

Abortive Infection of a Rabbit Cornea Cell Line by Vesicular Stomatitis Virus: Conversion to Productive Infection by Superinfection with Vaccinia Virus

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An abortive infection of a rabbit cornea cell line (RC-60) by vesicular stomatitis virus (VSV), yielding less than 1 PFU/cell, was converted to a productive infection, yielding 1,900 PFU/cell, when cells were superinfected with vaccinia. Studies on the synthesis of VSV-directed RNA in RC-60 cells suggest that the abortive infection by VSV alone may be due in part to (i) a limited production of 40S virion RNA and (ii) a markedly reduced activity of virion-bound transcriptase activity in RC-60 cells compared to the activity in mouse L cells, a permissive host for VSV. No recognizable VSV structures, except a small amount of viral core structures, were produced by the abortive infection. In contrast, double infection of RC-60 cells with VSV and vaccinia in the presence of hydroxyurea resulted in the production of infective B particles of VSV. Although the function supplied by vaccinia responsible for the productive replication of VSV in double infected RC-60 cells has not been identified, metabolic inhibitor studies indicate that continuous vaccinia-dependent RNA synthesis is required for maximal production of infective VSV. The possibility is considered that vaccinia may supply a product or function required for VSV replication which is ordinarily supplied by the host but which is lacking in RC-60 cells.

We reported earlier that the replication of vesicular stomatitis virus (VSV) is defective in a continuous line of rabbit cornea (RC-60) cells, yielding less than 1 infective virion per cell (9). We also observed that in cultures of RC-60 cells doubly infected with VSV and vaccinia, vaccinia was able to facilitate significantly the replication of VSV (9). This paper reports experiments dealing with the nature of defect of VSV replication in RC-60 cells and the role of vaccinia in the facilitation of VSV replication in these cells.

MATERIALS AND METHODS

Cell cultures. Mouse L cells (clone 929) and primary chicken embryo cells were propagated in Eagle minimal essential medium containing 4% calf serum as described previously (15). A rabbit cornea (RC-60) cell line, obtained from the American Type Culture Collection, Rockville Md., was grown and maintained in Eagle minimal essential medium supplemented with 10% fetal calf serum.

Viruses. A large-plaque mutant (L₁) of Indiana strain of VSV was grown in L cells (13), and vaccinia virus (strain Canada) was propagated in rabbit kidney (RK-13) cells (16). Virus suspensions were as-

sayed for PFU in primary chicken embryo cell monolayers as described before (16). Purified B and T particles of VSV used in some experiments were kindly supplied by E. Dubovi. VSV used as inoculum in these experiments was produced from dilute passage material to eliminate contamination with T particles.

Chemicals. [³H]uridine (specific activity, 27.88 Ci/mmol) was purchased from New England Nuclear, Boston, Mass. Hydroxyurea (A grade) was purchased from Calbiochem, and actinomycin D was made available through the courtesy of H. B. Woodruff of Merck, Sharp and Dohme Co.

Incorporation of [³H]uridine into VSV-directed RNA in RC-60 cell cultures infected with VSV alone or simultaneously with VSV and vaccinia. Cell monolayer cultures in 60-mm Petri dishes (2 × 10⁶ cells) were infected singly with VSV at an input multiplicity of infection (MOI) of 5 to 10 or simultaneously infected with VSV and vaccinia at an input MOI of 5 to 10 for each virus. After adsorption for 2 h at 37 C, the cells were washed twice with medium to remove unadsorbed virus, refed with medium, and reincubated at 37 C. The cell cultures were treated with 2 ml of medium containing actinomycin D (5 μg/ml) for 30 min prior to the addition of 2 ml of medium with [³H]uridine (50 to 100 μCi/ml) plus actinomycin D. The cultures were incubated at 37 C for 60 min and then processed to isolate RNA as described below.

Isolation and analysis of RNA from infected

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cultures. RNA was extracted from cell cultures using the phenol-sodium dodecyl sulfate procedure described in detail elsewhere (9). The extracted RNA was precipitated with ethanol and dissolved in 0.01 M Tris buffer, pH 7.4, containing 0.1 M NaCl and 0.001 M EDTA and stored at -70°C . The RNA was analyzed using sucrose density gradient centrifugation by layering 0.5 ml of RNA sample and L cell ribosomal RNA marker onto a 10 to 30% (wt/vol) sucrose gradient containing 0.5% sodium dodecyl sulfate. The tubes were centrifuged in an SW41 rotor at 34,000 rpm for 4 h at 22°C . The gradients were scanned at 260 nm in a Gilford recording spectrophotometer by pumping the gradient through a flow cell. Fractions (0.4 ml) were collected, and trichloroacetic acid-precipitable radioactivity was determined as described previously (9).

VSV-directed RNA synthesis induced by virion-bound polymerase. The virion-bound RNA-dependent RNA polymerase activity was determined *in vivo* under the conditions described by Marcus et al. (1). Cultures of RC-60 or L cells (2×10^6 cells per culture) were infected with VSV at an input MOI of 40 and allowed to adsorb for 45 min at 37°C . The cultures were then washed twice with medium to remove unadsorbed virus, and refed with 2 ml of medium supplemented with 4% fetal calf serum containing 5 μg of actinomycin D and 100 μg of cycloheximide per ml. Mock-infected control cultures were also treated with actinomycin D and cycloheximide as described above. At various times after infection, the acid-precipitable radioactivity per culture was determined as follows. The cell cultures were washed twice with phosphate buffered saline, pH 7.2, the cells were scraped into 1 ml of Hanks' solution, and the cell suspension was subjected to sonic oscillation for 2 min. The determination of acid-precipitable radioactivity was done as described previously (9).

Preparation and analysis of [^3H]uridine-labeled viral structures synthesized in RC-60 cells infected with VSV or simultaneously infected with VSV and vaccinia. Cell cultures (2×10^6 cells per culture) were infected with VSV alone or simultaneously infected with VSV and vaccinia in the usual manner. After 2-h adsorption at 37°C , the cultures were washed twice with medium, refed with 3 ml of medium containing 0.05 M hydroxyurea and 20 μCi of [^3H]uridine per ml, and reincubated at 37°C . After 24 h, the fluids from infected cultures were collected and centrifuged at low speed to remove whole cells or cell debris. The supernatant fluid was then centrifuged at $100,000 \times g$ for 60 min at 4°C to sediment viral structures. The pellet was resuspended in 0.01 M Tris buffer, pH 7.5, containing 0.1 M NaCl and 0.001 M EDTA, and subjected to sonic oscillation for 60 s. This material was then layered onto a 5 to 40% (wt/vol) sucrose density gradient and centrifuged for 100 min at $75,000 \times g$ at 4°C by the procedure of Marcus and Sekellick (2). Fractions were collected, and acid-precipitable counts were determined as described previously (9).

RESULTS

Single-cycle replication of VSV in rabbit

cornea cells simultaneously infected with VSV and vaccinia. The replication kinetics of VSV in RC-60 cells doubly infected with VSV and vaccinia were determined as follows. Cell cultures (2×10^6 cells per culture) were simultaneously infected with VSV and vaccinia at an input MOI of 5 to 10 for each virus. Control cultures were singly infected with VSV, also at an input MOI of 5 to 10. After adsorption for 2 h at 37°C , the infected cells were washed twice, refed with medium containing 0.05 M hydroxyurea, and reincubated at 37°C . This concentration of hydroxyurea inhibited vaccinia yield by more than 90% without affecting either the replication of VSV or the ability of vaccinia to facilitate VSV replication. At various times after infection the cells were scraped into the medium, the cell suspension was disrupted by sonic oscillation for 2 min, and total virus yield was determined by PFU assay in primary chicken embryo cell monolayers (15). No significant amount of progeny virus was synthesized in RC-60 cells infected with VSV alone (Fig. 1). However, in cultures simultaneously infected with VSV and vaccinia, VSV progeny appeared

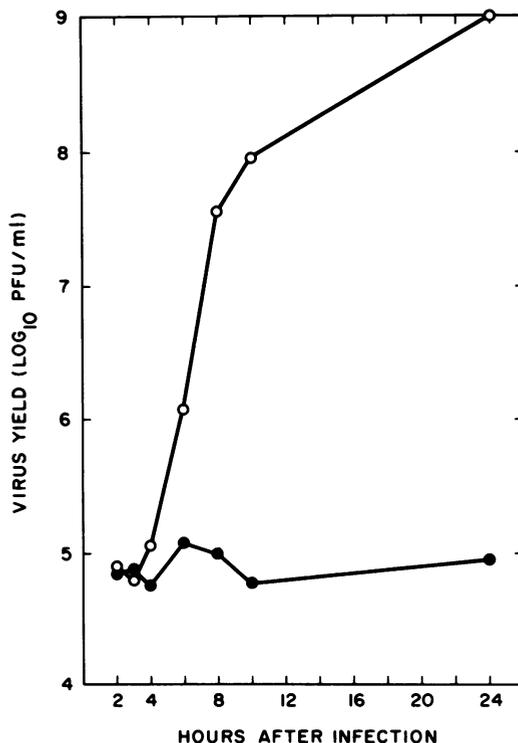


FIG. 1. Single-cycle replication of VSV in RC-60 cell cultures. VSV yield in cultures infected with VSV alone (●) or in cell cultures simultaneously infected with VSV and vaccinia (○). Singly or doubly infected cell cultures were treated with 0.05 M hydroxyurea at 2 h after infection.

between 4 and 6 h after infection and continued to be synthesized for at least 24 h, when the experiment was terminated. Calculations showed that in RC-60 cells infected with VSV alone, virus (PFU) yields per cell were less than 1. In contrast, yields of about 1,900 PFU per cell were obtained in cultures simultaneously infected with VSV and vaccinia. The cytopathic effects in singly and doubly infected cells could not be distinguished. In both the abortive and productive infections, cell destruction was detected as early as 6 h after infection, and by 24 h the cell monolayer was completely destroyed. The infectivity of VSV produced in doubly infected cells was not neutralized by anti-vaccinia rabbit serum, indicating the absence of pseudotype VSV particles with vaccinia antigens which could be detected by this procedure.

The possibility was eliminated that the presence of interferon (produced constitutively or as a result of some persistent viral infection) might be responsible for the abortive infection of RC-60 cells with VSV alone. Culture fluids from 7-day-old monolayers of uninfected RC-60 cells and disrupted cell suspensions (1.2×10^6 cells per ml) were tested for ability to inhibit VSV replication in a line of rabbit kidney cells (RK-13) which is permissive for VSV and sensitive to rabbit interferon (9, 16). There was no difference in VSV replication in RK-13 cells treated in this manner and in control, untreated RK-13 cells.

Influence of time of superinfection with vaccinia on VSV yields in RC-60 cells. We reported previously that there was a rapid loss of a functional VSV genome in RK-13 cells pretreated with homologous interferon, as determined by the rescue of VSV by superinfection with vaccinia (11). This loss of rescuability of the VSV genome in interferon-treated RK-13 cells was correlated with the loss of ability of the VSV genome to direct RNA synthesis (11). Experiments were done to determine how long the VSV genome remains functionally stable in RC-60 cells by utilizing the abortive nature of VSV replication in these cells and the ability of superinfection with vaccinia to facilitate VSV replication. RC-60 cell cultures (2×10^6 cells) were infected with VSV at an input MOI of 5 to 10 in the usual manner. At the time of, or at various times after, VSV infection, the cultures were superinfected with vaccinia at the same input MOI. After virus adsorption for 2 h at 37 C, the cultures were washed twice and refed with medium containing 0.05 M hydroxyurea to inhibit vaccinia replication. Cells and fluids were harvested 18 h after superinfection with vaccinia and assayed for total VSV yield by

PFU assay in primary chicken embryo cell monolayers. A VSV yield of 7.4×10^4 PFU/ml was obtained when RC-60 cells were infected with VSV alone. In contrast, a VSV yield of 8.0×10^8 PFU/ml was obtained from cells simultaneously infected with VSV and vaccinia (0 h); this represented maximal (100%) facilitation (Fig. 2). When vaccinia superinfection was carried out 4 h after infection with VSV, the ability of vaccinia to facilitate VSV replication decreased by more than 60%. Superinfection with vaccinia at 6 or 7 h after infection with VSV resulted in a further decrease in the facilitation of VSV replication (less than 10% of control). These results suggest a rapid loss of functional VSV genome in RC-60 cells infected with VSV alone.

Effect of actinomycin D on the ability of vaccinia to facilitate VSV replication in RC-60 cells. Cell cultures (2×10^6 cells) were singly or doubly infected with VSV and vaccinia in the usual manner. Actinomycin D ($5 \mu\text{g/ml}$) was added to the medium at the time of infection or at various times after infection. This concentration of actinomycin D inhibited the incorporation of [^3H]uridine into trichloroacetic acid-precipitable material by more than 90% within 30 min. Cells and culture fluid were harvested 18 h after infection, and total virus yields were determined as described above. As

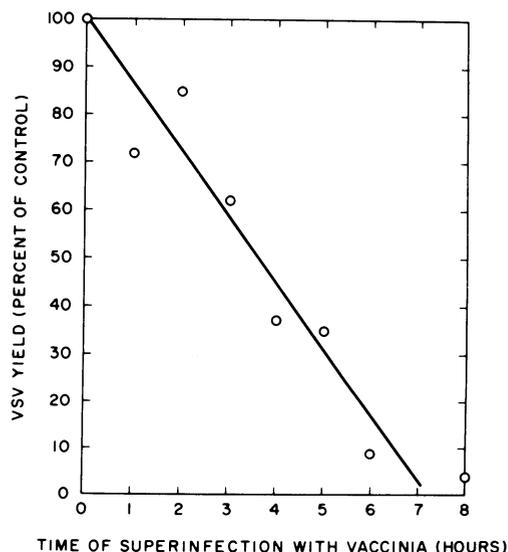


FIG. 2. Effect of time of superinfection with vaccinia on facilitation of VSV replication in RC-60 cell cultures. Cell cultures were treated with 0.05 M hydroxyurea after a 2-h adsorption period with vaccinia at 37 C. VSV yield in cell cultures infected simultaneously with VSV and vaccinia (0 h) was 8.0×10^8 PFU/ml. Total VSV yields were determined 18 h after infection.

expected, infection of RC-60 cells with VSV alone did not result in significant replication of infective virus (Table 1, group 1). Vaccinia replication was reduced by 3 log when actinomycin D was added at the time of infection (Table 1, compare groups 2 and 3). Double infection of cells with VSV and vaccinia in the absence of actinomycin D resulted in 40,000-fold facilitation of VSV yield (Table 1, compare groups 1 and 4). In contrast, when actinomycin D was added at the time of double infection, only marginal facilitation (15-fold) of VSV yield was observed (Table 1, compare groups 1 and 5). When actinomycin D was added to doubly infected cultures at later times (up to 6 h after infection), significant facilitation was observed, although PFU yields of VSV did not reach the levels obtained in the absence of actinomycin D (Table 1, compare group 4 to groups 6 to 10). These observations suggest that continuous vaccinia DNA-dependent RNA synthesis may be required for maximal facilitation of VSV replication by vaccinia in RC-60 cells.

VSV-directed RNA synthesis in RC-60 cells with and without superinfection with vaccinia. Cell cultures (2×10^6 cells) were infected with VSV alone or were doubly infected with VSV and vaccinia in the usual manner. The cultures were labeled with [^3H]uridine for 60 min starting either at 3 or at 5 h after infection; RNA was extracted and analyzed on a sucrose gradient as described above.

In cultures infected with VSV alone, four species of RNA, 40, 34, 18 and 12S, could be distinguished between 3 and 4 h after infection (Fig. 3a). Similar species of VSV-directed RNA have been reported by other workers in different cells (4, 6, 7, 14). In cultures doubly infected with VSV and vaccinia, and also labeled between 3 and 4 h after infection, the two major species of RNA detected were the 32 to 34S and 15S species (Fig. 3a); very few counts were detected in the 40S region of the gradient. It should be noted that the distribution of radioactivity of RNA labeled between 3 and 4 h after infection with vaccinia alone (Fig. 3a) was identical to the profile of RNA extracted from uninfected cultures (not shown in Fig. 3a).

The sucrose gradient profiles of RNA labeled with [^3H]uridine between 5 and 6 h after infection of RC-60 cells with VSV alone or with VSV and vaccinia (Fig. 3b) were different than the RNA profiles observed between 3 and 4 h after infection (Fig. 3a). It should be noted that the ordinate scale of counts per minute is 10-fold higher at the later labeling time (5 to 6 h) than at the earlier period. In cultures infected with VSV alone, only two species of RNA, 30S and 15S, could be detected between 5 and 6 h after infection (Fig. 3b). The number of counts present in the 40S region of the gradient at 5 to 6 h was approximately the same as the number present at the earlier labeling period (Fig. 3a). In cells doubly infected with VSV and

TABLE 1. Effect of time of addition of actinomycin D on the ability of vaccinia to facilitate VSV replication in RC-60 cells

| Expt group | Virus infection (MOI = 5) | | Interval (h) between virus infection and addition of actinomycin D (5 $\mu\text{g}/\text{ml}$) | Virus yield ^a | | |
|------------|---------------------------|-----|---|--------------------------|-------------------|---|
| | Vaccinia | VSV | | Vaccinia (PFU/ml) | VSV (PFU/ml) | -Fold increase over singly infected VSV control (group 1) |
| 1 | - | + | 0 | | 4×10^4 | |
| 2 | + | - | | 1.8×10^7 | | |
| 3 | + | - | 0 | 1.8×10^4 | | |
| 4 | + | + | | | 1.6×10^9 | 40,000 |
| 5 | + | + | 0 | | 6×10^5 | 15 |
| 6 | + | + | 1 | | 7.2×10^6 | 180 |
| 7 | + | + | 2 | | 8.2×10^7 | 2,050 |
| 8 | + | + | 3 | | 2.8×10^8 | 7,000 |
| 9 | + | + | 4 | | 3.0×10^8 | 7,500 |
| 10 | + | + | 6 | | 6.4×10^8 | 16,000 |

^a Total virus yield at 18 h.

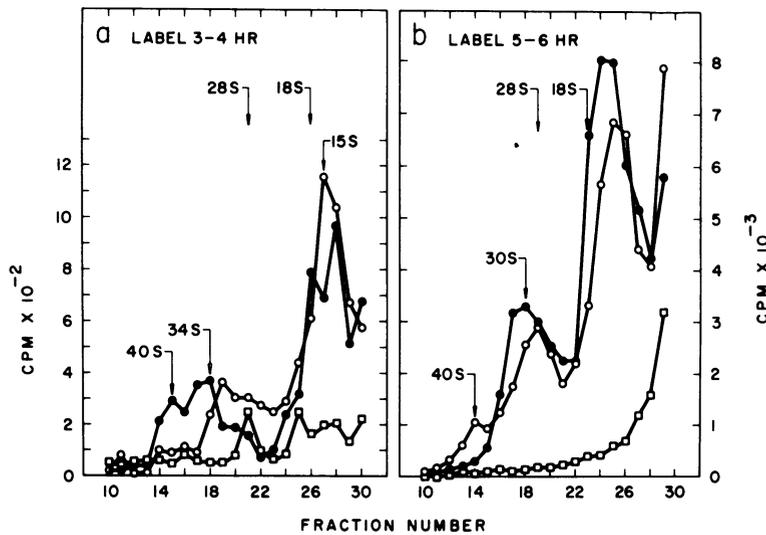


FIG. 3. Sucrose density gradient analysis of RNA synthesized in RC-60 cell cultures singly infected with VSV (●), vaccinia (□), or simultaneously infected with VSV and vaccinia (○). The RNA was labeled with [^3H]uridine in the presence of actinomycin D, extracted, and layered on a sucrose gradient (10 to 30%, wt/vol) with 28S and 18S RNA markers as described. The gradients were centrifuged in an SW41 rotor at 34,000 rpm for 4 h at 22 C. (a) [^3H]uridine incorporation measured from 3 to 4 h after infection; (b) [^3H]uridine incorporation measured from 5 to 6 h after infection. The distribution of radioactivity of RNA from uninfected control cultures exposed to actinomycin D was identical to the profile of RNA extracted from cells infected with vaccinia alone.

vaccinia, increased amounts of the 40S RNA species were detected between 5 and 6 h after infection, as well as the 30S and 15S species seen at the earlier labeling time. The increase in 40S RNA in cells simultaneously infected with VSV and vaccinia is not surprising since this RNA species is incorporated into infective virions, and the marked increase in PFU of doubly infected cells has been demonstrated (Fig. 1). These results suggest that a limited synthesis of 40S RNA in RC-60 cells infected with VSV alone may be, at least in part, responsible for the low production of infective virions under these conditions. The possibility that the 40S RNA seen in the abortive infection may be complementary plus strand RNA will be considered below.

Virion-bound transcriptase activity of VSV in RC-60 and mouse L cells. Virion-bound transcriptase activity was determined in RC-60 and mouse L cells in the presence of actinomycin D and cycloheximide by the procedure of Marcus et al. (1) as described in detail above. Viral RNA was first detected in RC-60 cells infected with VSV in the presence of actinomycin D between 2 and 3 h after infection and reached a peak 4 to 5 h after infection (Fig. 4a). However, when the virion transcriptase activity was measured in the absence of secondary RNA transcription, that is, in the presence of actinomycin D and cycloheximide, the RNA

synthesized represented only about 5% of the total synthesized in the presence of actinomycin D alone (Fig. 4a). A comparative study of the transcriptase activity of VSV in RC-60 and L cells showed that the virion-bound enzyme activity in RC-60 cells (in the presence of actinomycin D and cycloheximide) was only

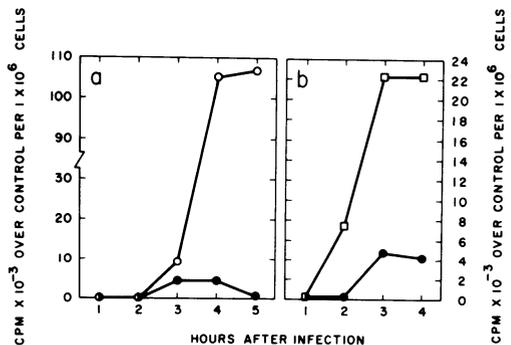


FIG. 4. Incorporation of [^3H]uridine into acid-precipitable material in actinomycin D-treated cultures infected with VSV (MOI = 40) in the presence or absence of cycloheximide. (a) Incorporation of label measured at various times after infection of RC-60 cell cultures with VSV in the absence (○) or presence (●) of cycloheximide as described. (b) Incorporation of label measured at various times after infection of mouse L cells (□) and RC-60 cells (●) with VSV in the presence of actinomycin D and cycloheximide as described.

20% of the activity seen under similar conditions in L cells (Fig. 4b). These data imply a reduced virion transcriptase activity in RC-60 cells but do not eliminate the possibility of a more rapid destruction of the transcribed RNA product in RC-60 cells than in L cells.

Synthesis of viral structures in RC-60 cells infected with VSV alone or simultaneously infected with VSV and vaccinia. Experiments were carried out to determine what RNA-associated structures could be detected in RC-60 cells infected with VSV alone or simultaneously infected with VSV and vaccinia. After adsorption of virus in the usual manner, the cells were washed and refed with medium containing 0.05 M hydroxyurea and [³H]uridine (20 μ Ci/ml) as described above. After 24 h at 37 C, the fluid was collected from the two groups of cultures and assayed for infective VSV. As expected, the yield of VSV from singly and doubly infected cultures were 6.8×10^4 and 1.0×10^9 PFU/ml, respectively. Each infected fluid was then concentrated by centrifugation as described above, and the [³H]uridine-labeled structures in the concentrates were separated by velocity sedimentation in 5 to 40% sucrose gradients. It should be noted in analyzing the results that the total amount of acid-precipitable radioactivity present in concentrated material from cultures infected with VSV alone was less than 1% of the radioactivity present in concentrates from cultures doubly infected with VSV and vaccinia.

The distribution of [³H]uridine-labeled structures from singly and doubly infected cultures after velocity sedimentation is shown in Fig. 5. The profile of radioactivity from RC-60 cells infected with VSV alone showed only one peak which occurred near the top of the gradient (fraction 34 to 35). This peak lacked infectivity and corresponded to peak VSV-IV, containing viral cores described by Marcus and Sekellick (2). In contrast, two peaks were obtained when concentrates from doubly infected cultures were sedimented under similar conditions (Fig. 5). A large peak (fraction 12) corresponded to the location of purified B particle marker particles; this peak contained almost all of the infectivity in the gradient. The smaller peak (fraction 34 to 35) contained less than 10% of the radioactivity of the B particle peak and lacked infectivity. This peak corresponded to the VSV core peak obtained from cells infected with VSV alone. Marker long T particles (50% the length of B particles) characteristic of the L₁ strain of VSV formed a peak in a control gradient at fraction 19. The absence of detectable T particles in the gradients in Fig. 5 is apparent. Electron micro-

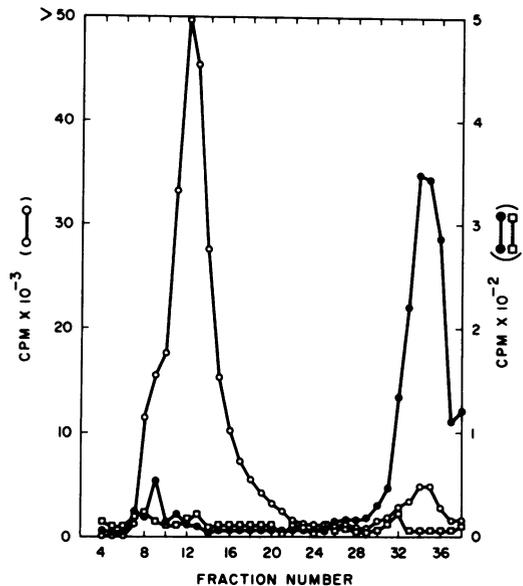


FIG. 5. Sucrose density gradient analysis of [³H]uridine-labeled structures released into the medium of RC-60 cell cultures infected with VSV alone (●) or simultaneously infected with VSV and vaccinia (○). The concentrated material from infected as well as from mock-infected (□) culture fluids was placed on a 5 to 40% sucrose gradient and centrifuged in an SW41 rotor at 25,000 rpm for 100 min at 4 C as described. Purified B and long T particles of VSV included in these gradients as markers appeared as single peaks in fractions 12 and 19, respectively. The acid-precipitable counts in each fraction were determined as described in the text.

scope examination confirmed the exclusive presence of typical B particles in the large peak (fraction 12) from cells doubly infected with VSV and vaccinia. However, the paucity of material in the smaller peak from doubly or singly infected cultures made it impossible to visualize any definite structures in this material by electron microscopy.

DISCUSSION

VSV has an extraordinarily broad range of vertebrate and invertebrate cells in which it will replicate (12). The abortive replication of VSV in the RC-60 line of rabbit cornea cells is one of the few examples of restrictive conditions for this virus. The experiments reported in this paper indicate that although there was less than 1 PFU produced per infected RC-60 cell, there was significant synthesis of several viral RNA species. The major defect observed was in the continued synthesis of 40S virion RNA. Although 40S RNA was detected early in infection (3 to 4 h), there was no significant increase in

this RNA species at later labeling times (5 to 6 h). The possibility exists that some, if not all, of this 40S RNA is complementary to virion RNA; this would account for the absence of significant numbers of progeny virions. In addition, the reduced amount of primary transcription product which accumulates in RC-60 cells infected with VSV alone in the presence of actinomycin D and cycloheximide may also contribute to the defectiveness of VSV replication. Only 20% of the activity seen under similar conditions in L cells was expressed by virion-bound enzyme in RC-60 cells (Fig. 4). Interpretation of these data cannot distinguish between the possibility of (i) reduced virion transcriptase activity in RC-60 cells and (ii) more rapid destruction of the primary RNA transcriptase product in RC-60 cells than in L cells.

No evidence of B or T particle synthesis was obtained by the fractionation of [³H]uridine-labeled structures from RC-60 cells infected with VSV alone (Fig. 5). Only structures corresponding to viral cores (2) could be identified near the top of the sucrose gradients. The presence of these structures implies that some viral structural proteins were being synthesized during the abortive replication. Experiments are in progress to identify the structural VSV proteins present in RC-60 cells infected with VSV alone.

Another example of a defective cell system for VSV is the restricted replication of this virus in a human lymphoblastoid cell line (Raji) reported by Nowakowski et al. (3). Restriction in the Raji cells, as in RC-60 cells, was characterized by a limited production of 40 to 42S virion RNA. Unlike RC-60 cells, in Raji cells primary transcription of VSV-specific RNA in the presence of actinomycin D and cycloheximide was not restricted compared to primary transcription in permissive cells. The results in Raji cells, as well as our results in RC-60 cells, suggest the possibility that there may be host factors which control the production or accumulation of 40S virion RNA as well as certain essential virion proteins.

When double infection of RC-60 cells with vaccinia and VSV was carried out in the presence of hydroxyurea, the abortive infection, which yielded less than 1 PFU per infected cell, was converted to a permissive infection yielding about 2,000 PFU of VSV per infected cell. Under these conditions, no evidence of T particle production was detected and only a small amount of viral core material was found, roughly the same amount seen in the abortive infection.

The facilitation of VSV replication in RC-60 cells by vaccinia did not require DNA synthesis by the poxvirus. This independence from vaccinia DNA synthesis was previously noted in the ability of vaccinia to rescue VSV in a permissive rabbit kidney cell line (RK-13) or mouse L cells treated with homologous interferon. On the other hand, by the use of actinomycin D it was shown that vaccinia DNA-dependent RNA synthesis was essential for providing permissive conditions for VSV in RC-60 cells. Further, continuous vaccinia DNA-dependent RNA synthesis seemed to be required for maximal facilitation of VSV. This requirement was in marked contrast to the vaccinia RNA synthesis necessary for the rescue of VSV in RK-13 or L cells treated with homologous interferon. In these instances, only 1.5 to 2 h of vaccinia-DNA-dependent RNA synthesis was required for maximum rescue of VSV (10). The addition of actinomycin D to the doubly infected cultures at any time after 2 h had no depressing effect on VSV yield. These findings suggest a more rapid breakdown of the essential vaccinia-coded RNA product in RC-60 cells than in interferon-treated RK-13 or L cells under conditions of rescue of VSV.

The RC-60 cell system, in which abortive infection by VSV is converted to a productive infection by superinfection with vaccinia, permits study of the interaction of the two viruses without the use of interferon-treated cells. Whereas the exact mechanism by which vaccinia infection of RC-60 cells is able to convert an abortive VSV infection to a highly productive infection is not known, it seems likely that vaccinia may supply a product required for VSV replication, a product which is ordinarily supplied by the host cell but which is lacking in RC-60 cells. This system may be another illustration of the importance of host cell functions in viral replication and provide an example of one virus, vaccinia, which can supply such a function for the replication of VSV, an unrelated virus. The possible involvement of host cell functions in the replication of VSV has been suggested in the case of host-range mutants of this virus (6).

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