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Ribonucleic Acid Synthesis of Vesicular Stomatitis Virus

I. Species of Ribonucleic Acid Found in Chinese Hamster Ovary Cells Infected with Plaque-forming and Defective Particles

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Plaque-forming B particles of vesicular stomatitis virus (VSV) induce the synthesis of virus-specific ribonucleic acid (RNA) in Chinese hamster ovary cells, whereas defective T particles do not. Infection with low input multiplicities of B results in the formation of four species of RNA. During infection with high multiplicities, RNA synthesis begins with mainly these four species of RNA but gradually shifts to a new pattern of RNA synthesis involving five other species of RNA. The change can also be induced by superinfection with T at 2.5 hr after infection with a low multiplicity of B. T added at the same time as B prevents virtually all RNA synthesis. Synthesis of the first group of RNA species correlates with the formation of B particles, whereas synthesis of the second group correlates with the formation of T particles. The various species of RNA formed after infection with VSV particles include single-stranded RNA, a completely double-stranded RNA, and RNA with partially double-stranded regions. These observations begin to establish a molecular basis for understanding the ability of T particles to interfere with the growth of B particles.

Biochemical studies on the replication of vesicular stomatitis virus (VSV) are complicated by the fact that crude virus preparations contain not only bullet-shaped, infectious B particles but also defective T particles which interfere specifically with the growth of B (5, 8). Separation of the two particles by rate zonal centrifugation has shown that B and T are antigenically identical (9) and have the same polypeptides (11, 17); however, T contains only one-third the length of ribonucleic acid (RNA) found in B (4, 7).

Previously it was shown that T particles do not replicate in the absence of B but do arise from plaque-purified clones of VSV (18). Depending on the relative input ratio of B to T added to cell cultures, T can either partially inhibit the production of B while causing a large yield of T, or T can completely prevent the synthesis of both B and T particles. Because interference by T is an intracellular event requiring functional T RNA (8), we examined virus-specific RNA synthesis to understand better the interference phenomenon on a molecular level. In a previous report, Schaffer et al. (16) showed that VSV-specific RNA synthesis can be detected in infected cells and they partially characterized the RNA species which are formed.

MATERIALS AND METHODS

Cells and media. Chinese hamster ovary (CHO) cells (15), obtained from T. T. Puck, were used for all of the experiments. In this laboratory CHO cells were grown in continuous suspension culture maintained at 10^5 to 4×10^5 cells/ml in Eagle medium modified for Spinner culture with added nonessential amino acids and 7% fetal calf serum. Monolayer cultures of CHO cells were made by seeding $\sim 7.0 \times 10^5$ cells per 60-mm plastic Falcon petri plate and incubating them at 37 C in a humidified atmosphere of 5% CO_2 for 24 hr prior to use. The modified Eagle medium with added $CaCl_2$ at 1.8 mM was used for monolayer cultures and for all infections.

Viruses. The strain of VSV used in all these experiments was the large plaque variant of the Indiana serotype isolated and described by Wagner et al. (18). Plaque assay of VSV was done by established methods (18) with the appropriate medium and serum on CHO monolayers.

To prepare virus stocks, CHO cells were concentrated to 4×10^6 to 40×10^6 cells/ml and infected with ~ 0.01 plaque-forming unit (PFU)/cell for diluted passage or ~ 20 PFU/cell for undiluted passage. After a 30-min attachment period, the cells were diluted to 1.2×10^6 cells/ml and incubated at 37 C. VSV was harvested from the medium at 10 to 12 hr after infection for a diluted passage stock and at 16 to 20 hr after infection for an undiluted passage stock. Diluted passage stocks contained 0.5×10^9 to $2 \times$

10^9 PFU/ml, whereas undiluted passage resulted in production of mainly T particles and 0.1×10^8 to 3×10^8 PFU/ml. Two successive undiluted passages in CHO cells resulted in no growth of either B or T particles.

The methods for harvesting VSV and for purifying B and T have been described (9). Instead of 0 to 50% sucrose gradients for separating B from T, 5 to 40% sucrose gradients made up in reticulocyte standard buffer [RSB: 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.4; 0.1 M NaCl; 0.0015 M $MgCl_2$] were used. Small samples of B and T were stored at −70 C and were thawed just before use.

Incorporation of radioactive precursor into virus-specific RNA. CHO cells were concentrated 10-fold from suspension cultures, infected with B particles at the desired input multiplicity, and immediately thereafter exposed to 10 μg of actinomycin D per ml. After attachment at 37 C for 30 min, the suspended cells were either allowed to continue at 4×10^6 cells/ml or were diluted to 1.2×10^6 cells/ml and further incubated at 37 C. Radioactive uridine was added at the indicated times. To measure total incorporated ^{14}C-uridine, 0.1 to 0.2 ml of the infected cell culture was diluted into 1 ml of 5% trichloroacetic acid and filtered through an HA filter (Millipore Corp., Bedford, Mass.). The filter was washed with 5% trichloroacetic acid, dried, and counted in a Nuclear Chicago planchet counter.

Sucrose gradient fractionation of cytoplasmic RNA. Cytoplasmic extracts of infected CHO cells were made by standard techniques (14). RNA was solubilized by the addition of 1% sodium dodecyl sulfate (SDS) to cytoplasmic extracts at 22 C. The extracts were either stored at −20 C or immediately layered onto 15 to 30% sucrose-SDS gradients containing 0.01% SDS in SDS buffer (0.01 M Tris, *p*H 7.4; 0.1 M NaCl; 0.001 M ethylenediaminetetraacetate). Centrifugation at 22 C was done in either a Spinco SW27 or an IEC SB110 rotor for the desired length of time and speed to insure the display of 28*S* and 18*S* ribosomal RNA. For example, appropriate conditions were $58{,}000 \times g$ for 18.3 hr for an SW27 rotor or $60{,}000 \times g$ for 17.25 hr for an SB110 rotor. After centrifugation, the gradients were separated into fractions; absorbance at 260 nm was monitored on a Gilford recording spectrophotometer. Where indicated, hydrolysis with 50 μg of ribonuclease per ml was performed on half of each sucrose fraction by the method described by Baltimore (1). Radioactivity from sucrose gradient fractions was precipitated by trichloroacetic acid in the presence of carrier yeast RNA and processed as described elsewhere (Huang and Baltimore, *submitted for publication*). ^{14}C was counted on a Nuclear Chicago planchet counter and ^{3}H was counted in a Beckman LS250 scintillation counter. The pellet of every sucrose gradient was also assayed and the radioactivity was found to be negligible.

Materials. ^{14}C-uridine at ~50 mc/mmole and ^{3}H-uridine at >17 c/mmole were purchased from New England Nuclear Corp., Boston, Mass. Actinomycin D was a gift from Merck & Co., Inc., Rahway, N.J. Bovine pancreatic ribonuclease was obtained from Worthington Biochemical Corp., Freehold, N.J. and SDS was from Matheson & Co., Inc., East Rutherford, N.J.

RESULTS

Cumulative incorporation of uridine by cells infected with B or T. Studies of virus-specific RNA synthesis with crude preparations of VSV were not reproducible. At input multiplicities of 10 or greater, infected CHO cells usually did not incorporate any more uridine than uninfected CHO cells which were exposed only to actinomycin. However, if CHO cells were infected with purified B particles at multiplicities of 0.1 to 20, there was detectable virus-specific RNA synthesis. Figure 1 shows the accumulation of acid-precipitable radioactive uridine into CHO cell cultures treated with actinomycin D and either uninfected, infected with B at an input multiplicity of 1, or infected with T. T particles alone did not stimulate viral RNA synthesis, whereas B particles stimulated viral RNA synthesis up to 6 hr after infection.

To examine virus-specific RNA made during infection, cytoplasmic extracts were prepared after 6.5 hr of infection from the cell cultures shown in Fig. 1. Uninfected cells or cells infected only with T particles showed no detectable labeled RNA sedimenting faster than 4*S* (Fig. 2a). The pattern of labeled RNA from cells infected with B alone shows incorporation of radioactivity into RNA sedimenting at 28*S* and 13*S* (Fig. 2b). Although not shown here, almost all of the RNA could be hydrolyzed by ribonuclease (*see* Fig. 4a, b, c). From the broad distribution of the radioactive peaks when compared to the absorbance peaks of ribosomal RNA, it is obvious that the two peaks of radioactivity in Fig. 2b do not represent homogeneous species of RNA.

In this SDS buffer system, RNA extracted from B and T particles sediment at 40*S* and 19*S*, respectively. In experiments such as that depicted in Fig. 2b, neither 40*S* nor 19*S* peaks of radioactive RNA were evident, although a shoulder at 40*S* was often discernible. However, radioactive 40*S* RNA was readily found in extracellular fluids associated with B particles. RNA may be incorporated into B particles soon after its synthesis and be rapidly released from the cells.

Effect of time and multiplicity on VSV-specific RNA synthesis. To detect variations in RNA synthesis during the replication of VSV, cells were infected with B at an input multiplicity of 1 or 20. RNA made during the early (0.5 to 2.5 hr postinfection), middle (2.5 to 4.5 hr postinfection), and late (4.5 to 7.5 hr postinfection) periods of synthesis was separated on sucrose gradients. Both total labeled RNA and labeled ribonuclease-resistant RNA were de-

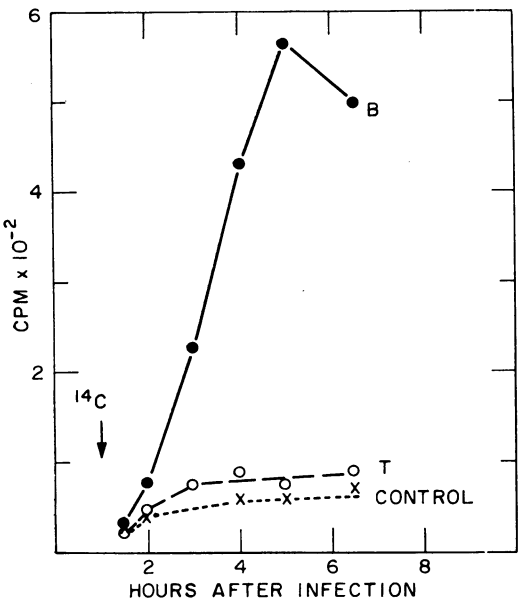

FIG. 1. *Cumulative incorporation of ^{14}C-uridine by cells infected with B or T. Three samples of 4 × 10^6 CHO cells were either mock-infected, infected with B at an input multiplicity of 1, or with T at a concentration which completely inhibited replication of B. After the attachment period, each sample was diluted with medium to 3 ml. At 1 hr postinfection, ^{14}C-uridine was added to a final concentration of 0.07 μc/ml. At the indicated times, 0.2 ml of the culture was analyzed for total radioactivity.*

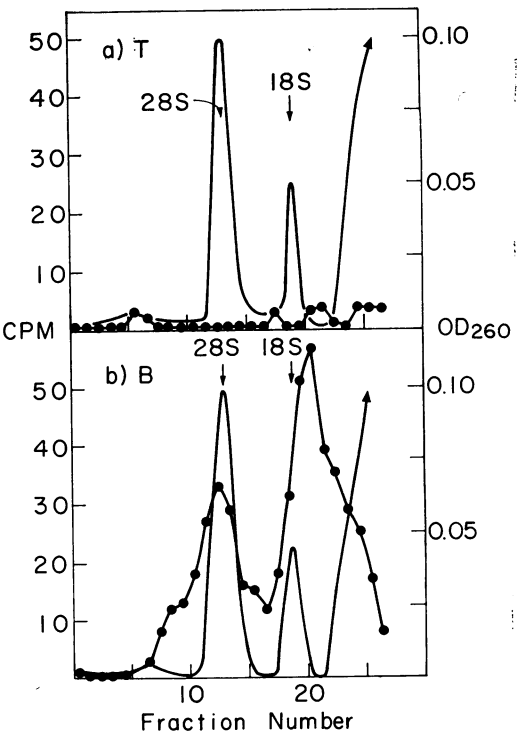

FIG. 2. *Sucrose gradient patterns of RNA synthesized by cells infected with B or T. CHO cells from the three samples shown in Fig. 1 were harvested at 6.5 hr postinfection, and the cytoplasmic extracts were fractionated by sucrose gradient centrifugation. The uninfected cells were identical to the cells infected with T particles so that their pattern is not shown. Symbols: ●——●, total ^{14}C; ——, absorbancy at 260 nm.*

termined for each fraction of the gradients. The initial accumulation of uridine into total viral RNA was faster with cells infected at a multiplicity of 20 than at a multiplicity of 1, but cells at both multiplicities eventually achieved the same degree of incorporation (Fig. 3).

At the low multiplicity, RNA synthesis was most rapid after the early period, and at all times incorporation of uridine was mainly into RNA sedimenting in the 28*S* and 13*S* regions (Fig. 4a, b, c). In addition, after the early period a minor radioactive peak appeared at 40*S* and a shoulder was found on the heavy side of the 13*S* peak (Fig. 4b and c). Virtually no ribonuclease-resistant RNA was detectable at any time.

At the high multiplicity there was a progressive change in the pattern of radioactive RNA (Fig. 4d, e, f). At the earliest time, 28*S* and 13*S* RNA predominated, but later 19*S* and 6*S* species of RNA dominated the pattern, and the 28*S* and 13*S* RNA species decreased markedly. In addition, there was an increased amount of ribonuclease-resistant RNA made during each successive period which presented a bimodal distribution at 15*S* and 19*S*. In several experiments the 15*S* ribonuclease-resistant RNA incorporated more uridine than the 19*S* ribonuclease-resistant species during the early and middle periods, with the reverse occurring during the late period.

The variations in RNA synthesis at the two multiplicities were also reflected in the release of B and T into the extracellular medium. To measure radioactivity in B and T, the medium from cells labeled during the middle and late periods was combined. The virions were pelleted and then separated in sucrose gradients. The ratio of the final yield of B to T from the infection with a multiplicity of 1 was 37:1, whereas at a multiplicity of 20 the ratio was 1:7. The yield of B at 7.5 hr after infection was also measured by plaque assay and found to be 1.5 × 10^9 PFU/ml for the low multiplicity infection and 2.1 × 10^8 PFU/ml for the high multiplicity infection.

RNA synthesis after superinfection with T.

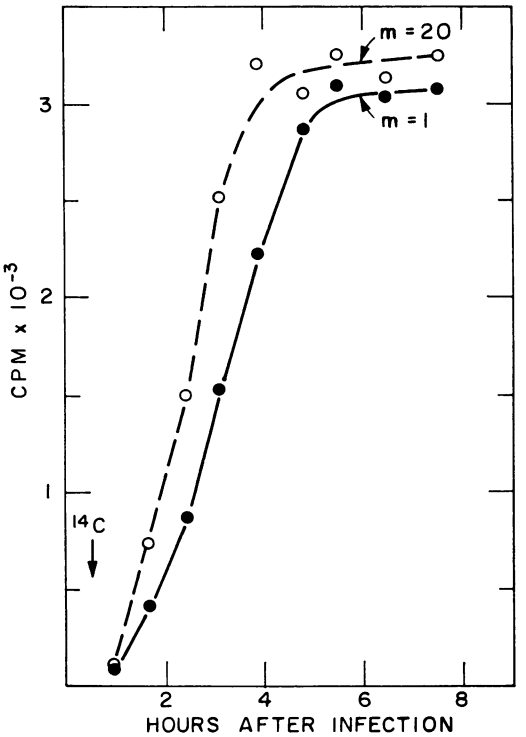

FIG. 3. *Cumulative incorporation of ^{14}C-uridine by cells infected at two different multiplicities. Two samples of 1.3 × 10^8 CHO cells were infected with B at an input multiplicity of 1 or 20. After the attachment period, the samples were washed two times with 35 ml of Earle saline and resuspended in 32 ml of fresh medium. At the time of resuspension, 2 ml was removed from each sample and exposed to 0.4 μc of ^{14}C-uridine per ml. At the indicated times, 0.1 ml from each of the 2-ml cultures was assayed for total radioactivity.*

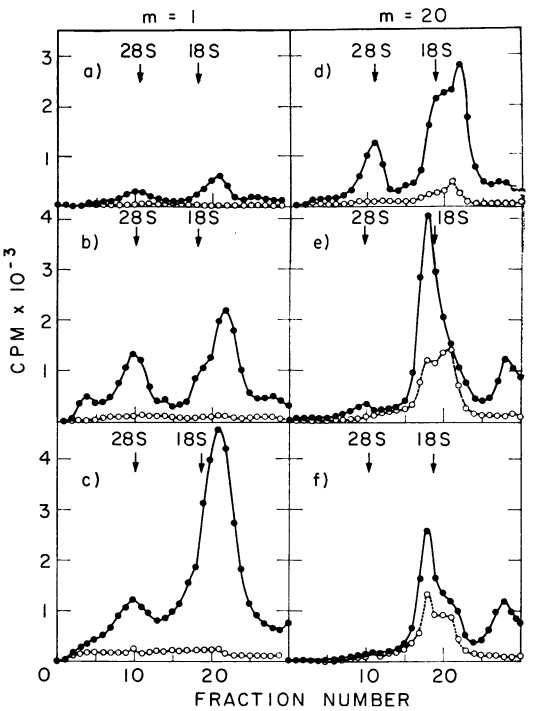

FIG. 4. *Sucrose gradient patterns of RNA synthesized by cells during three different periods of infection with B at an input multiplicity of 1 or 20. From the experiment shown in Fig. 3, the two 30-ml samples of infected CHO cells were each further divided into three portions. One portion of cells infected at each of the two multiplicities was exposed to 0.4 μc of ^{14}C-uridine per ml for each of the indicated periods. Cells were harvested at the end of each labeling period, and the cytoplasmic extracts were fractionated by sucrose gradient centrifugation. (a and d) RNA synthesized from 0.5 to 2.5 hr postinfection; (b and e) RNA synthesized from 2.5 to 4.5 hr postinfection; (c and f) RNA synthesized from 4.5 to 7.5 hr postinfection. Symbols: ●, total ^{14}C; ○, ribonuclease-resistant ^{14}C.*

Inhibition of the growth of B and the production of 19*S* RNA observed with cells infected with high multiplicities suggested the possibility that interference caused by T particles may also be reflected in RNA replication. Therefore, the patterns of RNA synthesis were examined with cells infected at a multiplicity of 1 which were also infected with T either at the same time as B or at 2.5 hr after attachment (Fig. 5). This experiment was performed at the same time as the experiment depicted in Fig. 4; therefore amounts of incorporated uridine are directly comparable. However, the differences of scale between Fig. 4 and 5 should be noted.

When cells were co-infected with B and T, the total accumulation of uridine was inhibited by approximately 90% when compared to cells infected with only B. The pattern of RNA synthesis after superinfection showed a small amount of 19*S* RNA and 6*S* RNA (Fig. 5a, b, c). This inhibition by T may explain the lack of viral RNA synthesis when cells were infected with crude preparations of VSV containing both B and T.

In contrast to the small amount of uridine incorporation when T particles were added at 0 hr, superinfection with the same amount of T at 2.5 hr only slightly decreased total incorporation of uridine during the middle period and inhibited incorporation about 50% during the late period. Also, superinfection with T caused a marked shift in the pattern of RNA synthesis from the profiles shown in Fig. 4b and 4c to a pattern resembling infection at high multiplicity. Figure 5d shows the expected 28*S* and 13*S* RNA synthe-

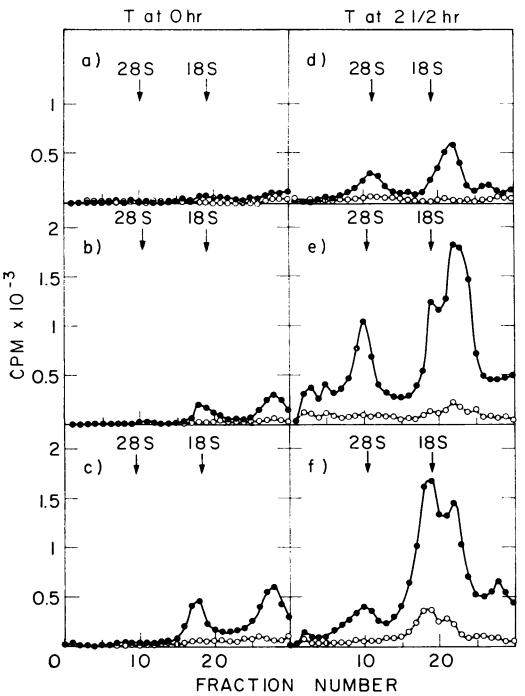

FIG. 5. *Sucrose gradient patterns of RNA synthesized by B-infected cells after superinfection with T at 0 hr or 2.5 hr. This experiment and the experiments shown in Fig. 3 and 4 were done at the same time, and similar procedures were used except for the additions of T particles. Two samples of 1.2 × 10⁸ CHO cells were infected with B at an input multiplicity of 1. One of the samples was co-infected with T. After attachment, both samples were washed, resuspended, and divided into three portions. Portions of cells co-infected with B and T were exposed to ^{14}C-uridine for the same three successive labeling periods shown in Fig. 4. Portions of cells infected with B alone were similarly labeled except that the two portions labeled for the last two periods were infected with T at 2.5 hr after infection with B, by adding an amount of T equivalent to the amount that was added to the cells co-infected with B and T. (a and d) RNA synthesized from 0.5 to 2.5 hr postinfection; (b and e) RNA synthesized from 2.5 to 4.5 hr postinfection; (c and f) RNA synthesized from 4.5 to 7.5 hr postinfection. Symbols: ●, total ^{14}C; ○, ribonuclease-resistant ^{14}C.*

sized during the early period (prior to addition of T). During the middle period (after exposure to T), 19*S* RNA was present in addition to the 28*S* and 13*S* RNA found in the control cells (cf. Fig. 5e and 4b). Late in infection, 19*S* was the predominant RNA species and inhibition of 28*S* and 13*S* RNA synthesis was quite evident (Fig. 5f). As seen during a high multiplicity infection with B alone, there is a bimodal distribution of ribonuclease-resistant RNA at 15*S* and 19*S* after superinfection with T, which is most pronounced during the late period (Fig. 5f).

The almost complete inhibition of 28*S* and 13*S* RNA synthesis when T was added at 0 hr was also reflected by a lack of detectable label incorporated into extracellular particles and an inhibition of greater than 99.99% of plaque-forming B. The partial inhibition of 13*S* and 28*S* RNA synthesis and enhancement of 19*S* RNA synthesis when T was added 2.5 hr after B also correlated with a ratio of uridine incorporation into B and T particles of 5:1, as compared to 37:1 produced by cells infected with B alone. Despite this shift to T production by the addition of T, the 7.5 hr yield of B was not markedly reduced when measured either by total radioactivity in B or by plaque assay.

Virus-specific RNA made during a 3-min exposure to ^{3}H-uridine. To enhance detection of double-stranded RNA species which may be sites of RNA synthesis, infected cells were exposed to radioactive precursor for 3 min at 3.75 hr postinfection. At a multiplicity of 1, two major heterogeneous classes of RNA were labeled: a class of ribonuclease-sensitive molecules sedimenting at 13*S* and a class of partially ribonuclease-resistant molecules sedimenting at 23 to 35*S* (Fig. 6a). In contrast, at a multiplicity of 15, most of the radioactive RNA sedimented in the region of 13 to 19*S*, including two peaks of ribonuclease-resistant RNA at 15*S* and 19*S* (Fig. 6b).

When T particles were added to cells at 2.5 hr after infection with B at a multiplicity of 1, there were, as expected, elements of both the high- and low-multiplicity infection. Label was found in both the 13 to 19*S* and 23 to 35*S* regions and both regions contained ribonuclease-resistant RNA (Fig. 6c).

If cells infected at a low multiplicity were labeled for longer times, such as 15 min, incorporation into the heterogeneous ribonuclease-resistant 23 to 35*S* material was not evident. In cells infected with a high multiplicity, however, even after 2 hr of labeling the 13 to 19*S* region contained labeled ribonuclease-resistant RNA (Fig. 4d, e, f).

Replicative intermediates and double-stranded RNA. The heterogeneous 23 to 35*S* RNA is implicated as a replicative intermediate by its preferential labeling during a short pulse of uridine, its heterogeneous sedimentation pattern and its ribonuclease resistance (cf. ref. 2). To distinguish between replicative intermediates and pure double-stranded RNA, precipitation by high concentrations of salt was used. Single-stranded RNA and replicative intermediates precipitate

in 2 M LiCl, whereas double-stranded RNA remains in solution (1).

At the low multiplicity, all of the RNA labeled within 3 min precipitated in LiCl and presented a sucrose gradient pattern similar to Fig. 6a. Therefore, at a multiplicity of 1 only single stranded RNA and partially double-stranded RNA became labeled in 3 min.

However, at a multiplicity of 20 with a 3-min exposure to radioactive uridine, precipitation by LiCl revealed that the 13 to 19S region contained both precipitable, partially ribonuclease-resistant molecules (Fig. 7b) and a fairly homogeneous, soluble, completely double-stranded 13S species (Fig. 7a). In contrast to previous findings with poliovirus that the double-stranded RNA is virtually unlabeled after a short exposure to uridine (2), the VSV-specific 13S double-stranded RNA seemed to be labeled preferentially with a

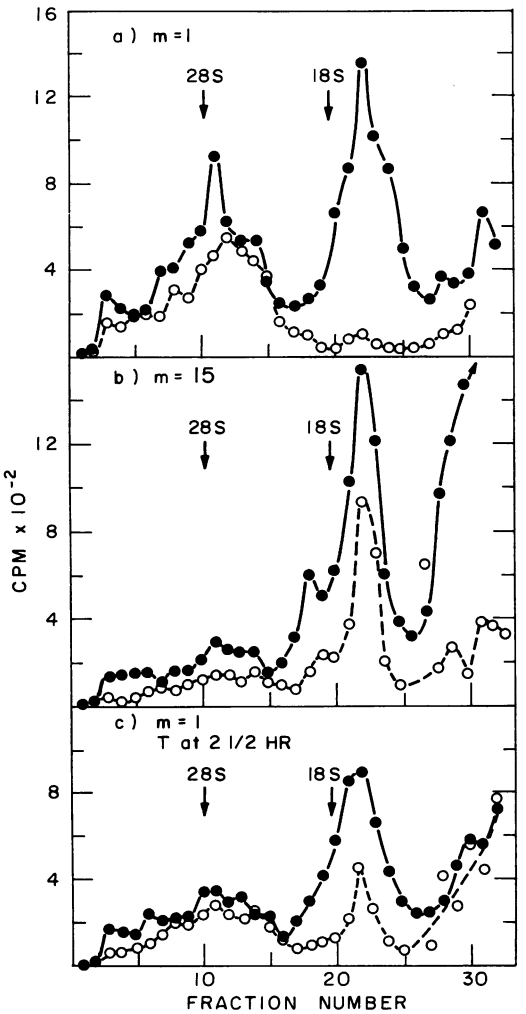

FIG. 6. *Sucrose gradient patterns of RNA synthesized by VSV-infected cells during a 3-min exposure to radioactive precursor. Two samples of 4 × 10⁷ CHO cells were infected with B at an input multiplicity of 1 and one sample of 4 × 10⁷ cells was infected with B at an input multiplicity of 15. At 2.5 hr postinfection, an amount of T equivalent to the amount used for the experiment shown in Fig. 5 was added to one of the samples infected with B at an input multiplicity of 1. All three samples were exposed to 25 μc of ³H-uridine per ml at 3.75 hr postinfection for 3 min. Incorporation was terminated by harvesting the cells into iced Earle saline. Cytoplasmic extracts were made and fractionated by sucrose gradient centrifugation. Symbols: ●, total ³H; ○, ribonuclease-resistant ³H.*

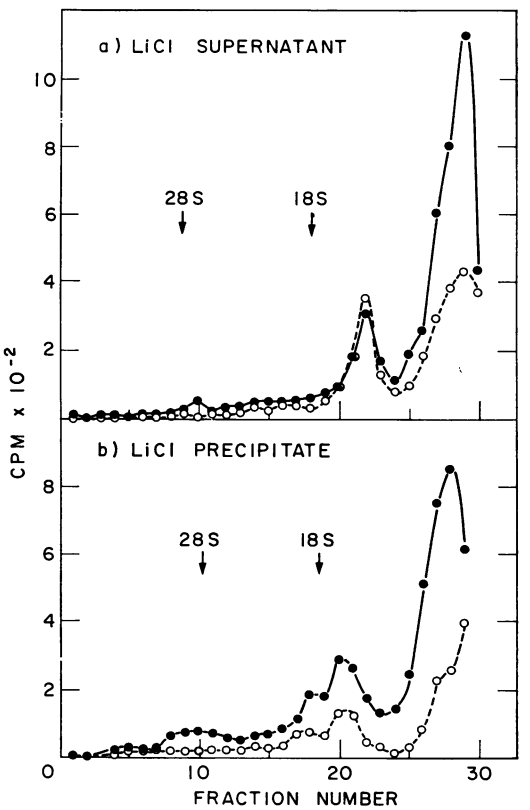

FIG. 7. *LiCl-soluble and -precipitable RNA from cells infected with B at an input multiplicity of 20. CHO cells at 4 × 10⁷ cells/10 ml of medium were infected with B. After 4 hr of infection, 30 μc of ³H-uridine per ml was added for 3 min. Incorporation was terminated as described in Fig. 6. After the cytoplasmic extent was solubilized by SDS, the extract was made 2 M with respect to LiCl. Previously described methods (1) were used for LiCl precipitation. CHO ribosomal RNA was added to the supernatant fluid just prior to sucrose gradient fractionation. Symbols: ●, total ³H; ○, ribonuclease-resistant ³H.*

3-min exposure compared to a 2-hr exposure of uridine (Fig. 6b and 4e).

DISCUSSION

Replication of VSV RNA in CHO cells consists of the synthesis of at least nine major RNA species which can be divided into two groups. Group I includes the RNA made when cells are producing mainly plaque-forming B particles: single-stranded 40*S*, 28*S*, and 13*S* RNA, and partially double-stranded 23 to 35*S* RNA. Group II includes the RNA made when cells are producing more T than B: single-stranded 19*S* and 6*S* RNA, partially double-stranded 19*S* and 15*S* RNA, and completely double-stranded 13*S* RNA. The stable, single-stranded RNA of groups I and II have been found also in Vero cells infected with VSV (16).

Although nine RNA species already seems exorbitant, it is not clear that any of these is homogeneous and some are almost certainly heterogeneous. The 13*S* single-stranded RNA which predominates during a low multiplicity infection, for instance, clearly includes multiple species. Also, other species may be present in small amounts. Analysis by acrylamide gel electrophoresis is in progress to resolve these questions, but it is already evident that VSV replication involves more RNA species than any other single-stranded RNA virus whose mechanism of replication has been investigated.

Two types of RNA structures seem to be replicative intermediates: the 23 to 35*S* and 15 to 20*S* heterogeneous components. The products of neither of these have been identified, but it is noteworthy that a short exposure to uridine at low multiplicity labels the 23 to 35*S* heterogeneous RNA and the 13*S* single-stranded RNA species. If the 13*S* RNA species are products of the 23 to 35*S* replicative intermediate, then the mechanism of synthesis must be very different from that of poliovirus (2). The finding of complementary single-stranded VSV RNA in the 13*S* region (16) suggests that, as with Newcastle disease virus (3, 12), there may be complementary RNA acting as messenger RNA in this system and that the 23 to 35*S* replicative intermediate might be copying partial complementary strands of RNA from the 40*S* viral RNA. However, it also seems likely that the 23 to 35*S* region contains a replicative intermediate for the synthesis of 40*S* B RNA.

A significant result of these studies is that the species and amounts of RNA synthesized in VSV-infected CHO cells depend on at least three factors: the time after infection, the input multiplicity of B, and the ratio of B to T during infection. The results obtained thus far suggest that the changing pattern of RNA synthesis involves a shift from group I to group II. The inhibition of almost all RNA synthesis when T is added at zero time indicates a prerequisite for synthesis of group I RNA before group II RNA can be synthesized. This requirement may be for a replicase encoded by B particles.

In attempting to explain the shift from group I synthesis to group II synthesis, a striking fact is that 19*S* RNA is a major component of group II and also 19*S* RNA is found in T particles. Although it is not known how much of the intracellular 19*S* RNA is identical to the RNA found in T, it is possible that the concentration of intracellular T RNA determines the rate of shift from group I to group II. The concentration of intracellular 19*S* RNA could be increased either by synthesis of 19*S* RNA or by adding 19*S* RNA to cells by superinfection with T. There is at present no explanation for how 19*S* RNA becomes the major species at the expense of the group I RNA species. One possible model is that the 19*S* RNA has a higher affinity for replicase than the 40*S* B RNA and that it is therefore able both to replicate itself and, in effect, to inhibit the replication of B RNA. This hypothesis is similar to the explanation for the takeover of the Qβ replicase reaction by a smaller RNA (13).

One of the factors controlling the time of switchover from synthesis of group I to group II RNA is the input multiplicity of B. It is not known whether the shift to group II RNA synthesis is caused by the increased number of B particles or by contaminating T particles in the B preparation. The presence of less than one T particle per cell could lead to production of T by most of the cells in a culture in 6 hr, because cross-infection of cells occurs within 2 hr after infection (10). However, it seems that T RNA can also be derived from B RNA because preparations of a plaque-purified strain of VSV still contains B and T (18). Whether this happens with a high frequency during a single cycle cannot be known until there is a precise measure of contamination of B preparations by T.

Although the initial appearance of T particles in plaque-purified clones of VSV is not explained, the data strongly support the hypothesis that T particles are not formed by mere breakage of B virions but are unique particles, synthesized and released by infected cells (6). Moreover, the experiments with 3-min exposures to radioactive uridine suggest that T RNA may be synthesized on a separate replicative intermediate from the one for B RNA.

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