Inhibition of Pichinde Virus Replication by Actinomycin D

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SUMMARY

The yields of Pichinde virus, a member of the arenavirus group, were markedly inhibited when infected BHK 21 cells were incubated in the presence of 0.4 to 4 µg/ml of actinomycin D. Maximal inhibition was observed when actinomycin D was added after the adsorption of virus to cultures; however, addition of drug as late as 12 h after infection reduced the 24 h yield by 50%. Virus antigen synthesis, as measured by complement fixation and immunodiffusion, was not dramatically reduced by actinomycin D. The expression of virus antigens on the surface of infected cells was greater on cells treated with actinomycin D than on untreated cells. Putative defective particles with a density of Pichinde virus were not detected in fluids of cultures incubated with actinomycin D and 3H-amino acids. Actinomycin D appears to inhibit Pichinde virus late in the replicative cycle. The observations raise the possibility that the drug inhibits the synthesis of proteins of the host cell membrane which are required for virus maturation.

INTRODUCTION

The arenaviruses characteristically produce persistent infections in their natural host (Rowe et al. 1970). The viruses produce minimal cytopathic effect in vitro and carrier cultures can be established with relative ease (Lehmann-Grube, Slenczka & Tees, 1969; Staneck et al. 1972). The mechanisms by which virus genetic information is maintained in animals persistently infected with arenaviruses are not known. Cyclic infection of cells by lymphocytic choriomeningitis virus has been proposed as an explanation for persistence of this virus in mice (Hotchin, 1974). A requirement of this hypothesis is the regulation of virus synthesis, mediated through a gene product coded for by the virus or by the host cell, which results ultimately in the loss or complete repression of virus genetic information. Alternatively, virus genetic information could be maintained in the infected cells in some form such as integrated double-stranded DNA; a situation analogous to the persistent infections produced by oncornaviruses.

Since oncornaviruses have a double-stranded DNA phase in their replicative cycle, their replication is inhibited by actinomycin D which prevents transcription of RNA from DNA (Temin, 1963; Collett, Kieras & Faras, 1975; Hruska & Takemoto, 1975). Actinomycin D has been shown to inhibit a number of single-stranded RNA viruses including the arenaviruses (Barry, Ives & Cruickshank, 1962; Pfau, 1974; Diener & Smith, 1975; Yamazaki & Notkins, 1975). The yields of infectious arenaviruses were found to be enhanced with low concentrations of actinomycin D (Stanwick & Kirk, 1971; Carter et al. 1973) while the yield of infectious virus was found to be markedly reduced at high concentrations (Buck & Pfau,
The mechanisms of inhibition of single-stranded RNA viruses which do not appear to have a double-stranded DNA phase in their replicative cycle are poorly understood. The finding of RNA-dependent RNA polymerase activity but not RNA-dependent DNA polymerase activity associated with Pichinde virus (Carter, Biswal & Rawls, 1974) suggests that a DNA phase in the replication of arenaviruses probably does not occur.

The present study was undertaken to examine the inhibition of Pichinde virus replication by actinomycin D in more detail. Specifically, we sought to determine at what point in the replicative cycle the drug was inhibiting virus replication. We found that actinomycin D did not inhibit the synthesis of virus antigens detected in the cell cytoplasm or at the cell surface although the yield of virus particles appeared to be reduced.

**METHODS**

**Virus and virus assays.** Viruses were replicated in BHK 21 cells which were cultured in plastic flasks (Falcon) or roller bottles (Corning). Vero cells were used for virus assays. The cells were grown to confluency using Eagle's minimal essential medium supplemented with 10% heat-inactivated foetal calf serum, glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 0.075% NaHCO₃ and 10 mM-hydroxyethylpiperazine N'-2-ethanesulphonic acid (Hepes), pH 7.4. After infection, confluent monolayers were maintained on Eagle's medium containing 2% serum and 0.15% NaHCO₃.

Pichinde virus, strain AN3739, was used in this study (Trapido & Sanmartin, 1971). The virus was passaged 5 or 6 times in BHK 21 cells after receipt of the virus in our laboratory. The source of Sindbis virus as well as the method of assay of both Pichinde virus and Sindbis virus by plaque formation in Vero cells has been previously described (Mifune et al. 1971).

To propagate the Pichinde virus, confluent monolayers were infected at a multiplicity of infection of 1 to 3 p.f.u./cell unless otherwise indicated. Adsorption was for 60 min at 37°C, after which the cells were washed free of unadsorbed virus and overlaid with maintenance medium. Isotopically labelled virus was prepared utilizing 0.5 µCi/ml all-amino acid mixture to label proteins (New England Nuclear Canada, Montreal). Details of the labelling procedures as well as the techniques used to concentrate and purify the virus from culture fluids are described elsewhere (Ramos, Courtney & Rawls, 1972). Actinomycin D (Calbiochemical) was stored at -35°C in lyophilized form and solubilized as needed for each experiment.

**Antiserum.** The antiserum used was prepared by infecting adult LVG hamsters with 2.5 × 10⁴ p.f.u. of Pichinde virus intraperitoneally. The animals were exsanguinated 5 weeks later and the serum was stored at -35°C. Hamster globulin was prepared by a method previously described (Cherry, 1974) with the modification that three sequential precipitations at 50%, 40% and 35% of the final concentrations of saturated ammonium sulphate were carried out, with washing of the precipitate with ammonium sulphate solution of the precipitating concentration at each step. A sample of the globulin obtained as described above was conjugated with fluorescein isothiocyanate by the method of Cherry (1970).

For iodination, IgG was purified from the globulin by ion exchange chromatography on a DEAE cellulose column. IgG was eluted from the column with 0.05 M-sodium phosphate buffer, pH 7.5. The IgG thus obtained was concentrated to approx. 4 mg/ml and 40 µg portions were labelled with Na¹³¹I by the chloramine T method of Greenwood, Hunter & Glover (1963). Free iodine was removed by chromatography on a Sephadex G-25 column and the fraction containing protein typically contained greater than 95% TCA-insoluble radioactivity. Iodinated IgG was stored at 4°C and used within 72 h of labelling.
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**Antigen assays.** A microcomplement fixation test for complement fixing (CF) antigen was carried out as described elsewhere (U.S. Dept. of Health, Education & Welfare Publication, 1969). Hamster serum from animals infected with Pichinde virus was used at a constant concentration of 5 full units of antibody per well, and five 50 % haemolytic units of guinea pig complement were used. All dilutions were made in veronal buffered saline, pH 7.4, containing 0.1 % gelatin. In experiments designed to measure antigen units per milligram of protein, the average of 3 to 5 determinations was taken as a quantitative estimate.

Direct immunofluorescent staining for virus antigens in the cell cytoplasm was carried out using acetone-fixed cells grown on coverslips. Briefly, thrice washed coverslips were dried then fixed by immersion in cold acetone for 10 min. Staining for cytoplasmic antigen was carried out with FITC conjugated immune hamster globulin for 30 min at 37 °C after which the coverslips were washed with phosphate buffered saline (PBS) three times and mounted in buffered glycerol. The conjugated globulin was used at a dilution of 1:64 and was shown to be specific.

Surface antigens were detected by immunofluorescence and by 125I IgG binding to viable cells. Surface antigens were detected on the surface of cells grown on coverslips by a modification of the method described by Rutter & Gschwender (1973). Briefly, coverslip cultures were washed three times with phosphate buffered saline then reacted for 15 min at 20 °C with immune hamster serum. The cultures were then washed three more times and fixed for 10 min in 10 % phosphate buffered formalin solution, pH 7.2, on ice. After fixation the coverslips were washed twice then reacted with FITC conjugated rabbit anti-hamster IgG globulin (Cappel) for 20 min at 20 °C. Coverslips were washed three times and mounted in buffered glycerol. Surface antigens were also detected using cells which had been monodispersed with trypsin. The trypsinized cells were washed once with buffered saline containing 2 % foetal bovine serum which had been heat-inactivated and sampled into 15 x 75 mm glass test tubes at 1 x 10^5 cells/tube. The cells were reacted for 30 min at 37 °C with fluorescein conjugated immune hamster globulin, washed three additional times, suspended in a drop of buffered glycerol and mounted on slides.

Further assessment of antigens on the surface of cells was obtained by reacting the cells with 125I-labelled IgG. The cells were monodispersed with trypsin, and samples containing 10^6 cells collected in siliconized tubes. The cells were pelleted at 1000 rev/min for 3 min, the supernate drained from the tubes and 50 μl of 125I-labelled IgG added. After 30 min at 37 °C the cells were washed five times with buffered saline containing 2 % foetal bovine serum which had been heat inactivated. During the final wash, the cells were transferred to new tubes and after the cells were pelleted, the supernates were removed and the radioactivity remaining in the cells was determined in a Beckman automatic gamma-counter. Estimates of protein concentration were made by the method of Lowry et al. (1951), using a bovine serum albumin standard.

**RESULTS**

**Effect of actinomycin D on the yield of infectious virus**

Previously, actinomycin D was shown to inhibit the replication of Pichinde virus in Vero cells (Mifune et al. 1971). We initially examined the effect of actinomycin D on the replication of Pichinde virus in BHK 21 cells. Monolayers of BHK 21 cells on coverslips were incubated with 1 to 3 p.f.u./cell and excess virus was washed from the monolayer after adsorption. The monolayers were then covered with medium containing different concentrations of the drug. Samples of medium were removed at 24 and 48 h after infection and assayed for infectious virus while the cells were examined for cytoplasmic antigen by immunofluorescence.
Simultaneously, uninfected monolayers were examined after 24 h for incorporation of $^3$H-uridine into acid-insoluble material. The replication of Sindbis virus was examined in parallel cultures.

The 24 h yield of infectious Pichinde virus was reduced by about 75 % in the presence of 0.4 μg/ml of actinomycin D and 92 % in the presence of 1 μg/ml of the drug (Fig. 1). Greater inhibition was observed after 48 h of incubation than after 24 h. The decrease in virus yield was not linearly related to the concentration of actinomycin D but paralleled the decrease in $^3$H-uridine incorporation in uninfected cells. In contrast to virus yield, the percentage of cells synthesizing virus antigen was not decreased by the increasing concentrations of actinomycin D. Neither the yield of infectious Sindbis virus nor the number of cells containing virus antigens were reduced by the drug (data not shown).

The time dependence of actinomycin D addition relative to the virus growth cycle was
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Fig. 2. The effect on virus yield of the addition of actinomycin D (2 µg/ml) at different times after infection. ●---●, No actinomycin D; ○--○, after adsorption; □--□, 4 h after infection; ▲--▲, 8 h after infection; △--△, 12 h after infection.

examined. BHK 21 cells were infected with 3 p.f.u./cell and 2 µg/ml of actinomycin D was added at various intervals after infection. Samples of culture fluids were removed at intervals and assayed for virus. The results are shown in Fig. 2. It is apparent that infectious virus was produced in the presence of the drug. Maximum inhibition of virus was observed when actinomycin D was added immediately after the period of virus adsorption; however, reduced yields were observed when the drug was added as late as 12 h after infection, the latest period examined. The degree of inhibition decreased as the duration between infection and the addition of actinomycin D increased; mean inhibitions of 3 experiments were 99%.
Table 1. Effect of multiplicity of infection on inhibition of Pichinde virus by actinomycin D

<table>
<thead>
<tr>
<th>Multiplicity (p.f.u./cell)</th>
<th>Fluid or cell associated</th>
<th>No act. D</th>
<th>Act. D (1 µg/ml)</th>
<th>Act. D (4 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yield</td>
<td>Inhibition (%)</td>
<td>Yield</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td></td>
<td>2·4 x 10^8</td>
<td>97</td>
<td>5 x 10^8</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1 x 10^6</td>
<td>99</td>
<td>1·1 x 10^8</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>6 x 10^6</td>
<td>97</td>
<td>7 x 10^5</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>9·3 x 10^7</td>
<td>98</td>
<td>9·1 x 10^5</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>3 x 10^7</td>
<td>98</td>
<td>1·2 x 10^5</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>3·6 x 10^7</td>
<td>98</td>
<td>7·8 x 10^6</td>
<td>98</td>
</tr>
</tbody>
</table>

* Virus yield expressed as p.f.u./ml measured after 48 h of incubation.

Table 2. Inhibition of Pichinde virus by actinomycin D at 24 and 48 h after infection in relation to culture conditions

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Concentration act. D</th>
<th>Cells/culture*</th>
<th>Virus yield (24 h)</th>
<th>Cells/culture*</th>
<th>Virus yield (48 h)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p.f.u./culture*</td>
<td>p.f.u./cell</td>
<td>p.f.u./culture*</td>
<td>p.f.u./cell</td>
</tr>
<tr>
<td>Exponential phase</td>
<td>0·00</td>
<td>22</td>
<td>600</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>0·4</td>
<td>3·7</td>
<td>22</td>
<td>5·9</td>
<td>2·5</td>
</tr>
<tr>
<td></td>
<td>1·0</td>
<td>3·5</td>
<td>22</td>
<td>6·3</td>
<td>2·6</td>
</tr>
<tr>
<td></td>
<td>4·0</td>
<td>3·4</td>
<td>28</td>
<td>8·2</td>
<td>2·5</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>0·00</td>
<td>100</td>
<td>160</td>
<td>1·6</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>0·4</td>
<td>23</td>
<td>12</td>
<td>0·5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1·0</td>
<td>32</td>
<td>10</td>
<td>0·3</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>4·0</td>
<td>25</td>
<td>3</td>
<td>0·1</td>
<td>20</td>
</tr>
</tbody>
</table>

* Cell and virus titres per culture x 10^5.

89 %, 84 % and 50 % when actinomycin D was added at 0, 4, 8 and 12 h after infection, respectively.

The results of a representative experiment designed to determine the effect of m.o.i. on inhibition by actinomycin D are presented in Table 1. The degree of inhibition of infectious virus was similar at all multiplicities examined and the yields of both cell-free and cell-associated virus were reduced.

To examine the possibility that inhibition of virus replication was related to the cell cycle, the extent of inhibition of virus replication by actinomycin D was compared in cultures in the exponential phase of growth and in cultures in stationary phase. Flasks were seeded with BHK 21 cells and incubated at 37 °C. The numbers of cells in the cultures were enumerated daily with the aid of a haemocytometer. One set of flasks which contained dividing cells and one set of flasks in which the cell numbers were not increasing were infected with 3 p.f.u./cell of Pichinde virus. Actinomycin D was added to some of the cultures after the adsorption period and the culture fluids were assayed for virus after 24 and 48 h of incubation. Inhibition of virus by actinomycin D was observed in both exponentially growing cells and in cells in stationary phase (Table 2). The yield of virus/cell was approx. tenfold greater in rapidly dividing cells than in cells which were not dividing.
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**Effect of actinomycin D on antigen synthesis**

Immunofluorescent staining of acetone fixed cells indicated that Pichinde virus antigens were synthesized in the presence of actinomycin D (Fig. 1). Estimates of the quantity of antigens produced were obtained by infecting monolayers of BHK 21 cells grown in 75 cm² flasks. The infected monolayers were incubated for 48 h in the presence of different concentrations of actinomycin D. The monolayers were washed and the cells then disrupted in a small vol. of buffer and assayed for protein content and CF antigen activity. Cells incubated in the absence of actinomycin D contained 7 CF units/mg of protein while 4.9, 4.6 and 4.5 CF units/mg of protein were obtained from monolayers incubated in the presence of 1, 2 and 3 μg of actinomycin D, respectively. Thus, there was approx. 35% less antigen synthesized in the presence of 1 μg of actinomycin D than in the absence of the drug and higher concentrations of the drug did not produce a further reduction in antigen synthesis.

The identity of the antigens produced in the presence of actinomycin D was examined by immunodiffusion. Two specific antigens are detected in cells infected by Pichinde virus. As can be seen in Fig. 3, both of these antigens were detected in lysates of infected cells which had been incubated in the presence of actinomycin D.

To further assess the effect of actinomycin D on antigen synthesis, infected cells were examined for surface antigens by immunofluorescent staining. Viable cells on coverslips and
Fig. 4. Immunofluorescent staining of the surface of cells infected with Pichinde virus. BHK 21 cells were infected with Pichinde virus, incubated 24 h at 37 C, trypsinized and stained with FITC conjugated IgG from immune hamsters. (a) Cells incubated in the presence of 1 µg/ml of actinomycin D; (b) no actinomycin D.

Table 3. The effect of actinomycin D on the expression of surface antigens on the surface of infected cells

<table>
<thead>
<tr>
<th>Cells examined</th>
<th>Immune serum</th>
<th>Non-immune serum</th>
<th>Immune serum</th>
<th>Non-immune serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected cells + actinomycin D*</td>
<td>3 + (80 %)†</td>
<td>-</td>
<td>3 + (70 %)</td>
<td>-</td>
</tr>
<tr>
<td>Infected cells, no actinomycin D</td>
<td>1-2 (50 %)</td>
<td>-</td>
<td>2 + (55 %)</td>
<td>-</td>
</tr>
<tr>
<td>Control cells + actinomycin D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control cells, no actinomycin D</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* BHK 21 cells were infected with 3 to 5 p.f.u./cell and incubated in the presence or absence of 1 µg/ml of actinomycin D for 24 h.
† Estimated intensity of the fluorescence and, in parentheses, the % of cells stained.
‡ - indicates no reaction.

Viable cells which had been monodispersed by trypsinization were examined and similar results were obtained by both methods. Representative findings are illustrated in Fig. 4. The staining on cells incubated in the presence of actinomycin D was more diffuse and of greater intensity than the staining on cells not treated with the drug. The reactions are summarized in Table 3 and the observations suggest an increase of virus antigen at the surface of Pichinde virus-infected cells incubated in the presence of actinomycin D.

Quantitative estimates of the expression of virus antigen at the surface of infected cells...
Inhibition of Pichinde virus replication

Fig. 5. Measurement of surface antigens with 125I-labelled antibody. BHK 21 cells were infected with 1 to 3 p.f.u./cell and incubated in the presence or absence of 1 µg/ml of actinomycin D. At intervals the cells were monodispersed and reacted with anti-Pichinde virus IgG which had been labelled with 125I. Δ--Δ, Infected cells, no actinomycin D; ○—○, infected cells and actinomycin D; ▲—▲, uninfected cells, no actinomycin D; ●—●, uninfected cells and actinomycin D.

were sought by reacting the cells with immune IgG which had been labelled with 125I. The results are shown in Fig. 5. Radioactivity in amounts above background was detected on the infected cells from 6 to 12 h after infection. More 125I-IgG reacted with infected cells incubated in the presence of actinomycin D than with untreated cells.

Particle production in the presence of actinomycin D

Decreased production of infectious virus without a similar decrease in the synthesis of virus antigens could be accounted for by the production of defective virus particles. This possibility was assessed by comparing the yield of infectious virus and radiolabelled virus from cells either treated or not treated with actinomycin D. The results shown in Fig. 6
Fig. 6. BHK 21 cell monolayers in two 75 cm² flasks were infected with 3 p.f.u./cell of Pichinde virus. After adsorption, 1 µg/ml of actinomycin D was added to 1 flask while the other received no drug. Two uninfected flasks were treated similarly. To all flasks, 0·5 µCi/ml of 3H-amino acid mixture was added. The fluids were harvested after 24 h at 37 °C, precipitated with PEG, resuspended in TNE buffer and centrifuged through 20% sucrose on to a 50% sucrose cushion. The material at the interface was aspirated by side puncture and centrifuged on a continuous gradient. Fractions from the gradients were assessed for radioactivity and for infectious virus. The radioactivity plotted in the figure represents the cpm/min in the fraction of the material from the infected cultures minus the cpm/min of the corresponding fraction of the material from the control cultures. 

- Infectious virus, no actinomycin D; - - - - - , infectious virus, actinomycin D; - - - - - , 3H-amino acids, no actinomycin D; • - • , 3H-amino acids, actinomycin D.

indicate that both infectious virus production and the production of particles with a density similar to Pichinde virus were inhibited by actinomycin D.

Pichinde virus and actinomycin D

The reduced yield of Pichinde virus in the presence of actinomycin D does not appear to result from a direct interaction of the virus with the drug. A virus stock was diluted 1:50 in either medium alone or in medium containing 1 µg/ml of actinomycin D. The mixtures were incubated for 5 h at 4 °C and then assayed for infectivity. The titre of virus incubated in the
Inhibition of Pichinde virus replication

Table 4. Effect of passaging Pichinde virus in the presence of actinomycin D

<table>
<thead>
<tr>
<th>Passage no.</th>
<th>Virus titres*</th>
<th>Inhibition† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actinomycin D</td>
<td>No actinomycin D</td>
</tr>
<tr>
<td>1</td>
<td>$2.5 	imes 10^4$</td>
<td>$2 	imes 10^6$</td>
</tr>
<tr>
<td>2</td>
<td>$3.5 	imes 10^4$</td>
<td>$8 	imes 10^6$</td>
</tr>
<tr>
<td>3</td>
<td>$2.2 	imes 10^5$</td>
<td>$1 	imes 10^7$</td>
</tr>
<tr>
<td>4</td>
<td>$8.0 	imes 10^5$</td>
<td>$6 	imes 10^6$</td>
</tr>
<tr>
<td>5</td>
<td>$7.5 	imes 10^5$</td>
<td>$2.5 	imes 10^7$</td>
</tr>
<tr>
<td>6</td>
<td>$1.0 	imes 10^6$</td>
<td>$1.5 	imes 10^7$</td>
</tr>
<tr>
<td>7</td>
<td>$4.0 	imes 10^6$</td>
<td>$3.5 	imes 10^7$</td>
</tr>
<tr>
<td>8</td>
<td>$6.0 	imes 10^6$</td>
<td>$4.5 	imes 10^7$</td>
</tr>
<tr>
<td>9</td>
<td>$6.0 	imes 10^6$</td>
<td>$5 	imes 10^6$</td>
</tr>
</tbody>
</table>

* Virus titres expressed as p.f.u./ml.
† Yield of cultures containing actinomycin D compared with cultures which did not contain the drug.

Presence of the drug was $2.8 	imes 10^7$ p.f.u./ml while virus incubated in medium only titred $3.3 	imes 10^7$ p.f.u./ml.

Actinomycin D did not completely inhibit Pichinde virus production (see Fig. 2). If the inhibition of the virus was mediated through an interaction of the drug with virus products, it might be possible to select viruses resistant to the drug. This possibility was examined by repeatedly passaging the virus in the presence of 1 μg/ml of actinomycin D. Monolayers of BHK 21 cells were inoculated with virus and one set of cultures were incubated in the presence of actinomycin D while a parallel set of cultures served as controls. After 48 h, the culture fluids were harvested and used to inoculate another set of monolayers. After 9 passages the harvests from each passage were assayed. The results are presented in Table 4. A population of virus resistant to the drug did not emerge.

DISCUSSION

In addition to viruses which possess RNA-dependent DNA polymerase, the replication of a number of single-stranded RNA viruses is inhibited by actinomycin D. Actinomycin D inhibits the transcription of RNA from DNA, thus, inhibition of the yield of RNA viruses implies a requirement for host-cell gene expression in the replication of the viruses. Studies on influenza virus indicate that an event early in its replicative cycle occurs in the nucleus of the cell and actinomycin D blocks this event. Transcription of influenza virus RNA cannot be detected in cells infected with the virus and treated with actinomycin D during the first 2 h after infection (Bean & Simpson, 1973; Pons, 1973; Stephenson & Dimmock, 1975). The drug does not inhibit influenza virus replication if added late in the replicative cycle (Barry et al. 1962).

Consistent inhibition of RNA viruses reported to be sensitive to actinomycin D has not always been observed. The yield of poliovirus in the presence of actinomycin D may be somewhat reduced (Grado, Fischer & Contreas, 1965) and the degree of inhibition was found to vary with the strains of virus used (Schaffer & Gordon, 1966) and with the conditions of growth of the host cells (Cooper, 1966). An initial report suggested that lactic dehydrogenase virus was not inhibited by actinomycin D (DuBuy & Johnson, 1970); however in a more
recent study the yield of the virus was dramatically reduced by the drug (Yamazaki & Notkins 1975). Among the arenaviruses lymphocytic choriomeningitis virus and Pichinde virus were found to be inhibited by actinomycin D while minimal reduction of virus was produced by the drug in cultures infected with Junin virus (Martinez Segovia & Grazioli 1969; Coto & Vombergar, 1969). For the most part, the reasons for these discrepancies are not clear.

The mechanisms by which actinomycin D inhibits the different RNA viruses does not appear to be the same in all cases. In the case of poliovirus, it has been suggested that the reduction in virus yield does not relate directly to DNA-dependent RNA synthesis but to cell death which can be attributed to the toxicity of the drug (Guskey & Wolff, 1974). Inhibition of lactic dehydrogenase virus may be similar to influenza in that actinomycin D prevents the synthesis of virus RNA (Brinton-Darnell, Collins & Plagemann, 1975). However, unlike influenza virus an inhibitory effect of actinomycin D on the replication of lactic dehydrogenase virus can be observed when the drug is added relatively late after infection. The latent period of lactic dehydrogenase virus is about 9 h and actinomycin D added 9 h after infection produced a 75% reduction in virus yield as compared to a 98% reduction when the drug was added at 0 time (Yamazaki & Notkins, 1975). We observed a similar relation between inhibition and time of addition of actinomycin D with Pichinde virus; drug which was added at or near the end of the latent period, 8 h, resulted in about an 80% reduction of virus yield while a 99% reduction was obtained when the drug was added at 0 time. In contrast to the lack of virus RNA synthesis in cells infected with lactic dehydrogenase virus and incubated in the presence of actinomycin D, Pichinde virus RNA synthesis appears to occur under these cultural conditions (S. SenGupta, unpublished observations).

Our data indicates that actinomycin D does not completely inhibit Pichinde virus during the early stages of replication. Replication of the virus occurred in the presence of the drug, albeit to a lesser degree, and the latent period of the replicative cycle was not appreciably prolonged in cultures treated with the actinomycin D (Fig. 2). In addition, the RNA of Pichinde virus does not appear to have messenger function (W. C. Leung, unpublished observations). Since nearly normal levels of virus antigens were synthesized in the presence of actinomycin D, appreciable transcription from virus RNA must have occurred.

While our data does not exclude a direct effect of actinomycin D on the synthesis of a replicative form of the Pichinde virus genome as may be the case for Reovirus (Silverstein et al. 1974), there appears to be a relationship between host cell RNA synthesis and virus production. The yield of virus was found to be about tenfold greater from cells in exponential growth than from cells in stationary cultures and the degree of reduction of virus yield by actinomycin D paralleled the degree of inhibition of 3H-uridine incorporation into uninfected cells which were treated with the drug (Fig. 1). It is also possible that actinomycin D, at the concentrations used, reduced the yield of Pichinde virus by a non-specific toxic effect on the cells. However, the viability of the cells was adequate to support the synthesis of Pichinde virus antigens and Sindbis virus.

Actinomycin D resulted in a reduction of only 35% of virus antigen synthesis as estimated by complement fixation tests and both antigens normally found by immunodiffusion were detected in cultures treated with the drug. The antigens detected at the surface of cells infected with Pichinde virus were also found on infected cells which had been incubated with actinomycin D. In fact, there appeared to be more virus antigen expressed on the surface of drug-treated cells than on the surface of cells not treated with the drug. These observations suggest that actinomycin D may reduce the yield of Pichinde virus by inhibiting virus maturation. Our inability to demonstrate particles less dense than infectious particles by
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using radiolabelled amino acid precursors in drug-treated cultures tends to support this mechanism of inhibition over the induction by actinomycin D of putative defective virus particles.

The apparent differences in the expression of surface antigens as estimated by immunofluorescence and by reactions with \(^{125}\)I-labelled IgG within the first 48 h after infection is of interest (Table 3, Fig. 5). The differences between infected cells which were treated with actinomycin D and untreated cells were more apparent by immunofluorescence than by reactions with \(^{125}\)I-labelled IgG. The staining pattern of cells treated with actinomycin D was diffuse while the pattern of untreated cells was beaded, a pattern which may result from antibody-induced redistribution of surface antigens into clusters (Edidin & Weiss, 1972; Birdwell & Strauss, 1974). The apparent differences which we observed using the two techniques may, thus, be due more to a redistribution of antigen by antibody on the surface of untreated cells than to a markedly greater accumulation of virus antigen on the surface of actinomycin D treated cells. Ligand-induced redistribution of surface antigens requires a functionally intact cell membrane. Membrane proteins are supplied to the plasma membrane from a precursor pool which takes several hours to deplete (Ray, Lieberman & Lansing, 1968). If a functionally intact cell membrane is required for Pichinde virus maturation, reduced virus yields in the presence of actinomycin D may be mediated through the inhibition of synthesis of host cell membrane proteins.

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REFERENCES


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