The Influence of
Mitomycin C, Actinomycin D and Ultraviolet Light on the
Replication of the Viruses of Foot-and-Mouth Disease
and Vesicular Stomatitis

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Foot-and-mouth disease virus and vesicular stomatitis virus both contain RNA and multiply in the cytoplasm (Mussgay, 1958; Mussgay & Weibel, 1963). It would be expected that their replication would not be affected by actinomycin D, which interferes with the template function of DNA (Reich et al. 1961 a; Goldberg, Rabinowitz & Reich, 1962; Hurwitz et al. 1962; Kersten & Kersten, 1962), or by mitomycin C and u.v. light, which are known to destroy the cell genome (Lathja, 1960; Reich, Shatkin & Tatum, 1961 b; Kersten et al. 1964). This communication presents evidence which shows that the replication of foot-and-mouth disease virus is inhibited by all three agents, whereas vesicular stomatitis virus replication is unaffected by mitomycin C or actinomycin D.

Foot-and-mouth disease virus (type O) and vesicular stomatitis virus (type Indiana) were grown in monolayers of baby hamster kidney cells (BHK 21, clone 13; Macpherson & Stoker, 1962) and assayed by the plaque method (Mowat & Chapman, 1962). Single-step growth cycles were used for all the inhibition studies. Petri dishes (6 cm.) or medicine bottles (4 oz) containing monolayers of 10^7 BHK cells were washed twice with phosphate-buffered saline and virus added at a multiplicity of infection of 100 p.f.u./cell for foot-and-mouth disease virus and 5 p.f.u./cell for vesicular stomatitis virus. The cells were incubated for 10 min. at 37°C, unadsorbed virus poured off and the cell sheets washed. For foot-and-mouth disease virus studies the cells were washed with 0.15 M sodium acetate, pH 6.0 (2 x 5 ml.); as this virus is acid-labile (Thorne & Cartwright, 1958) this wash inactivated any extracellular virus. For the experiments with vesicular stomatitis virus the sheets were washed with phosphate-buffered saline (2 x 5 ml.). Ten ml. of Eagle's medium (or in some cases Earle's saline) were added and the incubation continued to 200 min. after infection with foot-and-mouth disease virus or 300 min. after infection with vesicular stomatitis virus. At these times the yield of virus was maximal.

Monolayers of BHK cells were pretreated with different concentrations of mitomycin C for 8 hr. The cells were then infected and the virus yield at 200 min. (foot-and-mouth disease virus) or 300 min. (vesicular stomatitis virus) was determined. The yield of foot-and-mouth disease virus was depressed by 90% with 75 μg. mitomycin C/10^7 cells and 99% with 150 μg./10^7 cells (equivalent to 7.5 μg./ml. and 15 μg./ml. respectively). By comparison the replication of vesicular stomatitis virus was only slightly affected; with 150 μg./10^7 cells, for example, the yield was depressed by 30% (Fig. 1 a).

The replication of foot-and-mouth disease virus was unaffected by 10 μg. actinomycin D/10^7 cells and this concentration has been used routinely to suppress cellular RNA synthesis so that viral RNA synthesis can be studied (Brown & Cartwright,
When monolayers of BHK cells were treated with different concentrations of actinomycin D immediately after infection it was found that concentrations above 10 μg./10^7 cells suppressed viral replication (Fig. 1b). The replication of vesicular stomatitis virus, however, was not affected; in fact, slight stimulation occurred with actinomycin D 10 to 20 μg./10^7 cells. The effect of actinomycin D on cellular RNA synthesis (as shown by uridine incorporation) and cellular protein synthesis (as shown by amino acid incorporation) was also studied. Cellular RNA synthesis was suppressed 99% by actinomycin D at a concentration of 10 μg./10^7 cells, whereas cellular protein synthesis was not affected by concentrations as high as 50 μg./10^7 cells.

In order to determine whether the effect of the actinomycin D was specific to any period within the growth cycle of foot-and-mouth disease virus, actinomycin D (50 μg./10^7 cells) was added to infected BHK cell monolayers at different times after infection and the yield of virus at 200 min. after infection was determined. Actinomycin D inhibited the replication only when added within 45 to 60 min. of infection (Fig. 2).

BHK cell monolayers were irradiated with u.v. light as follows. Cell sheets in Petri dishes (6 cm.) were washed with phosphate-buffered saline (2 x 5 ml.) and then drained. The uncovered Petri dish was placed at a distance of 25 cm. from the centre of a Hanovia 'Chromatolite' Cold Cathode Discharge Lamp (with filters fitted to restrict the wavelength of the emitted light to 2540 Å) and irradiated for different times.
before infection. The replication of both viruses was suppressed (Fig. 1c). It should be noted that short periods of irradiation stimulated the replication of vesicular stomatitis virus (cf. the effects of low concentrations of actinomycin D on the replication of this virus, Fig. 1b). U.v. light irradiation of BHK cells caused a suppression of both cellular RNA synthesis and cellular protein synthesis. The viability of BHK cells (as measured by Trypan Blue exclusion) after treatment with mitomycin D, actinomycin D and u.v. light was unaffected (Table 1).

![Graph showing the effect of adding actinomycin D at different times after infection on the yield of foot-and-mouth disease virus.]

Fig. 2. Effect of adding actinomycin D (50 μg./10^7 cells) at different times after infection on the yield of foot-and-mouth disease virus. ×——×, Virus yield at 200 min.; ○——○, virus-induced RNA polymerase (determined by incorporation of radioactive substrate into acid insoluble products); ·——·, growth cycle in absence of actinomycin D.

**Table 1. Effect of mitomycin C, actinomycin D and u.v. light on viability of BHK cells**

<table>
<thead>
<tr>
<th>Mitomycin C</th>
<th>Viable cell count</th>
<th>Actinomycin D</th>
<th>Viable cell count</th>
<th>U.v. light</th>
<th>Viable cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.2 x 10^7</td>
<td>Control</td>
<td>9.0 x 10^6</td>
<td>Control</td>
<td>1.2 x 10^7</td>
</tr>
<tr>
<td>100 μg./10^7 cells</td>
<td>1.1 x 10^7</td>
<td>20 μg./10^7 cells</td>
<td>9.1 x 10^6</td>
<td>1 min.</td>
<td>1.1 x 10^7</td>
</tr>
<tr>
<td>200 μg./10^7 cells</td>
<td>1.2 x 10^7</td>
<td>40 μg./10^7 cells</td>
<td>9.2 x 10^6</td>
<td>4 min.</td>
<td>1.2 x 10^7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 μg./10^7 cells</td>
<td>8.9 x 10^6</td>
<td>10 min.</td>
<td>1.3 x 10^7</td>
</tr>
</tbody>
</table>

Mitomycin C rapidly inhibits cellular RNA synthesis but suppression of protein synthesis is delayed (Suzuki & Kilgore, 1967), because it is an indirect effect caused by the inhibition of messenger RNA synthesis. Under the conditions used here, mitomycin C (200 μg./10^7 cells) suppressed cellular protein synthesis by 46% in 8 hr. At this concentration of the drug the replication of foot-and-mouth disease virus was inhibited by more than 99%, whereas the replication of vesicular stomatitis virus was suppressed by only 30%. In contrast, u.v. light suppressed the replication of both viruses by comparable amounts (90%). Under the conditions of u.v. light irradiation used here, both cellular RNA synthesis and cellular protein synthesis were depressed.
The suppression of cellular protein synthesis in this case was probably due to direct action of the u.v. light rather than to the suppression of cell messenger RNA synthesis (Kagawa, Kukutome & Kawade, 1967). Replication of foot-and-mouth disease virus appeared to require the synthesis of cell messenger RNA, whereas replication of vesicular stomatitis virus was only suppressed when cellular protein synthesis was itself directly blocked. This last step would be required for the synthesis of viral components. The results obtained with actinomycin D support this view.

Actinomycin D (50 µg./10^7 cells) inhibited the replication of foot-and-mouth disease virus by 90% but the replication of vesicular stomatitis virus was unaffected. At this concentration actinomycin D inhibited cellular RNA synthesis by 99% but cellular protein synthesis was unaffected. From these results it seems that cellular RNA synthesis (i.e. DNA transcription) is required for some step in the replication of foot-and-mouth disease virus and that this step occurs within 45 to 60 min. of infection (Fig. 2). Pons (1967) showed that actinomycin D inhibits the formation of the replicative form of influenza virus RNA. If the suppression of viral replication by actinomycin D is due to this mechanism, it is difficult to see why it is specific for certain viruses, as it is now accepted that the replicative form of viral RNA is an intermediate in the replication of all RNA viruses. In an earlier report on the action of actinomycin D on influenza virus replication, Ho & Walters (1966) showed that the compound inhibits the formation of influenza-virus-induced RNA polymerase. In agreement with this, Polatnick & Arlinghaus (1967) showed that actinomycin D inhibits the formation of RNA polymerase induced by foot-and-mouth disease virus. Under the growth conditions used here, the viral polymerase was first detected at 45 to 60 min. after infection (Fig. 2 and Black, unpublished results). If actinomycin D inhibited the formation of the viral RNA polymerase, this result would be in agreement with the present observation that actinomycin D had no effect on the replication of the virus when added later than 45 min. after infection. Unfortunately a direct comparison of this observation with the results reported by Polatnick & Arlinghaus (1967) is not possible because the growth-cycle characteristics described by these authors are markedly different from those reported here. Recent work (Black, unpublished results) has shown that under the growth conditions used here actinomycin D (50 µg./10^7 cells) inhibited the formation of RNA polymerase induced by foot-and-mouth disease virus but only if it was added within 60 min. of infection.

REFERENCES


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