RNA and Protein Synthesis in Rhinovirus Infected HeLa Cells (36380)

DONALD C. THOMAS, ROBERT M. CONANT, AND VINCENT V. HAMPARIAN
(Introduced by H. G. Cramblett)

The Departments of Medical Microbiology and Pediatrics, The Ohio State University College of Medicine and the Children's Hospital Research Foundation, Columbus, Ohio 43205

There are no reports concerning macromolecular synthesis in rhinovirus infected cells. Previously, we reported that rhinoviruses will replicate in suspended cultures of dividing HeLa cells (10). The suspended cell system is useful for preparing large quantities of concentrated virions and for studying macromolecular synthesis. In this report, we describe the effect of rhinovirus infection on label accumulation into RNA and protein and the subsequent synthesis of viral RNA and protein.

Materials and Methods. Cells and virus. Methods for growing HeLa cells in suspension and for propagation of rhinoviruses have been described elsewhere (10). Rhinovirus type 14, strain 1059 (3–5) was used for these studies. Plaque and infectivity assays were performed as previously described (6).

Radioisotopes and assay procedures. Rhinovirus was added to HeLa cells at multiplicities of infection (MOI) of either 2–4 or 40–60 PFU/ml. After adsorption for 30 min, either ^{3}H-uridine (0.5 μCi/ml) or ^{3}H-leucine (0.5 μCi/ml) was added to cultures to label RNA and protein, respectively. To increase ^{3}H-leucine incorporation, cells were suspended in medium containing 1/10 the normal concentration of the amino acid. Samples were removed at intervals, poured over frozen Earle's balanced salt solution (EBSS) to stop label incorporation and clarified by low speed centrifugation. The cell pellet was washed and brought back to original volume with EBSS (pH 7.4) and frozen. Tricholoroacetic acid (TCA)-insoluble radioactivity was assayed in a Packard liquid scintillation counter.

Use of actinomycin D. Uninfected HeLa suspension cultures were treated with various levels of actinomycin D to determine optimal concentration for shut-off of RNA synthesis (Fig. 1). Although 10.0 μg/ml caused a rapid shut-off of RNA synthesis, cell viability was also decreased. At a concentration of 5.0 μg/ml, RNA synthesis was reduced approximately 90% in 1 hr and 98% in 2 hr; this concentration was used in experiments employing actinomycin D.

Results. RNA synthesis in infected cells. Figure 2 shows the accumulation of ^{3}H-uridine into RNA of uninfected and infected cells at low MOI. In both cases, RNA synthesis continued at similar levels for 5 hr. After this time, accumulation of label into infected cells was inhibited and a gradual loss of radioactivity was observed through 10 hr.

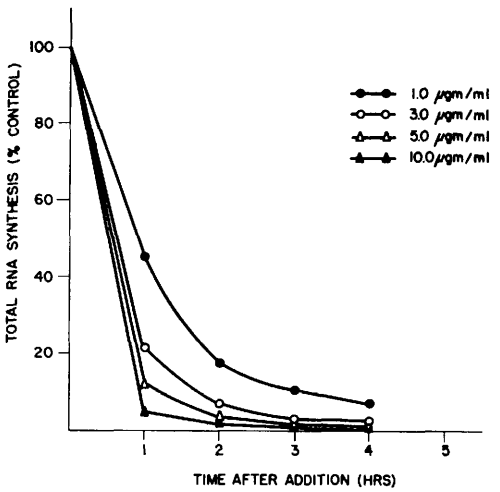

FIG. 1. ^{3}H-Uridine incorporation into actinomycin D treated cells. Suspension cultures of HeLa cells (5.0×10^5/ml) were treated with different levels of actinomycin D and labeled with ^{3}H-uridine. Samples were removed at various times after additon of the antibiotic, and assayed for TCA-precipitate radioactivity.

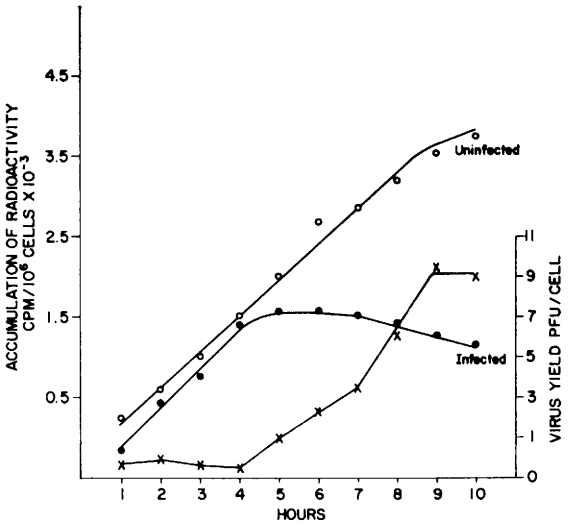

FIG. 2. ^{3}H-Uridine accumulation into rhinovirus infected cells. Suspension cultures of HeLa cells (5.0×10^5/ml) were infected with rhinovirus (MOI = 2–4 PFU/cell), and labeled with ^{3}H-uridine (sp act 20.0 Ci/mmole). Aliquots taken at various times after infection were assayed for TCA-precipitable radioactivity and plaque forming units. (×) PFU/cell.

The loss of TCA-precipitable radioactivity very late in infection may be due to lysis or degradation of RNA. Cell lysis may not account for all loss since extracellular virus does not increase significantly until 10–12 hr after infection (10). Accumulation of label into uninfected cells continued linearly to 9 hr and then remained constant.

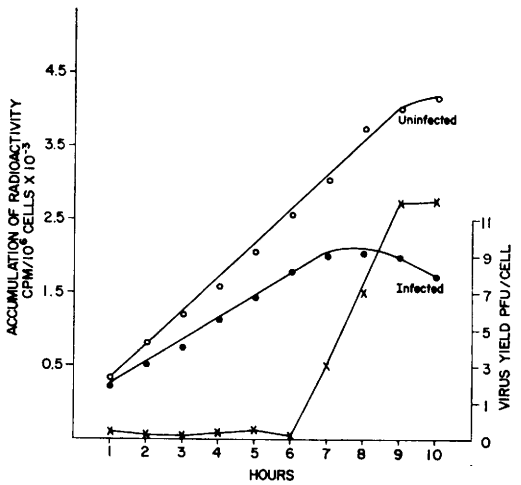

FIG. 3. ^{3}H-Leucine incorporation into rhinovirus infected cells. Cells were infected and treated as stated in Fig. 2, except ^{3}H-leucine (sp act 2.0 Ci/m mole) was added. (×) PFU/cell.

Protein synthesis in infected cells. In contrast to uptake in uninfected cells, accumulation of labeled amino acid into infected cells was reduced (Fig. 3). Accumulation continued at a reduced level for 7 hr, at which time the first significant increase in intracellular virus was detected. Intracellular virus peaked 9 hr postinfection, approximately 2-3 hr after accumulation of labeled amino acid had ceased.

RNA and protein synthesis in the presence of actinomycin D. These experiments (Fig. 4) were done with a high MOI (40–60); however, the results were similar to those obtained with an MOI of 2–4. Maximum ^{3}H-uridine accumulation into total RNA occurred at 4–5 hr after infection followed by a gradual loss of radioactivity through 8 hr. Maximum accumulation of label into viral RNA (measured by accumulation of ^{3}H-uridine into infected cells treated with actinomycin D) occurred at 4–5 hr after infection followed by a gradual loss of radioactivity. It is not known if the loss of radioactive RNA was due to lysis or degradation.

RNA synthesis was also investigated by pulse-labeling infected cells for 1 hr with tritiated uridine. The results indicated that uptake kinetics were similar to those ob-

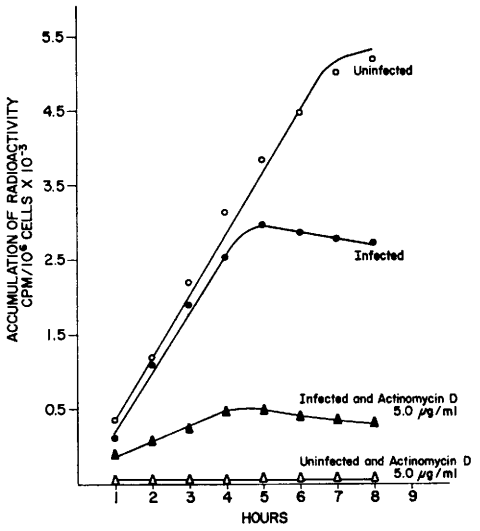

FIG. 4. ^{3}H-Uridine incorporation into rhinovirus infected cells treated with actinomycin D. Cells were infected with rhinovirus (MOI = 40–60 PFU/cell), treated with actinomycin D, and labeled with ^{3}H-uridine as stated in Fig. 2.

tained with continuous labeling.

High MOI (40–60) also did not significantly alter the pattern of ^{3}H-leucine accumulation into infected cells (Fig. 5). Maximum accumulation of label into proteins of infected cells occurred at 5–6 hr. Actinomycin D did not significantly alter the accumulation of label into infected cells. In addition, purified virus, prepared in CsCl gradients (10), was used in these experiments to avoid possible nonspecific effects of nonviral components in the inoculum (2).

Although not shown, the yield of infectious virus was not affected in experiments employing actinomycin D. In addition, the yield of cell associated virus was not increased by the higher MOI.

Discussion. Rhinovirus type 14 inhibited accumulation of ^{3}H-uridine into RNA late (about 5 hr) in the infectious cycle. Accumulation of label into rhinovirus RNA was maximal when virus induced inhibition of host cell RNA production was first detected. The transition from host to viral RNA synthesis appeared to be continuous. The loss of labeled RNA in infected cells might have been due to degradation of RNA. Probably, cell lysis did not account for this loss since it has been shown that extracellular virus does not increase significantly until 10–12 hr after infection (10). McCormick and Penman (8) reported a gradual inhibition of host cell RNA synthesis in either poliovirus or mengovirus infected HeLa cells. Maximum inhibition occurred late in the infectious cycle. Bablanian, Eggers, and Tamm (1) also demonstrated a similar inhibition of RNA synthesis in HeLa cells infected with poliovirus.

Rhinovirus infection appears to rapidly lower the level of ^{3}H-leucine accumulation into protein in HeLa cells. However, maximum inhibition occurs late in infection. Possibly, rhinovirus immediately affects the synthesis of host mRNA. In contrast, Penman and Summers (9) reported the inhibition of protein synthesis in poliovirus infected cells after a period of protein synthesis. They also reported that actinomycin D caused a more rapid decline of protein synthesis in infected than in uninfected cells. This reduction in protein synthesis was supposedly caused by inactivation of host cell mRNA, but apparently some host mRNA was produced and supported protein synthesis in the presence of virus (11). McCormick and Penman (8) have reported similar results in HeLa cells infected with mengovirus. However, in L-cells infected with mengovirus, actinomycin D did not affect protein synthesis. To explain these differences, they have suggested that the inhibitory mechanism suppressing host RNA synthesis in HeLa cells is either inoperative or insensitive to virus infection. However, protein synthesis in rhinovirus infected HeLa cells was not affected by actinomycin D. Thus, our results support the concept for the existence of 2 distinct viral functions: one that interrupts host protein synthesis and another that suppresses host RNA synthesis (7).

Patterns of label accumulation into RNA and protein are not affected by the multiplicity of infection. Possibly the low MOI used in our studies was sufficient to infect the majority of cells. Alternatively, the possibility exists that only a few cells in the population are susceptible to rhinovirus and higher multiplicities do not increase the proportion

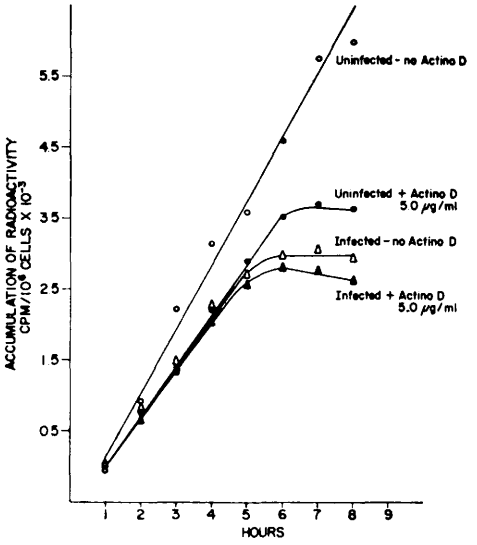

FIG. 5. ^{3}H-Leucine incorporation into rhinovirus infected cells treated with actinomycin D. Suspension cells were infected with rhinovirus (MOI = 40–60 PFU/cell), treated with actinomycin D (5.0 μg/ml), and labeled with ^{3}H-leucine as stated in Fig. 3.

of cells that become infected. Furthermore, the yield of infectious virus (PFU/cell) using a high MOI is not significantly increased.

Summary. Initial inhibition of label accumulation into RNA of rhinovirus infected cells occurs 5 hr postinfection when viral RNA synthesis is at a maximum. Infection lowers the level of label accumulation into protein with maximum inhibition occurring late in the cycle. Actinomycin D does not significantly affect protein synthesis in infected cells. These results support the hypothesis that two separate functions are responsible for inhibition of host RNA and protein synthesis.

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