these less prominent components might not be the result of either inadvertant degradation or aggregation occurring sometime between the removal of the tissue and the electrophoretic analysis of the RNA.

Aggregation as an artifactual source of RNA heterogeneity is quite unlikely since it has been repeatedly observed that when this occurs with the higher molecular weight fractions, it invariably leads to streaking of the bands with *loss* of the minor components secondary to apparent entrapment within the slower moving aggregates (*cf.* Plate 3). The EDTA present throughout the electrophoretic system minimizes aggregations dependent upon magnesium ion. Concerning the possibility of degradation, we consider the following arguments to be pertinent. Specimens that had been deliberately degraded (*e.g.*, heated at 100° for 10 min in solution B, or by prolonged storage at room temperature in solution B) contained broad, highly disperse zones of methylene blue staining material (which were not found in fresh or unheated RNA preparations) as well as a few new, discrete bands. (The report of McPhie *et al.* (1966) describes some of the specific degradation products obtainable by enzymatic digestion of ribosomal RNA.) On the other hand, the fact that the electrophoretic patterns are not altered by a 30-min incubation at 37° in the presence of Mg^{2+} and DNase argues for the stability of the RNA under these conditions. When whole cytoplasmic RNA (*i.e.*, unfractionated) is electrophoretically analyzed, the same minor components are present as are found after sucrose gradient fractionation (although they are less prominent because relatively smaller quantities must be applied to avoid aggregation). Again, the constant association of the minor components with specific RNA fractions derived from zone sedimentation argues against their arising during the sucrose gradient fractionation and subsequent handling of the RNA. Further confidence in their being present *in vivo* was obtained when it was found that the patterns were highly reproducible from preparation to preparation both from the same tissue as well as from different tissues of the rat.

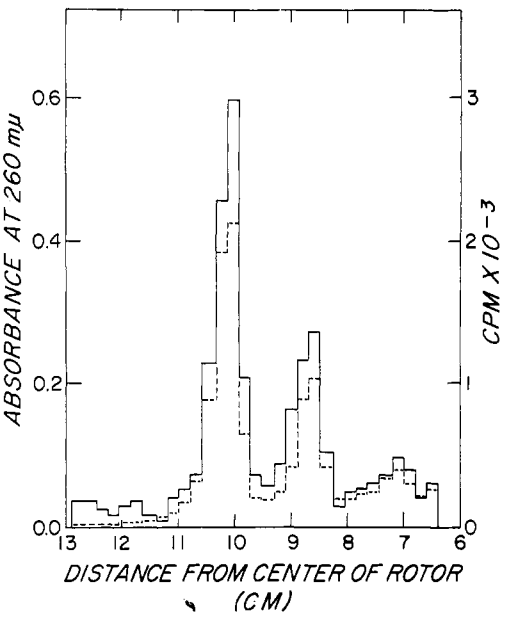

FIGURE 2: Analytical zone sedimentation of mouse liver crude nRNA, doubly labeled with [^{14}C]orotic acid and [^{3}H]uridine (not shown). In this case, the sucrose contained 0.025% Sarkosyl NL-97 in addition to the 0.1 M NaCl–1 mM EDTA (pH 6.2) buffer. Recovery of both RNA (135 μg) and radioactivity was 100%. Absorbance (———). ^{14}C (– – –).

Evidence for the existence of two of the less familiar species shown in Plate 1 has been described previously. The first of these is the low molecular weight fraction of microsomal RNA (5S, 7S, or transfer-like RNA) which has been found in a variety of tissues (*e.g.*, Rosset and Monier, 1963; Galibert *et al.*, 1965; Beney and Székely, 1966; Watson and Ralph, 1966; Bachvaroff and Tongur, 1966). This RNA is a prominent feature of the electrophoregram of fraction 6 from zone sedimentation and is clearly separated electrophoretically from sRNA in both 3.5% and 10% gels. However, it is not clear whether some of the less prominent electrophoretic classes of RNA that are found in our fraction 5 from zone sedimentation are also contained in the microsomal 5S RNA as isolated by others. The second observation for which some precedence exists is the prominent double band that comprises the bulk of the 18S class of RNAs (Plate 3). The complex character of 18S RNA is consistent with previous electrophoretic analyses in agar gel (Bachvaroff and McMaster, 1964) and with chromatographic analyses on methylated albumin columns (Drach and Lingrel, 1966). Thus, it is likely that in addition to the 4S, 18S, and 30S RNAs, there exist many species of cRNA of intermediate sedimentation and electrophoretic properties.

The Similarity of RNA from Different Tissues. We wondered whether some of these new species of RNA might be mRNA. However, on the basis of the present experiments, a definitive identification of any particular

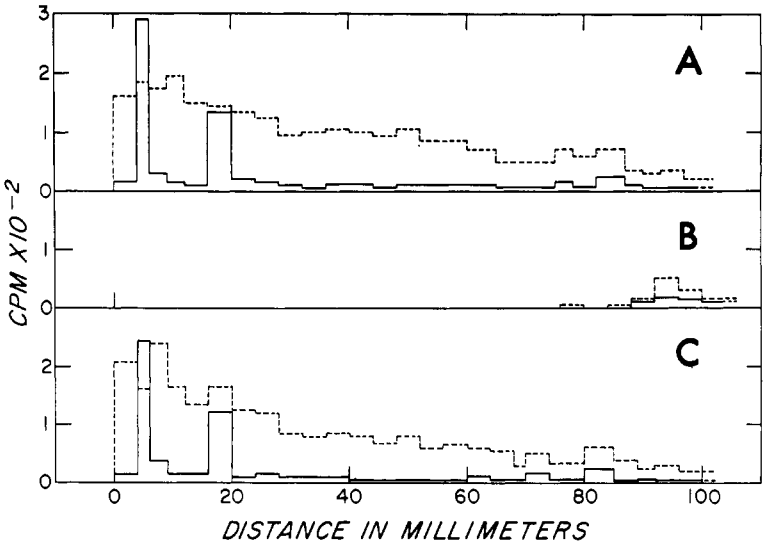

FIGURE 3: Electrophoretic analysis (in 3.5% polyacrylamide gel cross-linked with ethylene diacrylate) of mouse liver crude nRNA, doubly labeled with [^{14}C]orotic acid and [^{3}H]uridine. RNA isolated 18 hr after [^{14}C]orotic acid injection and 20 min after [^{3}H]uridine injection. (A) No preincubation of RNA. (B) RNA preincubated with RNase. (C) RNA preincubated with DNase; approximately 6 μg of RNA applied in each case. ^{14}C (———). ^{3}H (– – –). The two prominent ^{14}C peaks on the left of the figure in A and C are, from left to right, 30S and 18S RNA; the two small ^{14}C peaks on the right in A and C are, from left to right, microsomal 5S RNA and sRNA.

species as mRNA is not possible. Furthermore, there do not exist, at present, readily applied criteria for unambiguously identifying any particular species of RNA as messenger (Singer and Leder, 1966). Nonetheless, we had anticipated that the widely different biochemical functions carried on by liver, kidney, and brain might be reflected in different distributions of RNA species found in such tissues. Species of RNA unique to a given tissue would have been candidates for the role of mRNA. The remarkable similarity in the electrophoregrams of RNA from each tissue denied us the possibility of making an identification by this means. It is possible that many of the species may function as mRNA, but that the set of mRNA species present in the cytoplasm is much the same from tissue to tissue. Compared with the total variety of proteins present in cells, the number of proteins found nearly exclusively in one tissue or another may be very small. On the other hand, it is possible that no mRNA was demonstrated in these studies because the concentration of such species was too low.

In addition to the present findings of the similarity of RNA from various tissues and the report by Hirsch (1966) on similarities in nucleotide composition of the RNA from these same tissues, the protein composition of ribosomes from rat tissues has been found to be very similar (Low and Wool, 1967). Thus, the average chemical composition of ribosomes from different tissues is very much the same. Possible interpretations of this chemical similarity include (a) all ribosomes from these tissues are identical, and (b) the ribosomes within each tissue may not have the same composition (*e.g.*, different age, function) but each tissue has a similar assortment of such ribosomes.

The Classification of RNA Species by "S" Class. The existence of three major sedimenting classes of cRNA as observed in the ultracentrifuge has been the basis of classification of RNA into three major types. On the basis of the present results, it is clear that such restricted classification does not include the majority of species of cRNA. Thus to focus attention on three sedimenting classes of RNA is to miss and perhaps never seek for the biological specificity of the many species of RNA which probably exist. We interpret our results to indicate that although a classification based on sedimentation data has operational significance, it does not adequately describe the true state of heterogeneity of cRNA.

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