Recovery of the Nucleic Acids of Tobacco Mosaic and Potato X Viruses from Polyacrylamide Gel and Evidence for a Single Infectious Component in Each of the Two Viruses

Satyabrata Sarkar

Max-Planck-Institut für Biologie, Abteilung Melchers, Tübingen

(Z. Naturforsch. **28 c**, 618–625 [1973] ; received May 25, 1973)

RNA, tobacco mosaic virus, potato X virus, gel-electrophoresis, infectivity

A simple device is standardised for the elution of biologically active ribonucleic acid from polyacrylamide gel after electrophoresis. The pieces of gel containing the nucleic acid to be recovered are held in position by two short cylinders of foam rubber in a glass tube and the nucleic acid is concentrated over a sucrose-containing buffer layer by electrophoresis in a standard electrophoretic equipment. Using this method nucleic acids can be recovered undamaged almost quantitatively, as shown for microgram quantities of two viral RNAs by infectivity assay. The results offer additional experimental support to the single-component character of tobacco mosaic and potato X viruses.

Introduction

Advances in experimental methodology have revealed the multicomponent character of several viruses, where the viral genome occurs as more than one physically distinct units. Properties and behaviour of such viruses *in vivo* and *in vitro* have been reviewed by several workers [1–3]. The method of establishing the multicomponent nature of a virus consists basically of three steps: Separation of the components from one another, determination of the infectivity of each component on a suitable sensitive host and demonstration of a highly significant increase in specific infectivity by use of a proper mixture of two or more components. The opposite case, that a virus occurs as a single component in nature, can be proved only to the nearest approximation. The physical homogeneity attained during the purification of a virus is a matter of degree and is dependent upon the methods adopted.

The efficiency of plating in plant virus systems [4–6] being of the order of one in 50 000, a direct experimental proof of an one-component nature is difficult, in contrast to many bacteriophages, where a ratio of bacterium : bacteriophage close to 1 : 1 can be attained. The one component nature of tobacco mosaic virus, however, was identified by Zimmer [7], who demonstrated that the inactivation of TMV by ultraviolet light follows a one-hit curve. The results of many later workers, *e. g.* Mundry and Gierer [8], and the infectivity-dilution curves are in agreement with the one-component concept of TMV. However, it is also noteworthy, that 1. purified preparations of TMV always contain particles of different lengths in varying proportions, 2. particles shorter than 300 nm occur *in vivo* [9], 3. the infectivity of long particles can be increased by mixing with shorter ones [10, 11] and 4. the short particles observed both *in vivo* and during reconstitution *in vitro* appear to contain the 5′ OH end of the TMV-RNA [12, 13], indicating that the particles do not necessarily represent only a heterogeneous mixture of broken virions of TMV. It seems, therefore, desirable to check the one-component nature of TMV by an alternative method, if available. A method of quantitative recovery of viral RNA in a biologically active state developed now has been applied successfully to lend additional support to the single component concept. The argumentation can be formulated thus: If the infectivity of one highly uniform component remains unaltered after removal of all other associated components, the possibility that such a virus has a single component character would be very high. From this viewpoint the two elongated viruses, tobacco mosaic and potato X, were examined experimentally. Instead of attempting to purify virus particles of uniform length a purification of the total nucleic acids was undertaken. Electrophoresis in polyacrylamide gel is a

Requests for reprints should be sent to Satyabrata Sarkar, Max-Planck-Institut für Biologie, Abteilung Melchers, *D-7400 Tübingen*, Corrensstr. 41/42.

Abbreviations: TMV, tobacco mosaic virus; PVX, potato virus X; SDS, sodium dodecyl sulphate; Na_2EDTA, disodium salt of ethylene-diaminetetraacetic acid; TP, trishydroxymethyl aminomethane + phosphate buffer.

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

very efficient method of separating and measuring different kinds of nucleic acids [14–17]. However, available methods for recovery of high molecular weight nucleic acids quantitatively out of the gel in an undamaged state are not very satisfactory. Drastic methods for quantitative recovery, often adopted for measurement of radioactivity in labelled nucleic acids, do not preserve biological activity. Elution by electrophoresis, or electroelution [17], is probably the most gentle procedure and several experimental devices are now known, although many of them require special constructions [18–21].

A direct accumulation of eluted macromolecules in a dialysis bag is not always satisfactory, as has been discussed by other workers [17]. The procedure developed here is principally based on the method of Hjertén [22], in that the nucleic acid is collected over a sucrose solution, but it has the merit of being very simple and it can be adopted practically with the basic equipments required for routine electrophoresis.

Using this innovation, the high molecular weight RNAs of TMV and PVX were recovered from a uniform band in polyacrylamide gel after electrophoresis and the infectivity was compared with that of an aliquot of the corresponding total RNA without a prior electrophoretic purification. The experimental methods and the results are presented here, not only because they lend further support to the concept of a single-component character of the two RNA-containing viruses, but also for the probability of a useful application of the elution-technique in other investigations involving electrophoresis in supporting media. No indication of a possible heterogeneity of the infectious RNA(s) was found by measuring the biological activity of eluates from different regions within the RNA bands.

Material and Methods

Virus and test plants

The common strain *vulgare* and the *dahlemense* strain of TMV were multiplied in samsun tobacco (*Nicotiana tabacum* L. var samsun [23]) and PVX was multiplied on Xanthi tobacco (*N. tabacum* L. var Xanthi nc. [24]) in the greenhouse. Xanthi tobacco was chosen for PVX-multiplication, as there is hardly any risk of contamination with TMV, the latter producing only localised necrotic lesions on it. Three severe strains of PVX were available; one brought by Dr. Maria Kamienska-Zyła of the Virus Laboratory in Krakau, Poland, and two others were kindly supplied by Professor F. Nienhaus of the Institut für Pflanzenkrankheiten of the University of Bonn, Germany.

Virus extraction

TMV was purified from clarified sap by heat treatment and differential centrifugation as described earlier [25]. PVX was purified in the following way: About 80 g of systematically infected leaves of Xanthi tobacco were homogenised in a Waring blender with 240 ml cold distilled water containing 0.005 M sodium dithionite (E. Merck, Germany) and 0.01 M 2-mercaptoethanol and brought to pH 8 with 0.5 N NaOH. The homogenate was pressed through cheese cloth and the sap clarified by centrifugation at about $1500 \times g$ for 30 min, shaken with 1/4 vol. of a 1 : 1 mixture of chloroform and *n*-butanol [26] for 10 min. After 2 hours in an ice bath the phases were separated by centrifugation at $1500 \times g$ as above. The upper phase was centrifuged in rotor no. 30 of a Spinco preparative ultracentrifuge at about $35\,000 \times g$ for 2 hours, the sediment resuspended in 20 ml 0.01 M phosphate buffer (Sörensen) pH 7.0. After standing in ice overnight the preparation was centrifuged for 20 min at about $6000 \times g$ in a cooled Sorvall RC2B centrifuge and the virus recovered from the supernatant by centrifugation at about $35\,000 \times g$ as before. The virus was further purified by freezing and thawing and another cycle of low and high speed centrifugation. The purified virus had a ratio of extinction at 260 nm : 280 nm of 1.2 : 1 and the concentration was calculated [27] by taking an optical density of 1.0 at 260 nm as equivalent to 0.29 mg PVX per ml at pH 7.0. With TMV an OD of 1.0 corresponds to a concentration of 0.37 mg virus per ml.

Preparation of RNA

RNA was extracted from purified virus by a modified phenol method. 10 ml of virus suspension in 0.02 M phosphate buffer pH 7.3 was mixed with 100 mg each of bentonite, sodium salt of tri-isopropylnaphthalene-sulfonic acid and disodium salt of 1,5-naphthalene-disulfonic acid [28], 1 ml of 0.1 M disodium-EDTA, 0.5 ml of a 20% w/v solution of SDS and 500 mg sucrose (analytical grade). The mixture was shaken in a glass-stoppered cylinder for 10 min at room temperature with 10 ml of phenol reagent. The reagent [29] was prepared by mixing 480 ml of distilled phenol and 70 ml of distilled *m*-kresol with 200 ml of distilled water and dis-

solving 0.5 g 8-hydroxyquinoline in it. The phases were separated by centrifugation at about $1000 \times g$ for 20 min in SS-34 rotor of a Sorvall RC2-B centrifuge. The upper phase was taken out, shaken with 10 ml phenol reagent and centrifuged as above. The upper phase was shaken with 5 ml phenol reagent for a third time and the phases separated by centrifugation. Sodium chloride was added to the upper phase to give a final concentration of 0.3 M and then the nucleic acid was precipitated by the addition of two volumes of cold ethanol. After standing overnight at -10 °C the RNA was sedimented by centrifugation, washed with ethanol, taken up in 0.15 M sodium acetate pH 6.0 containing 0.5% SDS and dialysed against 1 liter of this solution for 12 to 24 hours at room temperature [30]. The RNA was recovered from the dialysate by precipitation with 2 vols. of ethanol (95 – 100% v/v), dried in vacuum and dissolved in a small volume of trisphosphate buffer [31] pH 7.7 containing 0.5% SDS and 5.0% sucrose. Concentrations were estimated by assuming that both TMV- and PVX-RNA at 10 μg per ml have an OD of 0.24 at 260 nm.

Gel electrophoresis

Gels were prepared from recrystallised acrylamide and bis-acrylamide, according to the method of Loening [31] in 12 cm long Plexiglas tubes of 6 mm internal diameter and run in trisphosphate buffer [31] of pH 7.7 containing 0.2% SDS (= E buffer). The RNA samples (usually 5 – 20 μg) were applied to the top of the gels after a prerun of 20 – 30 min and electrophoresed for 3 – 4 hours at a current strength of 5 mA per tube (about 60 – 70 V) at room temperature. The gels were scanned in a Chromoscan (Joyce Loebl Co., Gateshead, U.K.) at 265 nm and the nucleic acid peak was marked with India ink. Gels were then taken out, frozen in solid carbon dioxide and preserved at minus 10 °C.

Elution of RNA

Gels were usually cut into slices of 0.8 mm thickness and only the slices between the India ink marks were transferred directly to the elution tubes.

Fig. 1 illustrates the arrangement that was standardised after several preliminary experiments. The glass tube was closed at the bottom with a dialysis membrane held in place by one or two tightly fitting rubber rings. The bottom was filled with 1 ml of buffer S_{50} (0.1 M tris-phosphate buffer [31], pH 7.7 containing 50% sucrose w/v). Half a ml of buffer S_{10} (as above, but with 10% sucrose) was then carefully layered over the S_{50} buffer with the help of a Pasteur-pipette. The rest of the tube was carefully filled up with electrophoresis buffer E containing 5% sucrose (= E + S_5). It has been observed that the S_{10} and S_{50} buffers can be prepared without SDS without an appreciable loss of infectious RNA. A small cylinder (diameter about 7 mm)

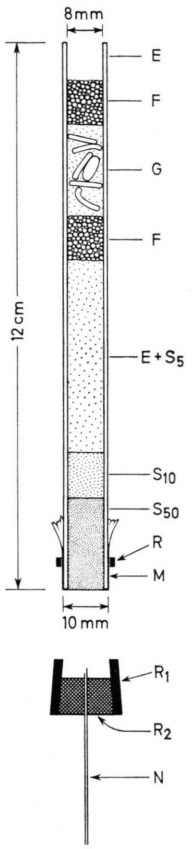

Fig. 1. Sectional view of arrangement for elution of nucleic acid from polyacrylamide-gel by electrophoresis. The glass tube is closed by a dialysis membrane (M), held tightly by a rubber ring (R). E = buffer for electrophoresis, F = foam rubber, G = gel slices. E + S_5, S_{10} and S_{50} are buffer solutions containing 5, 10 and 50% w/v sucrose respectively. The inverted rubber stopper (R_2) fitted with a rubber tube (R_1) and an injection needle (N) is pushed up to puncture the dialysis membrane (M) after electrophoresis to facilitate a dropwise collection of the contents of the elution tube.

of soft foam rubber cut out with a cork borer was soaked in buffer E + S_5 and pushed in position by means of a fine forceps and a mounted needle, so as to remain at a heigh of about 7 mm from the bottom of the tube. A part of the buffer solution in the upper region of the tube was removed and the slices of gel from which RNA was to be eluted were introduced from the top. A second cylinder of foam rubber was pushed in in the same way and the top was filled up with E buffer. To prevent a too rapid osmotic movement of water and salts through the dialysis membrane during electroelution, the lower ends of the elution tubes were allowed to dip into S_{50} buffer contained in the lower electrophoresis vessel. However, it was economical to use a relatively small volume of S_{50} buffer at the bottom of the lower electrophoresis vessel and to add carefully E-buffer (without sucrose) to form an upper layer.

Electrophoresis was routinely done at 8 mA per tube for about 5 hours at room temperature. After the run, the upper electrophoresis vessel was lifted, the major part of the E buffer in this vessel was removed by suction, the lower projecting parts of the tubes were gently washed with a jet of water and carefully wiped with absorbent tissue to remove excess water. Samples were collected dropwise from the bottom by puncturing or by pushing up a needle (No. 1) fitted in a rubber stopper as shown in Fig. 1. The contents of each tube were collected in 14 to 15 fractions of approximately equal volume. Most of the RNA accumulated in the S_{10} zone.

Infectivity assay

The SDS present in the eluates was found to interfere adversely with the biological assay procedure. It was therefore necessary to remove the SDS by precipitating the RNA with ethanol. Since only micrograms of RNA were present in the fractions, they were co-precipitated with added yeast RNA. Yeast RNA (Serva Entwicklungslabor, Heidelberg) was dissolved in $E+S_5$ buffer and diluted to a concentration of 1 mg RNA/ml. To each eluate of approximately 0.4 ml was added 0.1 ml of yeast RNA solution ($=100\,\mu g$ yeast RNA) and 1 ml of 95% ethanol, shaken well and kept at 4 °C for 10 to 15 hours. The precipitate was collected by centrifugation at about $1500 \times g$, suspended in 1 or 2 ml ethanol and after a couple of hours again collected by centrifugation. The tubes were partially dried by a gentle stream of nitrogen or air and then dried further by keeping the samples in a vacuum desiccator over dried silica gel for several hours, preferably in the cold. The sediment in each tube was then dissolved in 2 ml of 0.1 M tris-phosphate buffer [32] of pH 8.8 and tested for infectivity. Xanthi tobacco and *Chenopodium urbicum* were used as local lesion hosts for TMV- and PVX-RNA respectively. For the dahlemense strain of TMV the tobacco mutant "samsun EN" [33] was used. Inoculation was done in the usual way with a glass spatula [32]. The TMV lesions were counted 5 to 7 days after inoculation and the PVX lesions could be counted generally 8 days after inoculation.

Results

Direct sampling of yeast-RNA

In a preliminary experiment 1 mg of yeast RNA in about 30 μl $E+S_5$ buffer was added with a micropipette in the zone normally occupied by the RNA-containing gel slices in the elution tube as shown in Fig. 1. After electrophoresis 14 fractions were collected from the bottom, each fraction containing 30 small drops. The fractions were diluted with 3 ml each of TP-buffer [32] (pH 8.8). Measurement of optical density of the samples showed that more than 85% of the RNA was recovered in the fractions 3,4 and 5 (Fig. 2).

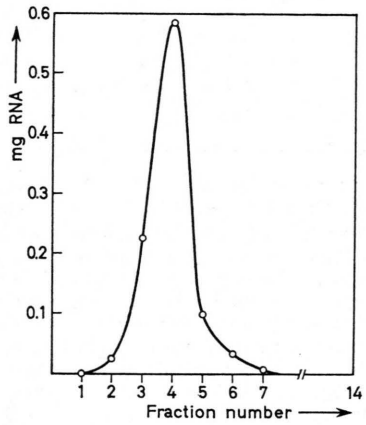

Fig. 2. Distribution of yeast RNA in the fractions collected after electrophoresis using the arrangement shown in Fig. 1. Yeast RNA (1 mg, in 0.1 ml $E+S_5$ buffer) was added with a micropipette directly to the zone where the gel slices (G) are shown in Fig. 1. After electrophoresis most of the RNA had accumulated in the lower part of the elution tube and the fractions 3, 4 and 5 collected from the bottom contained more than 85% of the input RNA.

Elution of TMV-RNA

In a typical experiment 8.8 μg TMV-RNA (dahlemense strain $E_{260\,nm}/E_{280\,nm}=2.16$) in 20 μl buffer was added to the top of 2.2% polyacrylamide gel and after a 3.5 hour electrophoresis only the uniform RNA band with a symmetrical scanning pattern in the Chromoscan at about 25 mm from the top of the gel was cut out in slices, as described under Material and Methods. No clear second RNA peak was detectable by scanning at 265 nm, although the possible presence of small amounts of other RNA species (presumably some aggregates and accompanying low molecular weight RNA species) was indicated by the uneven baseline. The gel slices were put between the foam rubber cylinders for elution of the RNA as shown in Fig. 1. Of the 15 fractions collected from the bottom, the fractions 3 to 6 contained most of the infectious material (Table I). To calculate the percentage of recovery of infectious

Table I. Infectivity of TMV-RNA eluates on Xanthi tobacco.

Fraction No.	Lesion number per leaf ± SE [a]	RNA conc. in inoculum [b]	Quantity of RNA in μg [c]
1	0	0	0
2	0	0	0
3	27.6± 7.7	0.21	0.42
4	202.5±33.8 [d]	1.56	3.12
5	163.8±21.8 [e]	1.26	2.52
6	46.4± 3.9	0.36	0.72
7	9.0± 1.7	0.07	0.14
8	6.1± 1.3	0.05	0.10
9	<2	—	—
10–15	<1	—	—

[a] Average of 8 test leaves each. SE = Standard error of the mean. [b] Calculated on the basis of infectivity, since the control sample containing 0.2 μg TMV-RNA/ml produced on the average 25.8 lesions per leaf. [c] The volume of inoculum being 2 ml in each case, the amount of RNA in μg is numerically double the concentration in the previous column. [d, e] The values 202.5 and 163.8 are derived from 20.25 and 16.38 lesions respectively obtained with a 1:10 dilution of the inocula. The corresponding undiluted inocula produced 156.5± 17.6 and 130.8±10.9 lesions respectively. Lesion numbers below 100 were preferred for calculations, as explained in the text.

RNA in the fractions the infectivity was compared with control samples of the same TMV-RNA that were not run in gels. 25 μl of TMV-RNA solution (= 11 μg RNA) was taken in a centrifuge tube, diluted with 1 ml of $E+S_5$ buffer, mixed with 100 μg yeast RNA and precipitated with 2 volumes of ethanol. Washing and drying was performed as usual and the final sediment was taken up in 5.5 ml tris-phosphate buffer (pH 8.8). Assuming a quantitative precipitation of TMV-RNA, this solution would have a maximum concentration of 2 μg TMV-RNA per ml. It was diluted further to 10^{-1} and 10^{-2} and tested on Xanthi tobacco leaves. The average number of lesions produced by the three solutions and the standard errors of the means were 159.4±20.7, 25.8±4.7 and 3.5±0.6 respectively. The numbers are not strictly proportional to the dilution factor but it is known, that lesion numbers below 10 and above 100 per test leaf are not always proportional to the concentration of inocula [32, 34]. For calculation of the concentration of RNA in the eluate fractions, therefore, the value 25.8 lesions for 0.2 μg RNA/ml was taken as standard. Since each eluate was taken up finally in a volume of 2.0 ml TP-buffer before bio-assay, the concentration of RNA in the fractions could be calculated from the average number of lesions as shown in Table I.

From Table I it can be seen, that the fractions 3, 4, 5 and 6 together can account for 6.78 μg RNA, this being 72% of the 8.8 μg used for gel electrophoresis. In other experiments, sometimes under slightly different conditions, the recovery was between 30 and 70%.

Infectivity distribution of PVX-RNA in eluates

Between 10 and 50 μg samples of PVX-RNA were run in gel and scanned as described for TMV-RNA. The RNA containing zone was cut into slices and elution was carried out as before. The distribution of infectivity is shown in Fig. 3. It is obvious, that

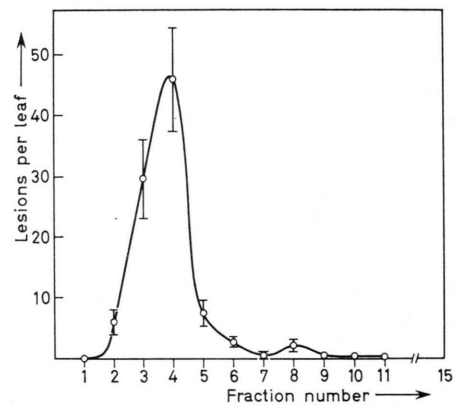

Fig. 3. Distribution of infectivity in the fractions collected after elution of PVX-RNA from polyacrylamide gel using the arrangement shown in Fig. 1. Lesion numbers are the averages of 8 test leaves (*Chenopodium urbicum*) each. The standard errors of the means are indicated. Most of the infectious material was recovered in the fractions 3 and 4.

most of the infectious material is contained in the fractions 3 and 4. For comparison, control samples of PVX-RNA were precipitated without passing through gel and tested on the local lesion host, *Chenopodium urbicum*. On the basis of infectivity, 37 to 85% of the PVX-RNA originally added to the top of the gel for electrophoresis in different trials could be accounted for in the fractions 3 to 6.

Evidence for homogeneity of the viral RNAs

Although the symmetrical scanning patterns of the viral RNAs speak for a homogeneous RNA species, the possible existence of two RNA components with almost equal electrophoretic mobilities deserves consideration. In other words, the resolving power of the electrophoretic method might not have suf-

ficed to separate two components with molecular weights of the order of, say, $1.9 \cdot 10^6$ and $2.1 \cdot 10^6$ daltons. With the RNAs of Bromegrass mosaic virus Lane [35] found, that the apparently uniform peak of the high molecular weight BMV-RNA (Bockstahler and Kaesberg [36, 37]) was really composed of two RNA species of mol. wts $1.09 \cdot 10^6$ and $0.99 \cdot 10^6$ daltons. If the bands of TMV- and PVX-RNAs each has two components, only one of them being infectious, the distribution of infectivity within the band would hardly be expected to coincide with the optical density pattern. Moreover, if the infectivity were dependent upon the presence of two RNA components of different molecular weights, a higher infectivity could be expected after mixing RNA-fractions from the faster and the slower ends of the RNA band. To test this possibility, the gel slices cut in succession from the band were grouped into 5 or 6 fractions as shown in Fig. 4. The eluate of

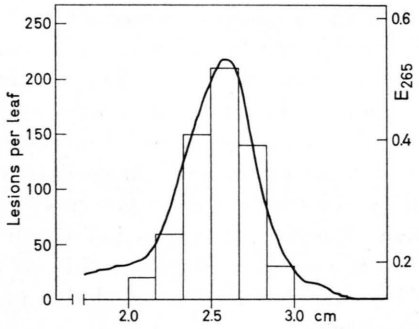

Fig. 4. Optical density pattern and distribution of biological activity of TMV-RNA after electrophoresis in 2.2% polyacrylamide gel. Abscissa: Distance in cm from the top of gel after electrophoresis for 3 hours. Ordinates: E_{265} values (right) relating to the drawn out curve and lesions per Xanthi leaf (left) of the six fractions cut out from gel. *Cf.* also Table II.

each was tested for infectivity after precipitating the viral RNA with added yeast RNA as described before and dissolving each in an equal volume (generally 4.0 ml) of tris-phosphate buffer of pH 8.8. Fig. 4 indicates a close relationship between the optical density and biological activity. Infectivity of some of the combinations of the fractions is shown in Table II. A mixture of equal volumes of fractions 2 and 4, for example, produced exactly the expected number of lesions, namely $1/2\ (L_2 + L_4)$, where L_2 and L_4 indicate the average number of lesions produced by the eluates from regions 2 and 4 of the RNA band. If one end of the band contained a qualitatively different kind of RNA with a

Table II. Distribution of infectivity within a TMV-RNA band after electrophoresis.

RNA-Fractions and some 1:1 mixtures	Average number of lesions ±SE *	
	Experimentally found	Calculated
1	20.8± 3.5	—
2	61.4±10.7	—
3	150.5±23.7	—
4	211.4±27.8	—
5	139.9±21.6	—
6	30.4± 6.8	—
1+6	23.8± 3.9	25.6
1+5	86.3±10.0	80.3
2+5	135.1±16.7	100.7
2+4	137.5±14.4	136.4
3+4	155.0±19.4	180.9
3+6	72.3±10.2	90.4

* Infectivity of six serial sections from the band of TMV-RNA in polyacrylamide-gel. None of the 1:1 combinations produced significantly more lesions than calculated. Details in text. The lesion numbers refer to those produced per whole leaf of Xanthi tobacco and are averages of 8 leaves in each case.

different molecular weight, and they were capable of mutually enhancing the infectivity, in a way similar to that known for multicomponent systems, the lesion number would have been higher than that expressed by the above formulation. This was tested for both TMV- and PVX-RNA and in no case a significant increase of infectivity was obtained by combining the RNA fractions from the two ends of the band.

Discussion

The elution method described here is essentially similar to that of Hjertén [23], in that the nucleic acid is electrophoresed to accumulate over a sucrose-containing buffer layer. The advantages of the present set up consist in the simplicity of the apparatus and the use of foam rubber to hold the gel slices easily in position. The good preservation of biological activity and the high percentage of recovery of nucleic acids indicate that the method could be widely used to suit specific requirements. A direct quantitative comparison with the data of Hjertén is not possible, as nucleic acids were not included in his experiments for biological assay.

The addition of yeast RNA for co-precipitation of viral RNA with ethanol does not affect the bio-assay of the two plant viruses tested here, but this practice may not be universally applicable. No attempt was

made to remove sucrose from the RNA samples recovered by precipitation with ethanol. An increased infectivity of viral RNA due to the presence of sucrose under certain conditions was reported [38], although this effect was hardly significant in the presence of phosphate [39]. Moreover, the control samples of TMV- and PVX-RNA in the present study were also mixed with a sucrose-containing buffer before treatment with ethanol. One disadvantage of electroelution from acrylamide gel lies in the accumulation of some contaminants along with the nucleic acids over the sucrose cushion, observed by many other workers. Whatever may be the nature of these contaminants, arising probably from incompletely polymerised acrylamide, they do not significantly affect the infectivity of the viral RNAs tested. However, it was observed that, if a large number of gel slices are stuffed in the elution tube, *e. g.*, when the gels were overloaded with a large amount of RNA, both the elution pattern and the percentage of recovery of infectious RNA are poor. A reestimation of the degree of recovery of the microgram quantities of viral RNAs by measuring their ultraviolet absorption was not possible due to the presence of such contaminants and also due to the proportionately much higher UV-absorption of the added yeast RNA. The foam rubber pieces get clogged with use; so that they have to be thoroughly cleaned and boiled with distilled water between experiments and preferably replaced by new ones from time to time.

The fact that often 70% or more of the infectivity could be recovered from the main peak alone (of high molecular weight RNA of TMV and PVX) fits the hypothesis that even if other nucleic acid components are present in the total RNA preparation they do not contribute significantly to the biological activity. Total unfractioned nucleic acid preparations from virus infected hosts are known to contain several kinds of virus-specific nucleic acids, some of which are present in very low concentrations and are detectable by labelling with radioactive precursors [40]. Some of these species of nucleic acids are intermediates in the replication process, others may have some specific function, but only when at least two of them are known to be encapsidated in the virions and mutually enhance the infectivity, they can be considered as constituting integral parts of a multicomponent system. In RNA preparations from purified virions of TMV small amount of a component with an electrophoretic mobility slower than that of the main RNA has been observed [41, 42]. It might be an artifact, since it is generally absent in most of our samples (*cf.* also ref. 43). The experimental data do not also support a possible existence of two RNA species (of TMV and PVX) with closely similar molecular weights. No evidence was obtained in favour of the observations of Fowlks and Young [44], who found two almost equally large separate TMV-RNA bands after electrophoresis.

In multicomponent type viruses the increase in infectivity by combining the proper components is of the order of ten times or more. If, therefore, TMV and PVX had at least two distinct components each, necessary for biological activity, the infectivity of the single RNA component isolated after gel electrophoresis could hardly be expected to be more than 10% of the unfractionated RNA. Although the present results at the nucleic acid level support the one-component hypothesis, the enhancement of infectivity by shorter nucleoproteid particles of TMV reported by other workers [10, 11] deserves further investigation. The present results indicate that it is possible to recover microgram quantities of biologically active viral RNAs using the elution procedure standardised here.

My sincere thanks are due to Professor G. Melchers, Direktor am Max-Planck-Institut für Biologie, for unrestricted experimental facilities and valuable discussions. Part of the work was done in co-operation with Dr. Maria Kamienska-Zyła of the Virus Laboratory, Polish Academy of Sciences, Krakau, during her short stay in our laboratory. I am also very thankful to Professor F. Nienhaus of the University of Bonn and to Professor C. Wetter of the University of Saarbrücken for the supply of inocula of potato virus X and seeds of *Chenopodium* species. The able technical assistance of Mrs. Renate Kaus, Mrs. Renate Binder and Miss Brigitte Fessler is also gratefully acknowledged. Thanks are also due to Mr. E. Freiberg for drawing the figures.

[1] J. B. Bancroft, The Molecular Biology of Viruses, Symp. Soc. Gen. Microbiol., L. V. Crawford and M. G. P. Stocker eds., pp. 229–247, Cambridge Univ. Press, London 1968.
[2] R. M. Lister, Proc. Fed. Amer. Soc. exp. Biol. **28**, 1875 [1969].
[3] A. van Kammen, Annu. Rev. Phytopath. **10**, 125 [1972].
[4] R. L. Steere, Phytopathology **45**, 196 [1955].
[5] H. Fraenkel-Conrat, S. Veldee, and J. Woo, Virology **22**, 432 [1964].

[6] I. Takebe and Y. Otsuki, Proc. nat. Acad. Sci. USA **64**, 843 [1969].
[7] K. G. Zimmer, Biol. Zbl. **63**, 142 [1943].
[8] K. W. Mundry and A. Gierer, Z. Vererbungslehre **89**, 614 [1958].
[9] J. Symington, B. Commoner, and M. Yamada, Proc. nat. Acad. Sci. USA **48**, 1675 [1962].
[10] H. R. Hulett and H. S. Loring, Virology **25**, 418 [1965].
[11] R. I. B. Francki, Virology **30**, 388 [1966].
[12] J. C. Thouvenel, H. Guilley, C. Stussi, and L. Hirth, FEBS-Letters **16**, 204 [1971].
[13] H. Guilley, C. Stussi, J. C. Thouvenel, P. Pfeiffer, and L. Hirth, Virology **49**, 475 [1972].
[14] E. G. Richards, J. A. Coll, and W. B. Gratzer, Analyt. Biochem. [New York] **12**, 452 [1965].
[15] U. E. Loening, Biochem. J. **102**, 251 [1967].
[16] D. H. L. Bishop, J. R. Claybrook, and S. Spiegelman, J. molecular Biol. **26**, 373 [1967].
[17] S. Hjertén, S. Jerstedt, and A. Tiselius, Analyt. Biochem. [New York] **27**, 108 [1969].
[18] M. Popescu, L. H. Lazarus, and N. Goldblum, Analyt. Biochem. [New York] **40**, 247 [1971].
[19] U. J. Lewis and M. O. Clark, Analyt. Biochem. [New York] **6**, 303 [1963].
[20] M. B. Mann and P. C. Huang, Analyt. Biochem. [New York] **32**, 138 [1969].
[21] D. K. Ray, R. M. Troisi, and H. P. Rappaport, Analyt. Biochem. [New York] **32**, 322 [1969].
[22] S. Hjertén, Biochim. biophysica Acta [Amsterdam] **237**, 395 [1971].
[23] G. Melchers, G. Schramm, H. Trurnit, and H. Friedrich-Freksa, Biol. Zentralbl. **60**, 524 [1940].
[24] W. N. Takahashi, Phytophathology **46**, 654 [1956].
[25] S. Sarkar, Z. Naturforsch. **15 b**, 778 [1960].
[26] R. L. Steere, Phytophathology **46**, 60 [1956].
[27] F. C. Bawden and A. Kleczkowski, Virology **7**, 375 [1959].
[28] J. H. Parish and K. S. Kirby, Biochim. biophysica Acta [Amsterdam] **129**, 554 [1966].
[29] K. S. Kirby, Biochem. J. **96**, 266 [1965].
[30] R. S. S. Fraser, Molec. Gen. Genetics **106**, 73 [1969].
[31] U. E. Loening, Chromatographic and Electrophoretic Techniques, Ed. I. Smith, **Vol. II**, pp. 437—442, William Heinemann Ltd., London 1968.
[32] S. Sarkar, Virology **20**, 185 [1963].
[33] G. Melchers, H. Jockusch, and P. v. Sengbusch, Phytopathol. Z. **55**, 86 [1966].
[34] B. Singer and H. Fraenkel-Conrat, Virology **14**, 59 [1961].
[35] L. C. Lane and P. Kaesberg, Nature [London] NB, **232**, 40 [1971].
[36] L. E. Bockstahler and P. Kaesberg, Virology **27**, 418 [1965].
[37] L. E. Bockstahler and P. Kaesberg, J. molecular Biol. **13**, 127 [1965].
[38] V. Santilli and J. R. Kongsvik, Plant Virology: Proc. 6th. Conf. Czechoslovak Plant Virologists, pp. 17—21, Olomouc, CSSR 1967.
[39] J. R. Kongsvik and V. Santilli, Virology **40**, 655 [1970].
[40] A. O. Jackson, M. Zaitlin, A. Siegel, and R. I. B. Francki, Virology **48**, 655 [1972].
[41] U. E. Loening, Biochem. J. **113**, 131 [1969].
[42] A. O. Jackson, D. M. Mitchel, and A. Siegel, Virology **45**, 182 [1971].
[43] D. W. Bradley and M. Zaitlin, Virology **45**, 192 [1971].
[44] E. Fowlks and R. J. Young, Virology **42**, 548 [1970].