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Replication of Measles Virus: Distinct Species of Short Nucleocapsids in Cytoplasmic Extracts of Infected Cells

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Cytoplasmic extracts of Vero cells infected with wild-strain Edmonston measles virus were found to contain two and probably three distinct species of nucleocapsids. Species sedimenting at 200 and 110*S* contained RNA which sedimented at 50 and 16 to 18*S*, respectively. The third nucleocapsid species which sedimented at 170*S* was not present in all experiments and was not characterized in detail. Essentially all 200 and 170*S*, as well as a portion of the 110*S*, nucleocapsids were membrane associated and probably present in part in cell-associated virions. Five of six plaque purified strains derived from wild-type Edmonston virus produced only 200*S* nucleocapsids. One of these five plaque-purified strains subsequently produced both 200 and 110*S* nucleocapsids after being passaged by using undiluted inocula. These results suggest that measles virus may produce distinct classes of defective virus containing short nucleocapsids and subgenomic viral RNA.

Measles virus has been classified as a paramyxovirus on the basis of its morphology (10, 18). Recent studies (3, 5, 16, 19) demonstrated that measles virions contain RNA with a sedimentation coefficient of approximately 50*S*, like that of other paramyxovirus virion RNAs (2, 4). Patterns of RNA synthesis in measles virus-infected cells were also found to be similar to patterns observed in cells infected with other paramyxoviruses (3, 11, 19; M. P. Kiley and F. E. Payne, *in* R. Barry and B. W. J. Mahy ed., Negative strand viruses, Academic Press, Inc., 1974 in press).

Viruses which appear to be variants of classical measles virus have been isolated from subacute sclerosing panencephalitis (SSPE) (12). To compare SSPE and other strains of measles virus and to understand their roles in the pathogenesis of SSPE, we are characterizing virus-specific components of cells infected with these viruses. The present report concerns the size and location of virus-specific structures and their RNA in the cytoplasm of measles virus-infected cells and their probable role in viral replication.

MATERIALS AND METHODS

Cells. The continuous line of green monkey kidney cells, Vero (20), was grown in Eagle minimal essential medium (MEM) containing 10% fetal calf serum (FCS). BSC-1 cells (6), also a green monkey kidney cell line, were grown in medium 199 containing 20% FCS and 0.1% yeast extract. All media contained 100 μg of penicillin and of streptomycin per ml.

Virus. Wild-strain Edmonston virus, originally obtained from H. M. Meyer, Jr., was propagated in BSC-1 cells. Plaque-purified strains were derived by plating wild-strain virus at limiting dilutions on BSC-1 or Vero cells. Plaques were picked, replated, and picked twice more before pools were prepared. Working pools were prepared by infecting BSC-1 or Vero cells with undiluted virus at a multiplicity of approximately 1 PFU/cell. When 80% or more of the cells showed cytopathic changes, cultures were frozen and thawed. Infected culture fluids were cleared by centrifugation and stored as samples at −60 C.

Labeling infected cells. Vero cell monolayers in 8-oz prescription bottles were infected with virus at a multiplicity of approximately 0.05 PFU/cell. After a 1-h adsorption period, 10 ml of MEM containing 2% FCS was added to each culture, and cultures were incubated at 37 C in 5% CO_2. At a time indicated for each experiment, cells were exposed to 25 μg of actinomycin D per ml (Calbiochem), and 2 h later they were exposed to 20 μCi of [5-^{3}H]uridine per ml (28 Ci/mmol, Schwarz/Mann). Cells were labeled in the continuing presence of actinomycin. At the end of the labeling period, cells were harvested, and a cytoplasmic extract was prepared. Mock-infected cells served as controls.

Cell fractionation. Cytoplasmic extracts were prepared essentially by the method of Penman et al. (15) as described previously (8). Medium was removed from cultures, and the cells were scraped into 5 ml of Tris-buffered saline (TBS: 0.01 M Tris-hydrochloride, 0.14 M NaCl, pH 7.4). Cells were pelleted at 1,500 × *g*

for 10 min, resuspended in 1 to 3 ml (depending on experimental conditions) of reticulocyte standard buffer (RSB: 0.01 M Tris, 0.01 M NaCl, 0.0015 M $MgCl_2$, pH 7.4) and allowed to swell for 20 min in an ice bath. Cells were disrupted by 15 strokes of a Dounce homogenizer. Nuclei were pelleted at 800 × *g* for 10 min, and the cytoplasmic extract was decanted.

Labeled structures in cytoplasmic extracts were examined by rate-zonal centrifugation in either 15 to 30% or 15 to 40% (wt/vol) 38-ml sucrose gradients prepared in RSB. Centrifugation was for 3 to 3.5 h at 95,000 × *g* and at 4 C in an SW27 rotor. Gradients were fractionated into 1.2-ml samples by using an ISCO density gradient fractionator (ISCO, Lincoln, Neb.) while absorption at 254 nm was continuously monitored. Gradient pellets were resuspended in 1.2 ml of RSB.

In some cases, membranes in cytoplasmic extracts were dissociated by making the extracts 0.5% with respect to the detergents deoxycholate (Sigma) and BRIJ-58 (Atlas Biochemical Industries) prior to centrifugation. Some extracts were analyzed on 15 to 40% sucrose gradients prepared in RSB-EDTA buffer (0.01 M Tris, 0.01 M NaCl, 0.02 M EDTA, pH 7.4) after treatment of the extract with 0.1 volume of RSB-EDTA buffer containing 0.2 M EDTA.

Extraction and analysis of RNA. Extraction of RNA from sucrose gradient fractions was performed as described previously (8). Appropriate fractions were diluted with RSB, made 1% with sodium dodecyl sulfate (SDS), and RNA extracted by addition of an equal volume of 90% phenol in RSB. After mixing and cooling in an ice bath, phases were separated by centrifugation at 1,500 × *g* for 10 min at 4 C. The aqueous phase was removed and re-extracted with one-third volume of 90% phenol, and the RNA was precipitated from this aqueous phase by addition of two volumes of cold ethanol. After at least 12 h at −20 C, the RNA precipitate was pelleted at 10,000 × *g* for 20 min, washed once with ice-cold 70% ethanol, and resuspended in NTE (0.1 M NaCl, 1 mM EDTA, and 0.01 M Tris, pH 7.4) containing 0.5% SDS. Samples in this buffer were layered on 17-ml 5 to 20% (wt/vol) sucrose gradients prepared in 0.1 M acetate buffer (pH 5.0), and were centrifuged for 12 h at 23,000 rpm in an SW27 rotor. ^{14}C-uridine-labeled RNAs prepared in uninfected Vero cells were included in each gradient as sedimentation markers. Samples were collected by puncturing the bottom of the gradient tube with a needle and collecting dropwise.

Radioactivity determinations. Acid-insoluble radioactivity was determined in the following manner. Samples were placed in test tubes and mixed with 200 μg of bovine serum albumin, and then an equal volume of 10% trichloroacetic acid was added. After 20 min at 4 C, precipitates were pelleted at 15,000 × *g* for 10 min, resuspended in NTE buffer, and re-precipitated with cold trichloroacetic acid. These precipitates were pelleted as above, dissolved in 0.3 ml of 7:3 NCS:toluene, and radioactivity was determined after the addition of 6 ml of a toluene-based scintillation cocktail.

Electron microscopy. Droplets of appropriate sucrose gradient fractions were placed on Formvar-carbon coated grids for 30 s. The grids were then floated with 3 to 4 drops of 1% ammonium acetate, the excess liquid was removed with filter paper, and the samples were stained with 1 drop of 2% phosphotungstic acid (PTA), pH 6.85, for 1 min before drying. The grids were examined with an AEI Corinth 275 microscope.

RESULTS

Cytoplasmic structures containing virus-specific RNA. As in other paramyxovirus infections, measles virus-specific RNA synthesis is revealed in the infected cell when host cell RNA synthesis is inhibited by treatment with actinomycin D. Such virus-specific RNA would be expected to be associated with subcellular structures which are intimately involved with measles virus replication. Accordingly cytoplasmic extracts of actinomycin-D-treated measles virus-infected cells which had been exposed to ^{3}H-uridine were examined for components associated with the radioactive label. Mock-infected cultures served as controls.

Vero cells were infected with wild-strain virus, and at 3 days postinfection they were labeled with ^{3}H-uridine for 5 h. Cells were treated with actinomycin D for 2 h before and during the labeling period. Cytoplasmic components of detergent-treated and untreated extracts were separated by centrifugation in a sucrose gradient.

Figure 1A shows the pattern of radioactivity obtained when a cytoplasmic extract of ^{3}H-uridine-labeled mock-infected cells was centrifuged as described above. This extract was detergent treated prior to centrifugation; however, untreated extract gave similar results. In this control culture no significant radioactivity was detected other than in the top three gradient fractions. Figure 1B depicts results obtained when a cytoplasmic extract of ^{3}H-uridine-labeled infected cells was subjected to centrifugation. This sample was not treated with detergent prior to centrifugation. Large amounts of ribonuclease-sensitive RNA sedimenting in the 30 to 70*S* region of the gradient were detected as well as a broad band of acid-insoluble radioactivity covering the region from 70 to 150*S*. The 110*S* region of this band was partially resistant to ribonuclease. The pellet from this gradient contained approximately 11,000 counts/min of acid-insoluble radioactivity. Figure 1C illustrates sucrose gradient analysis of a sample identical to that analyzed in Fig. 1B, but which was detergent treated prior to centrifugation. Under these conditions, the pellet from this gradient contained approximately 3,000 counts/min of acid-insoluble radioactivity. The peak of radioactivity in the 110*S* region of the gradient

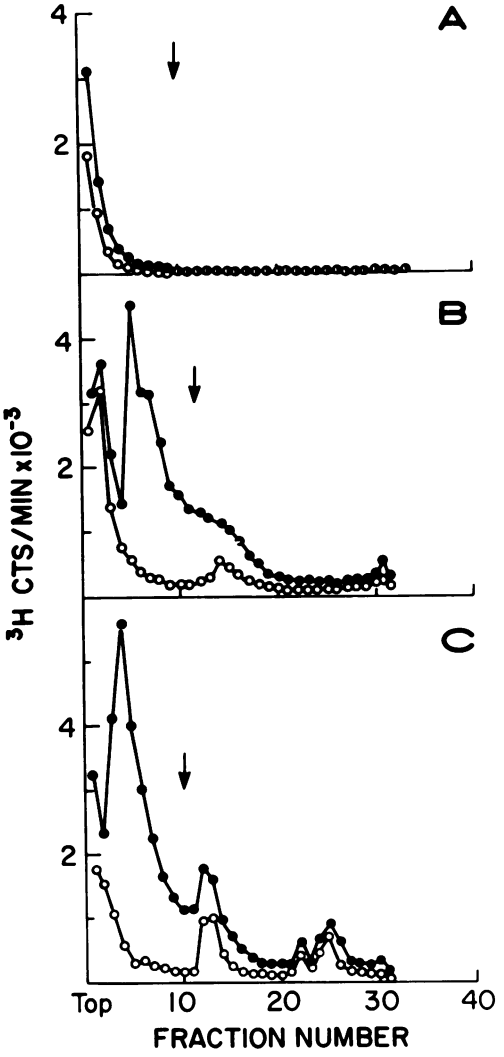

FIG. 1. *Velocity sedimentation analysis of ^{3}H-uridine-labeled structures from cytoplasmic extracts of measles-infected cells. Vero cells in 8-oz prescription bottles were infected at a multiplicity of 0.05 PFU/cell. At 3 days postinfection, cells were treated with 25 μg of actinomycin D per ml and 2 h later 20 μCi of ^{3}H-uridine was added per ml to each culture. After a 5-h labeling period, cells were harvested and a cytoplasmic extract was prepared. Extracts were layered onto 38-ml 15 to 30% (wt/vol) sucrose gradients and centrifuged at 27,000 rpm for 180 min at 4 C in an SW27 rotor. Fractions of 1.2 ml were collected while UV absorbance at 254 nm was continuously monitored. Trichloroacetic acid-precipitable radioactivity in 0.2-ml portions of each fraction was determined prior to (●) or after (○) treatment with 20 μg of pancreatic ribonuclease per ml for 20 min at room temperature. Sedimentation is from left to right and the arrow indicates the position of 80S ribosome-monomer in the gradient. A, Extract from mock-infected actinomycin-D-treated cells which was treated with detergent mixture prior to centrifugation; B, infected cell extract not detergent treated prior to centrifugation; and C, an extract identical to that in B except that it was detergent treated before centrifuging.*

was more prominent than in its counterpart in Fig. 1B, and two new peaks of radioactivity sedimenting at 170 and 200S were apparent, suggesting that a portion of those structures sedimenting at 110S and essentially all of those sedimenting at 170 and 200S had been associated with lipid-containing membranes.

As illustrated in Fig. 1C, treatment of the gradient fractions with 20 μg of ribonuclease per ml for 20 min revealed that the RNA of the 170 and 200S structures was 90 to 100% resistant to ribonuclease whereas that of the 110S structure was approximately 65% resistant. Further characterization of the 170S structures has been limited because they have not appeared in all experiments.

To examine further the nature of structures associated with ribonuclease-sensitive RNA, a cytoplasmic extract of labeled, infected cells was prepared essentially as described for Fig. 1C but the extract was first treated with 0.1 volume of 0.2 M EDTA and then analyzed in a 15 to 40% sucrose gradient prepared in RSB-EDTA buffer. This treatment rendered both the 110 and 200S structures almost completely resistant to ribonuclease treatment (Fig. 2). In other preparations, where 170S structures were present, they too were found to be stable to EDTA treatment, and their associated RNA was resistant to ribonuclease. Because EDTA treatment is known to dissociate polysomes, these results suggest that polyribosomes were the 100 to 200S structures containing ribonuclease-sensitive RNA which were seen in gradients of infected cell extracts not treated with EDTA.

The resistance to ribonuclease and the stability in EDTA of structures sedimenting at 110, 170, and 200S suggested that they were viral nucleocapsids. This suggestion was confirmed for the 110 and 200S structures by electron microscope examination of negative-stained samples from the gradients. The structures in Fig. 3A and C are from the 110S region and those in Fig. 3B and D are from the 200S region of the gradient. It can be seen that the herringbone character of these structures is identical to that previously reported for measles nucleocapsids (5, 10).

Observation of electron micrograph fields containing more than 30 of each type of nucleocapsid suggested that the two populations were distinct and quite uniform in length.

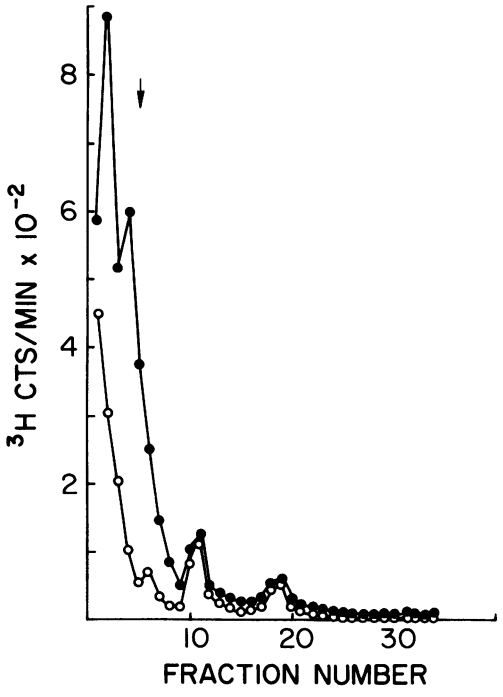

FIG. 2. *Effect of EDTA treatment on the ribonuclease sensitivity of 110S structures. Vero cells were infected, labeled, harvested, and analyzed as described in Fig. 1C with the following changes in protocol: the labeling period was 4.5 h and the cytoplasmic extract was made 0.02 M with respect to EDTA, and was centrifuged in a 15 to 40% sucrose gradient prepared in RSB-EDTA. Acid-insoluble radioactivity was determined on portions of each gradient fraction either before (●) or after (○) treatment with ribonuclease. Sedimentation is from left to right and the arrow represents the position of cellular 50S ribosomal subunit.*

Electron micrographs were enlarged to ×160,000 and lengths of eight randomly selected nucleocapsid filaments of each size class were measured. Lengths of the 110*S* nucleocapsids ranged from 135 to 150 nm, whereas lengths of those from the 200*S* region of the gradient ranged from 1,300 to 1,580 nm. Our procedure apparently led to relatively little breakage of the nucleocapsids. It can be seen in Fig. 3D that the helix of the longer nucleocapsid appears to be slightly unwound at irregular intervals. This type of unwinding was also observed by Nakai et al. (10).

Nucleocapsid RNA. Because electron microscope observations and sedimentation analysis indicated that nucleocapsids in cytoplasmic extracts of infected cells fell into at least two distinct categories, it was of interest to determine if RNA extracted from each nucleocapsid species would relate to the length of the nucleocapsids. RNA was extracted, by phenol-SDS treatment, from the 110 and 200*S* regions of the 15 to 30% gradients depicted in Fig. 1B and C, and was analyzed on 5 to 20% sucrose gradients. Vero cell RNA labeled with ^{14}C-uridine was employed as an internal marker in each gradient, and the 28 and 18*S* ribosomal RNAs were assumed to have molecular weights of 1.75×10^6 and 0.7×10^6, respectively. The 110*S* structures yielded RNA with a peak at 16*S* whether they were from the gradient depicted in Fig. 1B (Fig. 4A) or that in Fig. 1C (Fig. 4B). The 200*S* region from the gradient shown in Fig. 1C yielded 50*S* RNA (Fig. 4C). Since the gradients which served as sources of extracted RNA did not contain EDTA, the breadth of the base of both the 16 and 50*S* RNA peaks may be attributable to mRNA from contaminating polysomes. Molecular weights for 16 and 50*S* RNAs were estimated, employing the formula of Spirin (17), to be approximately 0.6×10^6 and 6.0×10^6, respectively. Since the 200*S* nucleocapsids were approximately 10 times as long as the 110*S* nucleocapsids, nucleocapsid length related to the size of RNA extracted from the nucleocapsids. The previously reported molecular weight of the measles virion RNA is 6.2×10^6 to 6.4×10^6 (5, 16).

Location of nucleocapsids and virus-specific RNA. No 200*S* nucleocapsids, and a relatively small amount of 110*S* nucleocapsids, were observed in the body of gradients (e.g., Fig. 1B) unless cytoplasmic extracts were treated with a mixture of deoxycholate and BRIJ-58 prior to centrifugation (e.g., Fig. 1C). Also, about four times as many counts appeared in pellets from nondetergent-treated samples as from those treated with detergent. Aggregation of nucleocapsids and their disaggregation by detergent did not appear to account for these results since electron microscope examination of pellets from extracts of infected cells, which had not been treated with detergent, revealed membrane fragments and no aggregates of nucleocapsids. Additional information regarding membrane-associated virus-specific structures was gained by examining the RNA species present in pellets from gradients of detergent-treated and nontreated cytoplasmic extracts. Pellets were resuspended in RSB buffer, and their RNA was extracted with phenol-SDS. Extracted RNA was then analyzed on 5 to 20% sucrose gradients employing ^{14}C-labeled Vero cell RNA as sedimentation markers. Figure 5A shows the distribution of RNA extracted from the pellet of a nondetergent (see Fig. 1B), treated, infected cell extract. This pellet yielded

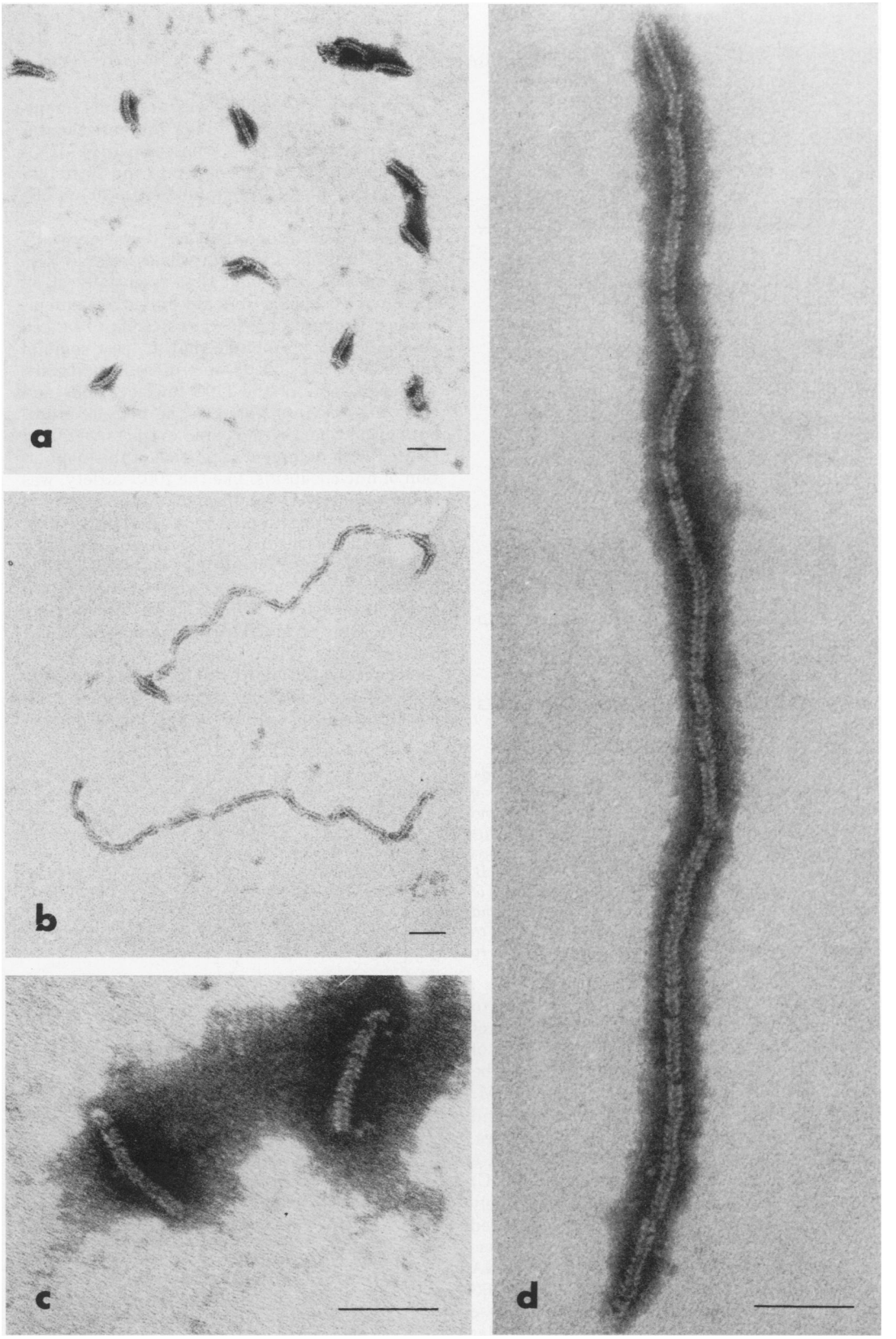

FIG. 3. *Electron micrographs of cytoplasmic nucleocapsids, negatively stained with 2% phosphotungstate. A, 110S nucleocapsids × 60,000; B, 200S nucleocapsids ×60,000; C, 110S nucleocapsids ×160,000; and D, 200S nucleocapsids ×160,000. Bar represents 100 nm.*

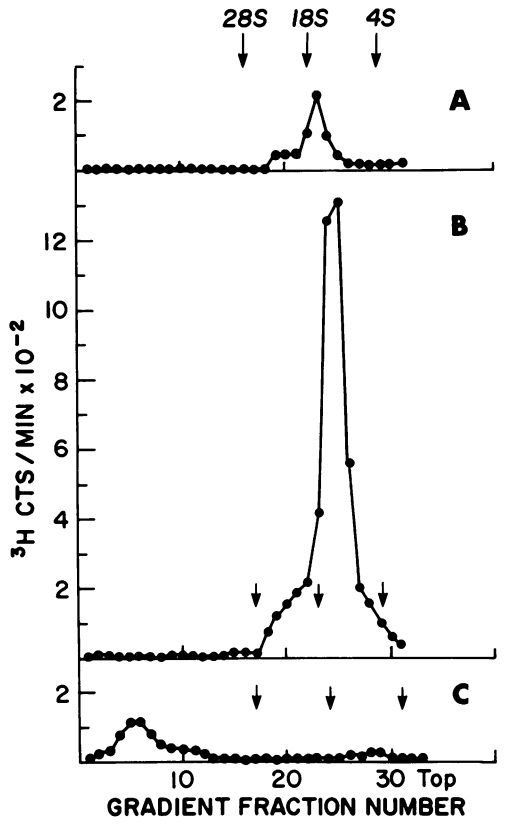

FIG. 4. *RNA from cytoplasmic nucleocapsids. ^{3}H-RNA was extracted from appropriate regions of the gradients depicted in Fig. 1 by phenol-SDS and was analyzed on 17-ml 5 to 20% sucrose gradients prepared in 0.1 M acetate buffer, pH 5.0. Panel A is the RNA extracted from the 110S peak in Fig. 1B; B, the 110S peak of Fig. 1C; and C, the 200S peak also of Fig. 1C. Centrifugation was for 13 h at 23,000 rpm and 4 C in an SW27 rotor. ^{14}C-Vero cell RNA was added to each tube as marker. Sedimentation is from right to left.*

four peaks of radioactivity corresponding to RNA species with sedimentation coefficients of 50, 30, 22, and 14 to 16S. RNA species of this size have been previously reported to be produced in measles-infected cells (3, 11, 19; M. P. Kiley and F. E. Payne, *in* R. Barry and B. W. J. Mahy ed., Negative strand viruses, Academic Press Inc., 1974 in press). RNA of a pellet from a detergent-treated (see Fig. 1C) cytoplasmic extract (Fig. 5B) was qualitatively and quantitatively different when compared with Fig. 5A. The pellet derived from a detergent-treated cytoplasmic extract yielded RNA which sedimented principally in the 10 to 16S and 20 to 22S regions of the gradient. These RNA species were present in much smaller amounts and little or no 50 or 30S RNA was found when the pellet was from detergent-treated extract. In results not presented it was determined that the RNase-sensitive RNA found in the 30 to 70S region of the 15 to 30% gradient in Fig. 1C contained little or no 50S RNA.

These observations indicate that essentially all 50S virus-specific RNA was associated with 200S nucleocapsids and that essentially all of these nucleocapsids were associated with membranes. Because measles virus tends to be cell associated, it is probable that at least some of the 200S nucleocapsids were present in virions. Although some of the 110S nucleocapsids and their 16S RNA appeared in the body of gradients even when cytoplasmic extracts were not treated with detergent, a portion of this population of nucleocapsids, like the 200S variety, was membrane associated. Judging from the size of 30S RNA, at least a portion of this species may have been present in 170S nucleocapsids which were exclusively membrane associated. The remaining major species of virus-specific RNA, i.e., 20 to 22S and 10 to 16S, was presumably mRNA, some of which was in membrane-bound polyribosomes.

Occurrence of short nucleocapsids in cells infected with various strains of measles virus. Information bearing on the significance of

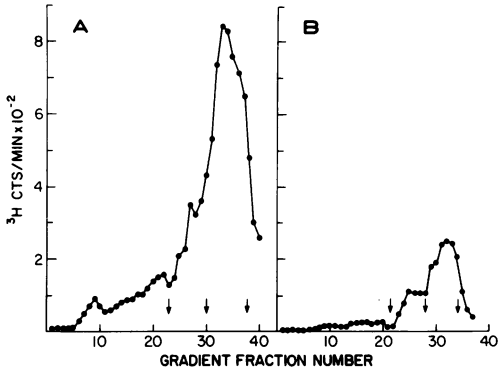

FIG. 5. *Analysis of ^{3}H-RNA from pellet fractions of detergent (deoxycholate and BRIJ-58) and nondetergent-treated infected cell cytoplasmic extracts. Pellets from the gradients depicted in Fig. 1B and C were individually suspended in RSB, and their RNA was extracted by phenol-SDS and analyzed on 5 to 20% sucrose gradients prepared in 0.1 M acetate buffer, pH 5.0. Conditions of centrifugation were as described in Fig. 4. Panel A represents RNA from the pellet of the gradient depicted in Fig. 1B, and panel B is the RNA from the pellet of the gradient depicted in Fig. 1C. Sedimentation is from right to left and arrows represent the position in the gradient of ^{14}C-labeled 4, 18, and 28S Vero cell marker RNA as in Fig. 4.*

the 110S nucleocapsids, which were observed in the above experiments, was forthcoming from examination of nucleocapsids in cells infected with different strains of measles virus. Only 200S nucleocapsids were observed in cells infected with low-passage virus, strain MUN-HT (12), which was isolated from a patient with SSPE. This observation suggested that production of 110S nucleocapsids was not obligatory to measles virus replication and that these short nucleocapsids might represent defective nucleocapsids containing RNA which is smaller than the viral genome.

Short nucleocapsids containing subgenomic RNA might be expected to occur under conditions, such as passage of virus using undiluted inocula, which would favor production of defective virus. Measles virion RNA that was smaller than 50S was recently reported to appear in virus stocks after repeated undiluted passage (3). The same report indicated that the smaller RNA may disappear from a measles virus stock after passage with diluted inoculum. Plaque purification of a virus represents an efficient means of diluted passage. Accordingly, we examined nucleocapsids produced by six different plaque strains which were derived from wild-strain Edmonston virus. Plaques of each strain were picked three times sequentially, and working pools were then prepared.

At 3 days after infection with a plaque-purified strain at approximately 0.05 PFU/cell, Vero cells were treated with actinomycin D and labeled with ^{3}H-uridine. Cytoplasmic extracts, after treatment with detergent and EDTA, were analyzed in 15 to 40% sucrose gradients prepared in RSB-EDTA. Cells infected with any one of five of the plaque-purified strains yielded 200S, but no detectable 110 or 170S, nucleocapsids as exemplified in Fig. 6. One of these strains produced 110S nucleocapsids after undiluted passage. The initial pool of the sixth plaque-purified strain produced both 200 and 110S nucleocapsids. Although other interpretations are possible, these observations are compatible with the suggestion that 110S nucleocapsids represent encapsidated-defective subgenomic viral RNA.

DISCUSSION

In the present study, cytoplasmic extracts of cells infected with wild-strain Edmonston measles virus were found to contain virus-specific RNA associated with polysomes, which were not prominent in gradient analyses, and nucleocapsids, which were present as two (200 and 110S) and probably a third (170S) distinct species. The largest nucleocapsids resembled those previously isolated from purified measles virions (5) and contained the putative complete viral genome as 50S RNA. Detailed characterization of the 170S nucleocapsids was not accomplished because they were not always present in infected cells. The 110S nucleocapsids, like the 200S species, had herring-bone morphology but they were shorter and contained correspondingly smaller RNA which sedimented at 16 to 18S.

Essentially all the 200 and 170S, as well as a portion of the 110S, nucleocapsids were membrane associated. The known tendency of measles virus to remain cell associated, and reports of both 50S and smaller species of RNA in measles virion preparations (3), suggest that at least a portion of the various species of nucleocapsids that were associated with membranes were contained in virions. Thus, previ-

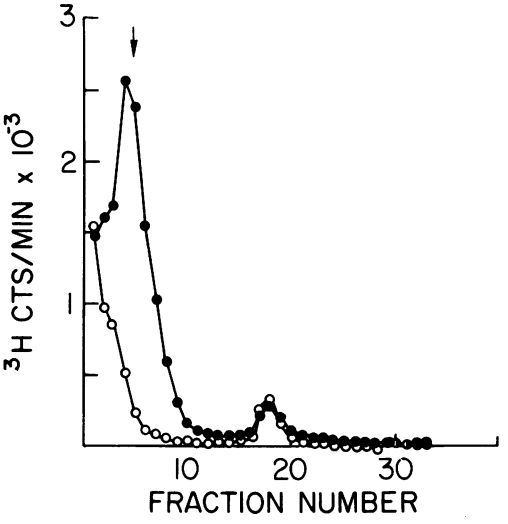

FIG. 6. *Nucleocapsids synthesized in cells infected with a plaque-purified strain of measles virus. Plaque-purified virus was prepared and Vero cells were infected at a multiplicity of approximately 0.05 PFU/cell. On day three postinfection cells were treated with 25 μg of actinomycin D per ml and 2 h later ^{3}H-uridine was added to a final concentration of 20 μCi/ml. After a 4-h labeling period, cells were harvested and a cytoplasmic extract was prepared by Dounce homogenization. The extract was made 0.02 M with respect to EDTA and was detergent treated prior to analysis on a 15 to 40% sucrose gradient prepared in RSB-EDTA buffer. Conditions of centrifugation are as described for Fig. 2. Acid-precipitable radioactivity was determined on a sample of each fraction prior to (●) and after (○) treatment with 20 μg of ribonuclease per ml for 20 min at room temperature. The arrow indicates the position of the 50S cellular ribosomal subunits.*

ous reports of RNA smaller than 50S in association with measles virions (3) may reflect incorporation of short nucleocapsids, with their relatively small RNA, into virus particles. In this regard, cytoplasmic extracts of cells infected with vesicular stomatitis virus were found to contain the same three species of nucleocapsids present in the virion population which contained both complete and defective particles (8). The intracellular nucleocapsids were presumably immediate precursors of virions. The findings by Kingsbury et al. (9) of subgenomic RNA associated with nucleocapsids of Sendai virions suggests that a similar phenomenon occurs with this virus.

Until the polarity and specificity of RNA found in the various cytoplasmic structures of measles-infected cells are established by nucleic acid hybridization experiments, the available data should be interpreted with reservation. However, measles virus like other paramyxoviruses (9) probably produces defective virus particles which contain subgenomic RNA. This suggestion is supported by the report that RNA smaller than 50S may disappear from a measles virus stock after passage with diluted inocula (3), and by our observation that short nucleocapsids were not produced by most plaque-purified stocks until they were passaged by using undiluted inocula.

Direct evidence of interference with measles virus replication by defective virus particles has not been reported. However, the possible role of defective interfering measles virus in SSPE has been considered (7). The possible involvement of measles virus subgenomic RNA and short nucleocapsids in the nonproductive infected cells from brains of patients with SSPE (1, 13, 14) is being investigated.

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