The Multiplication of Measles Virus in Human Amnion Cells in vitro

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(Accepted 13 November 1969)

SUMMARY

The multiplication of the EDMONSTON strain of measles virus in suspensions of human amnion cells was studied. The first new virus appeared in the cytoplasmic fraction of the cells 17 to 20 hr after infection. After another 7 hr the virus was liberated into the medium. The multiplication of the virus was not inhibited by actinomycin D as measured by virus growth, virus plaque formation, or change in sensitivity to u.v.-irradiation. The u.v.-irradiation of monolayers before infection did not reduce plaque formation.

INTRODUCTION

Measles virus is a lipid containing RNA-virus (Lain & Atherton, 1963; Norrby et al. 1964). In electron micrographs it resembles the paramyxoviruses Newcastle disease virus, Sendai virus and parainfluenza virus (Waterson et al. 1961). It also resembles them in inducing multinucleated giant cells and in multiplying in the presence of actinomycin D, suggesting that its multiplication is independent of host cell DNA (Parafanovich et al. 1965; Matumoto, 1966).

Experiments on the site of maturation of measles virus in the host cell are reported here, together with evidence confirming the insensitivity to actinomycin D.

METHODS

Virus and cells. The virulent EDMONSTON strain of measles virus was grown in human amnion cells of line U. The growth medium was lactalbumin hydrolysate 0.5% and bovine serum 5% in Hanks's balanced salt solution. The virus was titrated by a plaque method in the same cells (de Jong, 1964). The overlay medium was growth medium with yeast extract 0.1% and agar 0.9% (Difco Bacto-agar, washed after Dulbecco & Vogt, 1954). A second layer was applied 6 days after inoculation. Plaques were stained with neutral red 0.0025% and counted after an incubation period of 15 days at 37°C. Crude virus harvests contained from 5 to 8 x 10⁶ p.f.u./ml. and were used after removal of cell debris by low speed centrifugation.

Separation of cells into cytoplasmic and nuclear fractions. Cells were fractionated by a modification of the method of Fisher & Harris (1962). About 5 x 10⁶ cells were suspended in 2 ml. of double distilled water, centrifuged 2 min. at 500 g, resuspended in 0.5 ml. of a 0.1% solution of Tween 80, treated for 2 min. in an all-glass homogenizer of the Potter–Elvehjem type and diluted with 2.5 ml. of pH 7.4 buffer with CaCl₂ (Frenster, Allfrey & Mirsky, 1961; Ord & Stocken, 1961: 0.1875 M-sucrose; 0.02 M-glucose; 0.0285 M-NaCl; 0.008 M-MgSO₄; 0.0033 M-CaCl₂; 0.025 M-tris). The suspension was then separated by centrifugation (2 min. at 500 g) into a sedimented nuclear fraction and a supernatant.
cytoplasmic fraction. Microscopical examination of the nuclear fraction showed that, regardless of the time elapsed since infection, 70 to 80% of the cells yielded 'clean' nuclei and 10% yielded nuclei with attached membrane-like pieces. About 2 to 3% of the cells resisted fractionation and about 10 to 20% were not accounted for as nuclei or whole cells, so presumably were lysed.

Experiments with actinomycin D. Actinomycin D was a gift of Merck, Sharp and Dohme, New York. Protein and nucleic acid contents were estimated by the spectrophotometric method of Martin et al. (1961).

RESULTS

Titration of measles virus in nuclear and cytoplasmic fractions of infected human amnion cells

Suspensions of $2 \times 10^6$ cells were inoculated with measles virus and rotated slowly at 37°. The multiplicity of adsorption was 0.4, calculated from the infectivity of the extracellular fluid before and after adsorption. After adsorption the suspension was diluted with growth medium to $10^6$ cells/ml and incubated with magnetic stirring at 36° in an atmosphere of air + 10% CO₂. Samples of $5 \times 10^6$ cells were fractionated and titrated for virus content (Fig. 1).

The first new measles virus was detected in the cytoplasmic fraction 17 hr after infection. The infectivity of the nuclear fraction began to increase 3 hr later. This rise was secondary, however, and was due to contamination of the nuclear fraction with whole cells and cytoplasmic membranes. After maturation the virus was cell-associated for about 7 hr. After 48 hr the virus yield was only 3% of the maximal yield reached after 72 hr (see below).
Multiplication of measles virus

Influence of actinomycin D on the multiplication of measles virus in human amnion cells

Influence of actinomycin D on virus yield. The growth of measles virus is slow: de Jong (1964) reported maximal virus infectivities after 72 hr at 37 ° following infection of human amnion cells at a multiplicity of 1. At the same multiplicity we found that the yield at 3 hr and 48 hr was 0.5% and 3% of that at 72 hr. To test the effect of actinomycin D, the yield at 48 hr was studied, since at this time virus synthesis was most rapid and any delay would have been detected with great sensitivity. The drug was added 1 hr before virus inoculation and was present throughout the incubation period. After 2 days at 37° the cell cultures were frozen and thawed twice and titrated for infective virus by the plaque method. Actinomycin D in concentrations of 0.2 μg./ml. or more inhibited virus growth (Fig. 2). At these concentrations, however, the drug was toxic to the cells which rounded and detached from the glass. Concentrations as low as 0.03 μg./ml. significantly reduced the synthesis of both nucleic acid and protein (Fig. 2). This suggested that the observed inhibition of virus growth by higher concentrations of actinomycin D was due to adverse effects on cellular metabolism rather than to specific interference with multiplication of virus.

Influence of actinomycin D on plaque formation. The effect of actinomycin D was tested after different periods of exposure to the greatest concentration tolerated by the cells. After inoculation of virus, different concentrations of actinomycin D in growth medium, with or without agar, were poured on to the cells. After various periods the fluid medium was
replaced by an agar overlay without the drug or a second overlay without actinomycin D was
laid on the first. The plaque count was not significantly reduced in the presence of actino-
mycin D (Table 1).

**Influence of actinomycin D on the change of u.v.-sensitivity of plaque forming centres in the
course of infection.** An early event after infection is the decrease of the u.v.-sensitivity of the
cell as an infectious centre (Luria & Latarjet, 1947). This provides a means of detecting an
effect of actinomycin D exerted shortly after infection of the cell and long before the pro-
duction of complete virus. Monolayers in Petri dishes were inoculated with virus and supplied

![Table 1](image)

**Table 1. Influence of actinomycin D on plaque formation by measles virus in human amnion cells**

<table>
<thead>
<tr>
<th>Actinomycin D</th>
<th>Time of exposure</th>
<th>Number of plaques as percentage of control without actinomycin D ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 µg./ml.</td>
<td>5 hr</td>
<td>Cells killed</td>
</tr>
<tr>
<td>0.2 µg./ml.</td>
<td>5 hr</td>
<td>79 % ± 19 %</td>
</tr>
<tr>
<td>0.1 µg./ml.</td>
<td>17 hr</td>
<td>118 % ± 10 %</td>
</tr>
<tr>
<td>0.05 µg./ml.</td>
<td>6 days</td>
<td>75 % ± 16 %</td>
</tr>
<tr>
<td>0.03 µg./ml.</td>
<td>6 days</td>
<td>67 % ± 19 %</td>
</tr>
<tr>
<td>0.01 µg./ml.</td>
<td>6 days</td>
<td>115 % ± 9 %</td>
</tr>
</tbody>
</table>

![Table 2](image)

**Table 2. Influence of u.v.-irradiation (540 ergs mm.⁻² at 260 nm.) on plaque formation by measles virus in human amnion cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plaques/monolayer</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No u.v.-irradiation</td>
<td>125</td>
<td>100</td>
</tr>
<tr>
<td>Virus adsorption, then u.v.-irradiation</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>U.v.-irradiation, then virus adsorption</td>
<td>96</td>
<td>77</td>
</tr>
</tbody>
</table>

![Fig. 3](image)

**Fig. 3. Influence of actinomycin D on the u.v.-sensitivity of measles virus plaque forming centres.**
The number of plaque forming centres is expressed as percentage of control in absence of u.v.-
irradiation: % p.f.c. Exposure to u.v.-irradiation at the following times after adsorption for 1 hr
at 37°: (a) immediately; (b) 6 hr; (c) 17 hr. ○——○, No actinomycin D; ●——●, with actinomycin
D at 0.15 µg./ml. (b) or 0.10 µg./ml. (c).

with growth medium containing actinomycin D. At various times the medium was removed
and the cells washed twice with buffer to remove ultraviolet absorbing or fluorescent sub-
stances. The monolayers were irradiated with 9 ergs sec⁻¹ mm⁻² at 260 nm. wavelength
from an ultraviolet germicidal lamp. After irradiation an agar overlay was applied and the
dishes processed for plaque formation. Irradiation did not affect the capacity of cells to form plaques (Table 2). This result contrasts with data for influenza virus (Barry, 1964). With irradiation after virus inoculation (Fig. 3) there was no significant difference between curves in the presence or absence of the drug. The observation of a ‘shoulder’ after incubation for 17 hr (Fig. 3c) suggested that virus nucleic acid was replicating.

**DISCUSSION**

Measles virus, like most RNA viruses, multiplies in the cytoplasm. That multiplication is not dependent on functionally intact host cell DNA is shown by the lack of effect of the DNA-inactivating drug actinomycin D and of preceding u.v.-irradiation. Moreover, results with actinomycin D and with preceding u.v.-irradiation agree with data for paramyxoviruses and not with data for the myxoviruses of influenza and fowl plague (Barry, 1964; White & Cheyne, 1966). Electron micrographs also show resemblances between measles virus and paramyxoviruses rather than between measles virus and myxoviruses (Waterson et al. 1961).

The results with actinomycin D agree with those of Parafanovich et al. (1965), who obtained similar growth curves for measles virus in a line of human amnion cells in the absence or presence of 0.1 µg./ml. actinomycin D. Matumoto (1966), however, reported a 10 to 100 fold increase of measles virus production in the FL line of human amnion cells in the presence of 0.1 µg./ml. of actinomycin D; this might have been due to inhibition of interferon synthesis.

This work was submitted by J. C. de J. in partial fulfilment of the requirements for the degree of Doctor of Science at the University of Utrecht.

The technical assistance of Mr M. Harmsen and Mrs W. C. M. Harmsen-van Amerongen is gratefully acknowledged.

**REFERENCES**


(Received 25 June 1969)