A RE-EXAMINATION OF THE MOLECULAR WEIGHT OF POLIOVIRUS RNA

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SUMMARY: Poliovirus RNA was compared with tobacco mosaic virus (TMV), mammalian 28S and 18S ribosomal, and R17 bacteriophage RNAs. Acrylamide gel electrophoresis gave a linear relation between relative mobility of the standards and logarithm of molecular weight, and the mobility of poliovirus RNA corresponded to $2.56 \pm 0.13 \times 10^6$ daltons. Sucrose-gradient centrifugation in three solvents minimising secondary structures sedimented poliovirus RNA at a rate consistent with $2.1 - 2.6 \times 10^6$ daltons.

As discussed elsewhere (1), earlier estimates of the molecular weight of poliovirus RNA ($1.2 - 2 \times 10^6$ daltons) were mainly derived from rates of sedimentation in the ultracentrifuge, which are greatly affected by salt concentration and are presumably influenced by variable secondary structures. We have re-estimated this value by comparing poliovirus RNA with several RNAs of known size, using gel electrophoresis and zonal centrifugation in solvents that minimise secondary structure. We find that poliovirus RNA is some 25% larger than previously thought.

METHODS

Molecular weights of standard RNAs are: TMV, 2.0×10^6 (2); '28'S and '18'S ribosomal, 1.64 and 0.67×10^6 (3); R17, 1.1×10^6 (4) daltons. Ribosomal RNA was extracted from U cells (5) after 18 hr at 37° in 0.3 µC/ml uridine-2-^{14}C. Poliovirus strain *ts*$^+$ type 1 (6) was grown for 8 hr in U cells at 37°, with addition of actinomycin D (1 µg/ml at 3 hr) and uridine-2-^{14}C (0.2 µC/ml at 3.25 hr). After freezing and clarifying, virus was sedimented and the band of infectivity twice separated in CsCl gradients. *Nicotiana tabacum* L. leaves, infected with TMV (strain U1) for 5 days at 22°, were detached, lightly slashed and indirectly lit (2 days at 25°) in contact with 0.2 ml 100 µC/ml ^{3}H-adenosine and then ground in 0.02 M borate (pH 7.0). Debris was removed, virus sedimented, and the light-scattering band of radioactivity collected from a 10-40% sucrose gradient (2.5 hr at 43,000 x g);

it appeared pure, and homogeneous in particle length, in the electron microscope. Bacteriophage R 17 was grown for 8 hr in a 2 l. culture of hfr met$^-$ E. coli containing 200 μC ^{3}H-adenosine, and purified (7). When comparing RNAs, the source virions were mixed before extraction in order to treat the preparations identically. Extraction (8) was for 15 min at 20^o in 10% phenol + 1% sodium dodecyl sulphate + 0.01 M EDTA and 0.02 M phosphate (pH 7.0). Purified yeast RNA (15 μg/ml) and NaCl (to 0.2 M) were added, RNA twice precipitated in 66% ethanol and redissolved in 0.01 M tris (pH 7.4) + 0.01 M EDTA. Gel electrophoresis (9, 10) used recrystallised acrylamide and bisacrylamide in 7 cm gels, which were pre-electrophoresed (6 mA for 30 min) to remove ribonuclease. Gels were frozen and slices dried at 60^o with 10% piperidine in vials, swelled in 0.5 ml water and scintillation fluid added. Zonal centrifugation was through linear sucrose gradients (15 - 30%) in 0.1 M NaCl, 0.01 M EDTA, 0.01 M tris (pH 7.4), at 39,000 rpm in a Spinco SW/39 rotor; NaCl was omitted in some cases. In others, the RNA was pre-heated (1 hr at 60^o) in 6% (W/V) neutral HCHO (11, 12) with 6% HCHO also in the gradients, or treated with 5 vols of 99% dimethyl sulphoxide ($(CH_3)_2SO$) at 20^o for 30 min (13, 14), then centrifuged through 5-20% (W/V) sucrose gradients in $(CH_3)_2SO$ and 10 mM LiCl (65,000 rpm in a Spinco SW/65 rotor). Fractions collected from the bottom of the gradients were dried on filter paper, washed seven times in CCl_3COOH and ethanol, dried and counted in a Packard scintillation spectrophotometer, using dioxane-based fluid; $(CH_3)_2SO$ fractions were added directly to scintillation fluid.

RESULTS

Acrylamide gel electrophoresis consistently separated poliovirus and TMV RNAs (Fig. 1A). Fig. 1B and 1C are electropherograms of several RNA standards of known size, and Fig. 2 confirms the linear relation between their relative electrophoretic mobility and logarithm of molecular weight (9). By a small extrapolation, the molecular weight of poliovirus RNA is 2.65 x 10^6. These gels contained 2.4% acrylamide; gels containing 2.2%, 2.0% and 1.8% acrylamide also gave linear relations and values of 2.45, 2.70 and 2.50 x 10^6 daltons respectively, for poliovirus RNA. In a total of 6 gels, the range of molecular weight estimated for poliovirus RNA was 2.40 - 2.70 x 10^6, and the mean (with 95% confidence limits) was 2.56 ± 0.13 x 10^6 daltons.

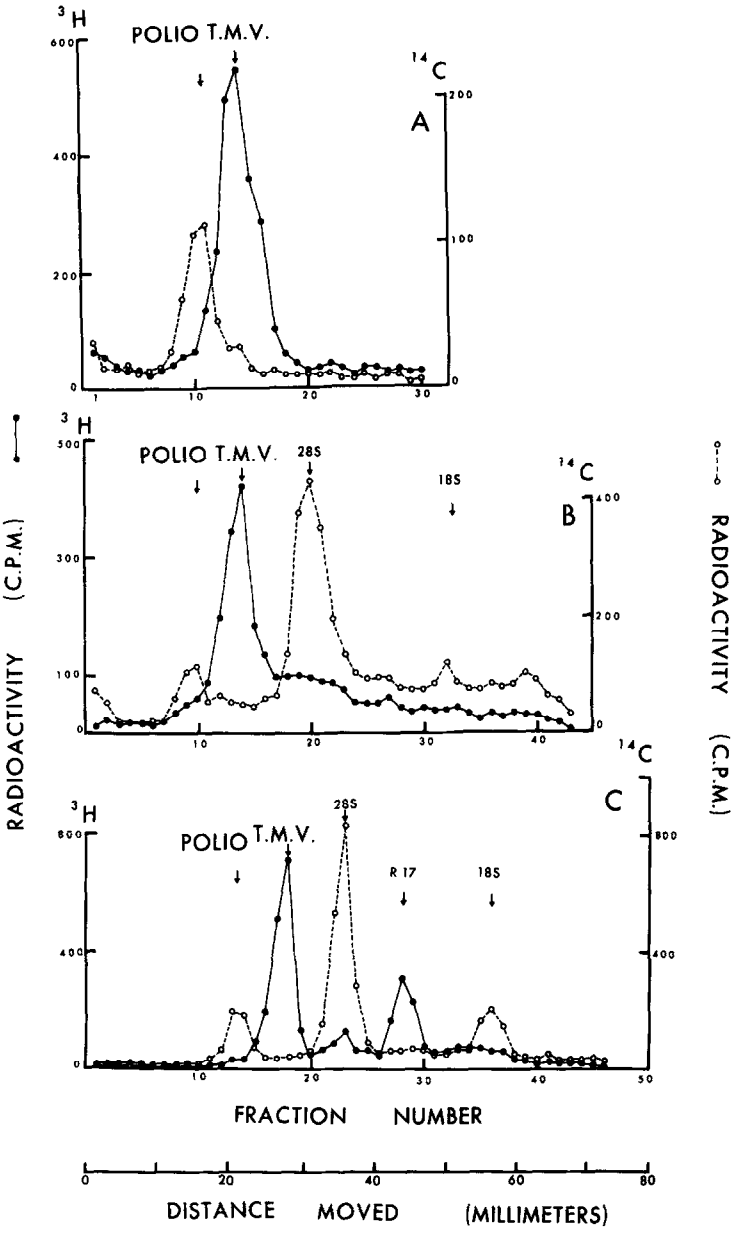

FIG. 1. Polyacrylamide gel electrophoresis of ^{14}C-poliovirus, ^{3}H-TMV, ^{14}C-ribosomal and ^{3}H-R17 RNA mixtures. Electrophoresis was for 4 hr in 2.4% gels, at 70 v and 6 mA/gel.

Sucrose gradient centrifugation through conventional gradients with or without NaCl also separated poliovirus and TMV RNAs (Figs. 3A, 3C). Poliovirus RNA sedimented the faster. However,

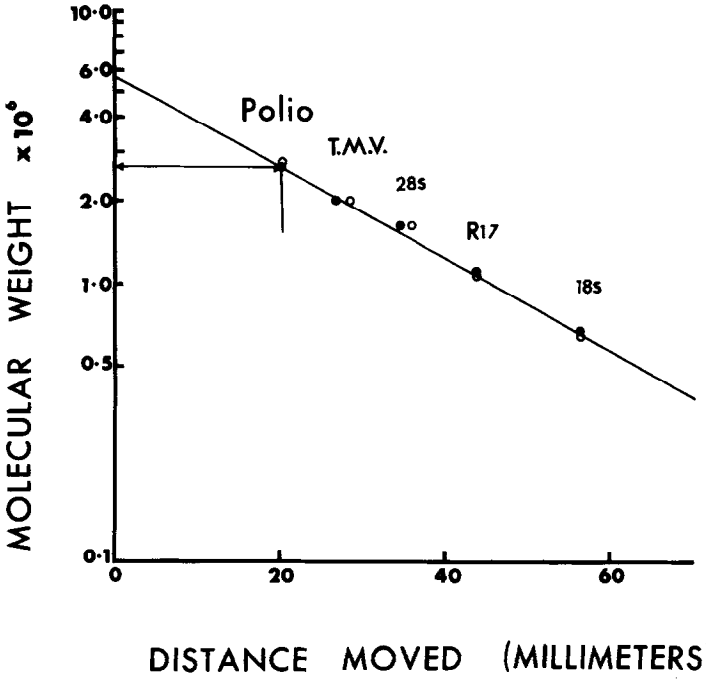

FIG. 2. Relation between molecular weight of standard RNAs and electrophoretic mobility (data from Fig. 1C and a concurrent replicate gel). The poliovirus mobility corresponds with a molecular weight of 2.65×10^6 daltons.

treatment with HCHO greatly decreased their difference in sedimentation rate, with or without NaCl (Figs. 3B, 3D). Thus either poliovirus RNA is normally more closely structured than TMV RNA, or else the secondary structures of TMV RNA are less vulnerable to HCHO. In either case, the two conformations differ, and the difference between their sedimentation rates in conventional gradients must be regarded as reflecting differences in conformation rather than size. Nevertheless, poliovirus RNA sedimented slightly faster than TMV RNA even in presence of HCHO. Treatment with $(CH_3)_2SO$ (Figs. 3E, 3F) also greatly decreased their difference in sedimentation rates, but poliovirus RNA again sedimented slightly faster in this denaturant. The sedimentation of both RNAs in $(CH_3)_2SO$ was significantly slower in presence of HCHO, suggesting that $(CH_3)_2SO$ alone may not completely remove secondary structure. These results, obtained by sedimentation of denatured RNAs, agree with those obtained by electrophoresis in that poliovirus RNA appears larger than TMV RNA. How much

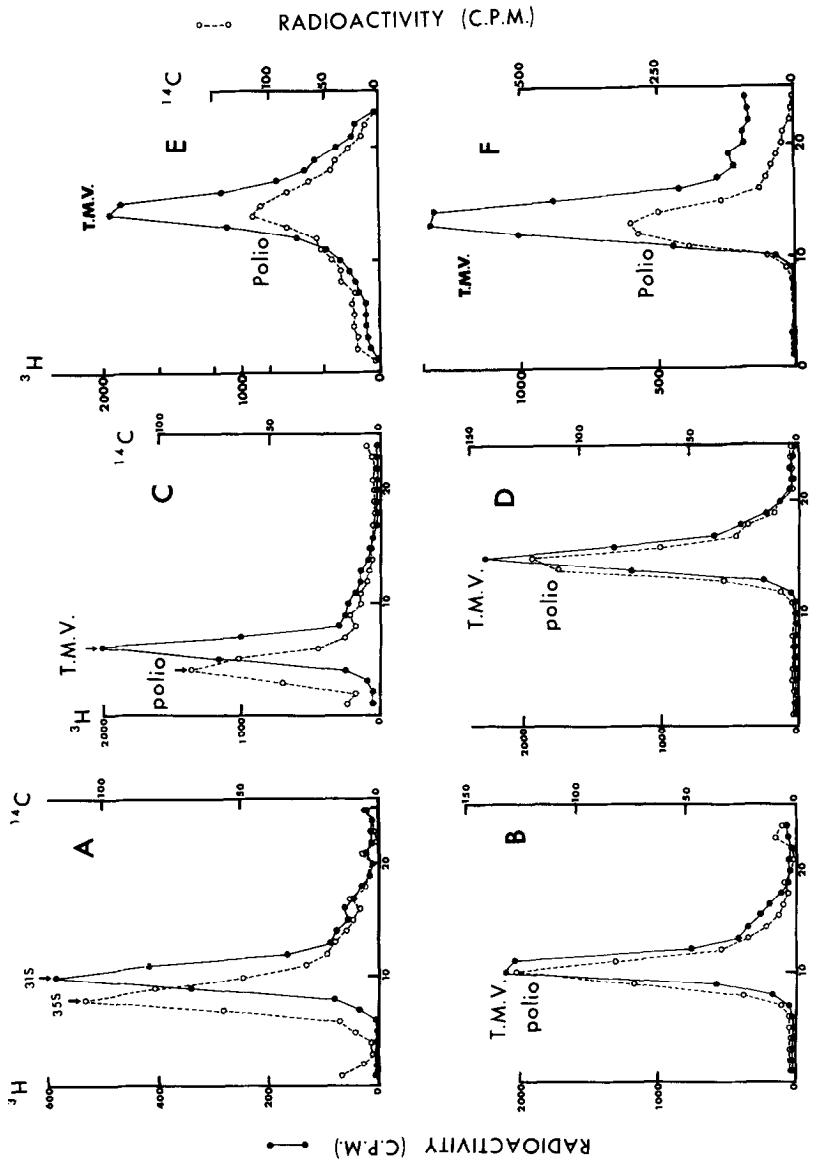

FIG. 3. Sucrose-gradient analysis of ^{14}C-poliovirus (O—O) and 3H-TMV (●—●) RNA mixtures. A: 0.1M NaCl, 4 hr; B: HCHO-treated + 0.1M NaCl, 7 hr; C: salt-free, 7 hr; and D: HCHO-treated, salt-free, 7 hr, all at 39,000 rpm; E: $(CH_3)_2SO$ alone, 5 hr at 65,000 rpm; F: $(CH_3)_2SO$ + HCHO, 7 hr at 65,000 rpm.

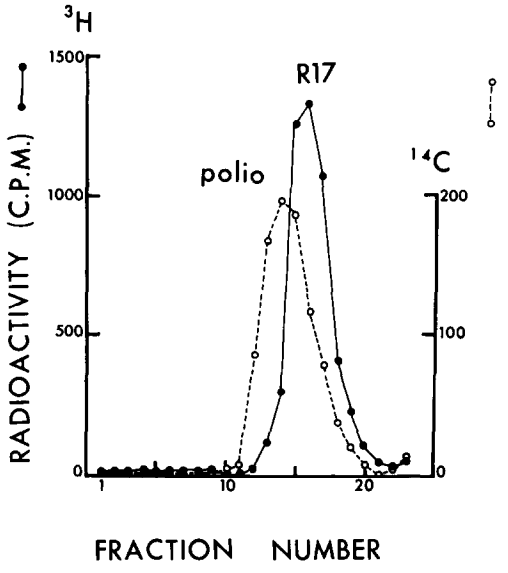

FIG. 4. Sucrose gradient analysis of ^{14}C-poliovirus (O–O) and ^{3}H-R17 (●–●) RNA mixtures; 7 hr at 39,500 rpm. The preparations were HCHO-treated but salt-free.

larger was difficult to estimate, however, as comparison with RI7 RNA (Fig. 4) showed that the resolution of denatured RNAs in 15-30% sucrose gradients was unexpectedly poor. The observed sedimentation rates were consistent with a molecular weight of poliovirus RNA between 2.1 and 2.6 x 10^6 daltons.

DISCUSSION

Both electrophoresis and gradient analysis show that poliovirus RNA is larger than the "accepted value" of 2 x 10^6 daltons; the sucrose gradient results suggest a value between 2.1 and 2.6 x 10^6 daltons, and the more precise electrophoresis data give 2.56 ± 0.13 x 10^6 daltons, some 25% larger than was previously thought. The base composition of poliovirus RNA (15) indicates an average molecular weight per sodium nucleotide of 350, so that poliovirus RNA contains 7320 ± 372 nucleotides, which can code for 2300-2600 amino acids. Granboulan and Girard (16) have very recently reported the length of both double- and single-stranded RNA of poliovirus to be 2.37 μm, equivalent to 7,500 ± 400 nucleotides or about 2.6 x 10^6 daltons. This value, reached by an independent method but using only R17 RNA as

standard, agrees very closely with the above results. These authors also found that poliovirus RNA sedimented faster than TMV RNA in sodium dodecyl sulphate-sucrose gradients, but the effects of removing secondary structure were not reported. Earlier estimates for the molecular weight of the similar encephalomyocarditis virus RNA (17, 18) were some 3×10^6 daltons, but Fenwick (19), using sedimentation in denaturing solvents, recently reports a value of about 2.4×10^6 for this virus RNA.

REFERENCES

1. Cooper, P.D. (1969). In "The Biochemistry of Viruses", Ed. H.B. Levy, Marcel Dekker, N.Y. p. 177.
2. Boedtker, H. (1960). J. Mol. Biol. 2, 171.
3. Petermann, M.L. and Pavlovec, A. (1966) Biochim. Biophys. Acta, 114, 264.
4. Gesteland, R.F. and Boedtker, H. (1964). J. Mol. Biol. 8, 496.
5. Pohjanpelto, P. (1961). Virology 15, 225.
6. Cooper, P.D. (1968). Virology 35, 584.
7. Watanabe, M. and August, J.T. (1967). In "Methods in Virology", Vol. III (Eds. K. Maramorosch and H. Koprowski), Academic Press, N.Y. p. 337.
8. Diener, T.O. and Schneider, I.R. (1968). Arch. Biochem. Biophys. 124, 401.
9. Bishop, D.H.L., Claybrook, J.R. and Spiegelman, S. (1967). J. Mol. Biol., 26, 373.
10. Loening, U.E. (1967). Biochem. J. 102, 251.
11. Hazelkorn, R. and Doty, P. (1961). J. Biol. Chem. 236, 2738.
12. Fenwick, M.L. (1968). Biochem. J. 107, 851.
13. Katz, L. and Penman, S. (1966). Biochem. Biophys. Res. Commun. 23, 557.
14. Strauss, J.H., Kelly, R.B. and Sinsheimer, R.L. (1968). Biopolymers, 6, 793.
15. Schaffer, F.L., Moore, H.F., and Schwerdt, C.E. (1960). Virology 10, 530.
16. Granboulan, N. and Girard, M. (1969). J. Virol. 4, 475.
17. Burness, A.T.H., Vizoso, A.D., and Clothier, F.W. (1963). Nature, 197, 1177.
18. Montagnier, L. and Sanders, F.K. (1963). Nature, 197. 1178.
19. Fenwick, M.L. (1968). Biochem. J. 107, 851.