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Infectivity and Sedimentation of Rhinovirus Ribonucleic Acid[1]

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Ribonucleic acid extracted from the virions of two human rhinoviruses is infective and is similar in size to poliovirus ribonucleic acid.

The ribonucleic acids of human rhinovirus types 2, 7, and 11 have been reported to be infective (2, 3, 5). Brown et al. (1) have recently reported that the virions of human rhinovirus type 2 and also of an equine rhinovirus contain ribonucleic acid (RNA) in the size range found for other picornaviruses. We have confirmed this with two human rhinoviruses and have also shown that the infectivity resides with this high-molecular-weight RNA. RNA was extracted from partially purified virions of rhinovirus type 2 (a substrain of type 2, HGP, obtained from R. R. Grunert of Stine Laboratory, Newark, Del., and which was plaque-purified) and rhinovirus type 14 (strain 1059 obtained from W. L. Davies of Stine Laboratory, Newark, Del.). The infectivity and relative sedimentation rate of each type of RNA were correlated after centrifugation through a sucrose gradient in dimethylsulfoxide (DMSO).

Details of the procedure used for virus growth and purification will be described elsewhere. Briefly, however, virus was grown in monolayer cultures of HeLa cells (Rhino-HeLa-cells, Flow Laboratories, Rockville, Md.) in the presence of modified McCoy's 5A medium containing 1% heat-inactivated fetal calf serum. The procedure for the partial purification of the virions employed the denaturation of cell proteins with fluorocarbon and sedimentation of the virions to a pellet by high-speed centrifugation. The virus was resuspended and finally sedimented in a sucrose gradient. The RNA was extracted from the virions with phenol at 45 C in the presence of 0.2% sodium dodecyl sulfate (SDS); 0.02 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.5; and 0.002 M ethylenediaminetetraacetic acid (EDTA). Yeast RNA (0.5 to 1.0 mg) and sodium chloride (final concentration, 0.2 M) were added to the aqueous phase obtained after the second phenol extraction, and the RNA was precipitated with 3 volumes of alcohol at −20 C. The RNA was dissolved in buffer containing 0.01 M Tris (*p*H 7.2), 0.1 M NaCl, 0.01 M EDTA, and 0.2% SDS; reprecipitated once with ethanol; and then redissolved in 0.5 to 1.0 ml of the same buffer.

The sedimentation of viral RNA was carried out in sucrose gradients in DMSO after denaturation with DMSO. (*See* legend to Fig. 1 for details.) Poliovirus RNA labeled with ^{14}C was included as a marker. The RNA from rhinovirus types 2 and 14 sedimented slightly slower than poliovirus RNA (Fig. 1a, b), 96 and 90% as fast, respectively. Since DMSO is reported to destroy the secondary structure of RNA (6), the distance sedimented by each RNA through the gradient is considered to be determined by its size. Assuming 2.6×10^6 daltons as the molecular weight of poliovirus RNA (4, 7) and a linear relationship between distance sedimented and the molecular weight under the conditions of our experiments, it follows that rhinovirus types 2 and 14 have molecular weights of 2.5×10^6 and 2.4×10^5 daltons, respectively.

To relate the profile of sedimentation of labeled RNA to its infectivity, gradients were run as described (legend to Fig. 1) but without added marker poliovirus RNA. Fractions were pooled and processed (*see* legend to Fig. 2), and the infectivity of the RNA in each pooled fraction was assayed on monolayer cultures of HeLa cells by using LTM buffer (*p*H 7.2) containing 1.2 mg of diethylaminoethyl (DEAE) dextran per ml as a diluent for the RNA (3). Figure 2 represents the infectivity of pooled fractions expressed as plaque-forming units (PFU) per milliliter of gradient. Most of the infectivity resided in the fractions which also contained most of the RNA.

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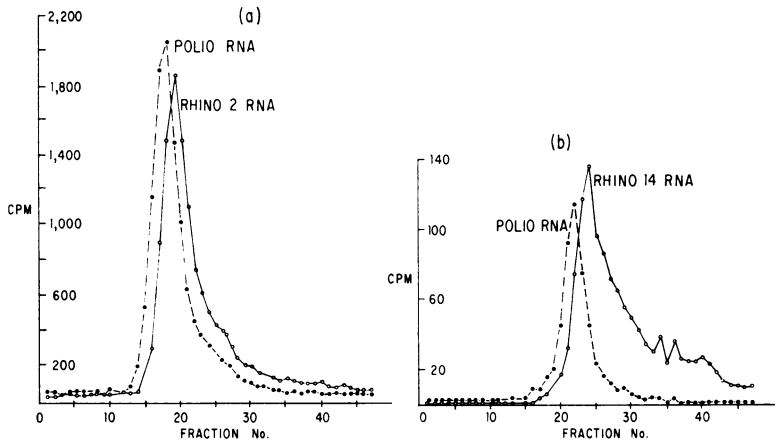

FIG. 1. *Sedimentation of rhinovirus RNA. A 50-μliter amount of a mixture of ^{3}H-rhinovirus RNA and ^{14}C-poliovirus RNA was denatured with 5 volumes of 99% DMSO (Matheson, Coleman and Bell) at room temperature for 0.5 hr. The mixture was layered on a 5 to 20% (w/w) sucrose gradient in 99% DMSO and centrifuged for 7 hr at 60,000 rev/min in an SW65 rotor at 20 C. Fractions were collected, and the radioactivity of each fraction was determined in a scintillation spectrometer.*

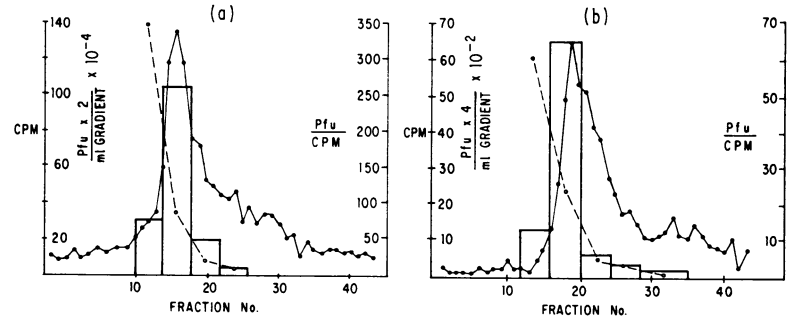

FIG. 2. *Sedimentation of infectious rhinovirus RNA. ^{3}H-labeled rhinovirus RNA was denatured, sedimented, and fractionated as described in the legend of Fig. 1. A sample of each fraction was counted to determine the profile of radioactive RNA. Fractions were pooled, and the RNA of each pool was precipitated at −20 C with alcohol in the presence of 0.5 mg of yeast RNA and was dissolved in 1.0 ml of water. Dilutions of RNA were made in LTM buffer (pH 7.2) containing 1.2 mg of DEAE dextran per ml (Pharmacia, Uppsala, Sweden). The infectivities of appropriate dilutions were assayed on monolayer cultures of HeLa cells (grown in modified McCoy's medium containing 10% calf serum). The monolayers were washed twice with phosphate-buffered saline without calcium or magnesium, the RNA was adsorbed for 0.5 hr at room temperature, and overlay [0.35% Ionagar no. 22 (Colab Laboratories Inc., Chicago, Ill.), 0.02% trypsin, 0.002% DEAE dextran, and 4.5% heat-inactivated fetal calf serum in McCoy's medium] was added. The plates were incubated for 3 to 5 days at 34.5 C for plaque development. (a) RNA of rhinovirus type 2; (b) RNA of rhinovirus type 14. Symbols: ●, counts per minute; ○, specific infectivity (PFU per counts per minute) of RNA. Histogram: infectivity per milliliter of pooled gradient fractions.*

It was repeatedly found that the highest specific infectivity was associated with the leading edge of the RNA profile (Fig. 2). This observation, together with the skewed appearance of the sedimentation peak which trails toward the slow side, indicates that some of the RNA is degraded during the preparation and purification of either the virus or the virion RNA itself. A less likely alternative is that some defective RNA is packaged within capsids during infection. It follows that our estimates of molecular weights based on sedimentation of labeled RNA may be slightly lower than the molecular weights of the infective molecules.

The infectivity recovered from RNA fractionated on sucrose gradients (in DMSO or in NaCl-Tris-EDTA-SDS buffer) was at least twice that of unfractionated RNA when both were assayed simultaneously. This might have been due to the presence of aggregates in the unfractionated

RNA. The ratio of PFU of virus to that of RNA was approximately 10^4 for both rhinovirus 2 and rhinovirus 14, in agreement with earlier reports (2, 3).

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LITERATURE CITED

1. Brown, F., J. F. E. Newman, and E. J. Stott. 1970. Molecular weight of rhinovirus ribonucleic acid. J. Gen. Virol. 8:145–148.
2. Dimmock, N. J. 1966. Biophysical studies of a rhinovirus. Nature (London) 209:790–794.
3. Fiala, M., and B. Saltzman. 1969. Enhancement of the infectivity of rhinovirus ribonucleic acid by diethylaminoethyl dextran. Appl. Microbiol. 17:190–191.
4. Granboulan, N., and M. Girard. 1969. Molecular weight of poliovirus ribonucleic acid. J. Virol. 4:475–479.
5. Ketler, A. V., V. Hamparian, and M. R. Hilleman. 1962. Characterization and classification of ECHO-28-rhinovirus-coryzavirus agents. Proc. Soc. Exp. Biol. Med. 110: 821–831.
6. Straus, J. H., Jr., R. B. Kelly, and R. L. Sinsheimer. 1968. Denaturation of RNA with dimethyl sulfoxide. Biopolymers 6:793–807.
7. Tannock, G. A., A. J. Gibbs, and P. D. Cooper. 1970. A reexamination of the molecular weight of poliovirus RNA. Biochem. Biophys. Res. Commun. 38:298–304.