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Simple Method for the Isolation of Encephalomyocarditis Virus Ribonucleic Acid

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A simple purification procedure for encephalomyocarditis virus is described, yielding material suitable for extraction of viral ribonucleic acid for use as messenger in animal cell-free systems.

Interest in encephalomyocarditis (EMC) virus and how best to obtain it pure in large amounts has increased with the demonstration that its ribonucleic acid (RNA) genome can be translated in animal cell-free systems (1, 4, 8, 14). To date purification has been as described by Faulkner et al. (6) and Burness (2), but the procedures are time-consuming, yielding, in our studies, low and variable recoveries (5 to 20%). Sodium dodecyl sulfate (SDS) has been used in the purification of poliovirus (7), and polyethylene glycol (PEG) has been used in the purification of a wide spectrum of viruses (9, 11, 15). Here we describe their use in a simple purification procedure for EMC virus requiring little skilled attention and the minimum use of high-speed centrifugation.

A summary of a typical purification is given in Table 1. After overnight growth of EMC virus in Krebs II mouse ascites tumor cells (12) and centrifugation at 800 × *g* for 3 min to remove cell debris, neutralized protamine sulfate (50 mg/ml; BDH Chemicals Ltd., Poole, England) in 50 mM tris(hydroxymethyl)aminomethane (Tris)-hyrdochloride buffer (*p*H 7.6) was added to the infected culture fluid (fraction 1, Table 1), with stirring at 0 C, to a final concentration of 2 mg/ml. The mixture was centrifuged 30 min later at 10,000 × *g* for 10 min. To the supernatant fluid (fraction 2, Table 1) PEG (30%, w/v; Carbowax 6000, G. T. Gurr Ltd., Searle Scientific Services, High Wy-

TABLE 1. *Purification of encephalomyocarditis virus*

Fraction	Description	Volume (ml)	Virus concn[a]		Protein concn[a]		Specific activity (HA/mg of protein)	Purification	Recovery[a] of virus (%)
			HA/ml	Total	Amt (mg/ml)	Total (mg)			
1	Clarified culture fluid	2,500	8,000	20[b]	3.5	8,750	2,300		
2	Protamine sulfate supernatant fluid	2,600	8,000	20					100
3	PEG concentrate	14	1.28×10^6	18	41.4	580	31,000	× 13.5	90
4	PEG concentrate from SDS	6.7	2.5×10^6	16.75	4.2	28	600,000	× 260	84
5	Sucrose	4	4×10^6	16	4.2	16.8	950,000	× 410	80[c]
6	Cesium chloride	2	5.5×10^6	11	4.85	9.7	1,100,000[d]	× 490	55

[a] Estimation of virus was by hemagglutination (HA; 11) and of protein by the method of Lowry et al. (10). Infectivity was not routinely measured, but in two preparations it paralleled the recovery of HA activity, whereas in a third it was within a factor of three of it.

[b] Values expressed × 10^{-6}.

[c] In 12 preparations the overall recovery of virus through steps 1 to 5 has varied from 50 to 100%.

[d] Analysis in the analytical ultracentrifuge, under the conditions of Burness and Clothier (3), showed that ≥ 95% of the material absorbing ultraviolet light at 260 nm in these preparations sedimented at 163 to 164*S* as intact virus in reasonable agreement with the figure of 162.35 reported by these authors.

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combe, Bucks, England) in water was added to 5%, and the mixture was held and centrifuged as above. The pellets were resuspended with gentle homogenization in 50 mM Tris-hydrochloride (*p*H 7.6), 10 mM β-mercaptoethanol (TM buffer). The resuspended virus (fraction 3, Table 1) can be stored at −70 C at this stage or processed immediately. After the addition of an equal volume of 0.1 M NaCl-0.1 M sodium phosphate-0.2 M sodium pyrophosphate (*p*H 8.0), solid trypsin (BDH Chemicals Ltd.,) was added to 0.5 mg/ml and the mixture was incubated for 10 min at 37 C. Thereafter, first SDS (10%, w/v; BDH Chemicals Ltd.) in 20 mM Tris-hydrochloride (*p*H 7.6) and then PEG (30%, w/v) in water were added slowly with stirring at room temperature to 1 and 5% (w/v), respectively. After 30 min, the mixture was centrifuged at 10,000 × *g* for 10 min at 25 C. The virus was resuspended in TM buffer to yield fraction 4 (Table 1). To dissolve the suspension of virus, 1 M Na_2HPO_4 (*p*H 8.7) was added to 0.2 M, and the *p*H was adjusted immediately to between 7 and 8 with 1 N acetic acid. Debris was removed by centrifugation at 10,000 × *g* for 10 min at 4 C. The supernatant fluid was layered over 20% (w/v) sucrose in 20 mM Tris-hydrochloride (*p*H 7.6) and centrifuged at 105,000 × *g* for 3 hr at 4 C. The virus was resuspended in TM buffer (fraction 5, Table 1) for storage at −70 C, for extraction of the RNA, or for further purification if virus of the highest purity with respect to protein is required. For purification on cesium chloride, fraction 5 virus, suspended in TM buffer, was dissolved by the addition of 1 M Na_2HPO_4 (*p*H 8.7) followed by 0.5 M NaH_2PO_4 to adjust the mixture to *p*H 8.0 and placed over layers of 50% (w/v) and 40% (w/v) cesium chloride in 20 mM Tris-hydrochloride (*p*H 7.6). Centrifugation was for 18 hr at 50,000 × *g* at 4 C. Fractions containing virus (clearly visible as a band at a density of 1.33 to 1.34 g/cm^3) were diluted at least threefold with 50 mM Tris-hydrochloride (*p*H 7.6) and centrifuged for 3 hr at 105,000 × *g*, and the virus pellets were resuspended in TM buffer to yield fraction 6 (Table 1).

Only virion capsid proteins were readily detectable on electrophoresis of fraction 5 virus on SDS-acrylamide gels, and the slight further purification on cesium chloride provided material (Fraction 6, Table 1) of purity comparable (Fig. 1) to that produced by previous methods (6, 8). RNA extracted from fractions 5 and 6 on an analytical scale for electrophoresis on SDS-acrylamide gels as described by Skehel (13) yielded only one species of RNA, and this migrated a distance consistent with its being intact viral RNA of molecular weight 2.7 × 10^6 daltons. When isolated on a preparative scale (8), this RNA was as active as that from virus purified as described previously (6, 8), in supporting the synthesis of EMC-specific polypeptides in the cell-free system (4, 8). The products formed in the cell-free system in response to the RNA prepared from virus purified by the different methods appeared identical as judged by a comparison of their trypic peptides (4) or of the series of unique polypeptides resolved in recent analyses of material of this type on SDS-acrylamide gels (1; Kerr et al., FEBS Symposia, Varna, 1971, *in press*).

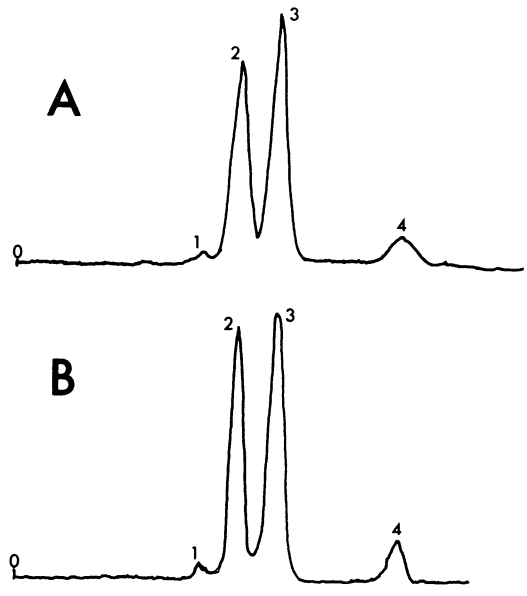

FIG. 1. *Comparison by electrophoresis on SDS-acrylamide gels of ^{35}S-methionine-labeled virion polypeptides from EMC virus purified as described here (A) and previously (B). The copies of the densitometer tracings shown are from autoradiographs of the ^{35}S-methionine-labeled polypeptides from fraction 6 (Table 1) virus (A) and from virus purified through chromatography on calcium phosphate by the methods used previously (B). Electrophoresis on SDS-acrylamide gels and autoradiography were as already described (8). Under these conditions, two of the major virion polypeptides (α and β) described by Dunker and Rueckert (5) are not resolved and appear as a single peak, peak 2, in this figure. Peaks 1, 3, and 4 here correspond to polypeptides ε, γ, and δ of Dunker and Rueckert (5; Dobos and Martin, in preparation). As presented, electrophoresis was from left to right.*

It is clear that steps 1 to 5 provide a simple, rapid, and inexpensive means of achieving a 500- to 1,000-fold concentration and a 250- to 500-fold purification of the virus with minimal loss of material (Table 1). The procedures lend themselves easily to work on a large (several liter) or small (a few milliliters) scale and provide a convenient method for the accumulation of material

for extraction of viral RNA for use in studies in animal cell-free systems or for further purification on cesium chloride or calcium phosphate (2, 8) should this be required.

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