Replication of Reticuloendotheliosis Viruses in Cell Culture: Acute Infection

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Replication of reticuloendotheliosis viruses (REV) in cultures of chicken and duck fibroblasts leads to some cell death soon after infection. This cell killing was used to develop a plaque assay for Trager duck spleen necrosis virus (TDSNV) on duck embryo fibroblasts. A normal replicative cell cycle was required for normal virus production and the development of cytopathic effects in chicken cells exposed to TDSNV. The latent period was about two days. Stationary chicken embryo fibroblasts could be infected by REV; DNA synthesis was required, but protein synthesis was not.

Reticuloendotheliosis viruses (REV) are a newly described group of avian viruses whose virions contain RNA and a DNA polymerase (4, 7–11). There are four members of the REV group: Trager duck spleen necrosis virus (TDSNV), duck infectious anemia virus (DIAV), chicken syncytial virus (CSV), and REV strain T (REV-T). The virions of REV have C-type morphology and contain 60–70S RNA and a DNA polymerase. Although these properties are also characterisic of virions of avian leukosis-sarcoma viruses, there is no serological relationship between the virion structural proteins or the native DNA polymerases of REV and avian leukosis-sarcoma viruses (4, 8, 9). In addition, there is apparently less than 5% nucleic acid sequence homology between the RNAs of REV and avian leukosis-sarcoma viruses (7).

This paper describes the kinetics of the replication of REV in cell culture soon after infection, which involves cell killing, plaque assays for DIAV and TDSNV, and a requirement for early DNA synthesis and cell division for REV production and cell killing. A later paper will describe the chronically infected cultures.

MATERIALS AND METHODS

Cells and viruses. General descriptions have recently been published (7, 9). Fertile Muscovy duck eggs were obtained from a local farmer. Primary cultures of duck embryo fibroblasts were grown in modified Eagle minimal essential medium with 20% tryptose phosphate broth and 5% calf serum.

REV-T was a kind gift of A. Levine and was grown on chicken cells. CSV, from a stock cloned on duck cells, was a kind gift of H. G. Purchase and was grown on chicken cells. TDSNV was obtained from Rockefeller University through the kind agency of R. Nowinski

and was grown on chicken cells. Some was cloned by us on duck cells. DIAV was a kind gift of H. G. Purchase and was grown on chicken cells. All virus stocks were shown to be free of avian leukosis virus by tests using nucleic acid hybridization (7).

Cells were infected with REV, and virus was harvested and concentrated by using techniques previously described for Rous sarcoma virus (1).

Vesicular stomatitis virus (VSV), Indiana strain, was a kind gift of C.-Y. Kang (6). It was grown and assayed on chicken embryo fibroblasts.

Assay of REV. Since it had been reported that multiplication of REV in cell culture caused no visible effect on the cells (2, 17), a biochemical measure of REV production was developed. Virus was concentrated from 10 ml of cell-free supernatant medium, and 0.1 ml of Nonidet P-40 disruption medium was added to the pellet for 5 min at 0 C (S. Mizutani, C.-Y. Kang, and H. M. Temin, in Methods in nucleic acids and protein synthesis, in press). A sample (25 μliters) of disrupted virus was added to 100 μliters of a standard DNA polymerase reaction mixture containing 250 μg of calf thymus DNA per ml (16). The mixture was incubated at 38 C, and the amount of [³H]TMP incorporated into DNA at 0, 15, and 30 min was measured.

To determine whether this assay was linear with virus concentration, twofold dilutions of unconcentrated stocks of TDSNV, CSV, and REV-T were prepared, and their DNA polymerase activity was measured. The amount of incorporation into DNA after 15 and 30 min of incubation was linear with the dilution of virus with a deviation of about 20% (data not shown).

TDSNV, CSV, and REV-T were then plated on chicken, duck, Japanese quail, pheasant, and turkey cells to determine whether REV replicate on all of these cells and whether cytopathic effects occur. Virus growth was monitored by the production of sedimentable DNA polymerase activity. All of the reticuloendotheliosis viruses replicated in all of the cells tested. Virus production was greater in chicken, pheasant,

and turkey cells than in quail and duck cells.

Cytopathic effects of virus replication were observed in chicken and duck cells. This cytopathic effect was also seen with cloned TDSNV and with REV-T recovered from a spleen tumor in culture. Therefore, we believe the cytopathic effect is from the REV and not from an unknown contaminant virus. The cytopathic effect was very localized on duck cells. This effect was used to develop a plaque assay. This assay works well for TDSNV and DIAV and less well for CSV and REV-T.

Duck embryo fibroblasts (6 × 10⁵), third passage or later, were plated in 4 ml of Eagle medium with 20% tryptose phosphate broth in 60-mm plastic petri dishes (Falcon Plastics). After incubation overnight, the medium was removed, and 0.2 ml of medium containing virus was added. After incubation for 40 min at 38 C, 5 ml of Eagle medium with 20% tryptose phosphate broth, 2% calf serum, and 1.5% fetal bovine serum was added. After 3 or 4 days of incubation at 38 C, areas of approximately 10 to 20 dead cells could be counted under 25-power of a microscope. The number of these microplaques was proportional to the virus dilution. After 1 or 2 additional days of incubation, the dead cells sloughed off into the medium, and the plaques became difficult to see. The plaques did not grow larger, perhaps because of the requirement for a normal replicative cell cycle for cell killing to occur (see later).

Stability of infectivity of TDSNV. The plaque assay was used to show that the ability of TDSNV to form plaques on duck cells was stable to repeated freezing and thawing and to sonication of the virus. Infectivity was reduced by incubation at 37 C with a half-life of about 1 h. Infectivity was inactivated by ultraviolet irradiation at a rate similar to that of the inactivation of the B77 strain of avian sarcoma virus and much less than that of the inactivation of vesicular stomatitis virus (data not shown).

Chemicals. Cycloheximide was obtained from Calbiochem. Colcemid was obtained from Ciba Pharmaceutical Products, Inc. Cytosine arabinoside was a kind gift of the Upjohn Co.

RESULTS

Kinetics of replication of TDSNV in chicken cells. Cultures containing 8 × 10⁵ chicken embryo fibroblasts were exposed to TDSNV at a multiplicity of infection of about 1 PFU/cell. After adsorption, 5 ml of medium containing 6% calf serum was added to the cultures. Each day the medium was harvested from two cultures, and the number of cells per culture was counted. At the end of the experiment, the medium was assayed for sedimentable DNA polymerase activity (Fig. 1). Virus was first detected at 2 days after infection. A slight cytopathic effect was seen by microscopy at 3 days after infection, and a more pronounced cytopathic effect was seen by microscopy at 4 days after infection. Concomitant with this visible cytopathic effect, there was a decrease in
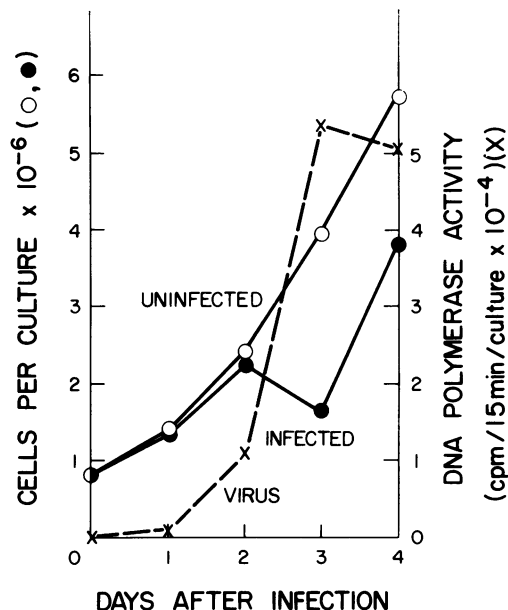

FIG. 1. *Growth curve of TDSNV on chicken embryo fibroblasts. Cultures containing 8 × 10⁵ chicken embryo fibroblasts in medium with no serum were exposed to 0.2 ml of TDSNV titering 5 × 10⁷ PFU/ml on duck cells or of medium from uninfected chicken cells. After incubation for 40 min, the inoculum was removed, and 5 ml of Eagle medium with 20% tryptose phosphate broth and 6% calf serum was added. Each day thereafter, the medium was harvested from two infected cultures and was frozen at −70 C, and after trypsinization the number of cells in each culture was counted with a Coulter counter. The average of values for two cultures is plotted. At the end of the experiment, the amount of extracellular sedimentable DNA polymerase activity per culture was determined.*

the number of cells relative to the mock-infected control cultures and the appearance of virus. (A similar time of virus appearance was found when PFU were assayed.) However, all of the cells in the infected cultures were not killed. The number of cells per culture then started to increase, and the cytopathic effect disappeared.

This experiment demonstrated cell killing by TDSNV soon after infection. The long latent period indicated that cell division might be necessary for normal virus production and cell killing, as it is necessary for normal virus production by Rous sarcoma virus (5, 14). This hypothesis was tested by inhibiting cell division by Colcemid and mitomycin C and by altering the serum concentration.

Effects of inhibitors of cell division on infection of chicken cells by REV. Chicken cells were exposed to TDSNV, and medium

containing serum and 0 or 10^{-7} M Colcemid was added. Since the cells treated with Colcemid did not divide, the initial number of cells in the Colcemid-treated cultures was made greater, so that by 3 days after infection there was approximately the same number of cells in the cultures with and without Colcemid. The presence of Colcemid resulted in over a 50-fold decrease in the amount of virus present at 3 days after infection (Table 1). Similar results were also obtained at 2 days after infection. Most of the cells in the cultures treated with Colcemid were rounded, apparently in "colchicine mitosis," and their number did not increase during the experiment. There was no direct effect of Colcemid on the infectivity or the DNA polymerase activity of TDSNV virions (data not shown).

Similar experiments were performed with CSV, DIAV, and REV-T. In all cases, the results were the same as with TDSNV. Virus production, measured by PFU or sedimentable DNA polymerase activity, was over 50-fold inhibited by 10^{-7} M Colcemid (data not shown).

As a control for possible nonspecific effects of Colcemid, chicken cells were exposed to 10^{-7} M Colcemid for 3 days, and then the production of VSV was measured in the presence of 10^{-7} M Colcemid. VSV production was not inhibited by these Colcemid treatments (Table 2).

Mitomycin C was used to study further the role of a normal replicative cell cycle on REV production, especially to determine whether the

TABLE 1. *Effect of Colcemid on infection of chicken embryo fibroblasts by TDSNV[a]*

Colcemid	No. of cells[b]	DNA polymerase activity[c]	Virus titer (PFU/culture)
Absent	2.8×10^6	3×10^4	4×10^5
Present	2.4×10^6	6×10^2	2×10^2

[a] Four cultures containing 6×10^5 chicken embryo fibroblasts and four cultures containing 2.4×10^6 chicken embryo fibroblasts were exposed to 0.2 ml of TDSNV titering about 5×10^6 PFU/ml on duck cells. After 40 min, the inoculum was removed, and 5 ml of Eagle medium with 20% tryptose phosphate broth, 2% calf serum, 1.5% fetal bovine serum, and no or 10^{-7} M Colcemid was added to the cultures with, respectively, 6×10^5 and 2.4×10^6 cells. Two and three days later, the medium was harvested and assayed for sedimentable DNA polymerase activity and PFU, and the number of cells was counted. Only the results for 3 days after infection are shown. The results for 2 days after infection were similar, but the amounts of virus were lower.

[b] Average of values for two cultures.

[c] Expressed as counts per minute per 15 min per culture.

TABLE 2. *Effect of Colcemid and mitomycin C on infection of chicken embryo fibroblasts by VSV[a]*

Treatment	Virus titer (PFU/culture)	No. of cells/culture[b]
None	3×10^7	2.0×10^6
Colcemid	2×10^7	1.8×10^6
Mitomycin	5×10^7	3.9×10^6

[a] Cultures containing 2.4×10^6 chicken embryo fibroblasts were exposed to 10 μg of mitomycin C per ml for 2 h. These and cultures containing 6×10^5 and 2.4×10^6 untreated chicken embryo fibroblasts were overlaid with 5 ml of Eagle medium containing 20% tryptose phosphate broth, 2% calf serum, and 1.5% fetal bovine serum. Colcemid (10^{-7} M) was added to the cultures with 2.4×10^6 untreated cells. Three days later, the number of cells in two cultures of each group was counted, parallel cultures were infected with 10^6 PFU of VSV, and 5 ml of Eagle medium with 20% tryptose phosphate broth, 2% calf serum, and 1.5% fetal bovine serum was added. The medium on the cultures pretreated with Colcemid also contained 10^{-7} M Colcemid. After incubation overnight, the supernatant media were harvested, and the VSV titer was determined.

[b] Average of values for two cultures.

effect of Colcemid on REV production was only a result of the infected cells being in mitosis.

Cells pretreated for 2 h with mitomycin C did not divide, and the production of TDSNV was inhibited (Table 3). Similar results were found with CSV, DIAV, and REV-T (data not shown).

As a control for nonspecific effects of mitomycin C, cells were exposed to mitomycin C and incubated for 3 days. Production of VSV was then measured. Pretreatment with mitomycin C caused no inhibition of VSV production (Table 2).

Therefore, treatment of chicken embryo fibroblasts with Colcemid or mitomycin C leads to inhibition of REV production.

Effect of different serum concentrations on infection of chicken cells by REV. Another way to control the amount of cell division by chicken cells exposed to REV is to vary the serum concentration in the medium. The amount of multiplication of chicken embryo fibroblasts is directly controlled by the amount of serum in the medium (13). In the absence of serum or another source of multiplication-stimulating activity, the cells are stationary in the IGI phase of the cell cycle (12, 15).

Stationary chicken cells were exposed to TDSNV, and medium with no serum or 6% calf serum was added. The cells with medium containing no serum remained stationary, and their number decreased as a result of cell detach-

TABLE 3. *Effect of mitomycin C on infection of chicken embryo fibroblasts by TDSNV[a]*

Mitomycin	No. of cells[b]	DNA poly- merase ac- tivity[c]	Virus titer (PFU/culture)
Absent	3.4×10^6	1.2×10^5	1.2×10^7
Present	2.7×10^6	1.3×10^3	2×10^4

[a] Cultures containing 2.4×10^6 chicken embryo fibroblasts were exposed for 2 h to 10 μg of mitomycin C per ml. Then these and cultures containing 6×10^5 untreated chicken embryo fibroblasts were exposed to 0.2 ml of TDSNV titering about 5×10^6 PFU/ml on duck cells and were overlaid with 5 ml of Eagle medium containing 20% tryptose phosphate broth, 2% calf serum, and 1.5% fetal bovine serum. Three days later, the media were harvested and assayed for sedimentable DNA polymerase activity and PFU, and the number of cells was counted.

[b] Average of values for two cultures.

[c] Expressed as counts per minute per 15 min per culture.

ment. The cells with 6% serum divided, increased in number, and were killed by TDSNV (see Fig. 1). Little virus was present at 3 days after infection in the cultures of stationary cells, whereas over 20 times more virus was present in the cultures of dividing cells (Fig. 2). When at 3 days after infection serum was added to the stationary cells, they divided, and virus was produced (Fig. 2).

Another experiment was performed in which the amount of cell multiplication was controlled by different concentratons of serum in the medium. The cell numbers in mock-infected cultures were directly proportional to the amount of serum in the medium (Fig. 3). The numbers of cells in the TDSNV-infected cultures were proportional to serum concentrations at levels up to 5% serum, but the numbers of cells were smaller than in the mock-infected cultures. Although there was less than a twofold difference between the numbers of cells at 3 days after infection in the cultures with 0.5 and 6% serum, the amounts of sedimentable DNA polymerase activity in the same cultures ranged from undetectable to 5×10^3 counts per min per 15 min per culture. The cytopathic effect was also greater in the TDSNV-infected cultures with more serum.

Similar experiments were carried out with TDSNV infection of duck embryo fibroblasts and CSV infection of chicken embryo fibroblasts. The results obtained were similar (data not shown).

These results indicate that stationary cells can be infected by REV and that a normal replicative cell cycle is necessary for normal REV production. Similar phenomena have been described for Rous sarcoma and murine sarcoma viruses (14).

Effect of inhibitors of DNA synthesis upon infection of stationary chicken cells by REV. To test the hypothesis that virus-specific DNA synthesis is required for REV infection, the following experiment was carried out. Stationary chicken embryo fibroblasts were exposed to REV and were incubated overnight with or without 2×10^{-4} M cytosine arabinoside. The cytosine arabinoside was then removed, medium with serum was added to the cells, and virus production was measured 3 days later. There was almost complete inhibition of detectable virus production in the cytosine arabinoside-treated cultures (Table 4). Control experiments showed a slight toxic effect of the cytosine arabinoside. There were 2.4×10^6 cells in
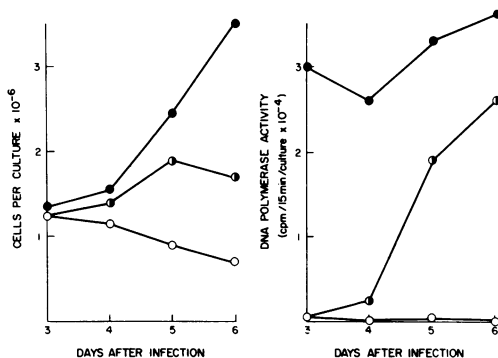

FIG. 2. *Effect of absence of serum on growth of TDSNV in chicken embryo fibroblasts. Cultures containing 6×10^5 or 2.4×10^6 chicken embryo fibroblasts in medium with no serum were exposed to 0.2 ml of TDSNV titering 10^7 PFU/ml on duck cells. Eagle medium (5 ml) containing 20% tryptose phosphate broth and 6% calf serum was added to the cultures with 6×10^5 cells, and the same medium with no serum was added to the cultures with 2.4×10^6 cells. Two days after infection, the medium was replaced with 5 ml of fresh medium with the same composition. Three days after infection, the medium was harvested and frozen, and the number of cells was counted in two cultures of each type. The medium on the remaining cultures was replaced with 5 ml of fresh medium with the same composition except for six of the previously serum-free cultures, to which 6% serum was added. On succeeding days, the medium was harvested and frozen, and the number of cells was counted in two cultures of each type. The medium on the remaining cultures was replaced with 5 ml of fresh medium of the same composition. At the conclusion of the experiment, the amount of extracellular sedimentable DNA polymerase activity was determined. Symbols: ●, cultures with 6% serum; O, cultures with 0% serum; ◐ cultures with 0% serum which were changed to 6% serum 3 days after infection.*
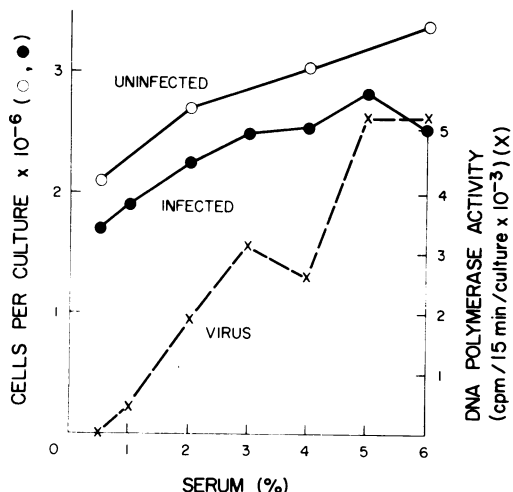
FIG. 3. Effect of different amounts of serum on production of TDSNV. Cultures containing 6 × 10⁵ chicken embryo fibroblasts in medium with no serum were infected with TDSNV titering 5 × 10⁶ PFU/ml on duck cells or were mock-infected, and 5 ml of medium with different concentrations of serum was added. Three days later, the medium was harvested, sedimentable DNA polymerase activity was determined, and the number of cells was counted. Symbols: ○, number of cells in mock-infected cultures; ●, number of cells in TDSNV-infected cultures; ×, sedimentable DNA polymerase activity in TDSNV-infected cultures.

the treated cultures and 5.2 × 10⁶ cells in the untreated cultures. (Experiments presented in Table 6 indicate that this amount of toxicity does not prevent REV production.)

Therefore, DNA synthesis other than normal S-phase cell DNA synthesis is apparently required for REV infection. Further studies using nucleic acid hybridization (C.-Y. Kang, unpublished data) have demonstrated the presence of virus-specific DNA in TDSNV-infected chicken embryo fibroblasts.

Effect of cycloheximide upon infection of stationary chicken cells by REV. Virions of REV contain RNA and a DNA polymerase, but they contain no detectable endogenous RNA-directed DNA polymerase activity (S. Mizutani, unpublished data; 7). To determine whether new protein synthesis was required for REV infection, the following experiment was performed. Chicken embryo fibroblasts were exposed to virus, and then cycloheximide (0.5 μg/ml) was added in the absence of serum. (This concentration of cycloheximide resulted in over 90% inhibition of [³H]leucine incorporation [S. Mizutani, personal communication].) After incubation overnight, the cycloheximide was removed, the cells were stimulated to

divide by the addition of serum, and the amount of virus production was determined. No toxic effects of the cycloheximide were observed, and the number of cells was the same in treated and untreated cultures at 3 days after infection. Normal virus production occurred in the cycloheximide-treated cultures (Table 5).

A second experiment involving successive treatments with cycloheximide and with cytosine arabinoside was performed to determine whether the virus-specific DNA synthesis took place in the presence of the cycloheximide. The results of this experiment (Table 6) indicate that over 70% of the apparent virus-specific DNA synthesis took place by 6 h after infection whether or not cycloheximide was present.

These experiments demonstrate that new protein synthesis is not required for REV infection.

DISCUSSION

Reticuloendotheliosis viruses are a newly described group of avian RNA viruses with a virion DNA polymerase. Replication of REV in avian embryo fibroblasts in culture leads to cell

TABLE 4. Effect of cytosine arabinoside upon infection of stationary chicken embryo fibroblasts by REV[a]

Virus	Cytosine arabinoside[b]	DNA polymerase activity[c]	Virus titer (PFU/culture)
CSV	0	18,500	2 × 10⁵
	+	0	0
DIAV	0	14,500	10⁶
	+	0	0
REV-T	0	77,500	10⁶
	+	0	0
TDSNV	0	48,000	10⁶
	+	400	0

[a] Cultures containing 10⁶ chicken embryo fibroblasts were prepared in 5 ml of medium with 0.4% calf serum. After 3 days of incubation, the medium was removed, and the cells were exposed to 0.2 ml of the different REV. After 40 min, the inoculum was removed, and 5 ml of Eagle medium with 20% tryptose phosphate broth and no or 2 × 10⁻⁴ M cytosine arabinoside was added. After 16 h, the medium was replaced with 5 ml of medium with 10⁻⁴ M deoxycytidine, 2% calf serum, and 1.5% fetal bovine serum. Three days later, the media were harvested, and the amounts of sedimentable DNA polymerase activity and the virus titers were determined. For the virus titers, 0 was less than 25 PFU/culture. For the DNA polymerase activity, assay, the background was 1,500 counts per min per 15 min per culture.

[b] +, Present; 0, absent.

[c] Expressed as counts per minute per 15 min per culture.

TABLE 5. *Effect of cycloheximide upon infection of chicken embryo fibroblasts by REV[a]*

Virus	Cyclohexi-mide[b]	DNA poly-merase activity[c]	Virus titer (PFU/culture)
CSV	0	14,500	3×10^5
	+	12,800	4×10^5
DIAV	0	35,000	4×10^6
	+	33,000	3×10^6
REV-T[d]	0	63,000	10^6
.	+	33,000	10^6
TDSNV	0	31,000	10^7
	+	37,000	3×10^7

[a] Cultures containing 6×10^5 chicken embryo fibroblasts were exposed to 0.2 ml of the different REV. After 40 min, the inoculum was removed, and 5 ml of Eagle medium with 20% tryptose phosphate broth and 0 or 0.5 μg of cycloheximide per ml was added. After incubation overnight, the medium was replaced with 5 ml of medium with 2% calf serum and 1.5% fetal bovine serum. Three days later, the media were harvested, and the amounts of sedimentable DNA polymerase activity and the virus titers were determined.

[b] +, Present; 0, absent.

[c] Expressed as counts per min per 15 min per culture.

[d] Separate experiment.

death and causes cytopathic effects soon after infection. This cytopathic effect allowed development of a plaque assay for the replication of TDSNV and DIAV in duck embryo fibroblasts. (REV plaques differ from those described by Graf [3] for subgroup B and D avian leukosis viruses. The REV plaques develop sooner, 3 or 4 days, are smaller, and appear even after daily changes of the medium.)

However, all of the chicken or duck cells in cultures exposed to TDSNV were not killed. Carrier cultures with no obvious cytopathology were established, but the mechanism for their maintenance has not yet been elucidated.

Even under conditions of rapid cell division, the latent period for virus production and the development of cytopathic effects was about 2 days. When cell multiplication was blocked by inhibitors or by the absence of serum, activation of virus production was greatly reduced. When cell multiplication was stimulated by higher concentrations of serum, virus production was increased. These results are consistent with a requirement for a normal replicative cell cycle for activation of normal REV production.

However, stationary chicken embryo fibroblasts could be infected with REV as shown by the production of virus after cell division was later stimulated. Infection of stationary chicken embryo fibroblasts required DNA synthesis but

not protein synthesis. These findings are consistent with the findings of a DNA polymerase in REV virions (7, 9, 10) and of DNA in TDSNV-infected chicken cells hybridizable to DNA made with Rous sarcoma virus-Rous-associated virus-0 DNA polymerase and TDSNV RNA (C.-Y. Kang, unpublished data). However, these findings make paradoxical the inability to detect endogenous RNA-directed DNA polymerase activity in disrupted REV virions (S. Mitzutani, unpublished data; 7). Perhaps some factors pre-existing in the newly infected cells alter the activity of the REV DNA polymerases.

Reticuloendotheliosis viruses are especially interesting in comparison to avian leukosis-sarcoma viruses. They both infect avian cells and have structurally similar virions, a DNA intermediate for replication, and a requirement for a normal replicative cell cycle for normal activation of virus production. However, reticuloendotheliosis viruses cause direct cell killing soon after infection and have no endogeneous RNA-

TABLE 6. *Effects of cycloheximide and cytosine arabinoside upon infection of stationary chicken embryo fibroblasts by TDSNV[a]*

Treatment[b]		DNA poly-merase activity[c]	Virus titer (PFU/culture)
Cyclohexi-mide	Cytosine arabino-side		
0	0	45,000	3×10^6
0	+	33,000	2×10^6
+	0	50,000	4×10^6
+	+	33,000	4×10^6

[a] Eight cultures containing 10^6 stationary chicken embryo fibroblasts were exposed to 0.2 ml of TDSNV titering 5×10^6 PFU/ml on duck cells. After 40 min, the inoculum was removed, the cultures were washed, and 5 ml of Eagle medium with 20% tryptose phosphate broth and 0 or 0.5 μg of cycloheximide per ml was added to each of four cultures. Six hours later, the media were removed from all cultures, and 5 ml of medium containing 0 or 2×10^{-4} M cytosine arabinoside was added to two cultures of each type. Seventeen hours later, the media on all cultures were replaced with 5 ml of Eagle medium containing 20% tryptose phosphate broth, 2% calf serum, 1.5% fetal bovine serum, and 10^{-4} M deoxycytidine. Three days later, the media were harvested, and the amounts of sedimentable DNA polymerase activity and virus titers were determined. (In parallel uninfected cultures, there was a slight toxic effect of the cytosine arabinoside. There were 3.7×10^6 cells in the cytosine arabinoside-treated cultures and 4.8×10^6 in the untreated cultures.)

[b] +, Present; 0, absent.

[c] Expressed as counts per minute per 15 min per culture.

directed DNA polymerase activity. In further work, we are attempting to understand these differences between the two groups of viruses.

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