MEASLES VIRUS RNA

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Summary. RNA has been isolated from measles virions which sediments with a coefficient of 52.2 S when compared with 50 S RNA from SV 5 virions.

Measles virus has been classified with paramyxoviruses largely on the basis of morphology (1). Recently, using a method similar to that used by Compans and Choppin for the simian parainfluenza virus SV 5 (2) and Newcastle disease virus (NDV) (3), Nakai et al calculated the length of measles nucleocapsids (4). Two estimates were made depending upon the type of correction applied to compensate for stretching; one was 1.06 μ and the other was 1.15 μ. Both measurements indicate that measles nucleocapsids are longer than SV 5 nucleocapsids, estimated to be 1.02 μ in length, and equal in length or longer than NDV nucleocapsids, 1.06 μ in length.

To test whether the measured increased length of measles nucleocapsids would be reflected in increased size of measles virus RNA, measles virus labeled with ^{14}C-uridine and SV 5 virus labeled with ^{3}H-uridine were mixed, the virions were purified by density gradient centrifugation and RNA was extracted. The sedimentation profiles of the two isotopes following fractionation of the RNA mixture on a sucrose gradient are shown in Figure 1. Experimental procedures are given in the legend. The fastest sedimenting measles RNA preceded SV 5 RNA in the gradient by one fraction. Taking the value 50 S determined by Compans and Choppin for SV 5 RNA (5) as the standard and using the method of Martin and Ames (6), a sedimentation coefficient of 52.2 S has been found for measles RNA. This RNA is sensitive to RNase and is presumed to be single stranded. According to the equation proposed by

Spirin (7) relating molecular weight to sedimentation coefficient, M.W. = 1550 $(S)^{2.1}$, measles virus contains an RNA of 6.22×10^6 daltons. This

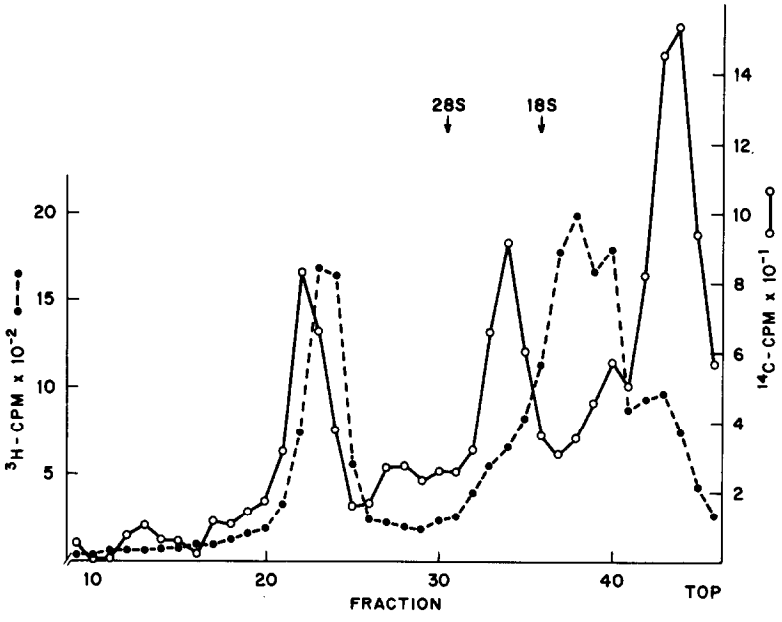

Figure 1. Cosedimentation of RNA extracted from SV 5 virus labeled with ^{3}H-uridine (●----●) and measles virus labeled with ^{14}C-uridine (o——o)

Medium from SV 5 infected MDBK cells (10) labeled as previously described (5) was generously provided by Dr. Purnell Choppin. Twenty milliliters of SV 5 medium was mixed with 40 ml medium from Vero cells (11) harvested 45 h after infection with measles virus. Eighteen hours after infection, actinomycin D had been added (2 μg/ml) and at twenty hours post infection, ^{14}C-uridine (50 μC/ml) had been added. The virus mixture was then centrifuged to a density interface (55%-20% w/w sucrose in 0.01 M Tris HCl, pH 7.2, 0.05 M NaCl) at 25,000 rpm, 60 min, in an SW 25.1 rotor. A 1-ml sample was withdrawn from each tube at the sucrose interface, diluted 1:2 with buffer and layered onto sucrose-D_2O gradients, density range 1.1 to 1.31, prepared in the Tris buffer described. The gradients were centrifuged at 48,000 rpm, 210 min, in an SW 50.1 rotor. One-milliliter fractions with average density 1.225 were removed through the side of each tube. The fractions were pooled, the volume was brought to 5 ml with Tris buffer and the virions were pelleted at 30,000 rpm, 30 min, in an SW 50.1 rotor. The pellet was dissolved in 0.1 ml 0.02 M EDTA, 1 % sodium dodecyl sulfate, 0.01 M PO_4, pH 7.2, and 0.1 % 2-mercaptoethanol. The solution was heated for 1 min in a 60° C water bath, then layered on a 15-30% linear sucrose gradient prepared in 0.1 M NaCl, 0.01 M Tris HCl, pH 8.5, 0.001 M EDTA, and 0.5 % sodium dodecyl sulfate. The gradient was centrifuged at 50,000 rpm for 1 1/2 h at 15° C in an SW 50.1 rotor. Fractions were collected from the bottom of the tube directly onto filter paper disks which were processed for measurement of acid-insoluble radioactivity by the method of Mans and Novelli (12) and counted in a Beckman Model LS 150 liquid scintillation counter. Vero cell ribosomal RNAs were centrifuged in a separate tube to provide additional sedimentation markers, and their position after centrifugation is noted in Figure 1.

agrees with the estimate of Nakai et al (6.2×10^6) based on their measurements of nucleocapsid length (4).

In the experiment shown, both measles and SV 5 infected media contained RNA sedimenting more slowly than 50 S. Introduction of an ammonium sulfate precipitation step in the preparation of virions (8) almost completely eliminated small molecular weight RNA labeled with ^3H (SV 5 preparation), but failed to eliminate the smaller RNA species from the measles preparation. The pattern of measles viral RNA obtained under uniform conditions is highly reproducible with respect both to S-rates of the principle peaks and to proportional amounts of radioactivity within the peaks. There is a large peak of 21 S RNA and secondary peaks of 32 S and 28 S RNAs. These coefficients are estimates relative to the sedimentation of Vero ribosomal RNAs presumed to be 28 S and 18 S.

The smaller measles RNAs seen were not the result of contamination with host RNA, as uninfected control preparations labeled under the same conditions in the presence of actinomycin were negative. At the present time it cannot be said whether these more slowly sedimenting measles RNAs represent non-virion RNA which has leaked into the medium from the cells but is in some way protected from RNase activity, or whether they might represent "subgenomic" RNAs, such as described by Kingsbury et al for Sendai virus (9), contained in "incomplete" virions. Measles RNAs with similar sedimentation characteristics have been isolated also from infected cytoplasm (Schlueder-berg, unpublished observations).

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References.

1. Waterson, A. P. Nature 193, 1163 (1962).
2. Compans, R. W., and Choppin, P. W. Proc. Natl. Acad. Sci. U.S. 57, 949 (1967).
3. Compans, R. W., and Choppin, P. W. Virology 33, 344 (1967).
4. Nakai, T., Shand, F. L., and Howatson, A. F. Virology 38, 50 (1969).
5. Compans, R. W. and Choppin, P. W. Virology 35, 289 (1968).

6. Martin, R. G., and Ames, B. N. J. Biol. Chem. 236, 1372 (1961).
7. Spirin, A. S. Progr. Nucleic Acid Res. 1, 301 (1963).
8. Klenk, H.-D., and Choppin, P. W. Virology 37, 155 (1969).
9. Kingsbury, D. W., Portner, A., and Darlington, R. W. Virology 42, 857 (1970).
10. Choppin, P. W. Virology 39, 130 (1969).
11. Shishido, A., Yamanouchi, K., Hikata, M., Sato, T., Fukuda, A. and Kobune, F. Arch. ges. Virusforsh. 22, 364 (1967).
12. Mans, R. J. and Novelli, G. D. Arch. Biochem. Biophys. 94, 48 (1961).