Growth Characteristics of Reovirus Type 2: Actinomycin D and the Preferential Synthesis of Viral RNA.* (31372)

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In a study of the effect of actinomycin D (AD) on cell cultures infected with reovirus type 2, it was reported previously that infected cells in the presence of 2 μg per ml of AD retained their capacity to produce viral antigen despite the inhibition of viral RNA synthesis(1). Furthermore, it was noted that the morphologic arrangement of the viral protein produced resembled that of an earlier period of infection. The present communication describes the preferential synthesis of reovirus RNA, and the formation of infectious virus, in the presence of low concentrations of the antibiotic.

Materials and methods. Cell cultures. The growth and preparation of RA (stable human amnion) and HeLa cells, and the infection procedures have been described previously(2). The growth and basic experimental media consisted of Eagle's basal medium (EBM) (3) plus 10% calf serum, and EBM plus 0.5% fetal calf serum, respectively. To facilitate detection of any changes in the RNA activity during infection, the latter medium was used to maintain the monolayer cell culture in a minimal state of metabolic activity.

Virus. Semipurified pools of reovirus type 2 (strain D-5) were passaged in HeLa cells and prepared as described previously(2).

Virus assay. Virus titration was performed by the immunofluorescent assay technique(4). The titer in RA cells of the seed virus was 2.1×10^8 infectious units (IU) per ml.

Infection procedure. Monolayers of HeLa cultures were infected with an exposure multiplicity of approximately 15 IU, per cell. After adsorption for 2 hours at 37°C, unadsorbed virus was removed by washing with balanced salt solution, the experimental medium was introduced, and the culture reincubated at 37°C.

Extraction of RNA. RNA synthesis was measured by the cumulative incorporation of uridine-H^3 (New England Nuclear Corp., specific activity 8100 mc/mM) into HeLa cells. The RNA was extracted by the method of Schmidt and Thannhauser(5). The amount of radioactivity was measured in a Packard liquid scintillation spectrophotometer and expressed as counts per unit of absorbancy at 260 mμ.

Antibiotic. Actinomycin D was generously supplied by Merck, Sharp and Dohme, Rahway, N. J.

Results. Effect of actinomycin on formation of reovirus. AD in varying concentrations was added to HeLa cell cultures 2 hours before infection and during the entire period of infection. At 24 hours post-infection (p.i.) the yields of infectious virus from antibiotic-treated and untreated infected cultures, respectively, were determined.

As seen in Table I, cultures treated with AD at a concentration of 0.1 μg per ml did not affect the yield of infectious virus. In several other experiments, as in this one, the yield of infectious virus was several times greater than the yield from untreated infected cultures. With concentrations of AD of 0.2 μg per ml and higher there was progressively greater inhibition of infectious virus forma-

TABLE I. Effect of Varying Concentrations of Actinomycin D on Production of Reovirus Type 2.

	Pre- and post-treatment*	
Concentration of actinomycin D (μg/ml)	Virus titer infectious units/ml $\times 10^4$	% virus inhibition
0†	980	—
.1	2065	0
.2	315	67.9
.3	147	85
.4	24	97.6
.5	37	96.3
1.0	3.5	100
2.0	7.5	100

* Pre-treatment for 2 hr and post-treatment for 24 hr after infection.

† Virus titer at 0 hr after infection was 11×10^4 IU per ml. All virus titers determined at 24 hr after infection.

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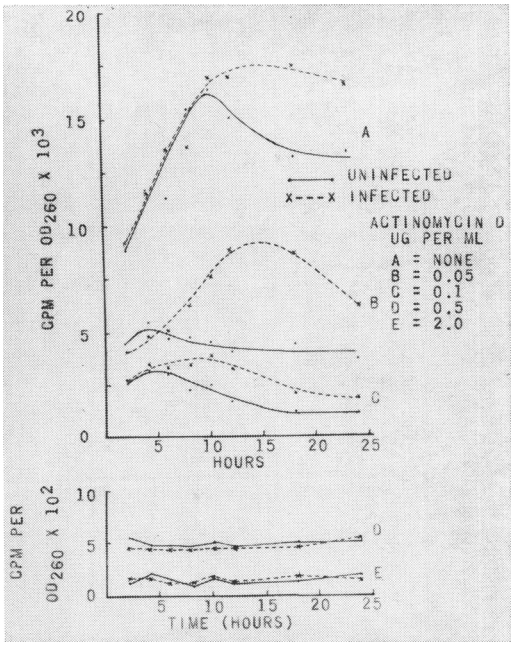

FIG. 1. Effect of varying concentrations of AD on RNA activity of uninfected and infected HeLa cells.

tion, until at 0.4 μg per ml the amount of virus produced was about 2% of that obtained from the untreated infected control.

Essentially similar results were obtained when cells were pretreated with AD for 4 hours before infection only. Under such conditions, the exposure of cells to 0.2 μg per ml of AD did not interfere with virus production, and again the yield of infectious virus was several times greater than that of control cultures. Similarly, higher concentrations of AD further reduced virus production.

RNA synthesis in presence of actinomycin. Monolayer cultures of HeLa cells were treated with varying concentrations of AD before and during infection and the synthesis of RNA was followed by addition of uridine-H^3 (0.25 μc per ml) at zero hour of infection. Total RNA was extracted and the amount of radioactivity incorporated at various times p.i. was determined (see *Materials and methods*).

The results (Fig. 1) indicate that the synthesis of RNA both in infected and in uninfected cultures not treated with AD proceeded at a similar rate for about 8 to 10 hours p.i. At this time period the incorporation of labeled precursor into the uninfected culture was halted, while the infected culture continued to take up uridine-H^3. When both infected and uninfected cultures were exposed to low concentrations of AD (0.05 μg and 0.1 μg per ml, respectively) which did not interfere with reovirus synthesis, the difference in the uptake of radioactivity between the two cultures could be clearly detected as early as 5 to 6 hours p.i., and was more marked at the lower concentration. Under these conditions, uninfected cells began to synthesize RNA at a decreasing rate at about 4 to 5 hours p.i. and very little net increase in radioactivity occurred after this period. However, infected cells continued to incorporate uridine; and in cultures treated with the lower concentration of AD (0.05 μg/ml) as much as a 2-fold increase in radioactivity was observed. Both the AD-treated infected and uninfected cultures exhibited a reduced capacity to incorporate the labeled uridine. In this instance, cell RNA synthesis in the uninfected cultures was markedly reduced by 45 to 85% of that of the control.

Treatment with higher concentrations of the antibiotic (0.5 μg or 2 μg per ml), which inhibit the synthesis of virus, did not produce a difference in incorporation of radioactivity between infected and uninfected cultures. At these concentrations of AD about 91 to 98% of the synthesis of RNA in uninfected cells was inhibited. The bulk of the label incorporated into the RNA of either uninfected or infected cells treated with AD was found to sediment as 4S soluble RNA in sucrose gradients.

Discussion. The present investigation indicates that the synthesis of reovirus RNA can be differentiated from cellular RNA synthesis by treating HeLa cells with low levels of actinomycin D. However, at higher concentrations of the antibiotic (>0.5 μg/ml), which inhibit cellular RNA activity to a greater extent (>95%), the preferential synthesis of viral RNA is not observed. In the light of the present evidence, and for the continued production of viral protein in the absence of viral RNA synthesis at high concentrations of AD (2 μg/ml) (1), it is clear that the mechanisms of inhibition of reovirus replication by the antibiotic are different from

those observed with a DNA-containing virus such as vaccinia(6,7). The conclusions that the inhibition by AD of reovirus replication is primarily a "toxic" effect on the cell, and that the antibiotic does not directly interfere with the synthesis of viral RNA, protein, or infectious virus, are strongly suggested by the progressive inhibition of cell RNA activity and the subsequent inability of the cell to synthesize viral RNA in the presence of increasing concentrations of AD. Furthermore, the reported failure of reovirus RNA to interact with AD(8) indicates that the effect of the antibiotic is on the host cell. Since the primary target of AD at low concentrations (<0.1 μg/ml) is the nucleus of the mammalian cell, resulting principally in the interruption of the formation of DNA-dependent ribosomal and messenger ribonucleic acids (9,10), and since the accumulated evidence favors the cytoplasm as the site of reovirus synthesis(2,11,12), it is reasonable to assume that under such conditions the replication of virus would not be inhibited. Because the cytoplasm is relatively unaffected(10,13) it is capable of supporting virus replication. Increasing the concentration of AD, which interferes markedly with all fractions of cell RNA activity, results in a more rapid deterioration of the cell's capacity to produce the complete particle, and only a limited amount of viral protein is synthesized(1). This incomplete sequence of virus production might be explained because of the relatively long latent period of the virus(2,11,12); and under these circumstances, the replacement of certain cellular enzyme systems ultimately required for some phase of virus formation may not occur in the presence of the antibiotic.

During the course of this investigation, Shatkin(14) and Kudo and Graham(15) reported results for the reovirus type 3-L cell system, which essentially agreed with those reported here. However, in the former system a higher concentration of AD (0.5 μg/ml) was found to permit normal output of virus and for differentiating between viral and cellular RNA synthesis. The difference in the concentrations used between the 2 systems may well reflect the relative sensitivities of the 2 cell lines to the antibiotic.

Summary. The effect of varying concentrations of actinomycin D on the synthesis of viral and cellular ribonucleic acids and on the replication of reovirus type 2 has been investigated. At low concentrations of the antibiotic (0.05 μg to 0.1 μg per ml) which allow maximal yields of infectious virus, the treated cultures exhibited a preferential synthesis of reovirus RNA and a suppression of cellular RNA activity.

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