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Properties of Visna Virus Particles Harvested at Short Time Intervals: RNA Content, Infectivity, and Ultrastructure

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The major RNA component of Visna virus harvested at short intervals of time (5 min) is not the 60 to 70*S* RNA but a molecule of higher electrophoretic mobility. This RNA has been isolated and characterized. Its sedimentation coefficient is identical to that of 30 to 40*S* RNA subunits obtained by heat denaturation of the 60 to 70*S* RNA. In 1.8% acrylamide gels without agarose the electrophoretic mobility of 30 to 40*S* RNA subunits present in rapidly harvested virus is slightly lower than that of the subunits obtained by denaturation of the 60 to 70*S* RNA; after heat denaturation the mobilities are identical. These free RNA subunits present in early virus particles assemble into a 60 to 70*S* RNA complex as shown by following the RNA content of early virus incubated at 37 C for various lengths of time. The rate of this maturation process is slow. There is no difference between the infectivity of immature and mature virus particles. Both particles have a dense core when examined in sections of virus pellets.

Visna virus is the agent of a slow demyelinating disease of the central nervous system of sheep (21). The resemblances between Visna virus and the RNA tumor viruses are numerous and include the presence of 60 to 70*S* RNA which can be dissociated into 30 to 40*S* subunits (2, 3, 10, 16). This RNA has an important secondary structure and is rather extensively "nicked" when extracted from particles harvested at long time intervals (3). It contains a poly(A) segment of large and homogeneous size (9). The virus particle, whose structure is very close to that of RNA tumor viruses (18, 26), contains an RNA-dependent DNA polymerase (15, 20, 23) and replicates through the intermediate of a DNA provirus (11). Lastly, Visna virus has been reported to transform murine cells in vitro (25). However, this virus does not induce tumors in its natural host, and in sheep cells it undergoes a typical lytic cycle with no sign of cell transformation.

There is evidence that free 30 to 40*S* RNA can be recovered, together with 60 to 70*S* RNA, from certain type C viruses. These included Maedi (17) and progressive pneumonia (24) viruses, which are closely related to Visna virus. It was also found (4, 5) that Rous sarcoma virus harvested at short intervals of time contains 30 to 40*S* RNA which is converted to 60 to 70*S* RNA during incubation of the virus at 37 C. These results prompted us to examine the RNA content, and some other properties, of Visna virus harvested at short intervals of time (5 min). We indeed found that these particles contain 30 to 40*S* RNA which is progressively transformed into 60 to 70*S* RNA, although at a much slower rate than that observed with Rous sarcoma virus (5). We also show in this article that rapidly harvested Visna virus has the same infectivity as virus harvested at hourly intervals and that its morphological appearance is already that of a dense core particle.

MATERIALS AND METHODS

Viruses. The K 796 strain of Visna virus (kindly supplied to us by G. Pétursson, University of Reykjavik, Iceland) was used throughout this work. The virus stock was grown on sheep choroid plexus cells at their 17th passage with a multiplicity of infection of 0.1 PFU/cell. The virus was titrated on the same cells by a plaque assay (28); its titer was 1.2×10^7 PFU/ml. Sendai virus (Harris strain) was a gift of D. Kolakofsky, Université de Genève, Geneva, Switzerland.

Reagents and buffers. Eagle medium was purchased from Eurobio, Paris, and sheep and calf sera from Sorga, Paris, France. Purified influenza virus used as a carrier was a gift from Institut Mérieux, Lyon, France. Diethylpyrocarbonate, *N,N,N',N'*-tetramethylethylenediamine, and ethylenediacrylate were from Eastman Organic Chemicals. Acrylamide and bisacrylamide were from Biorad. RNase-free sucrose, used for RNA work, was purchased from Schwarz-Mann. [^{3}H]uridine (20 Ci/mM) and carrier-free ^{32}P were from CEA, France. All other chemicals were obtained as reagent grade.

TNE buffer refers to the following solution: 0.01 M Tris-hydrochloride (pH 7.5), 0.1 M NaCl, and 0.001 M EDTA. TNE-SDS buffer refers to the same solution containing 0.5% sodium dodecyl sulfate (SDS). For the RNA work, the buffers and plastic pieces of equipment were treated with diethylpyrocarbonate

(22) and the glassware was heated at 180 C for 90 min to inactivate any contaminating RNase.

Radioactive labeling of virus RNAs. Sheep choroid plexus cells were used at passage levels not higher than twenty. The growth medium for these cells was Eagle minimum essential medium (MEM) supplemented with 15% of a mixture (2:2:1) of calf, lamb, and embryonic bovine sera. When confluent, the cells were infected at a multiplicity of infection of 0.1 PFU/cell. The medium used after infection was MEM supplemented with 2% lamb serum. When a cytopathic effect was first noticed, 1 mCi of [^{3}H]uridine (20 Ci/mM) was added per 75-cm^2 Falcon plastic culture flask containing 5 ml of MEM medium supplemented with 0.2% bovine serum albumin. The cultures were incubated at 37 C for 18 h and then harvested. The cells were rinsed three times with fresh medium to remove residual virus and then overlaid with 5 ml of medium which did not contain radioactive label. The harvests were repeated every 5 min or 2 h, depending on the experiment, for as long as 8 h. Each harvest was cooled to 0 C and kept at this temperature prior to virus purification. Care was taken during the harvesting period to avoid a change in the temperature of the cells. When labeling with ^{32}P, MEM lacking phosphate was used and 1.5 mCi of carrier-free ^{32}P was added per 75-cm^2 Falcon plastic culture flask containing 5 ml of medium. For the rest of this paper, virus harvested every 5 min will be referred to as early virus.

Sendai virus was grown in embryonated eggs in the presence of ^{32}P as described (14).

Virus purification; RNA extraction. Visna virus was purified as previously described (2) except for the last sucrose gradient which was omitted in most of the experiments. Briefly, the different steps of the purification were: (i) low speed centrifugation; (ii) centrifugation at 100,000 × *g* for 1 h to pellet the virus; (iii) centrifugation of the resuspended virus pellet (SW50 rotor, 50,000 rpm, 50 min) through a 20% sucrose solution on top of a 70% sucrose pad; (iv) isopycnic centrifugation of the virus in a 15 to 60% linear sucrose gradient. Purified influenza virus (MRC-2 strain, grown in embryonated eggs) was added to the culture medium at the final concentration of 1 μg/ml prior to the purification of early virus, to serve as a carrier. This precaution was necessary since in the initial steps of these purifications Visna virus particles were extremely diluted in a large volume of medium. RNA was extracted immediately after collecting the virus band from the sucrose gradient. The virus suspension was diluted with 1 volume of TNE-SDS buffer, diethylpyrocarbonate was added to a final concentration of 0.1% (vol/vol), and total yeast carrier RNA was added to a final concentration of 100 μg/ml. The suspension was then extracted for 10 min at room temperature by 1 volume of a mixture of phenol, metacresol, and 8-hydroxyquinoline (5 g of redistilled phenol, 0.7 ml of redistilled metacresol, 5 mg of 8-hydroxyquinoline) saturated with TNE-SDS buffer. The phenol phase was reextracted once with TNE-SDS buffer to ensure maximum recovery of RNA. The RNA in the combined aqueous phase was precipitated at −20 C by 2 volumes of ethanol after the addition of 0.1 volume of a 20% sodium acetate, pH 5.0, solution. The RNA was recovered by centrifugation at 1,000 × *g*. After drying, the RNA pellet was dissolved in the appropriate buffer. The same methods were used for the purification of Sendai virus and the extraction of its RNA. ^{32}P RNA of the Moloney strain of murine sarcoma leukemia virus [M-MVS (MLV)] grown on 78 A 1 cells was a gift from C. J. Larsen, Hôpital Saint-Louis, Paris, France.

Cellular RNAs. Choroid plexus cells were labeled with 0.1 mCi of [^{3}H]uridine (20 Ci/mM) per 75-cm^2 Falcon plastic flask. After overnight incubation at 37 C, cellular RNAs were extracted from the total cytoplasmic fraction as described (1).

RNA analysis. Sucrose gradients were as described (3). Electrophoresis in 1.7% acrylamide gels crosslinked with bisacrylamide and containing 0.5% agarose was according to Tiollais et al. (27). The gels were cast in glass tubes (0.5 by 12 cm). The electrophoresis buffer contained 89 mM Tris borate (pH 8.4) 1 mM EDTA, and 0.5% SDS. RNA samples were dissolved in 10 μl of half-strength electrophoresis buffer, mixed with 10 μl of half-strength buffer solution containing 0.1% bromophenol blue and 60% sucrose, and applied to the top of the gels. Electrophoresis was at 19 C with a current of 3 mA/gel until the tracer dye had reached the bottom of the gel. The gels were sliced and the radioactivity of each 1.5-mm thick section was measured after incubation for 1 h at 50 C in 3 ml of toluene-based scintillation fluid containing 20% NCS and 2% water. The counts recoveries for tritium were higher than 90%. For heat denaturation, the RNA samples (1 to 5 μg/μl) dissolved in 10 μl of half-strength electrophoresis buffer were placed in sealed capillary glass tubes, heated to 70 C for 3 min and quick cooled in an ice-water bath.

Electrophoresis in 1.8% acrylamide gels crosslinked with ethylene diacrylate was essentially as described by Duesberg (8). The gels were cast in Plexiglas tubes (0.6 by 6 cm). The electrophoresis buffer contained 40 mM Tris acetate (pH 7.0), 20 mM sodium acetate, 10 mM EDTA, and 0.2% SDS. RNA samples were dissolved in 50 μl of electrophoresis buffer diluted 1:10 except for the SDS and containing 5% glycerol and 0.1% bromophenol blue. Electrophoresis was at 5 mA/gel for 6 h. The radioactivity of 1-mm-thick sections was measured as described above for gels containing agarose. Counts recoveries were 90% or higher. For heat denaturation the RNA samples, dissolved in diluted electrophoresis buffer, were heated at 85 C for 50 s in sealed capillary glass tubes.

Electron microscopy. Medium from infected cell cultures was centrifuged at 10,000 × *g* for 10 min to remove cell debris. The supernatant was centrifuged at 100,000 × *g* for 1 h in the Spinco SW50 rotor. The virus pellet was fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, dehydrated, and embedded in araldite. Ultra-thin sections stained with uranyl acetate and lead citrate were examined with the use of a Philips EM 201 microscope.

RESULTS

RNA content of Visna virus harvested at 5-min intervals. Radioactively labeled Visna virus was harvested every 5 min as described.

After purification, an aliquot of the virus suspension was centrifuged in a 10 to 50% linear potassium tartrate gradient (data not shown). The virus banded at a density of 1.17 g/ml, a density identical to that of older virus. The RNA was extracted from the rest of the virus suspension as described. For the sake of comparison, ^{3}H-labeled RNA was also prepared from Visna virus harvested after an 18-h labeling period. Both RNA preparations were subjected to electrophoresis in mixed polyacrylamide agarose gels either native or heat denatured. Native ^{32}P-labeled M-MSV (MLV) RNA was added to the sample prior to loading on the gels, to serve as internal marker. The results of these experiments are shown in Fig. 1. Visna virus from an 18-h harvest (Fig. 1B) gave a single peak of RNA of lower mobility than M-MSV (MLV) RNA. This peak corresponds to the 60 to 70*S* RNA previously described (3). After melting this RNA was converted into 30 to 40*S* RNA subunits and a heterogeneous population of smaller molecules (Fig. 1D). On the contrary early Visna virus exhibited two separate peaks of native RNA (Fig. 1A). The minor peak migrated like 60 to 70*S* RNA, and the major one was a new species with electrophoretic mobility higher than that of M-MSV (MLV) RNA. The sedimentation properties of this RNA will be described in the following section. After heat denaturation the RNA of early virus (Fig. 1C) migrated ahead of M-MSV (MLV) RNA as a single homogeneous peak which corresponded to 30 to 40*S* RNA subunits. These experiments clearly show that the major RNA component of early virus is not the 60 to 70*S* RNA but an RNA species with a higher electrophoretic mobility.

Repeated 5-min harvests could select a population of virus different from the newly released virions normally present in hourly harvests. One way to ensure that this is not the case is to compare the recovery of radioactivity for virus obtained by rapid harvests with that of virus obtained by long harvests. In the experiment shown in Fig. 1, 2,900 counts/min were recovered per h of virus production in the 60 to 70*S* RNA of the virus harvested after an 18-h labeling period, as compared to 2,200 counts/min per h of virus production for the 60 to 70*S* and 30 to 40*S* RNAs of virus harvested every 5 min (see legend of Fig. 1). That these two values are in close agreement indicates that repeated 5-min harvests did not change the rate at which the virions are released from the cells. In the experiment the radioactive precursor was absent from the medium during rapid harvests, although it was present in the medium of the

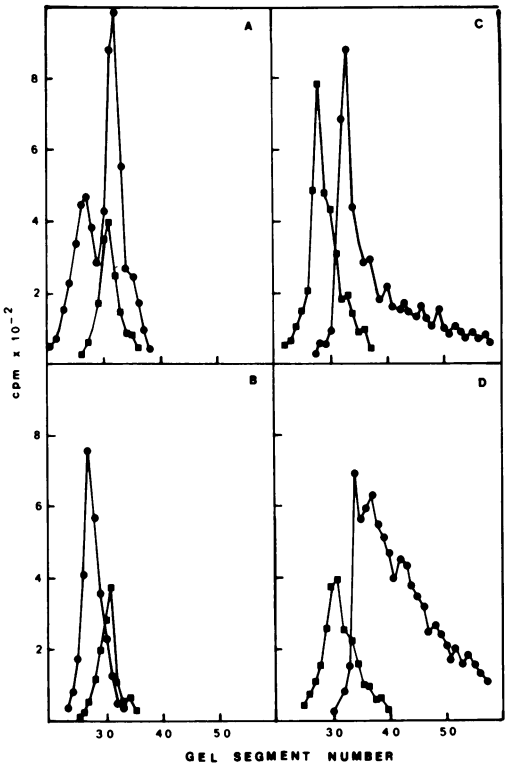

FIG. 1. *Electrophoresis in 1.7% acrylamide, 0.5% agarose gels of: (A) native ^{3}H-labeled RNA extracted from Visna virus harvested every 5 min; (B) native ^{3}H-labeled RNA extracted from Visna virus harvested after 18-h labeling period; (C) heat-denatured ^{3}H-labeled RNA extracted from Visna virus harvested every 5 min; (D) heat-denatured ^{3}H-labeled RNA extracted from Visna virus harvested after an 18-h labeling period. Two 75-cm² Falcon plastic culture flasks containing approximately 5 × 10⁶ cells were infected at a multiplicity of infection of 0.1 PFU/cell and labeled with 200 μCi of [^{3}H]uridine per ml (5 ml of medium per flask). The cultures were harvested after an 18-h labeling period. After rinsing, the cultures were harvested every 5 min for 6 h as described. The volume of the pooled 5-min harvests was 720 ml. The virus was purified from both the 18-h harvest and the pooled 5-min harvests, and the viral RNAs were extracted. 53,000 counts/min were obtained for the 60 to 70S RNA of the virus harvested after an 18-h labeling period and 13,000 counts/min for the 60 to 70S and 30 to 40S RNAs of the early virus. Approximately 3,000 counts/min of ^{3}H-labeled RNA were applied on top of each gel. ^{32}P-labeled RNA from M-MSV (MLV) (■) was added as an internal marker to the samples just before loading the gels. From each gel 70 segments were obtained and counted. Only those with counts higher than 40 counts/min have been used for plotting the results. Migration was from left to right.*

18-h harvest. The comparison of counts recoveries is nevertheless justified since we found in preliminary experiments (unpublished data)

that 5 h is required for maximum incorporation of [^{3}H]uridine by sheep choroid plexus cells, and that once the label has been removed the intracellular level of radioactivity remains constant for at least 10 h.

We then decided to investigate the possibility that the new RNA present in early virus consists of free 30 to 40S RNA subunits by examining its sedimentation and electrophoretic properties.

Sedimentation and electrophoretic properties of the RNA present in early virus. The two high-molecular-weight RNAs present in early Visna virus were separated on a preparative sucrose gradient (data not shown). Two RNA peaks were detected. The minor one sedimented in the 60 to 70S region of the gradient, whereas the major peak sedimented in the 30 to 40S region. The fractions corresponding to this last peak were pooled and the RNA was precipitated with ethanol. We first estimated the sedimentation coefficient of this RNA on a sucrose gradient using the following markers: 58S Sendai RNA 28, 18 and 4S cellular RNAs. The profile of the gradient is presented in Fig. 2. As shown in the insert of this figure, we found a sedimentation coefficient of 37S very close to that (36S) of the undegraded subunits obtained by complete melting of Visna 60 to 70S RNA (3).

We then directly compared this RNA with 30 to 40S RNA subunits in a double-label experiment. ^{3}H-labeled 30 to 40S RNA present in early virus and ^{32}P-labeled 30 to 40S RNA subunits obtained by heat denaturation of 60 to 70S RNA from virus harvested every 2 h were co-electrophoresed in 1.8% diacrylate cross-linked polyacrylamide gels which did not contain agarose (see Materials and Methods). These gels have a high resolving power allowing, for example, the separation of the a and b subunits of Rous sarcoma virus 60 to 70S RNA. However, in our hands, they do not allow the analysis of native Visna 60 to 70S RNA which barely penetrates the gel. Using this condition, the free subunits present in early Visna virus had a slightly lower electrophoretic mobility than the subunits obtained by heat denaturation of Visna virus 60 to 70S RNA (Fig. 3A). However, the two RNAs co-migrated exactly when the free subunits present in early virus were heat denatured prior to electrophoresis (Fig. 3B). We believe this is due to a difference between the secondary structures of the two RNA molecules, this difference having disappeared when both molecules have been subjected to the same denaturing treatment (see Discussion).

Intravirial conversion of 30 to 40S RNA to 60 to 70S RNA. Our results so far suggested

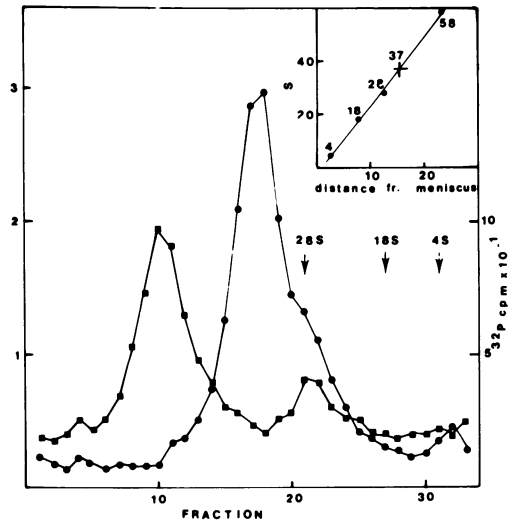

FIG. 2. *Sedimentation in a 5 to 20% linear sucrose gradient of native ^{3}H-labeled 30 to 40S RNA present in Visna virus harvested every 5 min (●). RNA extracted from purified early virus (34,000 counts/min obtained from 960 ml of medium) was separated on a preparative sucrose gradient into 60 to 70S and 30 to 40S components. The fractions corresponding to the 30 to 40S RNA peak were pooled and the RNA (21,000 counts/min) was precipitated with ethanol. The RNA was recovered by centrifugation and dissolved in TNE-SDS buffer, and 3,000 counts/min were mixed with 1,000 counts/min of ^{32}P-labeled 58S Sendai RNA (■) and layered on top of a 5 to 20% linear sucrose gradient in TNE-SDS buffer. The gradient was centrifuged at 50,000 rpm in the Spinco SW50 rotor for 60 min. The temperature during the run was +17 C. ^{3}H-labeled cellular RNA was centrifuged in a different tube of the same rotor. The two tubes gave the same number of fractions. The positions of cellular 28, 18, and 4S RNA are indicated by arrows. For the insert, the distances from meniscus were expressed as the number of fractions which separated the peaks from the top of the gradient.*

that free 30 to 40S RNA subunits present in freshly budded virions might be precursors for 60 to 70S RNA. To test the existence of such a precursor-to-product relationship it is necessary to follow the RNA content of virus particles at different times after their release from the cell. A similar study has been done with Rous sarcoma virus (5) by harvesting virus at different time intervals and examining its RNA content. However, in such an experiment each harvest in fact contains particles of all intermediate ages up to that corresponding to the time of the harvest. Therefore we preferred the alternative procedure which consists of incubating early virus at 37 C for different lengths of time and examining its RNA. In this last type of experiment we can observe the synchronous maturation of a population of early viruses.

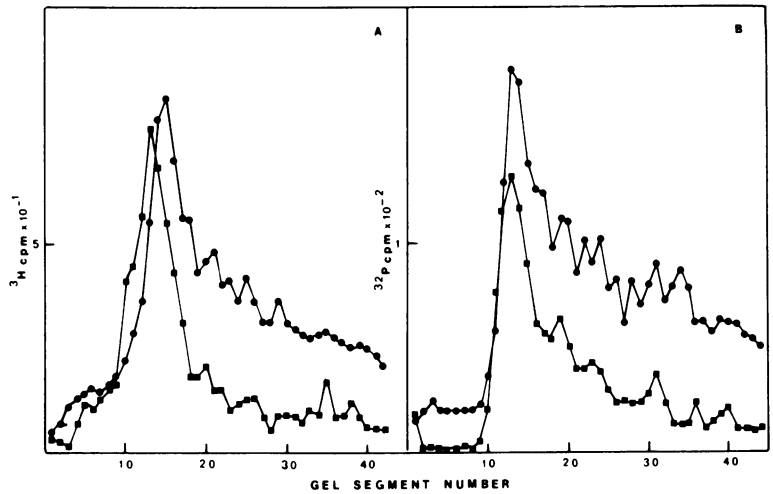

FIG. 3. *Electrophoresis in 1.8% diacrylate cross-linked polyacrylamide gels of the free 30 to 40S RNA subunits present in Visna virus harvested every 5 min (■, ^{3}H) and of the 30 to 40S RNA subunits obtained by heat denaturation of 60 to 70S Visna RNA (●, ^{32}P). The free 30 to 40S ^{3}H-labeled RNA subunits from virus harvested every 5 min were electrophoresed either in their native form (A) or after heat denaturation (B) together with heat-denatured 60 to 70S ^{32}P-labeled RNA extracted from virus harvested every 2 h. The 30 to 40S ^{3}H-labeled RNA was an aliquot (800 counts/min per gel) of the RNA preparation described in the legend of Fig. 2. ^{32}P-labeled 60 to 70S RNA was obtained from virus harvested every 2 h as described. From 40 ml of medium, 12,000 counts/min of 60 to 70S RNA were prepared and 3,500 counts/min were used per gel. The ^{3}H- and ^{32}P-labeled RNA samples were mixed just prior to electrophoresis. Radioactivity of 1-mm gel segments was determined as indicated for agarose-containing gels. Migration was from left to right.*

Since several 5-min harvests are required to obtain enough virus for RNA analysis, the simplest way to perform the experiment would have been to pool 5-min harvests kept at 0 C, shifting the temperature to 37 C and removing aliquots at different times. However, we decided to avoid the temperature shifts which could interfere with the putative maturation. In the end the experiment was performed as follows. The medium of [^{3}H]uridine-labeled infected cells was harvested every 5 min. Each harvest was collected in a separate vial and immediately incubated at 37 C in a water bath. The vials were removed from the water bath and quickly cooled after they had been incubated for 5, 25, 55, or 115 min. Some vials were immediately cooled to 0 C, instead of being immersed in the water bath, to obtain control early virus. Viruses were purified from the different samples and their RNA was extracted and analyzed in gel electrophoresis. Figure 4 shows the result of the experiment. Native RNA of early virus (Fig. 4A) exibited a pattern similar to that shown in Fig. 2A. By 10 min (Fig. 4B), an increase in the proportion of 60 to 70S RNA was observed. After 30 min (Fig. 4C), the 60 to 70S RNA became the major RNA. The intermediate species shown in this figure was not consistently found. After 1 h (Fig. 4D), the maturation was completed. All these RNAs were almost completely free of degradation as shown by their profiles after heat denaturation (Fig. 4F-J). This experiment demonstrated that 30 to 40S RNA subunits present in early Visna virus are precursors for the 60 to 70S RNA.

Infectivity of Visna virus harvested at 5-min intervals. At this point of our study, it was particularly interesting to compare the infectivity of immature early particles to that of older virus. Since RNA degradation, very likely accompanied by a loss of infectivity, has been observed for Visna virus harvested at long time intervals (3), it was essential to use for comparison a virus containing 60 to 70S RNA free from nicks. Figure 4F-J shows that early virus incubated at 37 C for 2 h meets this requirement. A modification of the plaque assay was also necessary in this experiment to prevent a maturation of early virus during the adsorption period which is normally performed at 37 C. The assay was modified as follows: after a 2-h adsorption period at 4 C, the cells were shifted to 37 C for 2 min to allow the adsorbed particles to penetrate and then were washed two times with cold medium. Agarose medium was added to the cells as usual. To obtain comparable results, the same technique was also used for testing the infectivity of early virus incubated at 37 C for 2

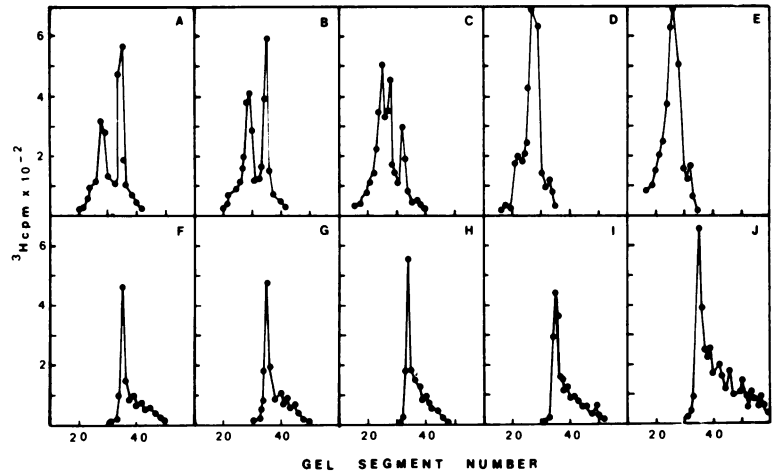

FIG. 4. *Electrophoresis in 1.7% acrylamide, 0.5% agarose gels of ^{3}H-labeled Visna virus harvested every 5 min and incubated at 37 C for various lengths of time. Each harvest was collected in a separate vial and immediately incubated at 37 C. The vials were quickly cooled after they had been incubated for various lengths of time and the harvests incubated for the same length of time were pooled. For each group of pooled harvests the volume of medium was 120 ml. Virus was purified from the different samples, and their RNA was extracted and analyzed in gel electrophoresis as described. Approximately 4,000 counts/min of viral RNA were obtained for each sample and 2,000 were loaded on each gel. (A–E) Native RNA from 5-min virus incubated at 37 C for 0 (A), 5 (B), 25 (C), 55 (D), and 115 (E) min. (F–J) Heat-denatured RNA from the same virus preparation. Migration was from left to right.*

h. The result of the experiment is shown in Table 1. No significant difference in titer was found between the two types of virus particles.

Electron microscopic study of freshly budded Visna virus. Previous studies (6, 7) had shown the existence in sections of infected cells of two types of extracellular particles. The first type has a centrally located dense core and resembles a type C particle very closely; the other type shows a clear granular center. The dense core particle has been associated with the infectious virion (6), whereas the significance of the clear particle has not been elucidated. Sarkar et al. (19) presented the hypothesis that the clear center particle observed in association with C type particles for several RNA tumor viruses could be an intermediate form between the bud and the mature C type particle. If this applies to Visna virus it was tempting to speculate that Visna clear-center particles possess free 30 to 40*S* RNA subunits whereas the 60 to 70*S* mature RNA is present in the C type particle. We thus decided to examine sections of early virus under the electron microscope. The virus was not purified for this experiment since we could not use carrier influenza virus to increase the yield of the purification. The culture medium from pooled 5-min harvests was processed as described above. Figure 5 shows an example of the particles that were observed. Clearly most of them were of the dense core type: out of 783 particles only 13 had a clear center.

TABLE 1. *Infectivity of early virus before and after a 2-h incubation at 37 C*[a]

Particle age	PFU/ml
5 min	2.1×10^6
2 h	1.6×10^6

[a] A cell culture (75-cm^2 Falcon plastic flask) was infected with 0.1 PFU/cell. At the time of maximal virus production (48 h after infection), the medium was discarded and the cells were rinsed three times with fresh medium to remove residual virus. Five ml of fresh medium was added and the harvests were repeated every 5 min for 30 min. The last 5-min harvest (5 ml) was divided into 2 aliquots. One aliquot was immediately chilled to 0 C, and the other one was incubated at 37 C for 115 min prior to chilling. The plaque assay was carried out as described in the text.

DISCUSSION

Our study of the RNA content of Visna virus particles (Fig. 1) shows that virus harvested every 5 min contains, in addition to some 60 to 70*S* RNA, an RNA species of lower molecular weight (Fig. 1A) which is absent from viruses harvested every 18 h (Fig. 1B). It must be pointed out that no information was obtained in these experiments concerning the small 4 to 8*S* viral RNAs since in our acrylamide agarose gels

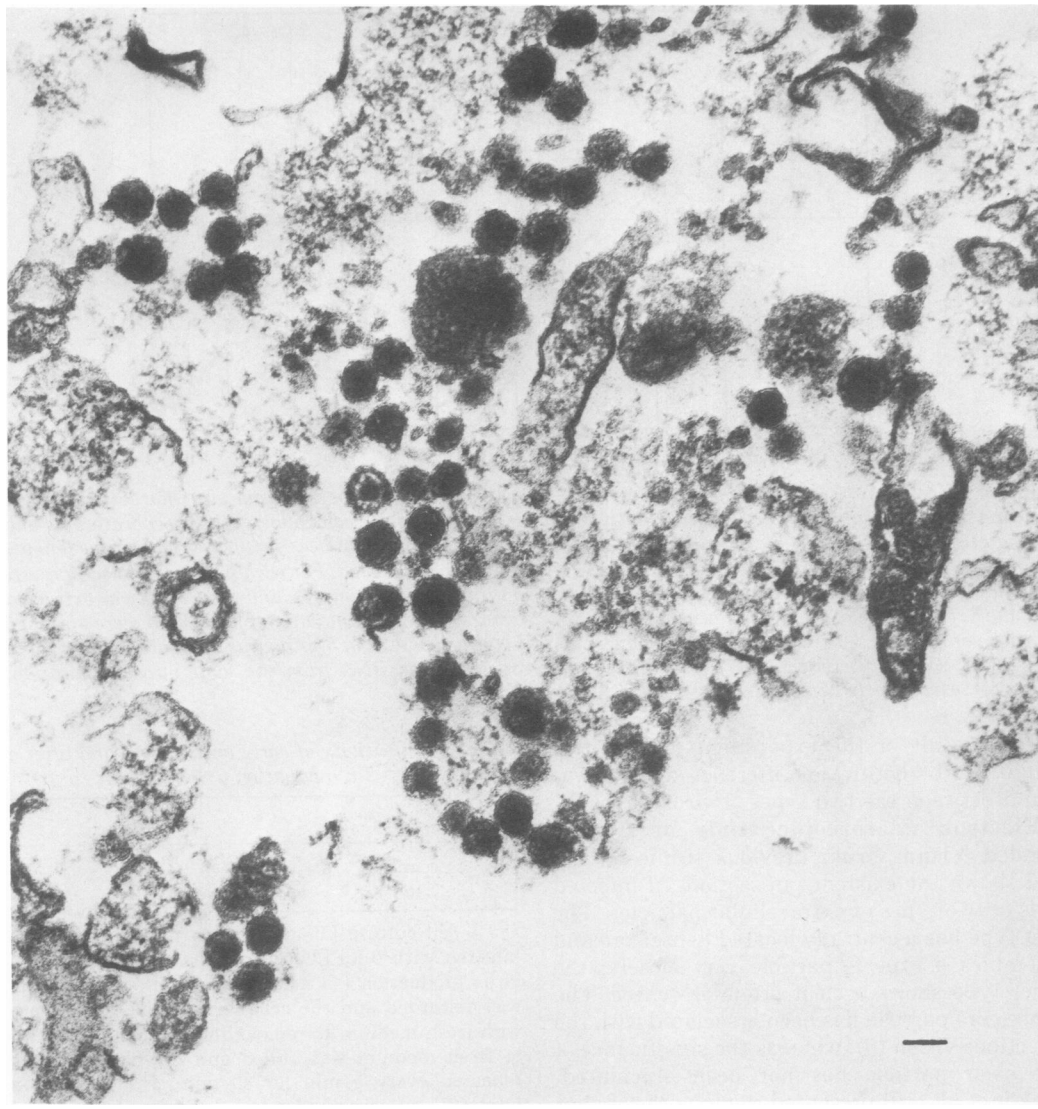

FIG. 5. *Electron microscopic aspect of Visna virus harvested every 5 min. Infected cell cultures were harvested every 5 min. The virus was concentrated from the culture medium by high speed centrifugation. The virus pellet was fixed, dehydrated, and embedded as described. Ultra-thin sections were made, stained with uranyl acetate and lead citrate, and examined in a Philips EM 201 microscope. The bar represents 1,000 Å.*

these molecules migrate faster than the tracer dye and are therefore lost during the electrophoresis. The results presented in Fig. 1 also confirm our previous observation (3) that Visna virus 60 to 70*S* RNA undergoes an extensive degradation during the incubation of the virus particle in the growth medium. This degradation can only be shown after complete melting of the molecule (Fig. 1C and D). It is also of interest to note that Visna virus 60 to 70*S* RNA is larger than the RNA of M-MSV (MLV) according to its electrophoretic mobility.

As already mentioned, the new RNA species present in early virus has the same sedimentation coefficient as subunits obtained by heat denaturation of 60 to 70*S* RNA. However, by using acrylamide gels of high resolving power a slight difference in electrophoretic mobility was found (Fig. 3A). This difference disappeared after heat denaturation of the free subunits present in early virus (Fig. 3B). A similar result has been obtained with Rous sarcoma virus (4), and it was suggested that the difference in electrophoretic mobility was due to the exist-

ence of small 4 to 5S RNAs associated with the 30 to 40S RNA in early particles (4). In connection with this it is interesting to note that no small RNAs have been found associated with the 60 to 70S RNA of Visna virus (10). Therefore we believe that the difference in electrophoretic mobilities shown in Fig. 3A is not due to 4 to 5S RNA associated with the free subunits present in early virus, but is the result of difference in the conformation of the two types of 30 to 40S RNA subunits. This is substantiated by the fact that after heat denaturation both RNAs had exactly the same electrophoretic mobilities (Fig. 3B). We were unable to rule out the possibility of the presence of a very small amount of 4 to 5S RNA associated with the free subunits of early virus because of the technical difficulties encountered in obtaining enough radioactivity in the RNAs of Visna virus harvested every 5 min.

In the experiment presented in Fig. 4, we were particularly interested in the exact rate of association of Visna virus 30 to 40S RNA subunits. We incubated early virus at 37 C for different lengths of time, instead of harvesting medium at different time intervals, to observe an almost synchronous maturation of the virus particles. During the course of the experiment we also avoided any temperature shift which could slow down the maturation process. Even with these precautions, Fig. 4 shows, for example, that the maturation of Visna RNA is still incomplete 30 min after the particle has been released from the cell. In contrast, Cheung et al. (5) have shown that almost no 30 to 40S RNA is present in Rous sarcoma virus harvested at 10-min intervals.

If, as suggested by some authors (4, 10), the small associated RNAs play a role in the association of the 30 to 40S RNA subunits within the 60 to 70S RNA complex, it is probably not surprising that the conversion of 30 to 40S RNA to 60 to 70S RNA should be slow with Visna virus (Fig. 4), which does not contain such small associated RNAs (10).

The formation of 60 to 70S RNA from 30 to 40S RNA subunits also demonstrates that the 30 to 40S RNA obtained by complete melting of 60 to 70S RNA is not the result of a cleavage of this large molecule.

Our study of the infectivity of early Visna particles before and after a 2-h incubation at 37 C (Table 1) shows that early particles are already infectious. This result suggests that early virus can attach to and penetrate the cells at a rate similar to that of mature virus. However, since we do not know the ratio of physical to infective particles for our virus population, we cannot conclude rigorously that these properties belong to particles containing free 30 to 40S RNA subunits.

Our observation that early virus particles possess a dense core when observed in thin sections of virus pellets does not exclude that the particles with a clear center observed in sections of infected cells are intermediate forms between the bud and the mature particle. However, if such a morphological maturation occurs, as suggested by some authors (19), it certainly does not correspond in time with the maturation of the RNA and has to be a much earlier event. An alternative interpretation is that the clear-center particles are in fact tangential sections of buds.

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