VACCINIA VIRUS DIRECTED RNA AND PROTEIN SYNTHESIS IN THE PRESENCE OF RIFAMPICIN

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Summary. The effects of rifampicin, an inhibitor of bacterial DNA transcription, on the formation of infectious vaccinia virus and viral particles, RNA and proteins were studied under single step growth conditions. Although virion formation was inhibited by more than 99%, the synthesis of early and late viral directed RNA species, as characterized by their sedimentation properties, was unaffected. Rifampicin did not prevent the synthesis of early and late viral proteins identified by immunodiffusion, disc gel electrophoresis and disc gel immunoelectrophoresis. The drug also did not inhibit the in-vitro activity of the virion associated RNA polymerase.

Rifampicin and related rifamycin derivatives prevent bacterial growth and phage replication by interacting with bacterial DNA-dependent RNA polymerase (1, 2, 3, 4). Although this class of antibiotics has relatively little effect on the activity of mammalian RNA polymerase (5, 6, 7), rifampicin prevents the growth of vaccinia virus and adenovirus (8, 9). It has been suggested that the antiviral action of rifampicin in animal cells results from interaction with virus-induced enzymes (8, 9). The report that rifampicin inhibits cytoplasmic uridine incorporation induced by wild-type vaccinia virus but not that induced by drug resistant mutants supports this suggestion (9).

The present experiments were designed to study the effects of rifampicin on: (1) vaccinia virus directed RNA and protein synthesis under single step growth conditions and (2) in-vitro activity of the template-associated RNA polymerase contained within the vaccinia virion.

Virus growth. We compared the relative effects of 100 μg/ml of rifampicin and 10 μg/ml of actinomycin D on vaccinia virus growth. An increase in infectious virus, usually detected at 5 to 6 hours, was not seen in the presence of either

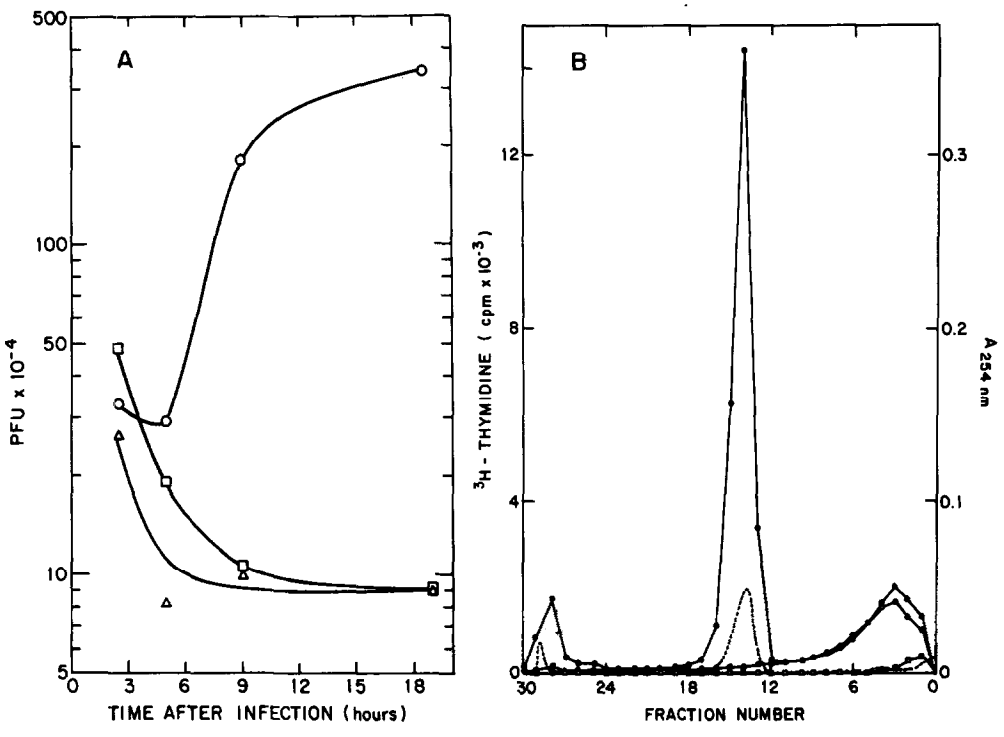

Fig. 1. Effect of rifampicin on virus growth. A. HeLa cells in suspension at a concentration of 4 X 10^6/ml were treated with 100 μg/ml of rifampicin or 10 μg/ml of actinomycin D. Purified vaccinia virus, strain WR, at a multiplicity of 30 PFU/cell was added after 10 minutes. At 30 minutes after infection the cells were washed 3 times with fresh medium containing the same drug concentrations and then resuspended in the same medium at a cell concentration of 4 X 10^5/ml. Samples were removed at appropriate times and infectious virus titers were measured by plaque assay on HeLa cell monolayers. 0, no drug; ▫ , 10 μg/ml of actinomycin D; △ , 100 μg/ml of rifampicin. B. HeLa cells treated with rifampicin and infected as in A were incubated with 0.2 μCi/ml of thymidine-methyl - ^{3}H (20 Ci/mmole Schwarz BioResearch Co.) from 1 to 19 hours after infection. Purified virus was then added as carrier and the entire suspension was frozen and thawed, subjected to sonication, digested with deoxyribonuclease and ribonuclease, sedimented through 36% sucrose and finally through a 25 to 40% sucrose density gradient, essentially as described by Joklik (12). The optical density of the carrier virus (----) and the amount of radioactive material in the fractions (——) are shown: ●, no drug; ▫ , 10 μg/ml of actinomycin D; 0, 100 μg/ml of rifampicin.

inhibitor, Fig. 1A. Rifampicin also inhibited, by more than 99%, the formation of ^{3}H-thymidine labeled viral particles, Fig. 1B. This inhibition was not due to a block in virus absorption nor caused by irreversible effects on the cells, since virus replication occurred when the drug was removed 7 hours after infection.

Virion RNA polymerase. The template-associated RNA polymerase activity of puri-

fied vaccinia virions was measured by the in-vitro incorporation of ^{3}H-UTP into trichloroacetic acid precipitable material. The quantity of RNA made in this system was calculated to be greater than the amount of template DNA present. Rifampicin, added either before or after the addition of the four nucleoside triphosphates, did not reduce this polymerase activity, Fig. 2. The polymerase activity of vaccinia virus cores, isolated from infected cells, was also not affected by rifampicin.

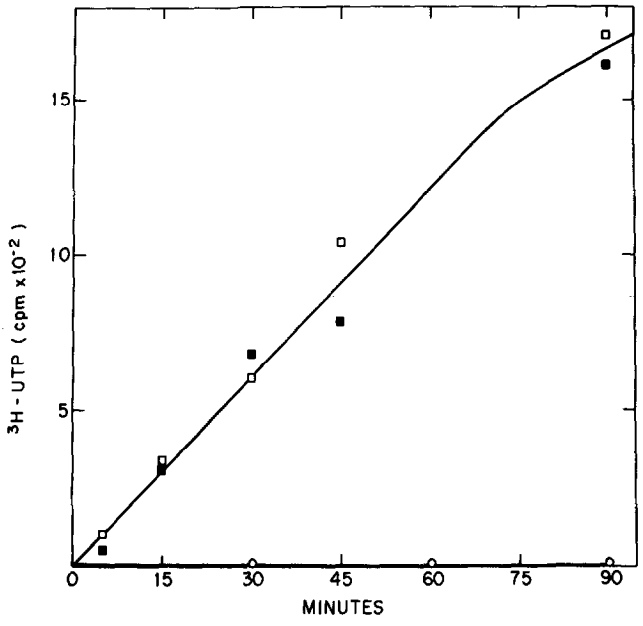

Fig. 2. Effect of rifampicin on RNA polymerase activity. Vaccinia virus was purified by sedimentation through 36% sucrose and repeated sedimentation into 25 to 40% sucrose gradients (12). The purified virus was disaggregated by sonication and incubated for 1 hour at 0 C in 0.05M Tris HCl, pH 9.0 containing 10mM mercaptoethanol as described (10). The virus was incubated without drug or with 10 µg/ml of actinomycin D or 100 µg/ml of rifampicin for 15 minutes at 37 C. Enzyme activity was measured with ^{3}H-UTP (77 mCi/mmole, Schwarz BioResearch Co.) using a previously described reaction mixture (17). □ , no drug; ■ , rifampicin; 0, actinomycin D.

Early and late viral RNA synthesis. Cellular RNA, except for the 4S transfer RNA, is not released into the cytoplasm in significant amounts during a 10 minute pulse with radioactively labeled uridine (13). This provides the basis for preferentially labeling vaccinia viral messenger RNA which is synthesized within the cytoplasm (13). Early vaccinia viral messenger RNA, which is made from par-

ental DNA templates during the first two hours of infection, has a lower sedimentation rate and hybridizes with fewer viral DNA sequences as compared with viral RNA made from progeny DNA at later times (14). Rifampicin, at a concentration of 100 μg/ml did not decrease the rate of synthesis of cytoplasmic RNA species with the sedimentation properties of early and late vaccinia messenger RNA, Fig. 3. In the presence of inhibitors of protein synthesis, early vaccinia

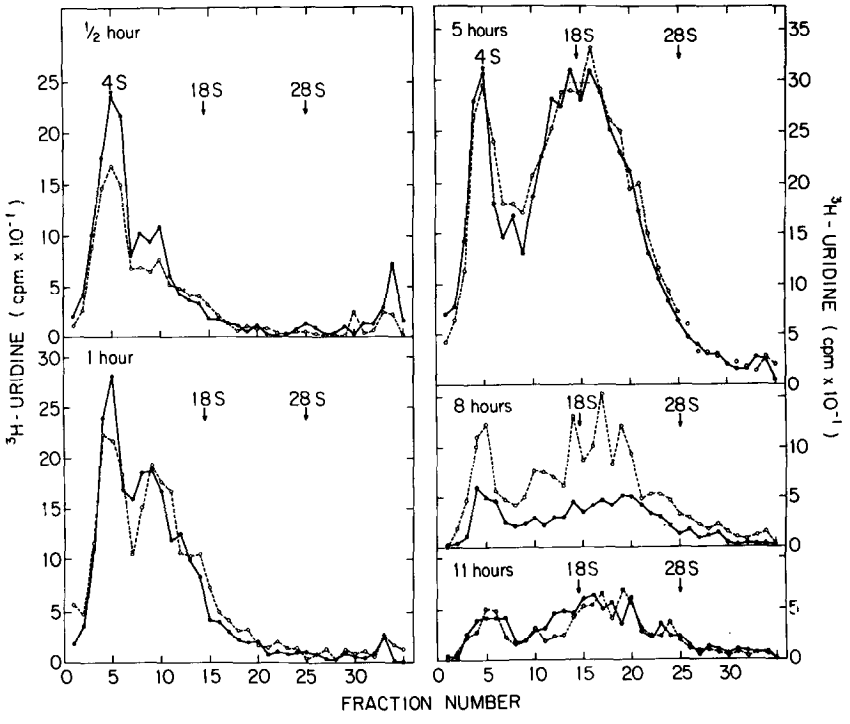

Fig. 3. Effect of rifampicin on the synthesis of early and late virus directed RNA. HeLa cells were treated with rifampicin and infected with vaccinia virus as described in Fig. 1. At intervals, 50 ml portions of cells were incubated with 200 μc of ^{3}H-uridine (20 Ci/mmole, Schwarz BioResearch Co.) for 10 minutes. The cells were rapidly chilled, washed and Dounce homogenized in 10mM Tris, 10mM NaCl, 1.5mM $MgCl_2$, pH 7.8. The cytoplasmic extract, obtained after centrifugation at 1,000 g for 5 minutes was made 1% in sodium dodecyl sulfate and sedimented through a 17 ml linear 15 to 30% sucrose density gradient, essentially as described by Becker and Joklik (17). The gradients were passed through an ultraviolet monitor at 254 nm and collected in 0.5 ml fractions. Samples were placed on filters and washed with 5% trichloroacetic acid prior to counting. ●, no rifampicin; ○, 100 μg/ml of rifampicin.

viral messenger RNA continues to be made for several hours at even higher than usual rates (15, 16). Rifampicin also did not affect the increased viral RNA synthesis occurring in the presence of 300 μg/ml of cycloheximide.

Decreased uridine incorporation into viral messenger RNA and 4S RNA was observed at a rifampicin concentration of 200 μg/ml. However, this concentration of rifampicin, which was greater than that needed to inhibit virus replication, markedly inhibited the growth of uninfected cells.

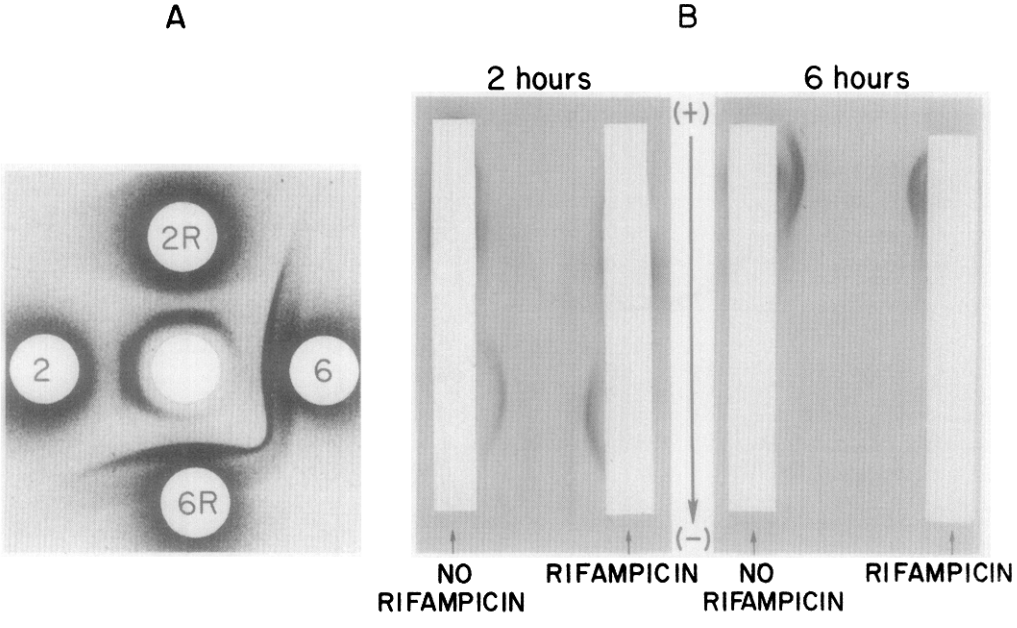

Fig. 4. Effect of rifampicin on early and late viral protein synthesis. HeLa cells were treated with rifampicin and infected with vaccinia virus as described in Fig. 1, except that the growth medium contained 10% of the usual concentration of amino acids. At 2 and 6 hours after infection, samples were removed and incubated with 0.5 μCi/ml of mixed ^{14}C-amino acids (Schwarz BioResearch Co.) for 30 minutes. Cytoplasmic extracts were then prepared and centrifuged at 100,000 g for 1 hour. The labeled proteins present in the supernatants were examined by: A. immunodiffusion (19) and B. disc gel-immunoelectrophoresis (18). In the latter procedure the gels were embedded in agar after polyacrylamide gel electrophoresis and antiserum was added to channels cut parallel to the polyacrylamide gels. Immunodiffusion lines were allowed to form in the agar. The antiserum was made by infecting rabbits with purified live vaccinia virus and does not react with HeLa cell proteins. Radioautographs of the immunoprecipitin lines formed by the 2 techniques are shown. The indicated numbers refer to the pulse-labeling times. Samples from cells treated with rifampicin are designated R.

Early and late viral protein synthesis. Different viral proteins are synthesized before and after replication of viral DNA, presumably because of the different messenger RNA species present at these times. The proteins made in the presence of 100 μg/ml rifampicin were identical, by immunodiffusion and disc gel-immunoelectrophoresis, to early and late proteins made in the absence of rifampicin, Figs. 4A, B. These proteins were still formed when rifampicin treatment was started 12 hours prior to infection. The sequential synthesis of 3 prominent vaccinia viral proteins has been shown using disc gel electrophoresis (18). These proteins were made, although in somewhat different proportions, in the presence of rifampicin, Fig. 5. The decrease in rate of viral protein synthesis, observed at 7 to 8 hours after infection, was accelerated in the presence of rifampicin.

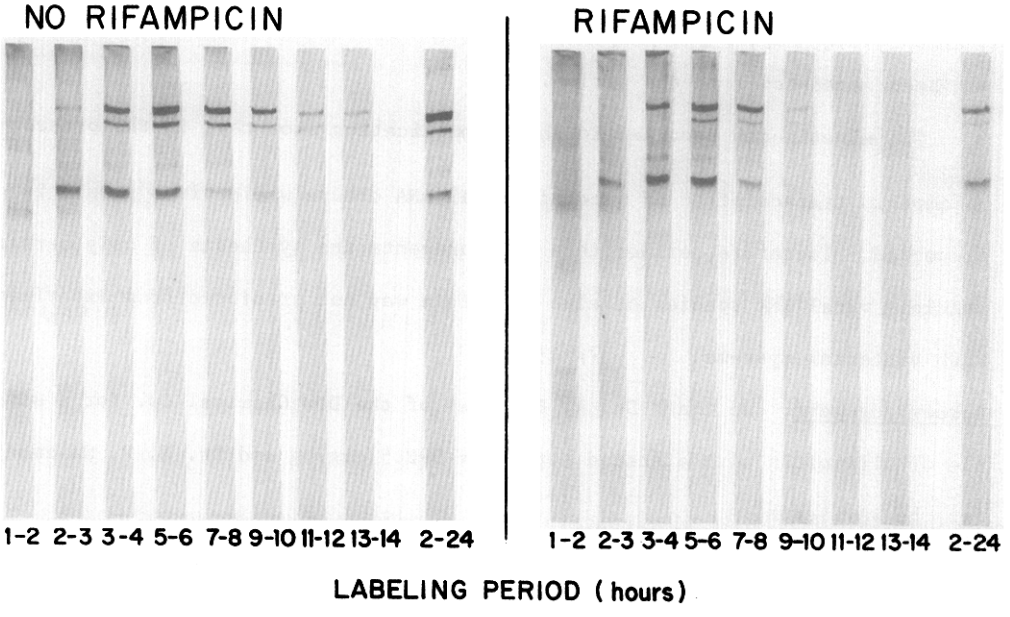

Fig. 5. Effect of rifampicin on the synthesis of late viral proteins. HeLa cells treated with rifampicin and infected with vaccinia virus, were incubated with ^{14}C-amino acids as described in Fig. 4. The cytoplasmic extracts prepared at the end of the labeling periods were centrifuged at 100,000 g and analyzed by disc gel electrophoresis (18). Radioautographs of the trichloroacetic acid washed gels are shown.

Discussion. Subak-Sharpe et al., using radioautographic methods, reported that rifampicin reduced vaccinia virus directed cytoplasmic uridine incorporation (9). We found that under conditions of single step vaccinia virus replication, a concentration of rifampicin which inhibited virion formation by more than 99% did not affect the rate of virus-directed cytoplasmic uridine incorporation. Furthermore, the RNA species synthesized in the presence of rifampicin had the sedimentation properties of early and late vaccinia messenger RNA suggesting that rifampicin did not prevent RNA synthesis from parental or progeny DNA templates. Our finding that early and late viral proteins were formed, further demonstrated that early and late RNA species were made.

The in-vitro activity of the DNA-directed RNA polymerase contained within the vaccinia virion was shown to be unaffected by rifampicin. It would be desirable to test the effects of the drug on a template-free form of this enzyme since rifampicin inhibits the E. coli RNA polymerase only prior to the formation of a stable complex with DNA (20). Unfortunately the vaccinia viral enzyme has not yet been separated from the virion.

The experiments reported in this communication show that in the presence of rifampicin transcription of vaccinia viral DNA occurs while virus growth is prevented. Therefore, either this drug prevents the synthesis of only certain vaccinia viral RNA species or else acts in a way not predicted from experiments with bacterial systems.

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