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Electrophoretic Separation of Viral Nucleic Acids on Polyacrylamide Gels

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A method for separating high molecular weight RNA or DNA by electrophoresis on polyacrylamide gels has been described.

Ten viral nucleic acid species and four *Escherichia coli* RNA species have been used to calibrate the system and demonstrate a general relationship between the logarithm of the molecular weight and the relative electrophoretic mobility.

The elution of viral RNA from gel slices and the demonstration of infectivity after electrophoresis are described.

1. Introduction

Chromatography on various columns (Mandell & Hershey, 1960) and centrifugation in linear gradients of sucrose (Britton & Roberts, 1960) have been the two procedures widely employed for the fractionation of RNA.

Electrophoretic separation of nucleic acids on starch columns was initially introduced by Paigen (1956) and used by Nomura, Hall & Spiegelman (1960) to identify the messenger RNA specific to viral infection. Agar columns have been similarly used (Tsanev, 1965) to separate ribosomal RNA components.

Most recently polyacrylamide gels have been adopted (Richards, Coll & Gratzer, 1965) since they possess the obvious advantage of controlled pore sizes. Polyacrylamide electrophoresis has been used to separate small (Gould, 1966) and large (Loening, 1967) molecular weight RNA.

The availability of different polyacrylamides provides a high degree of flexibility to this approach of RNA fractionation. It is our purpose here to illustrate the versatility of this method by exhibiting results obtained with a variety of viral RNA species.

2. Materials and Methods

(a) *Polyacrylamide gels*

In view of the quenching of ^{3}H counts by dried polyacrylamide gel slices, two types of gel were investigated: a bis-acrylamide cross-linked gel, which can be dissolved by hydrogen peroxide, and an ethylene diacrylate cross-linked gel which can be dissolved by alkali (Choules & Zimm, 1965). The lowest acrylamide concentrations yielding gels which can be readily manipulated have been found to be 2·2% for gels cross-linked with bis-acrylamide and 2·8% for those in which ethylene diacrylate was used.

Acrylamide and bis-acrylamide (Eastman Organic Chemicals) were recrystallized from chloroform and acetone, respectively. Ethylene diacrylate was obtained from the Borden Chemical Company.

Polyacrylamide 2·4% gels were prepared by mixing 4 ml. of an aqueous stock solution of 15% recrystallized acrylamide, 0·75% recrystallized bis-acrylamide with 12·45 ml. water and 8·33 ml. 3E buffer (0·12 M-Tris–0·06 M-sodium acetate–0·003 M-sodium EDTA made pH 7·2 with 6 ml. of glacial acetic acid). Air was removed from the mixture by evacuation and 0·02 ml. of *N,N,N′,N′* tetramethylethylenediamine and 0·2 ml. of fresh aqueous 10% ammonium persulphate solution added. After swirling, the mixture was transferred to Plexiglass tubes (0·7 cm internal diameter), with a rubber stopper in one end, and allowed to polymerize for 20 min in an upright position. The gel length was made exactly 5 cm. Due to extrusion of liquid during the polymerization, the gel surface was flat. After polymerization, the gels were transferred to 500 ml. of E buffer (one-third the concentration of 3E) containing 0·2% recrystallized sodium dodecyl sulfate. They were left for 72 hr in order to remove materials which absorb ultraviolet light (Fig. 1) and to swell to 150% of their original length (180% of their original volume). No further swelling is obtained if the gels are left for longer periods.

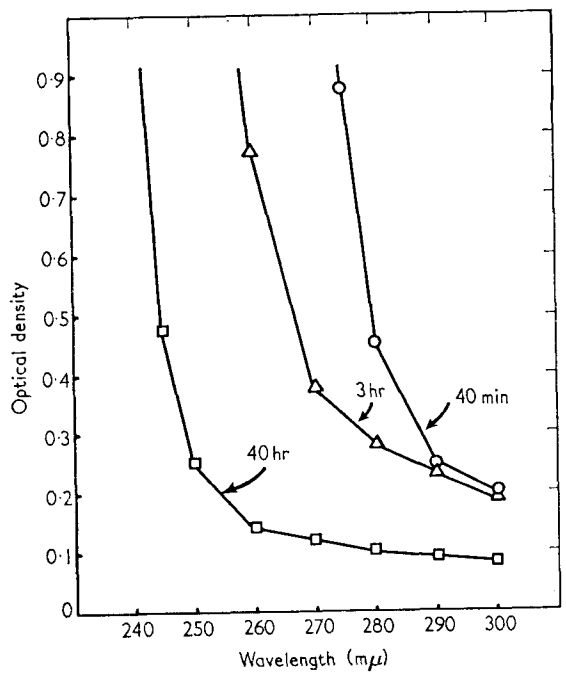

FIG. 1. Optical density of a bis-acrylamide 2·4% gel at various times after polymerization. A bis-acrylamide cross-linked 2·4% gel was prepared in a 1-cm quartz cell and scanned at intervals. After each scan, the gel was allowed to soak in E buffer containing 0·2% sodium dodecyl sulfate.

Diacrylate gels were prepared similarly except that to compare the behavior of RNA species in different gels, the gels were also prepared at different concentrations and the swelling period was omitted. The stock solution used for preparing diacrylate gels was 15% recrystallized acrylamide and 1·0% ethylene diacrylate. For gels having an acrylamide concentration greater than 4%, it is necessary to suck the gel up and down the tube gently after polymerization has commenced in order to prevent gel adherence to the tube walls and facilitate the final extrusion of the gel. At lower acrylamide concentrations and in contrast to glass tubes, the gels slip easily out of the Plexiglass tubes.

(b) *Electrophoresis*

The electrophoresis buffer was the buffer E containing 0·2% sodium dodecyl sulfate. Swollen gels were sucked into 0·7-cm (internal diameter) Plexiglass tubes and supported by a dialysis membrane stretched across the lower end. They were then pre-run at room

temperature for 30 min at 5 mA per tube and 50 v (5·5 v/cm) using a Buchler voltage and current regulated d.c. power supply set for current regulation. Samples of nucleic acid were added in E buffer containing 10% sucrose in *the minimum loading volume possible* in order to obtain good resolution. Excellent resolution is obtained with less than 15 μl.; samples of 100 μl. can be used, but yield a poorer resolution. The maximum amount of RNA which can be applied to one gel of the size specified is between 100 and 200 μg; excellent resolution is obtained with less than 20μg. Electrophoresis is carried out at room temperature, 5 mA per tube and 50 v for 90 min or more, as indicated.

After electrophoresis, the gels were transferred to a rectangular quartz cell (0·5 cm × 12·0 cm × 2·0 cm) and scanned by transmitted ultraviolet light in a Joyce high-resolution Chromoscan using a 266 mμ interference filter. At the end of the run the lengths of the gels in the cell were measured and subsequently, the gels were floated in water on a right-angle aluminum trough in order to obtain the natural gel length. The water was then removed and the gel frozen in the aluminum trough on powdered dry ice. After freezing, each gel was transferred to a carbon dioxide gas-cooled microtome, sequentially sliced and the 0·5-mm slices dried on filter paper, or placed in scintillation vials. Slicing is facilitated by the presence of sodium dodecyl sulfate in the gel, since the sodium dodecyl sulfate precipitates on cooling and inhibits the formation of large ice crystals. The resolution obtained between different RNA species is evidence to the fact that this method of freezing does not distort the RNA bands in the gel (*vide infra*).

(c) *Sources and methods of nucleic acid preparation*

RNA was extracted from [^{14}C]uridine-labeled satellite tobacco necrosis purified virus by the method of Reichmann (1965). ^{32}P-labeled single-stranded DNA and ^{3}H-labeled double-stranded DNA from ϕX174 were prepared and isolated by the procedures of Hayashi, Hayashi & Spiegelman (1963*a*).

Nucleic acid was extracted from [^{3}H]uridine-labeled *E. coli* K38 as follows. Exponentially growing cells (1×10^{8} ml.) in 50 ml. of 3XD medium (Fraser & Jerrel, 1953) were inoculated with 5 mc [^{3}H]uridine, incubated at 37°C with shaking to a concentration of 6×10^{8} cells ml. and harvested by centrifugation. The cells were washed by suspending in 50 ml. of 0·15 M-sodium acetate, recentrifuged and finally suspended in 10 ml. of 0·15 M-sodium acetate for phenol extraction as described below.

^{32}P-labeled MS2, R17, and Qβ viruses were prepared from exponentially growing *E. coli* Q13 in 50 ml. 3XD medium (modified to contain 1×10^{-4} M-phosphate and 0·04 M-Tris buffer (pH 7·3)). When the cells reached a density of 1×10^{8}/ml., [^{32}P]orthophosphate was added. The amount used varied from 1 to 50 mc, depending on the requirements of the experiment. The incubation was continued to a cell density of 4×10^{8}/ml. at which point the culture was inoculated with virus at a multiplicity of infection of 10. After lysis, bacteria and bacterial debris were removed by centrifugation at 10,000 *g* for 20 min and 15·5 g ammonium sulfate added per 50 ml. supernatant. After 1 hr at 4°C, the precipitate, containing most of the virus particles, was collected by centrifugation, suspended in 2 ml. TE buffer (0·01 M-Tris buffer (pH 7·5)–3×10^{-3} M-EDTA), and dialyzed overnight against the same buffer. The dialysate was then layered on a 5 to 20% linear gradient of sucrose containing TE buffer and centrifuged for 5 hr at 25,000 rev./min in a Spinco SW25 rotor. The 84 s viral peak (Overby, Barlow, Doi, Jacob & Spiegelman, 1966*a*) was identified by its position and radioactivity. The peak fractions were pooled and dialyzed against 0·15 M-sodium acetate. The MS2 and R17 viruses were grown in the presence of Qβ antiserum and the Qβ-virus in the presence of MS2 antiserum.

RNA was isolated from bacteria or purified virus† by a method based on that described by Kirby (1965). To 10 ml. of suspension was added 0·5 g tri-isopropylnaphthalene sulfonate (Eastman Organic Chemicals) and the mixture shaken at 4°C for 5 min. Ten ml. of a phenol–cresol mixture (500 g phenol, 70 ml. redistilled M-cresol, 0·5 g 8-hydroxyquinoline, and 55 ml. aqueous 0·15 M-NaCl) were added and the mixture shaken at 4°C for 10 min. After centrifugation, the aqueous phase was carefully removed,

† Abbreviations used: MS2, R17 and Qβ refer to the corresponding bacterial viruses. Plant viruses included TNV, tobacco necrosis virus; STNV, satellite tobacco necrosis virus; TYMV, turnip yellow mosaic virus; TMV, tobacco mosaic virus; BMV, bromegrass mosaic virus.

leaving most of the DNA at the interface. Sodium chloride was added to a concentration of 0·5 M and the solution was re-extracted with 10 ml. of phenol–cresol mixture at 4°C. To obtain *high molecular weight RNA*, the aqueous phase was mixed with 2·0 g of sodium benzoate and 0·15 vol. of M-cresol added. The transparent precipitate was recovered by centrifugation, dissolved in 0·5 M-NaCl and reprecipitated by M-cresol as before. The precipitate was then dissolved in cold 0·15 M-sodium acetate and precipitated with 2 vol. of alcohol. To obtain *total RNA*, the aqueous phase from the second phenol–cresol extraction was mixed with 2 vol. of cold ethanol and the nucleic acid allowed to precipitate at −20°C for 1 hr. The precipitate was recovered by centrifugation, washed twice with cold 75% aqueous ethanol and once with ethanol, dried by evacuation and finally dissolved in either TM buffer (0·01 M-Tris (pH 7·4)–0·005 M-$MgCl_2$), E buffer or 0·15 M-sodium acetate (pH 6·5) and stored at −80°C.

Qβ, MS2, and R17 virus RNA was further purified by sedimentation for 12 hr at 25,000 rev./min in a 2·5 to 20% linear gradient of sucrose containing 0·15 M-sodium acetate. The peak viral RNA fractions were combined and alcohol-precipitated from 0·15 M-sodium acetate as described above.

The biological activity of viral RNA was assayed by the ability to infect spheroplasts and give plaque-forming units as detailed by Pace & Spiegelman (1966).

(d) *Measurement of radioactivity*

Membrane filters or gel slices dried on filter papers were counted in toluene-based BBOT (2, 5-bis [2(5-*tert*) butylbenzoxazolyl)]-thiophane) using a Packard liquid-scintillation spectrometer. Diacrylate gel slices were dissolved in 0·5 ml. of a mixture of alcoholic hyamine hydroxide and 1 M-piperidine (1 : 9 v/v) prior to counting in Kinard's scintillation fluid (Kinard, 1957). ^{32}P, ^{14}C and ^{3}H were counted at efficiencies of 85, 65 and 13%, respectively. When ^{32}P and ^{3}H were counted in the same sample, the ^{3}H counts were corrected for cross-over from the ^{32}P counts. Similarly, when a sample contained ^{14}C, ^{32}P and ^{3}H, the use of suitable gain and discriminator settings and the appropriate simultaneous equations permitted the detection of cross-over counts from one isotope to another and the observed counts for each were suitably corrected. The recorded counts in Kinard's scintillator are adjusted to 85%, 65% or 13% efficiency of counting for ^{32}P, ^{14}C or ^{3}H and all counts are corrected for background radiation.

3. Results

(a) *The effects of concentration and pre-swelling of diacrylate gels on the mobilities of RNA species*

Figure 2(a) and (b) show the movement of Qβ[^{32}P]RNA and tritium-labeled bacterial RNA components (23 s, 16 s, 5 s and 4 s) on unswollen ethylene diacrylate cross-linked gels at two concentrations (2·8 and 3·6%) Figure 3 shows the relationship between the distance traveled and gel concentration for all five RNA species. The effect of pre-swelling on the subsequent electrophoresis is also noted. It is evident, as has been observed by Loening (1967) with bis-acrylamide cross-linked gels, that the low molecular weight 4 s and 5 s RNA can be separated at high gel concentrations, whereas resolution of higher molecular weight species (23 s and 16 s RNA) requires lower gel concentrations. It is also clear that pre-swelling the gel results in a greater electrophoretic mobility for all the RNA components examined. It has been found that the observed mobilities vary with the time allowed for polymerization and the properties of the particular batch of recrystallized acrylamide. When polymerization times and swelling conditions are kept constant, quantitative agreements amongst repetitive runs are obtained.

(b) *Electrophoresis of the RNA of bacterial viruses on ethylene diacrylate 3·0% gels*

Mixtures of a ^{32}P-labeled viral RNA (MS2, R17, or Qβ) and ^{3}H-labeled bacterial

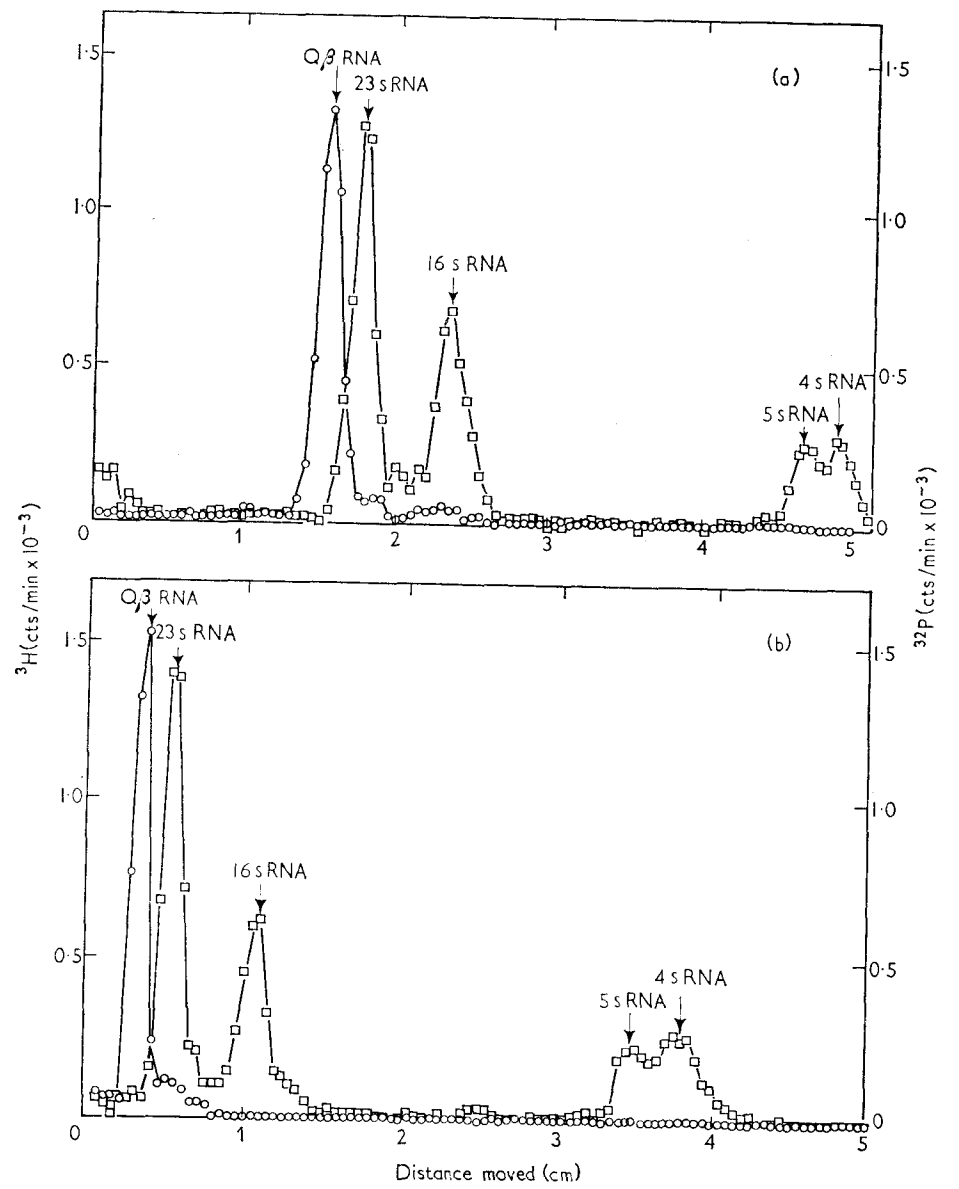

FIG. 2. Electrophoresis of a mixture of Qβ[^{32}P]RNA and *E. coli* bulk [^{3}H]RNA. Ethylene diacrylate cross-linked gels were prepared at (a) 2·8% and (b) 3·6% acrylamide concentrations, and pre-run for 30 min at room temperature, 5 mA/tube and 5·5 v/cm. A mixture of (—○—○—) Qβ [^{32}P]RNA ($6·72 \times 10^{3}$ cts/min, 0·08 μg) and (—□—□—) *E. coli* bulk [^{3}H]RNA (13×10^{3} cts/min, 2 μg) was prepared and run on the gels for 90 min as described in the text. After electrophoresis, the gels were sliced, dissolved in alkali, and counted in Kinard's scintillation fluid to obtain the profiles of the various components.

RNA were electrophoresed on ethylene diacrylate gels. Figure 4(a) and (b) show the behavior of Qβ RNA and MS2 RNA respectively. Using the 23 s peak as a reference, it is evident that MS2 RNA has a higher relative mobility than Qβ RNA, that MS2 runs closer to the bacterial 23 s than does Qβ—a difference which is highly

reproducible. It was found that after 90 minutes of electrophoresis, both MS2 RNA and R17 RNA are located 1·5 mm behind the 23 s RNA, whereas Qβ RNA is 3·0 mm behind—equivalent to six fractions.

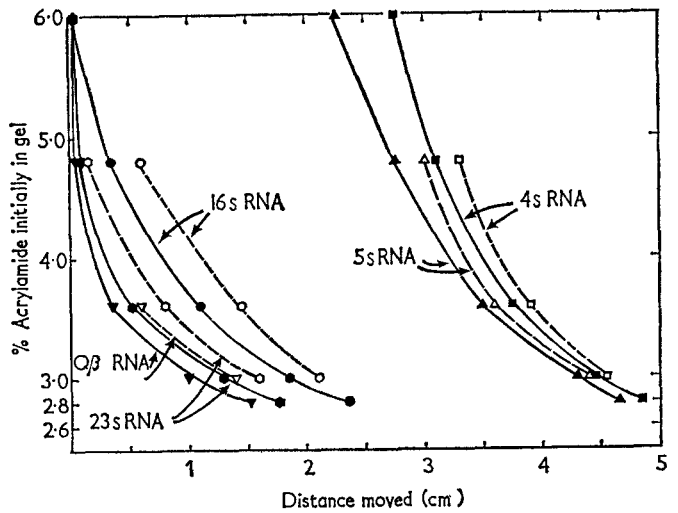

FIG. 3. Relationship between distance moved and gel concentration for five RNA species. Diacrylate gels were prepared at different acrylamide concentrations as described in the text. Gels were either pre-run for 30 min and used directly (continuous lines) or allowed to swell and then pre-run for 30 min (broken lines) as described in the text. A mixture of Qβ[^{32}P]RNA ($6{\cdot}7\times10^3$ cts/min, 0·08 μg) and *E. coli* bulk [^{3}H]RNA (13×10^3 cts/min, 2 μg) was run on the gels for 90 min and the position of the components determined as described in Fig. 2. The distance moved under standard conditions is plotted against the acrylamide concentration.

(c) *Electrophoresis of the RNA of bacterial viruses on swollen bis-acrylamide gels*

The separation of virus–bacterial RNA mixtures on electrophoresis in bis-acrylamide swollen 2·4% gels for the standard 90 minutes electrophoresis time is greater than for the diacrylate 3·0% gel as illustrated in Fig. 5(a) and (b). Again, no difference in the position of R17 and MS2 RNA was detectable; both are 2 mm behind the 23 s bacterial RNA, while Qβ RNA is 4 mm behind the 23 s RNA. It should also be noted that the mobility of high molecular weight RNA is slightly greater in a bis-acrylamide swollen gel than in a 3·0% diacrylate gel, and that 5 s and 4 s bacterial RNA species are not resolved from each other.

It may be noted that the mobility of Qβ RNA relative to the 23 s and 16 s RNA components was not detectably modified by the presence of 5×10^{-3} M-$MgCl_2$ or 5×10^{-3} M-EDTA in the gels.

(d) *Retention of biological activity after electrophoresis*

It was of obvious interest to see whether the biological activity of an RNA preparation survived the electrophoretic separation. Accordingly, 0·5 μg of purified ^{32}P-labeled Qβ RNA in 10 μl. E buffer was electrophoresed for 90 minutes on a swollen 2·4% gel and the slices put in cold vials containing 0·5 ml. of 5×10^{-3} M-EDTA (pH 7·4). The vials were capped and shaken overnight at 4°C. The liquid was frozen at −20°C and the overnight extraction repeated with a further 0·5 ml. of the EDTA solution. The liquid was pooled with the first eluant and samples taken for assays

of radioactivity and infectivity. The residual gel slice was dried on filter paper and put back in the dried vial and the residual radioactivity and hence the efficiency

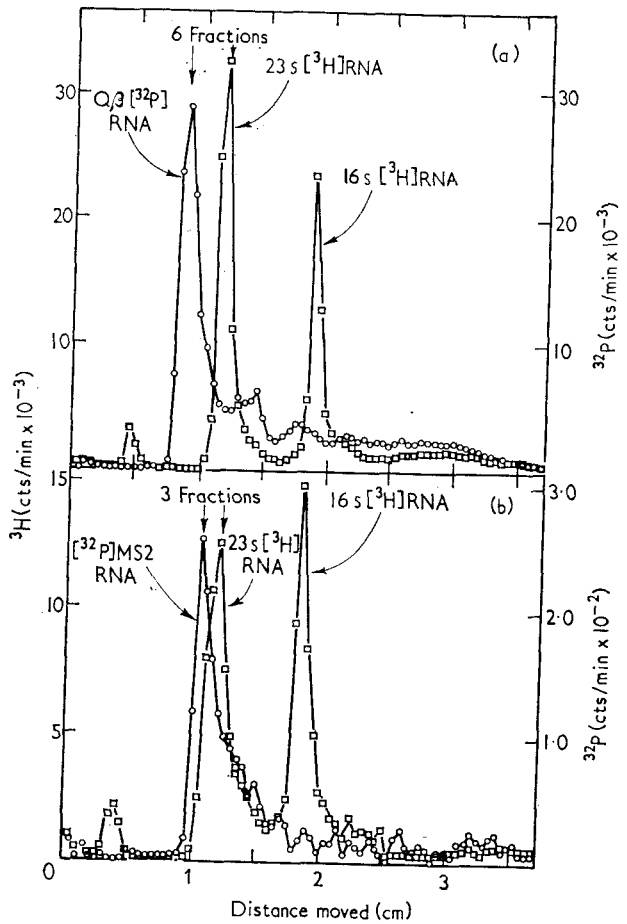

FIG. 4. Comparison of the electrophoresis of MS2 RNA, Qβ RNA and *E. coli* bulk RNA on ethylene diacrylate 3·0% gels. Mixtures of (a) Qβ[^{32}P]RNA ($2{\cdot}6\times10^5$ cts/min; 3 μg) and *E. coli* bulk [^{3}H]RNA ($2{\cdot}1\times10^5$ cts/min; 32 μg); or (b) MS2[^{32}P]RNA (2×10^3 cts/min; 0·06 μg) and *E. coli* bulk [^{3}H]RNA ($1{\cdot}6\times10^5$ cts/min; 25 μg) were run under standard conditions on ethylene diacrylate cross-linked 3·0% gels, cut, dissolved in alkali and counted in Kinard's fluid.

of extraction determined. It was found (Fig. 6) that 85 to 90% of the viral RNA was eluted by this procedure. Only the viral RNA in the main peak was infectious and its specific infectivity (plaque-forming units per μg) of $7{\cdot}4\times10^4$ compared favorably with the value of $6{\cdot}9\times10^4$ obtained for the original RNA.

(e) *Separation of fragments from intact Qβ RNA*

The Qβ RNA used in the experiment described above came from the 28 s region of a sucrose gradient. Nevertheless, non-infectious components possessing higher mobilities than the major peaks were evident.

The extent of this sort of contamination depends on the preparation and varies between 15 and 40%. To determine whether the smaller noninfectious component

pre-exists or is generated by the electrophoresis procedure, the following type of experiment was performed. Purified Qβ[^{32}P]RNA (10 μg) isolated from the 28 s peak tube of a sucrose gradient was layered on a gel and electrophoresed for 90

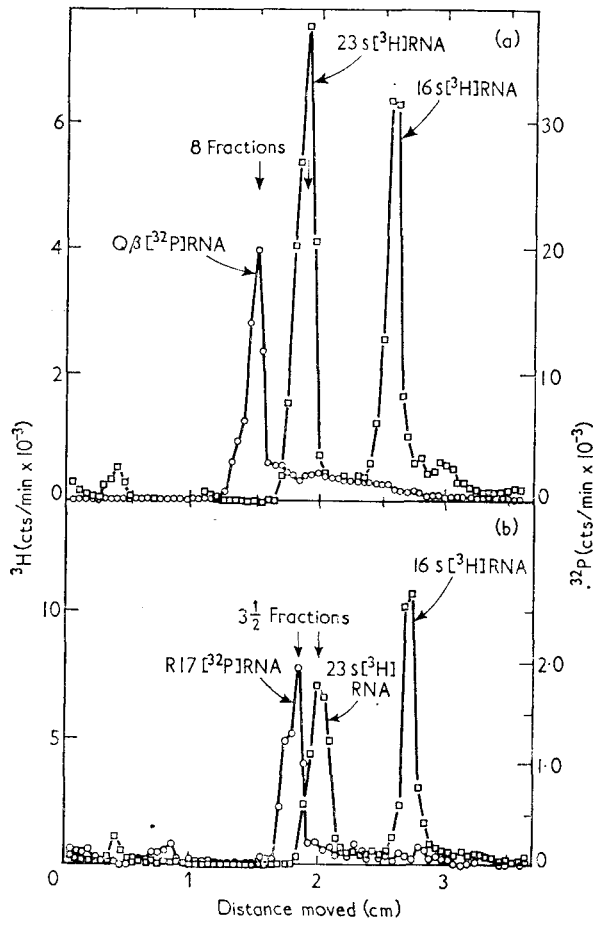

FIG. 5. Comparison of the electrophoresis of R17 RNA, Qβ RNA and *E. coli* bulk RNA on bis-acrylamide swollen gels. Mixtures of (a) Qβ[^{32}P]RNA ($1\cdot0\times10^5$ cts/min; 1·4 μg) and *E. coli* bulk [^{3}H]RNA ($3\cdot7\times10^5$ cts/min; 56 μg) or (b) R17 [^{32}P]RNA ($7\cdot5\times10^2$ cts/min; 0·01 μg) and *E. coli* bulk [^{3}H]RNA ($5\cdot3\times10^5$ cts/min; 80 μg) were run under standard conditions on bis-acrylamide cross-linked swollen 2·4% gels, cut, dried on filter paper and counted.

minutes. After slicing, one of the peak slices, as determined by a hand Geiger counter, was placed on top of a fresh gel and subjected to another 90-minute electrophoresis. Both gels were completely sliced and counted in order to determine the percentage of RNA in the peak fractions of each electrophoresis (after correcting for the transferred slice). It was found that 72% of the original RNA was present in the peak fractions of the first electrophoresis, whereas 95% of the RNA was present in the peak fractions of the second electrophoresis. It can be concluded that the electrophoresis *per se* does not substantially degrade the RNA.

It has been observed, by comparing different RNA species on polyacrylamide gels, that MS2 and R17 preparations contain less degraded material when originally

prepared (10 to 20% of the total preparation) than does Qβ. Furthermore, it is our experience that RNA prepared by the tri-isopropylnaphthalene sulfonate–phenol–*m*-cresol procedure is always less (about 10%) degraded than that prepared by the more usual sodium dodecyl sulfate–phenol method.

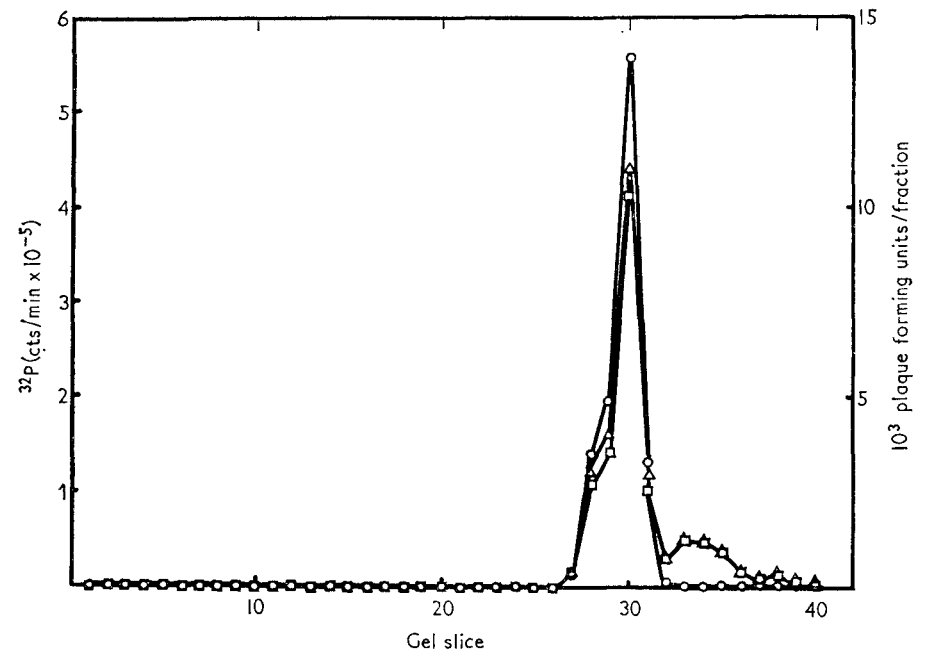

FIG. 6. Demonstration of biological activity after electrophoresis. Qβ[^{32}P]RNA ($1\cdot1\times10^6$ cts/min; 0·5 μg) was subjected to electrophoresis on a bis-acrylamide (swollen 2·4%) gel under standard conditions, sliced and each slice eluted twice with 0·5 ml. of 5×10^{-3} M-EDTA. The eluted acid-insoluble radioactivity was determined (□) and the residual acid-insoluble radioactivity in the gel slice to determine the original content of ^{32}P (△). The ability to infect spheroplasts to give plaque-forming units was determined in duplicate on portions of each eluant and the total plaque-forming units per eluant calculated (○).

(f) *Electrophoresis of plant viral nucleic acids*

A mixture of bacterial [^{3}H]RNA, Qβ virus [^{32}P]RNA and [^{14}C]satellite tobacco necrosis virus RNA was prepared and electrophoresed on 3·0% ethylene diacrylate gels. The peaks were identified by their *radioactivity* (Fig. 7(a)) and optical density (Fig. 7(b)). The satellite tobacco necrosis virus RNA was located 2·0 mm in front of the 16 s bacterial RNA after 90 minutes of electrophoresis.

When turnip yellow mosaic virus RNA was run on a bis-acrylamide 2·4% swollen gel with or without *E. coli* bulk RNA and scanned for ultraviolet absorption, it was found that the turnip yellow mosaic virus RNA ran substantially behind the 23 s *E. coli* ribosomal RNA (Fig. 8).

The bromegrass mosaic virus was a particularly interesting object of analysis since three RNA components possessing sedimentation coefficients of 14·2 s, 22·1 s and 26·6 s have been reported by Brockstahler & Kaesberg (1965). Electrophoretic separation also revealed three components. Further, their relative electrophoretic mobilities with respect to a marker (satellite tobacco necrosis virus RNA) agreed

quite well (see Table 1 and Fig. 11) with the average of the molecular weights estimated by these authors.

Finally, to illustrate the resolving power of the electrophoretic procedure, a mixture

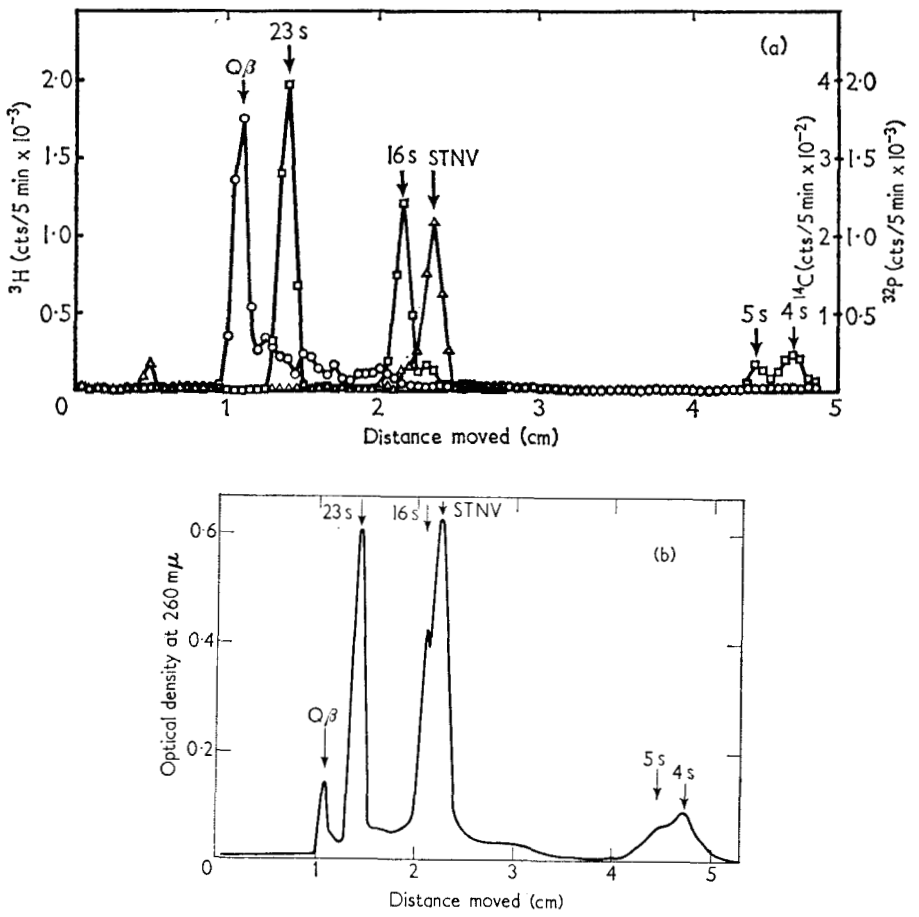

FIG. 7. Separation of STNV [^{14}C]RNA, $Q\beta$ [^{32}P]RNA and *E. coli* bulk [^{3}H]RNA. (—△—△—) STNV [^{14}C]RNA (6×10^2 cts/min; 12 μg) was mixed with (—○—○—) $Q\beta$ [^{32}P]RNA ($2{\cdot}5\times10^3$ cts/min; 1 μg) and (—□—□—) *E. coli* bulk [^{3}H]RNA (5×10^3 cts/min; 8 μg) and subjected to electrophoresis under standard conditions on an ethylene diacrylate cross-linked 3·0% gel. The gel was scanned in a 5-mm quartz trough for ultraviolet absorption using a 266-mμ interference filter. The absorption profile was calibrated using solutions of known absorption at 266 mμ and for convenience it is represented as the optical density equivalent of a 1-cm solution at 260 mμ (b). After scanning the gel was sliced, dissolved in alkali and counted in Kinard's solution to obtain the radioactivity profile (a).

containing five viral RNA species and bulk RNA of *E. coli* was electrophoresed on one gel with results as shown by the optical density tracing of Fig. 9.

(g) *Electrophoresis of ϕX174 DNA*

A mixture of 23 s, ^{32}P-labeled ϕX174 circular single-stranded and 21 s ^{3}H-labeled double-stranded circular form DNA was subjected to 180 minutes of electrophoresis on a bis-acrylamide swollen 2·4% gel. The separation is shown in Fig. 10.

TABLE 1

Properties of the nucleic acid species

Nucleic acid species		Base ratio (mole %) C	A	G	U(T)	Molecular weight	Sedimentation $S_{20,w}$	Salt	REM	References
ϕX174 – RF	DNA	21·8	28·2	21·8	28·2	Probably $3{\cdot}4\times10^6$	21·0	0·15 M-NaCl	0·4	a, b
ϕX174	DNA	18·5	24·6	24·1	32·8	$1{\cdot}7\times10^6$	23·8	0·2 M-NaCl	0·6	c
TYMV	RNA	38·1	22·6	17·2	22·1	$2{\cdot}3\times10^6$	28	0·1 M-Tris	0·8	d, e
							22	0·01 M-Tris		
TMV	RNA	20·0	28·0	24·0	28·0	$2{\cdot}0\times10^6$	28·2	0·15 M-NaCl	1·0	d, f, g
							22	0·01 M-Tris		
TNV	RNA	22·0	28·0	25·5	25·7				1·6	h
$Q\beta$	RNA	24·7	22·1	23·7	29·1	9×10^5	28·9	0·1 M-NaCl	1·6	i
MS2	RNA	24·5	22·4	27·5	25·6	$1{\cdot}05\times10^6$	25·8	0·1 M-NaCl	1·8	i, j
R17	RNA	24·9	23·1	26·3	25·7	$1{\cdot}04\times10^6$	26·0	0·1 M-NaCl		k, l
							23·1	0·05 M-NaCl	1·8	
							20·4	0·02 M-NaCl		
							16·5	0·01 M-NaCl		
BMV 1	RNA	19·4	28·2	27·0	25·4	$1{\cdot}07\times10^6$	26·6	0·1 M-KCl	2·0	m
23 s *E. coli*	RNA	21·5	25·4	33·5	19·6	$1{\cdot}1\times10^6$	23·5	0·1 M-NaCl	2·0	n, o
BMV 2	RNA	20·5	25·2	28·0	26·3	$7{\cdot}6\times10^5$	22·1	0·1 M-KCl	2·4	m
16 s *E. coli*	RNA	22·7	24·8	31·0	21·5	$5{\cdot}6\times10^5$	16·3	0·1 M-NaCl	2·75	n, o
STNV	RNA	22·1	28·0	25·0	24·9	$3{\cdot}9\times10^5$	13·5	0·1 M-NaCl	3·0	p
BMV 3	RNA	20·3	25·5	29·6	24·6	$3{\cdot}4\times10^5$	14·2	0·1 M-KCl	3·3	m
"5 s" *E. coli*	RNA	28·5	18·5	34·8	16·6	$3{\cdot}6\times10^4$	4·5	0·15 M-NaCl	~4·9	q
4 s *E coli* transfer	RNA	28·9	20·3	32·1	15·0	$2{\cdot}6\times10^4$	4·2	0·1 M-NaCl	~4·9	r, s

The base ratios, molecular weight as measured by light scattering (except for ϕX174 replicative form–ϕX174-RF), sedimentation coefficient at various salt concentrations were obtained from the references indicated. The relative electrophoretic mobility (REM) was obtained by running mixtures of nucleic acids on bis-*acrylamide cross-linked swollen* 2·4% *gels* and determining the position of the bands from the optical density scan (see Fig. 7) or the radioactive profile (see Materials and Methods). The REM was then calculated by reference to 23 s *E. coli* RNA. Data on the molecular weight and sedimentation coefficient of the RNA from the tobacco necrosis virus strain used are not available. (a) Chandler, Hayashi, Hayashi & Spiegelman, 1963; (b) Hayashi, Hayashi & Spiegelman, 1963b; (c) Sinsheimer, 1959; (d) Haselkorn, 1962; (e) Markham & Smith, 1951; (f) Cooper & Loring, 1954; (g) Boedtker, 1960; (h) Markham, 1953; (i) Overby, Barlow, Doi, Jacob & Spiegelman, 1966b; (j) Strauss & Sinsheimer, 1963; (k) Mitra, Enger & Kaesberg, 1963; (l) Gesteland & Boedtker, 1964; (m) Brockstahler & Kaesberg, 1965; (n) Kurland, 1960; (o) Midgley, 1962; (p) Reichmann, 1964; (q) Rosset, Monier & Julien, 1964; (r) Tissières, 1959; (s) Dunn, Smith & Spahr, 1960.

4. Discussion

The distance moved in a gel under standard conditions, the molecular weight of an RNA or DNA species, its base-ratio and $S_{20,w}$ as determined at various salt concentrations are summarized in Table 1. The logarithms of the molecular weights

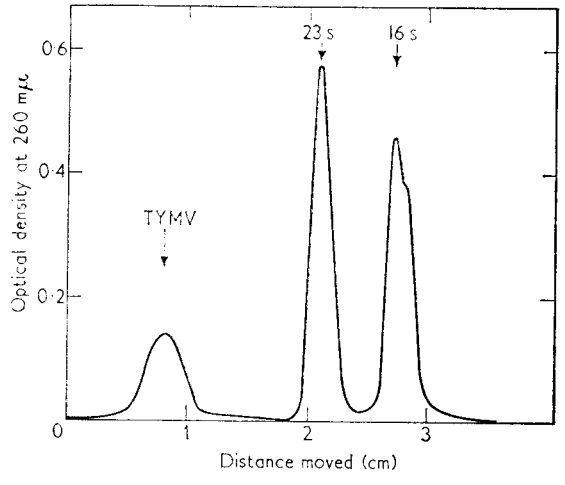

FIG. 8. Separation of TYMV RNA and *E. coli* bulk RNA. TYMV RNA (5 μg) with or without *E. coli* bulk RNA (10 μg) was subjected to electrophoresis under standard conditions on bis-acrylamide swollen 2·4% gels and scanned for ultraviolet absorption as described in Fig. 7. The profile of the mixed sample is given.

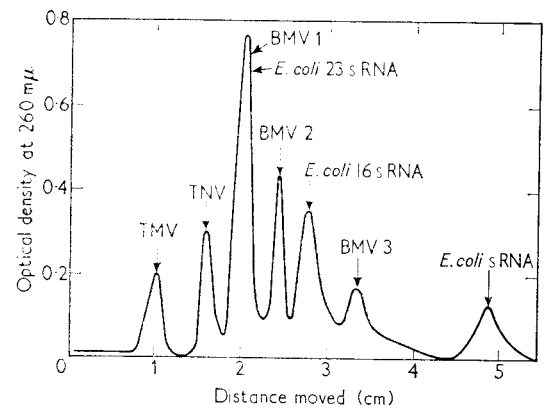

FIG. 9. Separation of TMV, TNV, BMV and *E. coli* bulk RNA. A mixture of TMV RNA (5 μg), TNV RNA (5 μg), BMV RNA (10 μg) and *E. coli* bulk RNA (10 μg) was subjected to electrophoresis on bis-acrylamide swollen 2·4% gels for 90 min and scanned for ultraviolet absorption as described in Fig. 7.

are plotted against the corresponding electrophoretic mobilities in Fig. 11. As with gel filtration of proteins (Andrews, 1965), the relative electrophoretic mobility appears to be linearly related to the logarithm of the molecular weight of an RNA species for high molecular weight RNA between 3×10^5 and $2{\cdot}3\times10^6$ for bis-acrylamide cross-linked swollen 2·4% gels. For unswollen gels, there may be a linear relationship down to $2{\cdot}6\times10^4$ molecular weight (see Fig. 3). The effects

on the relative electrophoretic mobility of base composition and secondary structure must await further data and the determination of the specific volumes of the RNA's *under the conditions used*. It is of interest to note that both

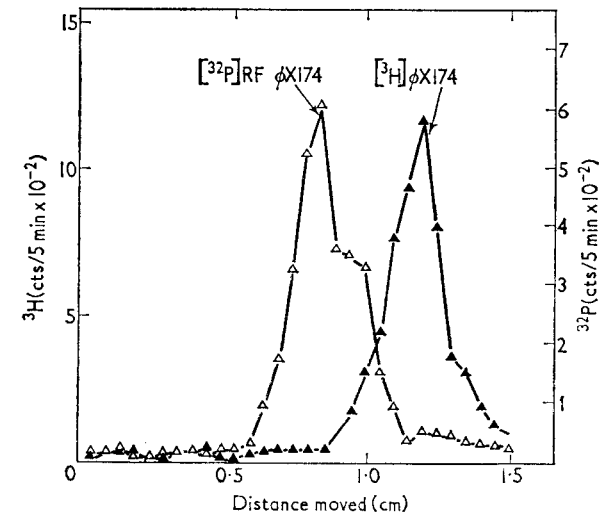

FIG. 10. Separation of φX174 single and double-stranded DNA. φX174 circular 23 s single-stranded [^{32}P]DNA (6×10^2 cts/min) was mixed with 21 s φX174 replicative form double-stranded, circular, [^{3}H]DNA ($2{\cdot}8\times10^3$ cts/min) and subjected to electrophoresis on bis-acrylamide swollen 2·4% gels for 180 min. The gels were sliced and counted.

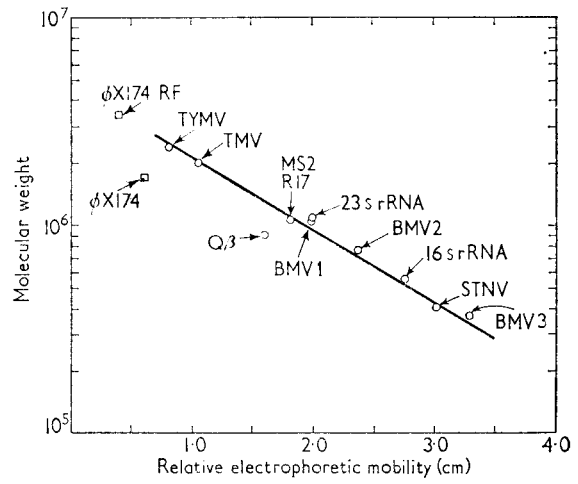

FIG. 11. Relationship between molecular weight and electrophoretic mobility. The relative electrophoretic mobility is plotted against molecular weight for all the nucleic acid species examined (see Table 1 for details).

E. coli ribosomal RNA species have a higher relative electrophoretic mobility than one would expect from their molecular weight. Although a determination of the molecular weight of tobacco necrosis virus RNA by light scattering is not available, if its behavior on gel electrophoresis is similar to that of the other plant viruses,

then its molecular weight is about $1{\cdot}3 \times 10^6$. Both the single-stranded form of ϕX174 and Qβ RNA do not appear to agree with the linear relationship found for the other virus nucleic acids. Finally, it must be emphasized that under the conditions used, the relative electrophoretic mobilities are highly reproducible, and can be very accurately determined by using mixed RNA samples labeled with different isotopes. This will be demonstrated in a subsequent communication concerning the relative electrophoretic mobility of various bacterial ribosomal RNA species. Further, the resolution obtainable is superior to the presently available methods which employ either sucrose gradient centrifugation or column chromatography.

It will be noted (Fig. 6) that electrophoresis separates smaller molecular weight material from the 28 s infectious RNA. This, together with the fact that re-electrophoresis of intact infectious Qβ RNA does not generate further degradation products, is of practical significance. It suggests that electrophoretic separation can be used to supplement the usual sucrose gradient procedures to remove hydrogen-bonded fragments which may remain with the large components as suggested by Spirin (1962) and Haselkorn (1962). Moreover, it is our experience that Qβ RNA purified by gel electrophoresis not only is more infectious with spheroplasts, but also has a more efficient priming activity with the Qβ replicase (Haruna & Spiegelman, 1965).

Of great interest are the different mobilities of the replicative form DNA and single-stranded DNA of ϕX174 (Fig. 11). This suggests that gel electrophoresis can be used to identify and fractionate the Hofschneider structure observed with bacterial RNA viruses both *in vivo* (Hofschneider, 1963) and *in vitro* (Mills, Pace & Spiegelman, 1966). Experiments along these lines have been performed and will be detailed elsewhere. They indicate that both the Hofschneider structure and the more recently reported complex of Franklin (1966) are readily identified. The mobilities of the two complexes are sufficiently different from each other and from that of infectious 28 s viral RNA to permit satisfactory separation on the gel.

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