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The Fractionation of High-Molecular-Weight Ribonucleic Acid by Polyacrylamide-Gel Electrophoresis

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1. Gels were prepared with recrystallized acrylamide and bisacrylamide. Electrophoresis was in tris–sodium acetate–EDTA buffer for 0·5 to 3 hr. Gels were scanned at 280 or 265 mμ. Techniques are described for slicing and radioactive counting. 2. The mobility of RNA was inversely related to the sedimentation coefficient and varied with gel concentration. Electrophoresis in 2·2–2·6% gels gives a fractionation similar to density-gradient centrifugation. It shows the two ribosomal RNA components, the 45 s precursor, transfer RNA and minor components. In 5% and 7·5% gels, 4 s and 5 s RNA separated and ribosomal RNA was excluded. 3. The resolution is greater and more detailed than by centrifugation, and many samples can be analysed simultaneously and rapidly.

The fractionation of RNA by electrophoresis in supporting gels offers the possibility of more extensive and precise separations than are possible by density-gradient centrifugation. Excellent separations of ribosomal RNA components (23–30 s and 16–18 s according to species) and of various minor components have been obtained by electrophoresis on agar gels, as described by Tsanev (1965). Polyacrylamide-gel electrophoresis, which has been so successful in the fractionation of proteins (Fredrick, 1964) and in which the gel pore size can be closely controlled, has been little used for RNA. Separations of the smaller RNA molecules have been described by Richards, Coll & Gratzer (1965) and of partial digests of ribosomal RNA by Gould (1966). G. G. Brownlee, J. Hindley & F. Sanger (personal communication) are using the method for the separation of 5 s from 4 s RNA.

It appears that, although dilute gels for the fractionation of high-molecular-weight RNA can be made, as shown by the separation of the ribosomal sub-units by Hjerten, Jerstedt & Tiselius (1965*a*,*b*), intact ribosomal RNA tends to 'stick' to the gel surface. This problem has now been overcome by the use of purified reagents in the preparation of the gels and thorough deproteinization of the RNA. Excellent separations are then obtained and the gels are sufficiently transparent to be scanned in ultraviolet light. The present paper describes the details of the method and some preliminary results illustrating the resolution obtainable.

METHODS

Recrystallization of the monomers. Acrylamide (Eastman–Kodak Organic Chemicals, Rochester, N.Y., U.S.A.) was dissolved in A.R. benzene at 70°, filtered hot and cooled slowly to 0°. The crystals obtained were washed repeatedly with heptane and dried *in vacuo* to remove the benzene. Methylene bisacrylamide (Koch–Light Laboratories Ltd., Colnbrook, Bucks., or British Drug Houses Ltd., Poole, Dorset) was dissolved in water at 80–90°, decolorized with activated charcoal and filtered through filter-paper flock. The crystals obtained on cooling were washed with ice-cold water.

The above methods were used for all the results reported below. Since then it has been found that purer preparations can be obtained more easily as follows. A 70 g. sample of acrylamide is dissolved in 1 l. of chloroform at 50°. The solution is filtered hot, without suction. Crystals are obtained at −20° and recovered by filtration in a chilled filter funnel. The crystals are washed briefly with cold chloroform or heptane or both, and dried. Bisacrylamide is dissolved in acetone (approx. 10 g./l. at 40–50°) and filtered hot. The solution is slowly cooled to −20°; the crystals can be recovered and washed with cold acetone either by centrifugation or by filtration. (This method avoids the polymerization of bisacrylamide that occurs in water, leading to unsatisfactory cloudy solutions unless filter-paper flock is used.)

Stock solutions containing 15% (w/v) of acrylamide and suitable concentrations of bisacrylamide in water could be stored without apparent change for at least 1 month at 5° in the dark.

Both compounds are exceedingly poisonous and suitable precautions must be taken against contact with the skin or inhalation of the light crystals.

Preparation of the gels. For the preparation of dilute gels in which the final acrylamide concentration is between 2% and 5% (w/v), the bisacrylamide concentration used was 5% of that of the acrylamide. For gel concentrations between 5% and 8% (w/v), the bisacrylamide concentration was 2·5% of the acrylamide. For brevity, gel concentrations are described by their percentage content of acrylamide only.

Suitable volumes of the acrylamides, buffer (see below)

and water to provide the desired final concentrations were mixed and degassed at room temperature *in vacuo* for about 20 sec. *NNN'N'*-Tetramethylethylenediamine (0·033 ml.) and 10% (w/v) ammonium persulphate (0·33 ml.) were added/g. of acrylamide present. The solution was mixed, excessive aeration being avoided, and rapidly pipetted into the vertical tubes or the flat-plate apparatus described below, both filled to within 1·5 cm. from the top. Water was carefully layered over the solutions by using a pipette with a short length of thread, to ensure a flat gel surface. Dilute gels containing 2·2–3·0% (w/v) of acrylamide set in 10–20 min. at room temperature; gels containing 7·5% (w/v) of acrylamide set in about 1 min. These gels set more rapidly and had a smaller pore size (as indicated by the mobilities of a number of proteins) than corresponding gels made with the unpurified monomers.

Water was difficult to layer over the dilute acrylamide solutions. Heptane was tried as an alternative, but caused irregular polymerization of the gel surface. Attempts were also made to cast the gels in the complete absence of air, under heptane or light petroleum, but the gels then became optically inhomogeneous and could not be scanned.

Buffers. The buffers used in most of the examples shown here contained (final concentrations): tris, 0·04 M; sodium acetate, 0·02 M; sodium or potassium EDTA, 2 mM; acetic acid was used to adjust the pH to 7·8 at about 5°. All reagents were of analytical grade; the tris was British Drug Houses Ltd. 'specially purified' or Sigma Chemical Co. (St Louis, Mo., U.S.A.) Trisma. Acetate was used as the anion instead of chloride to avoid the formation of hypochlorite by electrolysis; sodium acetate was added to maintain the secondary structure of the RNA.

Several other buffers were tried in preliminary experiments. In general, it was found that a chelating agent such as EDTA or pyrophosphate was required to prevent the RNA from remaining at the gel surface. The chelating agent could be added in small amount to the RNA or layered over the gel, and need not be present in the bulk buffer solution. Mg^{2+} ions could be added with little effect; the 'sticking' seems to be due to heavier metal ions.

The use of EDTA, which has a high electrophoretic mobility, precludes the use of the discontinuous buffer system described by Ornstein (1964). Adequate zone-sharpening of the RNA layered over the gels was obtained, in agreement with the results of Hjerten *et al.* (1965*b*).

Procedure for electrophoresis. In most cases vertical tube equipment similar to that described by Davis (1964) was used. Perspex (Plexiglas) tubes ($\frac{1}{4}$ in. internal diam. × $2\frac{1}{2}$ or 5 in. long) were used; these facilitated later removal of the gels, since polyacrylamide does not adhere to Perspex as it does to glass. Rubber rings were inserted into the bases of the tubes after polymerization of the gels, to prevent the soft gels from sliding out. A 200 ml. volume of buffer, with platinum electrodes, was used in each buffer compartment for a set of eight tubes. The buffer was normally used once only. Electrophoresis was carried out at about 5° in a refrigerator; the gels warmed several degrees during the run. Up to 10 v/cm. at 5 mA/gel was applied when the tris–acetate buffer described above was used. The current was normally applied for up to 1 hr. to remove the polymerization catalysts and other impurities. The RNA sample [5–100 μg., dissolved in 10–100 μl. of the buffer containing 5% (w/v) of sucrose] was then layered over the gels and electrophoresis continued. Suitable separations were obtained in 0·5–3 hr., depending on the length of the gel and the resolution desired. With ribosomal RNA in dilute gels, the 28 s and 18 s components could be seen as refractive bands after a few minutes' electrophoresis.

After the run, the rubber rings were removed and the gels gently blown out of the tubes with a rubber teat. The dilute gels could not be handled with forceps without damage, and were best picked up by sucking into tubes of the same diameter.

Gels have also been prepared as flat plates in an apparatus similar to that described by Narayan, Vogel & Lawrence (1965). Gels 3 mm. × 8 mm. in section and 12 cm. long could be dried on silicone-treated water-repellent filter paper in a warm oven without distortion of the RNA bands. Radioautographs of ^{32}P- or ^{14}C-labelled RNA were then readily obtained. Gels of larger section, e.g. 4 mm. × 15 mm., allow more than 300 μg. of RNA to be fractionated. They can be scanned and cut in the same way as the cylindrical gels described below, and are suitable for the analysis of RNA of low specific radioactivity.

Scanning of ultraviolet absorption. The gels prepared with acrylamide recrystallized from benzene, and from which u.v.-absorbing materials had been removed by the electrophoresis, had an extinction value less than 0·3 at wavelengths down to 270 mμ. Below this wavelength the extinction rose rapidly, and measurements at 260 mμ were not possible. The gels were scanned by using a Joyce–Loebl Chromoscan instrument fitted with a Hanau deuterium lamp and an interference filter with a maximum transmission at 280 mμ. The gels were held in a parallel-sided quartz container and scanned with a light-beam about 0·8 mm. × 0·05 mm. Small irregularities in the gels and dust etc. caused fluctuations in the background of about 0·01 extinction unit. This limits the sensitivity of detection to about 0·05 μg. of RNA in a band 1 mm. wide. The extinctions of the gels were slightly higher within 1·5 cm. of the ends than in the middle; this could be up to 0·1 unit, but was usually less. The refractive effect due to a sharp band of RNA did not contribute to the extinction. This was checked with a control scan of a gel at longer wavelength or in white light; such a scan can also be used to correct for small irregularities in the gels.

Under these conditions of scanning, in which the light is not monochromatic and mainly at a wavelength to one side of the absorption maximum of RNA, the measured extinction is not proportional to RNA concentration. For convenience, the system was calibrated with RNA solutions of known absorption at 260 mμ. The scales on the ordinates of the scans show the extinction at 260 mμ calculated from the measured extinction at 280 mμ. The instrument was set to expand the scanned length of the gel by the factor 3.

Gels prepared with the acrylamide recrystallized from chloroform are more transparent at lower wavelengths and can be scanned at 260 or 265 mμ.

Scanning of radioactivity. The dilute gels used for the fractionation of high-molecular-weight RNA are too soft to be sliced with a wire slicing device. After several attempts, the following method was adopted for transverse slicing. An indian-ink line, at right angles to the length of the gel, was injected near each end of the gel with a fine syringe needle, to act as accurate markers for the registration of the extinction and radioactivity scans. After optical scanning, the gel was placed in a horizontal open aluminium trough, with the top end of the gel (the origin) lying against

a rubber stopper to maintain its shape and the other end free. The trough was then placed horizontally on powdered solid CO_2 to freeze the gel from the sides and below only. No solid CO_2 was spilled into the trough over the gel. This procedure allows for expansion of the gel during freezing without longitudinal distortion of the RNA bands or of the markers. In transverse section, the gel is then pear-shaped, owing to the upward transverse expansion. The gels were then sliced on an apparatus essentially similar to the McIlwain chopper (McIlwain & Buddle, 1953; available from H. Mickle, Gomshall, Surrey); the blade of the cutter was tilted so that the leading cutting edge was vertical; there is then no tendency for the blade to push the gel and the slicing is accurately at right angles to the gel length. The gel was frozen on to the platform with solid CO_2 and allowed to thaw slightly before cutting. Slices were cut 0·7mm. thick. For counting ^{32}P or ^{14}C, the slices were dried on to adhesive labels, which were then stuck to counting planchets, or on filter paper, which was then counted in toluene scintillator. For counting ^{3}H (experiments not illustrated in this paper), the slices were heated at 60° in 0·5ml. of 10% (v/v) piperidine in scintillation vials for several hours and allowed to dry. Then 0·5ml. of water was added, which caused the gel slices to swell to many times their normal volume, followed by 15ml. of a water-miscible scintillation fluid. This caused the gels to shrink, and more than 90% of the hydrolysed RNA was dissolved in the scintillator.

Preparation of RNA. Pea-seedling root tips were homogenized in buffered sucrose medium (Loening, 1965) and the homogenate was centrifuged at 1000*g* for 5min. This sedimented the cell debris and nuclear material and left the bulk of the cytoplasmic fractions in suspension. The debris fraction was resuspended in the same medium. Sodium dodecyl sulphate (0·5%) and sodium 4-aminosalicylate (5%, w/v) were added to both fractions (Kirby, 1965) and the suspensions shaken with phenol containing 0·1% (w/v) of 8-hydroxyquinoline at 0–5°. The phases were separated by centrifugation at 1000*g* for 10min. and the phenol extraction was repeated once or twice. The RNA was precipitated from the final supernatant by the addition of 2% (w/v) sodium acetate and 2·5vol. of ethanol at −20°. To remove DNA, the precipitate was dissolved in the homogenizing medium and recrystallized deoxyribonuclease (Sigma Chemical Co.) (10μg./ml.) added. The solution was incubated at 5° for 30min. followed by the addition of sodium dodecyl sulphate and deproteinization with phenol. The final RNA precipitate was reprecipitated from 0·3M-sodium acetate twice, washed once with ethanol and partially dried for a few minutes *in vacuo*. It was then dissolved in the electrophoresis buffer containing 5% (w/v) of sucrose to give a final concentration between 0·5 and 2·0mg./ml.

In later experiments, sodium tri-isopropylnaphthalenesulphonate (2–5%, w/v) was used instead of sodium dodecyl sulphate and 4-aminosalicylate, and the phenol contained in addition 10% (w/v) of *m*-cresol (Kirby, 1965). After one or two such phenol extractions, the supernatant was made 3% (w/v) with respect to NaCl and the phenol extraction repeated. RNA was then precipitated by addition of 2vol. of ethanol.

RNA was prepared from *Escherichia coli* by Dr D. Bishop by the sodium dodecyl sulphate–phenol method. Rabbit reticulocyte polysomes were kindly given by Dr J. Bishop. RNA was prepared from these by the sodium dodecyl sulphate–4-aminosalicylate method and by the sodium tri-isopropylnaphthalenesulphonate–phenol–*m*-cresol method.

None of these methods completely removed low-molecular-weight ^{32}P-labelled impurities from ^{32}P-labelled RNA. Attempts were made to prepare and to further purify the RNA by using the guanidine chloride method of Cox & Arnstein (1963). Usually the preparations gave poor separations on the gels, with considerable trailing of the bands. This may be due to traces of protein or to aggregation of the RNA. The problem is being further investigated, since reprecipitation from guanidine chloride does remove ^{32}P-labelled contaminants.

RESULTS

The two ribosomal RNA components from higher organisms are referred to below as 28s and 18s and those from bacteria as 23s and 16s. Amino acid-transfer RNA is 4s and the RNA fraction described by Rosset & Monier (1963) is 5s. These designations are intended only for identification of the RNA components, and do not imply accurately measured sedimentation coefficients or electrophoretic mobilities.

Gel concentration. The separations obtained with reticulocyte RNA in gels from 2·2% to 7·5% are shown in Fig. 1. At the lower gel concentrations, the separations resemble those obtained by density-gradient centrifugation. It appears therefore that the mobilities of the RNA components are inversely related to their sedimentation coefficients. There is a clear separation of 28s, 18s and 4s RNA; 4s RNA is slightly separated from 5s RNA and a number of minor components are resolved. With increase in gel concentration the mobilities decrease, and the decrease is greatest for the higher-molecular-weight components. Therefore the separation between the 28s and 18s RNA is greater in the 2·4% gel than in the 2·2% gel; a minor component that appears as a shoulder on the 18s RNA in the 2·2% gel is resolved in the 2·4% gel. On the other hand, the 2·2% gel is better for the resolution of components heavier than 28s RNA (not present in this preparation). With increasing gel concentration above 2·4%, the separation between the 18s and the 4s and 5s components increases. The 28s RNA is excluded from gels above 3% and the 18s RNA above about 5%. The 4s and 5s RNA are best separated from each other in 7·5% gels, in which the higher-molecular-weight RNA is totally excluded; the 9s 'messenger' (see below) just enters the 7·5% gel.

Effect of impurities. Contaminants in the gel materials or in the RNA preparations can result in streaking and poor separations. It was frequently found that impure RNA entered the gels for about 2mm. and there became concentrated in a fine band that developed an irregular surface. This effect

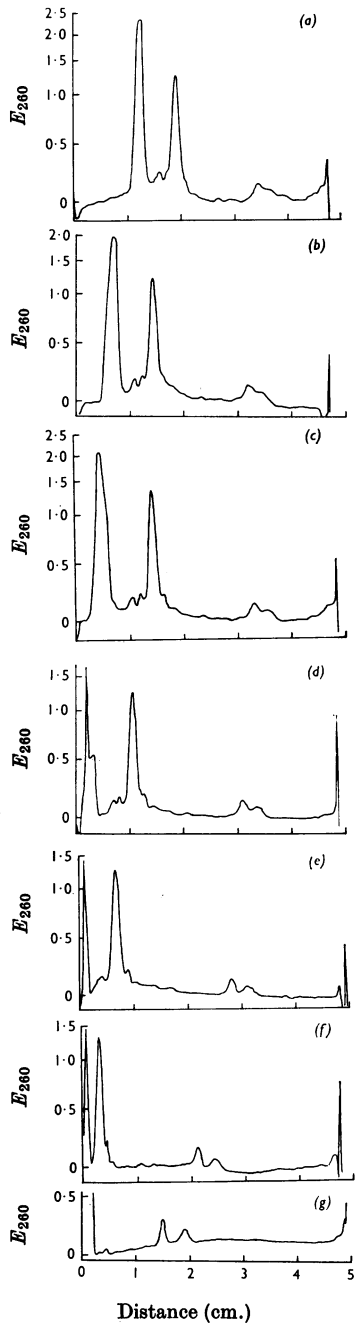

Fig. 1. Electrophoresis of RNA at different gel concentrations. RNA was prepared from rabbit reticulocyte polysomes by the sodium tri-isopropylnaphthalenesulphonate–phenol–*m*-cresol procedure. In each case 40μg. of RNA was applied/gel; gel concentrations were: (*a*) 2·2%; (*b*) 2·4%; (*c*) 2·6%; (*d*) 3·0%; (*e*) 3·5%; (*f*) 5·0%; (*g*) 7·5%. Electrophoresis was carried out for 65min. at 50v in buffer as described in the text.

was visible after 10–15min. electrophoresis. The band caused this region of the gel to constrict. RNA trailed out of the constricted region during electrophoresis. The trouble was accentuated at high RNA concentrations above 20μg./gel. Thorough deproteinization was found to be essential for clear separations. This was more difficult to achieve with the reticulocyte polysome RNA than with pea-seedling preparations. Usually the sodium dodecyl sulphate methods failed to give sufficiently pure reticulocyte RNA. The sodium tri-isopropylnaphthalenesulphonate–phenol–*m*-cresol extraction consistently gave RNA that was clear in solution and showed no streaking even at high concentrations of 100μg./gel.

Examples. (1) Reticulocyte RNA. The trace component seen in Fig. 1 in the 2·2% gel at 2·7cm. and in the 2·4% gel at 2·3cm. presumably corresponds to the supposed 9s messenger RNA for haemoglobin isolated by Burny & Marbaix (1965). This is shown more clearly in Fig. 2, in which more RNA was applied to the gel. A longer gel was used to compensate for the loss in resolution at the higher RNA concentration. The 'messenger' is now seen to include at least two components at 4·4 and 4·7cm., and perhaps one at 4·9cm. The amount of this messenger and of the other minor components in relation to the main ribosomal components varied in different preparations of polysomes. A buffer containing phosphate and pyrophosphate was used in this case; the separation obtained is similar to or identical with that obtained with the usual tris–acetate buffer.

(2) *E. coli* RNA. Fig. 3 shows the fractionation

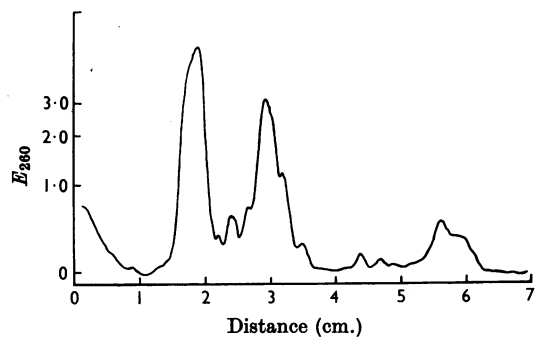

Fig. 2. Electrophoresis of rabbit reticulocyte polysome RNA. A different batch of polysomes from those in Fig. 1 was used; RNA was prepared by the sodium dodecyl sulphate–4-aminosalicylate method; 100μg. was applied. The gel concentration was 2·2%. Electrophoresis was carried out for 10min. at 40v and then for 2·5hr. at 60v (approx. 4mA). The buffer contained: $Na_2H_2P_2O_7$, 15mM; K_2HPO_4, 15mM; EDTA, 3mM; KOH was added (approx. 20mN) to give pH8 at 5°.

of *E. coli* RNA labelled with [^{14}C]uracil, kindly given by Dr D. Bishop. This illustrates the precision with which the radioactivity and extinction scans coincide; an error of one slice leads to the discrepancy shown in the 4s RNA. The ribosomal RNA peaks do not trail, so that it should be possible to measure the radioactivity of polydisperse messenger RNA even in the presence of highly labelled ribosomal RNA.

The results from a 15min. labelling period after a 'step-down' incubation of the cells is shown in Fig. 4. The labelled messenger appears polydisperse, as on density-gradient centrifugation; considerable fractionation of the messenger should be possible. The radioactivity over the 23s peak is exactly coincident with the extinction peak, but the radioactivity over the 16s peak is broader than the extinction peak and just separated into two components. The reasons for this are not known; the point here is to illustrate the resolution obtainable. Less than 3·5cm. of gel length was used in the fractionations in Figs. 3 and 4; the separations can be greatly extended in longer gels.

(3) Pea-seedling RNA. Rapidly labelled cytoplasmic RNA from pea-seedling root tips is shown in Fig. 5. The 18s RNA has a higher specific activity than the 28s RNA, as was described by Henshaw, Revel & Hiatt (1965) in rat liver. The radioactivity is not exactly coincident with the 18s peak, like the lighter component of the labelled *E. coli* 16s RNA. Henshaw *et al.* (1965) considered this RNA to be messenger RNA either attached to the 18s ribosomal RNA or of similar sedimentation coefficient. If the RNA found in the pea seedlings is similar to that in liver, then the separation obtained in the gels shows that it is not attached to 18s RNA (the complex would be heavier) but is a separate molecule.

RNA prepared from the cell-debris–nuclear fraction after the same [^{32}P]phosphate incubation shows a similar labelling in the peak lighter than 18s (Fig. 6). The 28s RNA is more highly labelled than in the cytoplasm, consistent with its accumulation in the nucleus. The highest specific activity is in the supposed ribosomal precursor (just visible as an extinction peak), reported as 45s by Scherrer, Latham & Darnell (1963). The high sedimentation coefficient indicates that this is either a high-molecular-weight RNA or that it is a more tightly

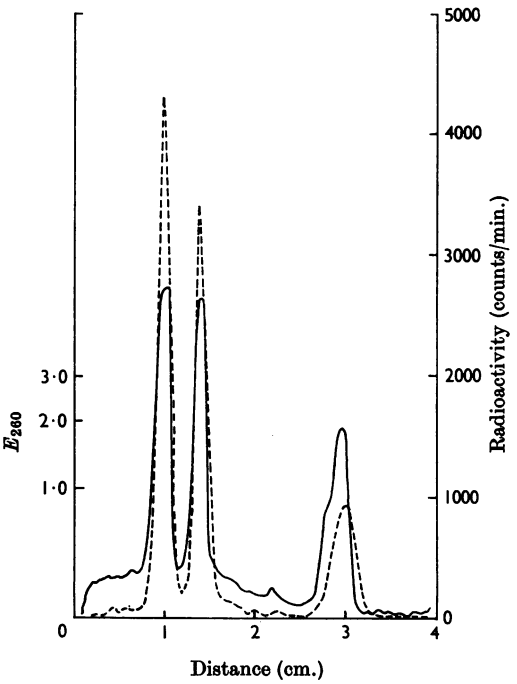

Fig. 3. Electrophoresis of *E. coli* RNA labelled with [^{14}C]-uracil. Exponential-phase growing cells were incubated in [^{14}C]uracil, and nucleic acids were extracted by the sodium dodecyl sulphate–phenol method. DNA was removed with deoxyribonuclease and the RNA reprecipitated from 4M-guanidine chloride with 1vol. of ethanol. Electrophoresis was carried out for 50min. at 50v in 2·4% gel. ——, E_{260}; ----, radioactivity.

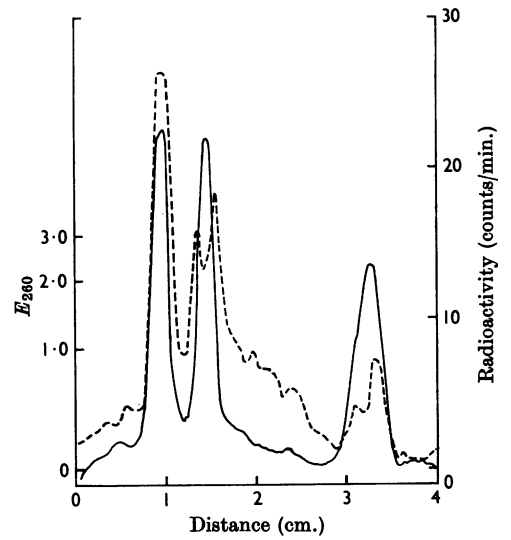

Fig. 4. Electrophoresis of *E. coli* RNA labelled with [^{14}C]-uracil after a 'step-down'. Cells were labelled with [^{14}C]uracil for 15min. after a 'step-down' incubation as described by Hayashi & Spiegelman (1961). RNA was extracted as described in Fig. 3. Electrophoresis was carried out for 60min. at 60v in 2·4% gel. ——, E_{260}; ----, radioactivity.

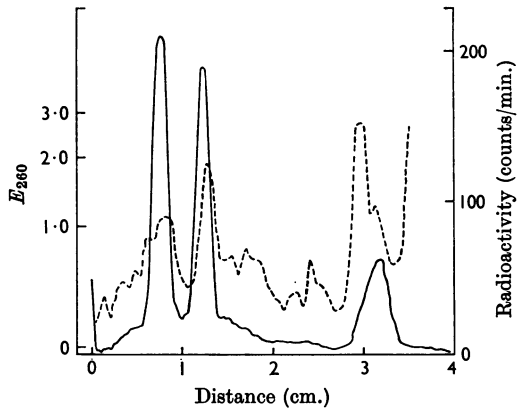

Fig. 5. Electrophoresis of pea root-tip cytoplasmic RNA. Pea seedlings were labelled for 15min. in [^{32}P]phosphate (approx. 1mc/25ml. of water; 30 seedlings) followed by 30min. in unlabelled potassium phosphate (20μM). Root tips were cut 7mm. long. They were homogenized and RNA was extracted from the cytoplasmic fraction as described in the text, by using the sodium dodecyl sulphate–4-aminosalicylate method. Electrophoresis was carried out for 60min. at 60v in 2·4% gel. ——, E_{260}; ----, radioactivity.

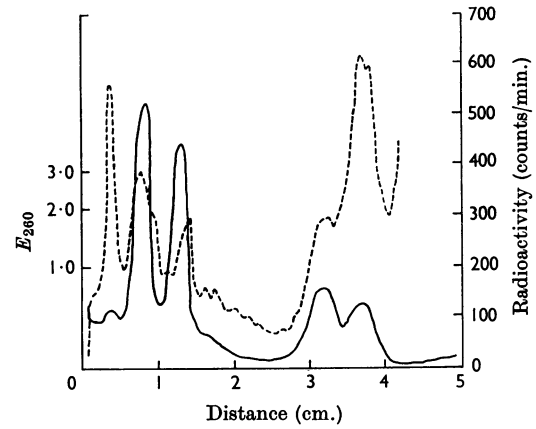

Fig. 6. Electrophoresis of pea root-tip cell-debris–nuclear RNA. Details were as given in Fig. 5: RNA was extracted from the 1000g sediment as described in the text. ——, E_{260}; ----, radioactivity.

coiled form of ribosomal RNA. If the latter were the case, then it would be expected to have a higher electrophoretic mobility in the gels than ribosomal RNA, since a more compact molecule should be less delayed by gel filtration. Since its mobility is low, it appears that the ribosomal precursor has a higher molecular weight than ribosomal RNA (assuming that the pea-seedling material is similar to the mammalian).

There are ^{32}P-labelled impurities shown in both Figs. 5 and 6, moving ahead of the 4s RNA. These largely 'chase out' after longer incubations. Fig. 6 also shows labelled deoxyribonucleotides as a second peak ahead of the 4s RNA.

(4) 4s and 5s RNA. 5s RNA was found by the gel electrophoresis method in several different classes of organism: bacteria (*E. coli*), fungus (*Trichoderma*), higher plant (pea), insects (*Drosophila* and *Smittia*), bird (pigeon) and mammal (rabbit). The mobilities of the 5s RNA varied in different species, whereas that of the 4s RNA was similar in all. The degrees of separation of the 4s from 5s RNA therefore varied. *Smittia* 5s RNA had the highest mobility and did not completely separate from 4s RNA in 5% gels, but did so in 7·5% gels. Reticulocyte (rabbit and pigeon) 5s RNA had the lowest mobility, and was present in the greatest relative amount. In all cases the 5s RNA peak was sharper than the 4s RNA peak. This is to be expected if the 5s RNA is a single molecular species and the 4s RNA is a complex mixture.

Electrophoresis of DNA. Plant DNA prepared with the RNA from whole cells by omitting the deoxyribonuclease digestion, and mouse DNA kindly given by Dr P. M. B. Walker and Dr Anne McLaren, entered the gels as sharp peaks with a very low mobility. Some fibrous material was also left on the origin and could be detected by staining with acridine orange. Mouse DNA that had been treated ultrasonically to give a molecular weight of about 500000 moved as a very diffuse band with an average mobility greater than that of ribosomal RNA.

DISCUSSION

Since the separations obtained in these experiments are similar to those obtained by density-gradient centrifugation, it is clear that the electrophoretic mobility is in general inversely related to the sedimentation coefficient of the RNA. This compares with the results found for 2–6s RNA fragments by Richards *et al.* (1965). The fractionation thus depends on molecular filtration, and the pore sizes of the gels can be adjusted to provide a degree of filtration suited to the molecular weight of the RNA, as shown in Fig. 1. Differences in the ratios of charge to mass of different RNA molecules are likely to be too small to affect the electrophoretic mobilities.

Changes in the secondary structure of the RNA should affect the mobilities, however, since the effective volumes of the molecules would be expected to change. It is thus possible that some fractionation according to the composition of the

RNA could be obtained, dependent on the ionic environment. The unfolding of an RNA molecule should lower its mobility so that it will move with molecules of higher molecular weight on the gels but with molecules of lower molecular weight on density-gradient centrifugation. A combination of the two methods can therefore be used to distinguish a change in structure from a change in weight of the RNA. This leads to the suggestion made in the Results section, that the ribosomal RNA precursor of 45s RNA is in fact a larger molecule and not a more compact form. The effects of known changes in RNA structure on the mobilities in the gels is being further investigated.

The examples shown here were chosen to illustrate the potentialities of the method. It is suggested that the gel electrophoresis provides a convenient alternative to density-gradient centrifugation for analytical purposes. Large numbers of samples can be analysed rapidly and the resolution is better than on gradients. Polyacrylamide may have the advantage over agar electrophoresis in that there is no risk of ribonuclease contamination, and that the pore size of the gels can be controlled over a wide range. The method can probably be adapted for preparative separations as has been done for proteins; this leads to the possibility of preparing large amounts of purified RNA components rapidly.

While this work was being completed, I heard that Dr U. Grossbach was also fractionating RNA on polyacrylamide gels. I thank him and Dr W. B. Gratzer, Dr H. Gould and Dr E. D. Richards for many interesting and helpful discussions. I also thank Professor R. Brown for his interest. The work was supported by grants from the Agricultural Research Council (London) and the National Institutes of Health (Bethesda, Md.).

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