

# Reovirus: Effect of Noninfective Viral Components on Cellular Deoxyribonucleic Acid Synthesis

ROGER HAND AND IGOR TAMM

*The Rockefeller University, New York, New York 10021*

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We determined the effects of noninfective reovirus components on cellular deoxyribonucleic acid (DNA) synthesis. Reovirus inactivated by ultraviolet light inhibited cellular DNA synthesis, whereas reovirus cores and empty capsids did not. Both cores and empty capsids were adsorbed to cells. Adenine-rich ribonucleic acid (RNA) from reovirus, adsorbed to cells in the presence of diethylaminoethyl-dextran, produced a partial inhibition of DNA synthesis. RNA was synthesized in the presence of actinomycin D after infection with ultraviolet light-irradiated reovirus, and this RNA synthesis was not due to multiplicity reactivation of virus infectivity. These data suggest that viral structural proteins do not inhibit DNA synthesis and that the inhibition produced by ultraviolet-irradiated virus may be mediated in part or in toto by a newly synthesized viral product.

Infection of L cells with reovirus results in inhibition of cellular deoxyribonucleic acid (DNA) synthesis. This cut-off occurs 6 to 8 hr after infection, before the onset of cytopathic effect (11). Previous work has shown that decreased thymidine incorporation in infected cells reflects a real decrease in DNA synthesis (10), and that a DNA precursor block is not involved in the cut-off (9, 13). Neither the entry of cells into the DNA synthetic phase of the cell cycle (11) nor DNA chain growth (15) is inhibited by infection. These findings suggest that reovirus infection blocks initiation of synthesis of new DNA chains (9, 10, 15). Since infection does not produce a concomitant decrease in cellular protein synthesis (8, 11, 31), the action of reovirus on DNA replication is direct and specific. We therefore wished to determine which virus function or product causes this inhibition. Cox and Shaw (6) have observed that irradiation of reovirus with ultraviolet (UV) light does not abolish its inhibitory effect upon cellular DNA synthesis. This suggests that either a component of the virus is capable of causing inhibition or that the ribonucleic acid (RNA) genome can function partially after UV irradiation and give rise to an inhibitory component.

We have performed experiments that explore these two possibilities in some detail. We have investigated the kinetics of inhibition of cellular DNA synthesis caused by UV-irradiated reo-

virus as well as by various noninfective viral components. We have also determined whether RNA synthesis occurs after infection of cells with UV-irradiated virus.

## MATERIALS AND METHODS

**Cells and virus.** The characteristics of the mouse fibroblast line, L929, and its growth in monolayer and suspension cultures (14) as well as the characteristics of reovirus type 3, Dearing strain, and its plaque assay for infectivity have been described (12, 14).

**Purification procedures.** The purification of cell-associated reovirus, released reovirus, empty capsids (particles deficient in viral nucleic acid), and virus cores (particles with the outer layer of capsomers removed by proteolytic digestion) was performed as described previously (14), with minor modifications. Empty capsids were recentrifuged after initial isolation in cesium chloride solutions of density 1.30 g per cubic centimeter (cc) and desalted by passage through columns of Sephadex G-25 equilibrated with phosphate-buffered saline (PBS). The empty capsids were used immediately for experiments. Their structural integrity was verified by electron microscopy during each experiment. Storage of empty capsids at 4 C for more than several hours, or desalting by dialysis, resulted in their breakdown. Viral RNA was prepared from purified virus using the method of Oda and Joklik (24). Adenine-rich RNA was separated from the double-stranded genome RNA on 5 to 20% sucrose density gradients. Isopycnic centrifugation in cesium sulfate of adenine-rich RNA does not result in a sharp band, probably because of diffusion of

these relatively low-molecular-weight molecules (25); therefore, this procedure was not used.

Figure 1 shows the absorbance at 260 nm of each individual fraction after an initial rate zonal centrifugation of purified reovirus RNA in sucrose. The pattern is similar to that obtained by other investigators (3). Fractions 4 to 6 were precipitated with 2 volumes of absolute ethanol at  $-20^{\circ}\text{C}$ , and the precipitates were pooled and redissolved in TNE: 0.01 M tris-(hydroxymethyl)aminomethane (Tris) (pH 7.4), 0.2 M NaCl, and 0.01 M ethylenediaminetetraacetate (EDTA). The redissolved fractions were centrifuged again in a 5 to 20% sucrose gradient for 16 hr at 50,000 rpm in an SW56 rotor. A homogeneous peak was found one-third of the distance from the top of the gradient by absorbance measurements. The peak fractions were again precipitated by the addition of 2 volumes of absolute ethanol and stored at  $-20^{\circ}\text{C}$  until use. This preparative purification procedure may not have completely separated fragments of double-stranded genome RNA from the adenine-rich RNA.

**Radioactive labeling of virus and viral components.** To prepare reovirus with its RNA labeled,

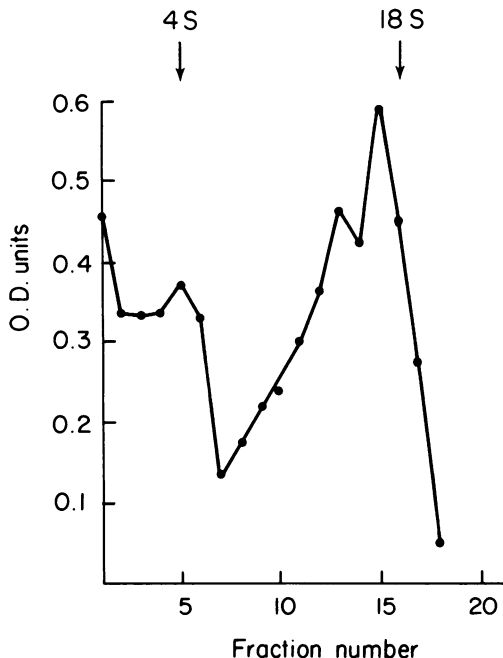


FIG. 1. Rate zonal centrifugation of RNA extracted from purified reovirus. RNA was layered atop 5 to 20% sucrose gradients in TNE and centrifuged for 16 hr at 50,000 rpm in an SW56 rotor in a Beckman L2 65B ultracentrifuge. Fractions of 0.25 ml were collected by puncture of the bottom of the tube, and the absorbancy at 260 nm of each fraction was determined after dilution to 1 ml with TNE. Labeled marker RNAs from L cells were run in separate tubes; their position relative to the reovirus RNA is therefore only approximate.

$^3\text{H}$ -uridine (1.0  $\mu\text{Ci}/\text{ml}$ , 28 Ci/mmmole) was added to infected cultures from 2 hr after infection until harvest 18 to 20 hr after infection. The virus was purified and used directly or converted to cores by protease treatment. Infected cells labeled with  $^3\text{H}$ -amino acid mixture, 0.25  $\mu\text{Ci}/\text{ml}$ , from 2 hr after infection until harvest 20 hr after infection yielded radioactively labeled empty capsids. Infected cells labeled with  $^3\text{H}$ -adenosine (1.0  $\mu\text{Ci}/\text{ml}$ , 24.5 Ci/mmmole) yielded radioactively labeled adenine-rich RNA. All isotopes were purchased from Schwarz BioResearch, Orangeburg, N.Y.

**Irradiation of virus.** Preparations of stock reovirus or purified reovirus were adjusted to the titer of infectivity appropriate for the experiment by diluting 1:20 or more. The virus suspensions were exposed to UV light from a General Electric G 15 T8 Sterilamp delivering 57.7 ergs per sec per  $\text{mm}^2$  at a distance of 10 cm. An exponential decrease in infectivity to 1/1,000th of the original titer resulted from exposure to increasing doses up to 6,000 ergs/ $\text{mm}^2$ . At doses beyond 6,000 ergs/ $\text{mm}^2$ , further inactivation occurred at a non-exponential rate.

**Estimates of the amounts of virus components.** Infectivity was assayed by plaque tests. The virus particle to plaque-forming unit (PFU) ratio was determined by estimating the amount of purified virus spectrophotometrically using 5.42 optical density units at 260 nm as equivalent to 1 mg of reovirus or  $1.13 \times 10^{13}$  reovirus particles (29). Particle-to-PFU ratios were usually in the range of 100:1. Whole virus particles are converted to cores with 80 to 95% efficiency by treatment with chymotrypsin (17, 28); thus the number of cores in a suspension could be estimated from the number of particles in an identical suspension of virus that had not been treated with chymotrypsin. Purified empty capsids contained less than 1% of nucleic acid as determined by the optical density ratio, 260 nm:280 nm. The amount of protein in the purified preparation was estimated by optical density at 280 nm, and the number of particles was derived using the amount of protein as 85% of the weight of an equivalent number of virus particles. The amount of adenine-rich RNA in a purified preparation was estimated spectrophotometrically by its optical density at 260 nm. Adenine-rich RNA forms 5% of the virus particle or 35% of its nucleic acid (23). The figure 5% was used to derive the number of virus particles that would contain the amount of adenine-rich RNA that was obtained by extraction and purification.

**Determination of rate of cellular DNA synthesis.** Cells were infected with reovirus or treated with the indicated reovirus component. Control cultures were exposed to an identical amount of medium or buffer. The adsorption period was 2 hr, except that in experiments with adenine-rich RNA, adsorption was for 20 min in PBS containing diethylaminoethyl (DEAE)-dextran, 0.3 mg/ml. The inoculum was then replaced with Eagle's minimal essential medium with 5% fetal calf serum. Samples of cells were pulse-labeled with  $^3\text{H}$ -thymidine (0.1  $\mu\text{Ci}/\text{ml}$ , 20–25 Ci/mmmole, Schwarz BioResearch). Fifteen-min pulses (in some experiments, 30-min pulses) were given hourly

from 4 to 10 hr after the beginning of the experiment. By 4 hr, DNA synthesis had returned toward normal after having been perturbed by exposure of the cells to suboptimal amounts of medium during the adsorption period. The inhibition of DNA synthesis by reovirus did not begin until 5 to 6 hr after infection and was marked by 9 to 10 hr after infection (9, 11). The acid-precipitable radioactivity incorporated by the cells during each pulse was determined by liquid scintillation counting.

**Preparation of RNA from cytoplasmic extracts.** Experimental or control cultures were labeled with  $^3\text{H}$ -uridine (1.0  $\mu\text{Ci/ml}$ , 28 Ci/mmmole). The cells were washed, resuspended in reticulocyte standard buffer for 20 min at 4 C, and disrupted with 25 strokes of a tight-fitting Dounce homogenizer. The intact nuclei were removed by centrifugation at 1,000 rpm for 5 min. The cytoplasmic extract was decanted, and sodium dodecyl sulfate was added to 1%, EDTA to 0.01 M, and NaCl to 0.2 M. The cytoplasm was then extracted three times with phenol saturated with TNE. The aqueous phase was extracted with ether three times to remove the phenol, and the ether was removed by evaporation with nitrogen. The RNA was precipitated with 2 volumes of absolute ethanol and stored at  $-20^\circ\text{C}$  until analyzed. When the cytoplasmic RNA was extracted with chloroform-isoamyl alcohol (24), we obtained similar experimental results. The RNA was analyzed on 5 to 20% sucrose density gradients in TNE centrifuged at 50,000 rpm for 120 min in an SW56 rotor in an L2 65B preparative ultracentrifuge. Fractions were collected by displacement with 40% sucrose. In some experiments alternate fractions were treated with ribonuclease A. The acid-precipitable radioactivity of each fraction was determined by liquid scintillation counting.

## RESULTS

**Effect of UV-irradiated virus particles on DNA synthesis.** We first wished to confirm that UV-irradiated reovirus was able to inhibit cellular DNA synthesis. L cells were infected with a stock suspension of reovirus at a multiplicity of 100 PFU/cell (10,000 particles/cell) or exposed to an equivalent amount of virus previously irradiated with UV light which reduced its infectivity 1,000-fold. Figure 2 shows that UV-irradiated virus inhibited cellular DNA synthesis, but that it did not cause the marked inhibition produced by infective virus 8 to 10 hr after infection. The inhibition observed with UV-irradiated virus was not caused by residual infective particles in the inoculum, since at a multiplicity of infection of 0.5 PFU/cell (higher than that of the UV-irradiated suspension) DNA synthesis was inhibited at a much slower rate (Fig. 3).

UV-irradiated purified virus, either released or cell-associated, inhibited cellular DNA synthesis at a rate similar to infective purified virus. This showed that inhibition was not pro-

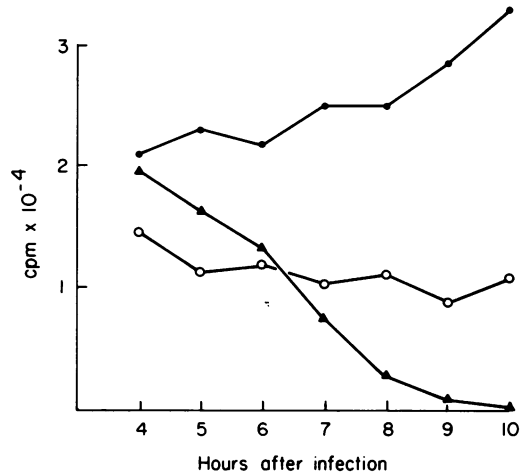


FIG. 2. Effect of UV-irradiated reovirus on cellular DNA synthesis. L cells in suspension culture were infected with UV-irradiated reovirus (infectivity decreased 1,000-fold) or infective reovirus, and pulse labeled with  $^3\text{H}$ -thymidine. Symbols: ●, control uninfected cells; ○, UV-irradiated reovirus, residual infectivity, 0.1 PFU/cell; ▲, infective reovirus, multiplicity 100 PFU/cell.

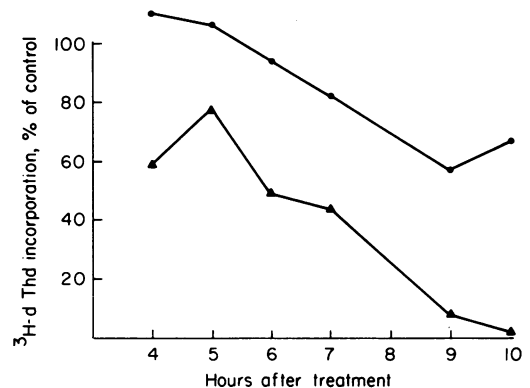


FIG. 3. Effect of low multiplicity reovirus infection on cellular DNA synthesis. Symbols: ●, 0.5 PFU/cell; ▲, 100 PFU/cell. (In Fig. 3-6, the data are plotted as percent of concomitantly pulsed controls.)

duced by a nonviral contaminant of the virus suspension.

**Effect of reovirus cores.** If a structural component of the virus causes the inhibition of DNA synthesis, it might be possible to identify it by selectively degrading the reovirus particle. Reovirus contains seven or eight proteins (14, 21, 29); three of these are in the outer coat of the virus and the remainder are associated with the core. The three outer coat proteins can be removed by treatment of the virus

with trypsin or chymotrypsin. Virus cores were prepared by treatment of purified virus with chymotrypsin at 37 C (100  $\mu$ g/ml for 30 min). They were then further purified by isopycnic centrifugation. A control suspension of infective virus particles was treated similarly, but not exposed to chymotrypsin. The chymotrypsin-treated suspension showed a band at density 1.43 g/cc (reovirus cores). The untreated suspension showed a band at density 1.37 g/cc (infective reovirus). The band fractions were dialyzed against PBS, assayed for infectivity, and inoculated into suspension cultures of L cells. Virus was inoculated at a multiplicity of 1,000 PFU/cell or 100,000 virus particles per cell. Cores had a residual infectivity which gave a multiplicity of 0.001 PFU/cell; we assume that the core particle-to-cell ratio approached 100,000:1. The cores of reovirus type 3 Dearing strain commonly show decreased infectivity (1, 17, 26). Figure 4 shows that the core particles did not inhibit cellular DNA synthesis at the high particle to cell ratio used. Infective reovirus at a multiplicity of 1,000 PFU/cell (100,000 particles/cell) inhibited DNA synthesis at a rate similar to that observed after infection at a multiplicity of 100 PFU/cell (10,000 particles/cell).

**Effect of empty capsids.** Cells infected with reovirus produce, in addition to progeny virus, empty capsids (particles deficient in viral RNA). These particles contain all the proteins of the virion and have a density of 1.30 g/cc (14, 29). We isolated these as a by-product of purification of cell-associated virus and further purified them by isopycnic centrifugation

in cesium chloride. Freshly purified preparations had approximately 1% of the infectivity of complete virus. This probably reflected contamination of the empty capsid band with infective virus.

We determined whether empty capsids were able to inhibit cellular DNA synthesis. L cells in suspension cultures were exposed to a multiplicity of 50,000 particles/cell. Since this was associated with an infective multiplicity of 0.5 PFU/cell, an equivalent number of empty capsids were irradiated with 20,000 ergs/mm<sup>2</sup>. This reduced infectivity by 10,000-fold. Another culture was infected with purified reovirus at a multiplicity of 167 PFU/cell (16,700 particles/cell).

Figure 5 shows that reovirus inhibited DNA synthesis at the expected rate. Empty capsids caused a slight inhibition which could be accounted for by the presence of residual infective virus (compare with Fig. 3) and was reproducible from experiment to experiment. After irradiation, empty capsids produced no inhibition. Similar results were obtained at a particle to cell ratio of 72,000:1.

**Effect of adenine-rich RNA.** The above experiments suggest that none of the proteins of the virus inhibits DNA synthesis. Neither empty capsids nor cores contain adenine-rich RNA that is present in complete virus particles (17, 20, 26). We therefore determined whether such RNA was able to inhibit DNA synthesis. L cells were exposed to adenine-rich RNA extracted from purified reovirus (*see above*). The amount of adenine-rich RNA inoculated was equal to the amount present in a reovirus sus-

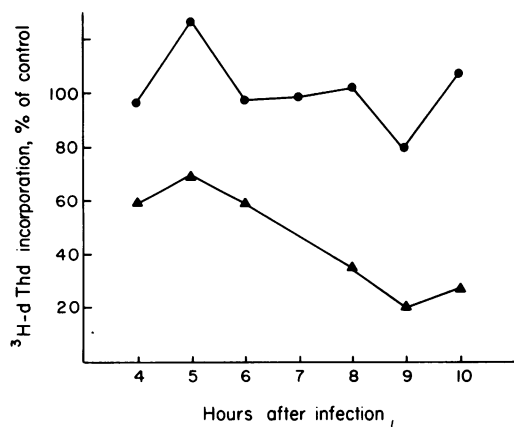


FIG. 4. Effect of reovirus cores on cellular DNA synthesis. Symbols:  $\blacktriangle$ , purified reovirus, multiplicity 100,000 particles/cell;  $\bullet$ , reovirus cores derived from an equivalent amount of purified virus.

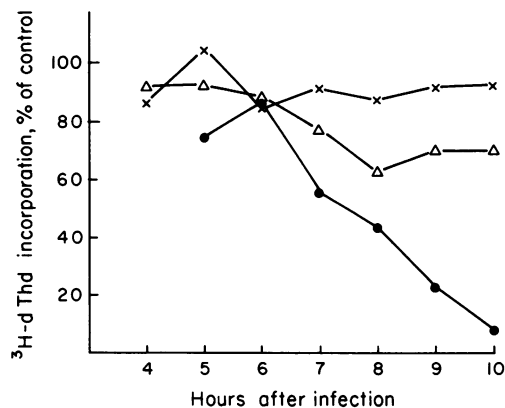


FIG. 5. Effect of empty capsids on cellular DNA synthesis. Symbols:  $\bullet$ , purified reovirus, multiplicity 16,700 particles/cell;  $\Delta$ , empty capsid preparation, 50,000 particles/cell;  $\times$ , UV-irradiated empty capsid preparation, 50,000 particles/cell.

pension giving a multiplicity of 600 PFU/cells or 60,000 particles/cell. Figure 6 shows that adenine-rich RNA adsorbed to cells in the presence of DEAE-dextran produced slight to moderate inhibition of DNA synthesis, much less than that produced by infection with complete reovirus at high multiplicities. This inhibition was reproducible in repeated experiments. We have not yet investigated the effect of higher concentrations of adenine-rich RNA on DNA synthesis, since the purpose of these experiments was to determine the effects of amounts of adenine-rich RNA that approached the amounts found in virus suspensions capable of inhibiting DNA synthesis.

**Adsorption of noninfective viral components.** Since the degree of inhibition of DNA synthesis produced by cores, empty capsids, and adenine-rich RNA may be related to the

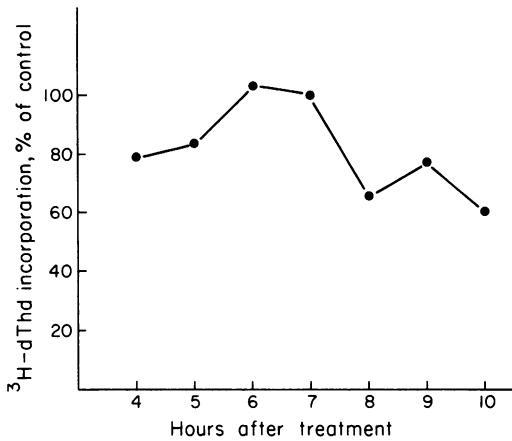


FIG. 6. Effect of adenine-rich RNA on cellular DNA synthesis. *L* cells in suspension culture were exposed to a concentration of adenine-rich RNA equivalent to that in a virus suspension giving 600 PFU/cell, and pulse labeled with <sup>3</sup>H-thymidine.

amount of these components adsorbed to the cells, we determined the extent of adsorption of these components to cells under the conditions of these experiments. The radioactively labeled components used in these adsorption studies were prepared as indicated above. Such labeled components were allowed to adsorb to the cells under the same conditions as in the experiments in which the kinetics of inhibition of DNA synthesis was determined. At the end of the adsorption period, the inoculum was removed and the cells were washed thoroughly in PBS. The amount of acid-precipitable radioactivity unabsorbed and adsorbed to the cells was determined. The percentage of radioactivity, and from this the number of particles adsorbed, was calculated using the specific activity of each labeled component. The results are shown in Table 1. Close to 80% of inoculated reovirus particles were adsorbed during a 2-hr period, and cores were almost as well adsorbed. These results are closely similar to those previously published (12, 17). Adenine-rich RNA was taken up by cells relatively well in the presence of DEAE-dextran. However, empty capsids were not well adsorbed, only 15% of the radioactivity becoming cell-associated in 2 hr. This may be related to the instability of these particles at physiologic salt concentrations. Even so, sufficient radioactivity was adsorbed to give a high particle-to-cell ratio. Adsorption of this many infective virus particles would have resulted in a marked inhibition of DNA synthesis.

**New RNA synthesis after infection with UV-irradiated virus.** Our data do not support the concept that a component of the input virus is responsible for the severe sustained inhibition of DNA synthesis seen after infection with complete reovirus. We therefore investigated whether any virus-specific RNA is made after infection of cells with UV-irradiated reo-

TABLE 1. Adsorption of reovirus or reovirus components to *L* cells

Inoculum	Counts/min adsorbed to cells	Counts/min remaining in inoculum	% Adsorption	Adsorption particles/cell
<sup>3</sup> H-uridine-labeled reovirus . . . .	4,398	1,332	78.0	78,000
<sup>3</sup> H-uridine-labeled cores . . . . .	3,084	2,378	56.5	56,600
<sup>3</sup> H-amino acid-labeled empty capsids . . . . .	1,575	8,067	16.3	16,300
<sup>3</sup> H-amino acid-labeled UV-irradiated empty capsids . . . . .	1,474	6,775	17.8	17,800
<sup>3</sup> H-adenosine-labeled adenine-rich RNA . . . . .	662	354	65.1	— <sup>a</sup>

<sup>a</sup> The amount of adenine-rich RNA adsorbed was equal to the amount of adenine-rich RNA present in a suspension of reovirus particles that would have given an adsorbed particle-to-cell ratio of 40,000:1.

virus. L cells in suspension culture were infected with reovirus at a multiplicity of 133 PFU/cell, or with a suspension containing an equivalent number of particles whose infectivity had been reduced to a multiplicity of 0.013 PFU/cell by UV irradiation, or with a 1:1,000 dilution of the infective suspension (0.13 PFU/cell). A control culture was mock-infected. Adsorption was for 2 hr, and 4.5 hr after infection actinomycin D (0.5  $\mu\text{g}/\text{ml}$ ) was added to all cultures to suppress cellular RNA synthesis without inhibiting reovirus RNA synthesis (18). The cultures were pulse-labeled with  $^3\text{H}$ -uridine from 5 to 8 hr after infection. RNA was extracted from the cytoplasm of the cells. Sucrose density gradient analyses of the extracted RNA are shown in Fig. 7. Cells infected with reovirus at a multiplicity of 133 PFU/cell showed a peak sedimenting at less than 18s and another peak between 18 and 28s. This is typical of the pattern of RNA produced 6 to 8 hr after reovirus infection representing synthesis of single- and some double-stranded RNA (18). Gradients from cells infected with

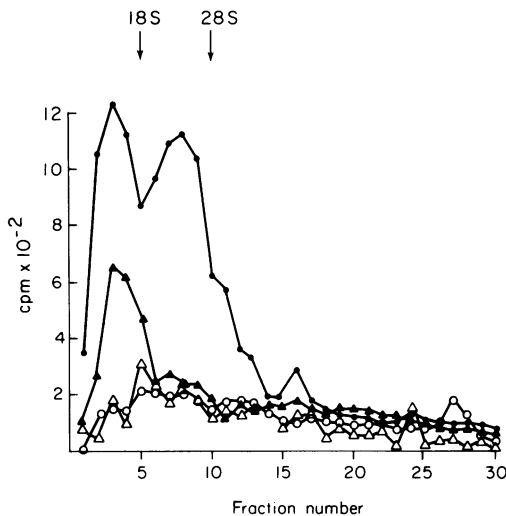


FIG. 7. Rate zonal centrifugation of  $^3\text{H}$ -uridine-labeled cytoplasmic extracts from cells infected with UV-irradiated reovirus. L cells in suspension culture were infected with reovirus or UV-irradiated reovirus. Actinomycin D was added 4.5 hr after infection, and the cells were labeled with  $^3\text{H}$ -uridine from 5 to 8 hr after infection. RNA was extracted from the cytoplasm of the cells and centrifuged on 5 to 20% sucrose gradients. Symbols:  $\circ$ , mock-infected control;  $\bullet$ , reovirus-infected, multiplicity, 133 PFU/cell;  $\Delta$ , reovirus-infected, multiplicity, 0.13 PFU/cell;  $\blacktriangle$ , infected with UV-irradiated reovirus, residual infectivity, 0.013 PFU/cell.

irradiated virus showed synthesis of RNA sedimenting at  $s$  values lower than 18. Gradients from the uninfected control culture and from the culture infected with reovirus at a multiplicity of 0.13 PFU/cell showed little acid-precipitable radioactivity. In replicate cultures not treated with actinomycin D, DNA synthesis was estimated by pulse labeling samples of cells with  $^3\text{H}$ -thymidine for 15 min at 8 hr after infection. DNA synthesis was 43.2% of control in the cells infected with reovirus at a multiplicity of 133; 34.5% of control in cells infected with UV-irradiated virus, and 91.1% of control in cells infected with virus at a multiplicity of 0.13 PFU/cell. The data show that an RNA species sedimenting at less than 18s is synthesized in cells infected with UV-irradiated reovirus, whereas little if any RNA sedimenting between 18 and 28s is made.

In a similar experiment, cells were infected with infective reovirus at a multiplicity of 450 PFU/cell or an equivalent inoculum of UV-irradiated virus whose residual infectivity resulted in a multiplicity of infection of 0.003 PFU/cell. Actinomycin D was again added 4.5 hr after infection, and the cells were labeled with  $^3\text{H}$ -uridine from 5 to 8 hr after infection. The RNA was extracted and sedimented on 5 to 20% sucrose gradients. After collection of the fractions from each gradient, alternate fractions were treated with ribonuclease A (100  $\mu\text{g}/\text{ml}$ , 37 C for 30 min.). The resulting gradient profiles are shown in Fig. 8. Infection with reovirus produced several species of RNA that were sensitive to ribonuclease A. Infection with UV-irradiated reovirus produced RNA again sedimenting less than 18s that was resistant to digestion with ribonuclease A. These data suggest that infection with UV-irradiated virus results in synthesis of either double-stranded RNA or adenine-rich RNA.

**Multiplicity reactivation.** A possible explanation for synthesis of RNA in cells infected with UV-irradiated virus would be reactivation of the virus through a multiplicity-dependent mechanism. Multiplicity reactivation of other strains of reovirus has been described (20). If reactivation of virus occurs after cells are infected with UV-irradiated virus, then an increase in infective virus should be detectable in such cultures after an interval sufficient for completion of a single growth cycle. We therefore determined the yield of infective reovirus produced by cultures of L cells infected at a multiplicity of 200 PFU/cell (particle-to-cell ratio, 20,000:1), or infected with a suspension containing an equivalent number of particles

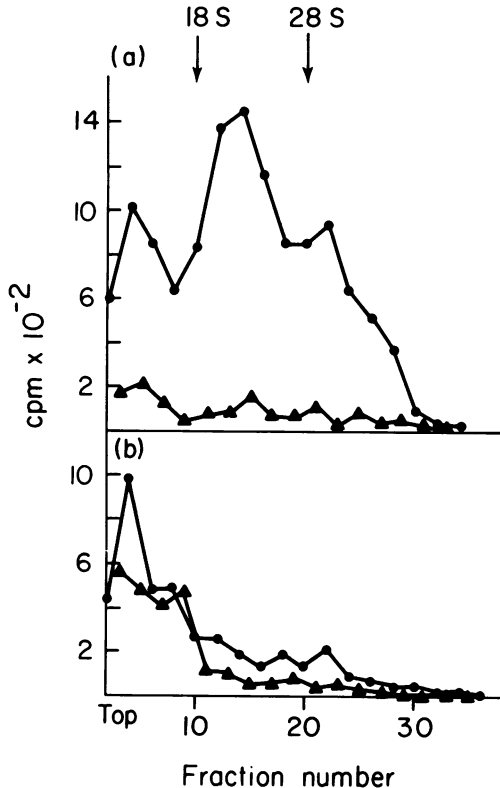


FIG. 8. Rate zonal centrifugation of <sup>3</sup>H-labeled cytoplasmic extracts from cells infected with UV-irradiated reovirus. (a) Infected with reovirus, multiplicity 450 PFU/cells. Symbols: ●, virus-infected; ▲, virus-infected and fractions treated with ribonuclease. (b) Infected with UV-irradiated reovirus, residual infectivity, 0.013 PFU/cell; ●, infected with UV-irradiated virus; ▲, infected with UV-irradiated virus and fractions treated with ribonuclease.

but inactivated 10<sup>4</sup>-fold by UV irradiation (particle-to-cell ratio 20,000:1). We also determined the yield in cultures infected with reovirus at a multiplicity of 0.2 PFU/cell (particle-to-cell ratio, 20:1). Cultures were harvested 18 hr after infection and infectivity was assayed. The results are shown in Table 2. Cells infected at low multiplicity produced less than 1/100th the amount produced when high multiplicity was used. Cells infected at a high particle-to-cell ratio with UV-irradiated virus produced very small amounts of virus which could be accounted for by the residual infectivity of the inoculum. The data show that virus whose infectivity had been reduced by UV irradiation was not significantly reactivated by inoculation into cells at high multiplicity, under the conditions of our experiments.

TABLE 2. Yield of reovirus after infection of cells with UV-irradiated virus

Inoculum	Yield <sup>a</sup> (PFU)
Irradiated reovirus <sup>b</sup>	5.63 × 10 <sup>3</sup>
Infective reovirus, multiplicity 200 PFU/cell	6.91 × 10 <sup>7</sup>
Infective reovirus, multiplicity 0.2 PFU/cell	4.86 × 10 <sup>5</sup>

<sup>a</sup> Total plaque-forming units in L cell monolayers (500,000 cells/plate) 18 hours after infection.

<sup>b</sup> Prior to irradiation infectivity was equivalent to 200 PFU/cell; this was reduced to 0.001 PFU/cell by irradiation.

## DISCUSSION

We have shown that reovirus inactivated by UV light inhibits DNA synthesis, whereas reovirus cores and empty capsids do not. Our data also suggest that adenine-rich RNA may produce a partial inhibition of cellular DNA synthesis, that RNA is synthesized in cells infected with UV-irradiated virus, and that this limited RNA synthesis is not due to reactivation of the UV-irradiated virus.

Our findings confirm and extend those of Cox and Shaw (6) who showed that UV-irradiated reovirus type 3 inhibits cellular DNA synthesis. Loh and Oie (19) reported that UV-irradiated reovirus type 2 produces a cytotoxic effect in HeLa cells and inhibits cellular macromolecular synthesis shortly after infection. Since they also showed that cellular protein synthesis is inhibited at the same time as DNA synthesis, reovirus type 2 probably inhibits DNA synthesis by a mechanism different from that operative in reovirus type 3 infection.

UV-irradiated reovirus type 3 seems to produce only a partial inhibition of DNA synthesis by 10 hr after infection, whereas infective reovirus produces a more complete cut-off of DNA synthesis by this time. This raises the possibility that sustained inhibition of DNA synthesis at the virus multiplicities used may require replication of the viral genome, which UV irradiation may prevent. The partial inhibition produced by UV-irradiated virus might be caused by a product of limited transcription of the UV-damaged genome as well as by input adenine-rich RNA. The more marked effect on DNA synthesis at early times produced by UV-irradiated virus is difficult to interpret, since this was seen at times relatively close to the adsorption period for the virus and cellular DNA synthesis may have still been deranged from suboptimal amounts of medium.

Our data suggest that none of the proteins of the virus particle causes the inhibition of DNA synthesis, since neither empty capsids nor cores produced the cut-off, despite adequate adsorption to the cells. The slight degree of inhibition of DNA synthesis produced by empty capsids can be explained by residual infectivity, since the kinetics of inhibition produced by empty capsids (Fig. 5) were similar to the kinetics of inhibition after infection at low multiplicity (Fig. 3). At low multiplicity, replication of the viral genome is probably necessary to produce inhibition of DNA synthesis, since the onset of this inhibition occurs during the logarithmic phase of virus growth. UV-irradiated whole virus in the empty capsid preparations did not inhibit DNA synthesis probably because the number of complete particles was insufficient to give rise to an inhibitory amount of product transcribed from UV-irradiated genomes.

Adenine-rich RNA, when adsorbed to the cells in the presence of DEAE-dextran, was able to produce a partial inhibition of DNA synthesis; however, the conditions of adsorption were not physiological. The effect of adenine-rich RNA was incomplete despite the fact that large amounts of this material were adsorbed to the cells. Inhibition of DNA synthesis by input adenine-rich RNA could only partially explain the effects produced either by infectious or irradiated virus.

We have not measured the effect of double-stranded reovirus RNA on cellular DNA synthesis. Such RNA produced inhibition of protein synthesis *in vitro* (7) and would probably inhibit DNA synthesis by this mechanism *in vivo* if it penetrated the cell membrane. Free input reovirus RNA has not been found in the cytoplasm of infected cells (4, 27), nor is cellular protein synthesis inhibited after infection (11). Thus, experiments measuring cellular DNA synthesis after uptake of free double-stranded RNA would bear little relation to the actual *in vivo* mechanism by which reovirus inhibits cellular DNA synthesis.

New RNA is synthesized in cells infected with irradiated virus when cellular RNA synthesis is inhibited by actinomycin D. This suggests that the RNA may be virus-specified. This RNA sediments at less than 18s, is resistant to ribonuclease A, and is produced at a time when cells infected with irradiated virus produce predominantly ribonuclease-sensitive RNA. This suggests that full transcription of viral genome RNA does not occur in cells infected with UV-irradiated virus. We do not know the nature of the RNA produced in cells

infected with UV-irradiated reovirus; however, there are several possibilities, one of which is that it may represent adenine-rich RNA. Bellamy et al. (2) and Nichols et al. (23) have suggested that adenine-rich RNA may be synthesized as a result of either a slippage mechanism during transcription or abortive attempts at initiation of transcription in infected cells. Such slippage or abortive transcription might well occur on genomes damaged by UV light. Another possibility is that abortive replication of the UV-damaged genomes might produce small fragments of double-stranded RNA. Such fragments might inhibit DNA synthesis. Further study is necessary to determine the nature of the RNA made in cells infected with UV-irradiated reovirus. The observation that UV-irradiated viral RNA may be partially functional is not new. Hupert et al. (16) and Clavell and Bratt (5) have shown that cells infected with UV-irradiated Newcastle disease virus synthesize virus-specific RNA.

In sum, our data suggest that there may be a two-stage mechanism for the inhibition of DNA synthesis. The initial inhibition can be produced by UV-irradiated virus, and may represent the combined effect of a nonprotein viral component such as adenine-rich RNA and a product of abortive transcription or abortive replication of the UV-irradiated genome. Alternatively, this initial inhibition may be caused exclusively by a *de novo* synthesized product. The sustained inhibition of DNA synthesis may require larger amounts of inhibitory component(s) than those introduced or made after infection with UV-irradiated virus. Such a complex multifactorial mechanism has precedent from the work with vesicular stomatitis virus. Wertz and Youngner (30) have shown that this virus inhibits cellular protein synthesis by two different mechanisms, an initial process mediated by a component of the input virus and a progressive mechanism that may require a functional viral genome.

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