The Effect of Heat-inactivated Murine Cytomegalovirus on Host DNA Synthesis of Different Cells

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SUMMARY
Heat-inactivated murine cytomegalovirus (MCMV) stimulates cellular DNA synthesis in WME, NMG, 3T3, Wg1A, chick and NIK-8 cells, but active or u.v.-irradiated MCMV does not. The stimulation of DNA synthesis in NIL-8 and chick cells was studied in detail. We found that both the nuclear and the mitochondrial DNA synthesis were stimulated in these cells. There was no virus DNA synthesis during the period we studied (48 h). The stimulation of nuclear DNA synthesis was about threefold in NIL-8 and 2.5-fold in chick cells as measured by the rate of incorporation of $^3$H-thymidine ($^3$H-dThd) in the CsCl fractions which banded at the density of cell DNA. The stimulation was about 9.5-fold in NIL-8 and 1.7-fold in chick cells as detected by autoradiography. There was a 3-fold and a 2.2-fold increase in the degree of incorporation of $^3$H-dThd into mitochondrial DNA of NIL-8 and chick cells, respectively. The amount of mitochondrial DNA obtained in infected cells of both kinds was about twice that in control cells. The synthesis of mitochondrial DNA was also stimulated by a factor of 2 in the thymidine kinaseless 3T3 cells which incorporate exogenous thymidine exclusively into mitochondrial DNA. There were no MCMV specific antigens detectable by immunofluorescence 5 h after infection, but diffuse nuclear fluorescence could be demonstrated 24 h after infection. Our results indicate that the heat-inactivated virus penetrates the cells, stimulates host DNA synthesis and induces synthesis of early MCMV antigens.

INTRODUCTION
The stimulation of host DNA synthesis by some viruses may be a key step in the malignant transformation of the cells. Several studies have been published on the ability of cytomegaloviruses (CMV) to stimulate host DNA synthesis (St. Jeor et al. 1974; Furukawa et al. 1975; Albrecht et al. 1976; Moon et al. 1976; Waner & Budnick, 1977; J. Boldogh et al. unpublished data), but the circumstances necessary for stimulation and the nature of the newly synthesized DNA were still not clear. Thus it was of interest to study this phenomenon using murine cytomegalovirus (MCMV) and cells with varying degrees of permissiveness for virus replication.

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METHODS

Cells. Primary who~' mouse embryo (WME) cells were prepared by trypsinization of 14 to 16 day mouse embryos (Tyler's original strain). Primary chicken embryo cells were prepared by trypsinization of 10 day Brown Leghorn embryos. Mouse normal mammary gland (NMG) cells were a gift from C. Dickson and are described elsewhere (Owens et al. 1974). The thymidine kinaseless mutants (tk-) of 3T3 cells were supplied by K. Quade and described elsewhere (Basilico et al. 1969). The NIL-8 cells were a clone of fibroblasts isolated by I. Macpherson from the NIL-2 Syrian hamster cell line described by Diamond (1967). The BALB/c 3T3 A31 is a mouse fibroblast cell line described by Aaronson & Todaro (1968) and the WgIA is a Chinese hamster fibroblast cell line described by Roscoe et al. (1973).

Dulbecco's modified Eagle's minimal essential medium (MEM) supplemented with 10% calf serum was used for all cell cultures, except for (tk-) 3T3 cells where the supplement was 10% foetal calf serum and 30 µg/ml 5-bromodeoxyuridine (BrdUrd; Sigma, St Louis, Mo. U.S.A.), and chick cells where 10% calf and 1% chicken serum were added to the medium.

Virus. The Smith strain of murine cytomegalovirus (MCMV) was kindly provided by Professor C. A. Mires (Guy's Hospital, London) and maintained in WME cells. The titre fluctuated between 10^6 and 10^7 p.f.u./ml. Virus stock was kept at −70 °C in medium with 10% calf serum.

Plaque titration. The procedure was as described for human cytomegalovirus (HCMV) (Gönczöl et al. 1975), except that the cultures were fixed and stained on the fourth day after infection.

U.v. irradiation of MCMV. Suspensions (4 ml) of MCMV in 9 cm Petri dishes were exposed to u.v. irradiation at a dose rate of 24000 ergs/mm².

Heat inactivation of MCMV. MCMV suspended in culture medium or PBS (in experiments where the virus was concentrated 100 times) was heat inactivated in a water bath at 56 °C for 30 min and then cooled on ice. There was no cytopathic effect (c.p.e.) or infectious virus production in WME cells infected with heat inactivated virus in the first 4 days after infection. On the fourth to fifth day c.p.e. was detectable and on the fifth to sixth day infectious virus was produced.

Measurement of total DNA synthesis of different cells. Cellular DNA synthesis was arrested by exposing the cells to low serum medium (0.3%) for 72 to 96 h before infection. At the time of infection the medium was poured off and reserved and the cells were infected with active virus, at a multiplicity of 10 to 12 plaque forming units (p.f.u.) per cell, or with u.v.-inactivated or heat-inactivated virus. The u.v.- and heat-inactivated viruses were prepared from the same stock as the infectious virus and consequently the number of particles was the same. Mock infection was carried out with culture medium already used for cell culture and contained the same serum concentration as the virus inoculum. After 1 h adsorption the inocula were removed and the original low serum medium was added to the cultures. The cells were labelled with 0.5 µCi/ml methyl-3H-thymidine (3H-dThd; sp. act. 21 Ci/mmol; Radiochemical Centre, Amersham, England) plus 2 µM-cold thymidine (Sigma), for 24 h either at the time of infection, or 24 or 48 h after infection. At the end of the labelling period the cells were washed with cold PBS followed by cold 5% trichloroacetic acid (TCA), fixed with methanol and then solubilized in 1 M-NaOH. The level of 3H was measured in Aquasol scintillation fluid (New England Nuclear, Boston, Mass., U.S.A.) in a Nuclear Chicago mark II scintillation counter.
Host DNA synthesis in MCMV-infected cells

Detection of the susceptibility of the different cells to MCMV. Confluent monolayers of the cells were infected with MCMV at a multiplicity of 4 to 5 p.f.u./cell. After 1 h adsorption the cultures were washed three times with PBS and fresh culture medium was added. On the fourth day after infection the virus yield was detected on WME cells by plaque titration.

Measurement of $^3$H-dThd uptake into the nuclear DNA of NIL-8 and chick cells. The DNA synthesis of NIL-8 cells was arrested by incubating the cells in low serum medium (0.25%) for 72 to 96 h. Chick cells were plated in medium containing 10% calf and 1% chicken serum but the medium was replaced with serum-free medium once the cells had settled. The cells were then incubated in the serum-free medium for 72 to 96 h. To minimize the stimulation of DNA synthesis by the procedure itself, the infection was carried out without removing the medium, using virus concentrated 100 times by pelleting at 30000 rev/min for 1 h at 4 °C in a MSE 8 × 50 ml angle rotor and resuspending in PBS. The virus suspension was heat inactivated at 56 °C for 30 min. Mock infection was done with PBS or with medium already used for cell culture and treated in the same way as the virus. The cells were labelled for 24 h either by adding $3\mu$Ci/ml $^3$H-dThd plus 2 $\mu$M-cold thymidine at the time of infection or 24 h post infection. At the end of the labelling period the cells were washed with PBS and lysed with a solution of 0.6% sodium dodecyl sulphate (SDS; Serva, Heidelberg, Germany) and 0.01 M-ethylenediaminetetraacetic acid (EDTA; Fisons Scientific Apparatus, Loughborough, Leics., England). The lysate was poured into a centrifuge tube, 5 mM-NaCl was added to a final concentration of 1 M and maintained at 4 °C overnight. The supernatant and precipitate were separated by centrifuging at 12000 rev/min for 1 h at 4 °C in a HB-Sorvall rotor (Hirt procedure, Hirt, 1967). The pellet was taken up in a solution of 0.15 M-NaCl and 0.1 M-EDTA (pH 8.0), treated with 2% SDS, with 1 M-NaCl and then with proteinase-K (Merck, Darmstadt, Germany). Protein was removed by extraction with chloroform–isoamyl alcohol mixture. The DNA was then centrifuged to equilibrium in a neutral CsCl gradient (initial density 1.744 g/ml) at 32000 rev/min for 60 h at 20 °C in a MSE 6 × 5 ml swing out rotor. The CsCl was obtained from Fisons Scientific Apparatus. The gradients were collected by bottom puncture and the levels of radioactivity determined as described for the measurement of total DNA synthesis.

Autoradiography of NIL-8 and chick cells. Cellular DNA synthesis was arrested and infection carried out as already described. Cells were labelled with 2 $\mu$Ci/ml $^3$H-dThd either at the time of infection or 24 h later for a 24 h period. At the end of the labelling period the cells were fixed as previously described, coated in Ilford K-5 emulsion (Ilford Ltd, Ilford, England), kept at 4 °C for 6 days and then developed. Randomly chosen microscope fields were examined and the percentage of labelled nuclei were calculated after counting 2000 cells.

Measurement of $^3$H-dThd uptake into the mitochondrial DNA of NIL-8 and chick cells. The Hirt supernatant was centrifuged at 38000 rev/min for 1 h at 4 °C in a MSE 10 × 10 ml angle rotor and the pellet was resuspended in a solution containing 10 mM-tris (pH 7.6) and 1 mm-EDTA, made 2 M in CsCl and left overnight at 5 °C. The solution, cleared of precipitated RNA by centrifuging at 11000 rev/min for 10 min at 4 °C in a HB-4 Sorvall rotor, was increased in density to 1.550 g/ml by addition of CsCl, and ethidium bromide (EBr; Sigma) was added to a concentration of 250 $\mu$g/ml (in a final volume of 2.5 ml). $^{32}$P-labelled polyoma DNA was added to the gradients as marker. The samples were then centrifuged isopycnically at 38000 rev/min for 24 h at 20 °C in a MSE 6 × 5 ml swing out rotor. Fractions were taken by bottom puncture and the levels of radioactivity determined. The fractions of the dense peaks of $^3$H and $^{32}$P were collected, dialysed against a solution of PBS and 0.01 M-EDTA and then sedimented through a 5 to 20% neutral sucrose gradient.
The sucrose was obtained from Schwartz/Mann, Orangeburg, New York, U.S.A. Sucrose gradients were centrifuged in a SW65 Beckman rotor at 48 rev/min for 3 h at 19 °C. Fractions were collected and radioactivity measured.

Measurement of the ³H-dThd uptake into nuclear and mitochondrial DNA of (tk⁻) 3T3 cells. The thymidine kinaseless mutants (tk⁻) of 3T3 cells were maintained in medium containing 3% foetal calf serum and 20 µg/ml BrdUrd for 3 days before the infection and then during the whole period of the experiment. Infection, labelling and the analysis of low and high mol. wt. DNA were carried out as with NIL-8 and chick cells.

Measurement of the total amount of mitochondrial DNA in NIL-8 chick and (tk⁻) 3T3 cells. Concentrations of mitochondrial DNA were measured by the EBr-DNA fluorimetric method (LePecq & Paoletti, 1966) with polyoma DNA (form I) as a standard. Fractions of the ³H peak of the sucrose gradients were collected and the concentrations of mitochondrial DNA measured in a spectrophotofluorometer (excitation 360 nm; emission 580 nm; concentration of EBr 5 µg/ml).

Detection of antigens in NIL-8 and chick cells infected with heat inactivated MCMV. The indirect immunofluorescent method was used. The immune serum was raised in young Tyler's mice which were inoculated intraperitoneally four times at weekly intervals with a virus suspension of 10⁶ p.f.u. in 0.1 ml of PBS. The virus suspension was a 10% homogenate in PBS of salivary glands of infected mice, provided by Professor C. A. Mims. The immunized mice were exsanguinated 20 days after the last injection. The pooled antiserum was stored at −70 °C. The antiserum reacted with MCMV antigens (MCMV infected WME cells at 3 days post infection) at a dilution of 1:60. It did not react with uninfected cells at a dilution of 1:5. In the experiment described in this paper, the serum was used at a dilution of 1:10.

RESULTS

Total DNA synthesis of different cells infected with active, u.v.-irradiated and heat-inactivated MCMV

It has been reported that active and u.v.-irradiated human cytomegalovirus (HCMV) stimulates DNA synthesis of permissive and non-permissive cells (St. Jeor et al. 1974; Furukawa et al. 1975; Albrecht et al. 1976; J. Boldogh et al. unpublished data) but heat-inactivated HCMV does not (St. Jeor et al. 1974; J. Boldogh et al. unpublished data). On the other hand, active and u.v.-irradiated MCMV does not appear to stimulate the DNA synthesis of fully permissive cells, but heat-inactivated MCMV does (Moon et al. 1976). We therefore decided to study the effect of active, u.v.-irradiated and heat-inactivated MCMV on the DNA synthesis of cells which exhibit a range of susceptibility to the virus.

Table 1 shows the changes in the incorporation of ³H-dThd into the total DNA of virus infected cells compared with incorporation into mock infected cells. The incorporation of ³H-dThd was lower or unchanged if the infection was carried out using active or u.v.-irradiated virus, but there was a 2 to 8-fold increase if the cells were infected with heat-inactivated virus. The increased incorporation of ³H-dThd could usually be detected on the second day after the infection or, in the case of chick cells, usually on the first day.

Table 2 shows the ability of MCMV to form plaques and replicate infectious virus on different cells. The NIL-8 and chick cells were the least susceptible for virus replication, therefore they were chosen for the next experiments.
Host DNA synthesis in MCMV-infected cells

Table 1. Effect of active, u.v.-irradiated (24000 ergs/mm²) and heat-inactivated (56 °C, 30 min) MCMV on the total DNA synthesis of infected cells*

<table>
<thead>
<tr>
<th>Cells</th>
<th>MCMV†</th>
</tr>
</thead>
<tbody>
<tr>
<td>WME</td>
<td>Active or u.v.-irradiated (max. stimulation)</td>
</tr>
<tr>
<td>NMG</td>
<td>Suppression or no change (max. stimulation)</td>
</tr>
<tr>
<td>3T3</td>
<td>Suppression or no change (max. stimulation)</td>
</tr>
<tr>
<td>Wg1A</td>
<td>Suppression or no change (max. stimulation)</td>
</tr>
<tr>
<td>Chick</td>
<td>Suppression or no change (max. stimulation)</td>
</tr>
<tr>
<td>NIL-8</td>
<td>Suppression or no change (max. stimulation)</td>
</tr>
</tbody>
</table>

* Effect detected by the incorporation of ³H-dThd into the total DNA of cells.
† Multiplicity of 10 to 12 p.f.u./cell before inactivation.

Table 2. Replication of MCMV on different cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Plaque formation*</th>
<th>Virus yield†</th>
</tr>
</thead>
<tbody>
<tr>
<td>WME</td>
<td>100</td>
<td>5.6 x 10⁶</td>
</tr>
<tr>
<td>NMG</td>
<td>15</td>
<td>2.8 x 10⁴</td>
</tr>
<tr>
<td>3T3</td>
<td>15</td>
<td>2.4 x 10⁴</td>
</tr>
<tr>
<td>Wg1A</td>
<td>ND†</td>
<td>2.0 x 10⁴</td>
</tr>
<tr>
<td>Chick</td>
<td>0.005</td>
<td>1.8 x 10³</td>
</tr>
<tr>
<td>NIL-8</td>
<td>0.3</td>
<td>1.5 x 10³</td>
</tr>
</tbody>
</table>

* Plaque assays were performed on the various cells, scoring plaque production 4 days after infection and expressing the result as the percentage of plaque formation on WME cells.
† Cells (5 x 10⁶) in 35 mm dishes were infected with MCMV at a multiplicity of 4 to 5 (as assayed on WME). Virus was harvested 4 days after infection and assayed on WME. The values given are the total p.f.u. per culture.
‡ ND = Not done.

³H-dThd uptake into the nuclear DNA of NIL-8 and chick cells infected with heat-inactivated MCMV

Fig. 1 shows the radioactive profiles of the fractions of a neutral CsCl gradient of high molecular weight DNA of NIL-8 and chick cells. Fractions 26 to 29 had the density reported for cellular DNA (1.692 g/ml) (Lawrence, 1971; Lee, 1972). There was no peak at the density reported for MCMV DNA (1.717 g/ml) (Mosmann & Hudson, 1973). The data indicate that heat-inactivated MCMV stimulates ³H-dThd uptake into the nuclear DNA; the incorporation was 5 times greater in NIL-8 DNA and 2.5 times greater in chick cell DNA than in mock infected cells. The chick cell results were obtained on the first day after infection and the NIL-8 results on the second day.

Autoradiography of NIL-8 and chick cells infected with heat-inactivated MCMV

The results are shown in Table 3. In both the virus-infected NIL-8 and chick cells the percentage of labelled nuclei was 9.5 and 1.7 times higher, respectively, than in mock infected cells.

³H-dThd uptake into the mitochondrial DNA of NIL-8 and chick cells infected with heat-inactivated MCMV

Fig. 2 (a) represents the profiles of radioactivity obtained in fractions from EBr–CsCl gradients of the low mol. wt. DNA of NIL-8 and chick cells. The dense peak of ³H is
Fig. 1. Heat-inactivated MCMV induced stimulation of nuclear DNA synthesis in NIL-8 and chick cells. The cells were arrested and then the infection was carried out with 100 times concentrated and heat-inactivated (56°C, 30 min) MCMV. Mock infection was done with culture medium already used for cell cultivation and treated as the virus suspension. The cells were labelled for 24 h with 3 µCi/ml 3H-dThd and 2 µM-cold thymidine at the time of infection or 24 h later. At the end of the labelling period the high and low mol. wt. DNA of the cells were separated by the Hirt procedure (Hirt, 1967). The high mol. wt. DNA (nuclear DNA) was then centrifuged to equilibrium in a neutral CsCl gradient. The fractions were assayed for radioactivity. Density: • virus infected cells; • mock infected cells: ○.

Table 3. Autoradiography of cells infected by heat-inactivated MCMV

<table>
<thead>
<tr>
<th>Cells</th>
<th>% of labelled nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day after infection</td>
</tr>
<tr>
<td>NIL-8</td>
<td>Mock infected</td>
</tr>
<tr>
<td></td>
<td>Virus infected</td>
</tr>
<tr>
<td>Chick</td>
<td>Mock infected</td>
</tr>
<tr>
<td></td>
<td>Virus infected</td>
</tr>
</tbody>
</table>

composed of the closed circular DNA molecules. The 32P-labelled polyoma DNA (form 1) bands in the same position. The fractions of the dense peak were collected, dialysed and then sedimented through a neutral sucrose gradient. The profiles of radioactivity thus obtained can be seen in Fig. 2 (b). 3H-labelled DNA sedimented at 37 to 39S, which are the sedimentation constants of closed circular mitochondrial DNA. The polyoma DNA was used as sedimentation marker (21S). The results show that the incorporation of 3H-dThd into the mitochondrial DNA of the virus-infected NIL-8 cells was about 3 times and that of the chick cells about 2.2 times higher than in mock infected cells. The data presented were obtained on the first day (chick cells) and on the second day (NIL-8 cells) after the infection.

3H-TdR uptake into the nuclear and mitochondrial DNA of (tk-) 3T3 cells after infection with heat-inactivated MCMV

In this study thymidine kinaseless (tk-) cell cultures, which do not incorporate exogenous thymidine into chromosomal DNA, were used to examine whether the enhanced incorporation of 3H-dThd into mitochondrial DNA, as an effect of virus infection, can be
Host DNA synthesis in MCMV-infected cells

Fig. 2. Heat-inactivated MCMV induced stimulation of mitochondrial DNA synthesis in NIL-8 and chick cells. Pre-treatment, infection and labelling of the cells are described in the legend of Fig. 1. At the end of the labelling period cells were harvested by the Hirt procedure. The low mol. wt. DNA, together with SP-labelled polyoma DNA (added as a closed circular 21S DNA marker), was then centrifuged to equilibrium in an EBr-CsCl gradient (a). The dense peak (arrow) from this gradient was dialysed and sedimented through a neutral 5 to 20% sucrose gradient (b). Fractions were assayed for radioactivity. Virus infected cells: ••••; mock infected cells: ••••; polyoma DNA: ▲—▲.

Fig. 3(a) shows the radioactive profile of EBr-CsCl gradients of the low mol. wt. DNA of the (tk-) 3T3 cells and the SP-labelled polyoma DNA. The fractions of the dense peak (supercoiled DNA) were collected, dialysed and sedimented through a neutral sucrose gradient. Fig. 3(b) shows the sedimentation profile of the 3H- and SP-labelled DNA. 3H-labelled DNA sedimented at 37 to 39S. Polyoma RNA was again used as a sedimentation marker (21S). The results indicate that the incorporation of 3H-dThd into the mitochondrial DNA (form I) was about two times higher in virus infected cells than in mock infected cells. Fig. 3(c) represents the profile of radioactivity obtained in fractions of the CsCl gradients of high molecular weight DNA. There was no radioactive peak in these fractions. The results summarized in Fig. 3 were observed on the first day after infection.
Fig. 3. Heat-inactivated MCMV induced stimulation of mitochondrial DNA synthesis in (tk−) 3T3 cells. The cells were arrested and then the infection and labelling were carried out as described in the legend of Fig. 1. At the end of the labelling period the cells were harvested according to the Hirt procedure. The low mol. wt. DNA, together with 32P-labelled polyoma DNA (added as a closed circular 21S DNA marker), was then centrifuged to equilibrium in an EBr-CsCl gradient (a). The dense peak (arrow) from the gradient was dialysed and sedimented through a neutral 5 to 20 % sucrose gradient (b). The high mol. wt. (nuclear) DNA was centrifuged to equilibrium in a neutral CsCl gradient (c). Fractions from the gradients were assayed for radioactivity. Virus infected cells: ••••; mock infected cells: ○○○○; polyoma DNA: ▲▲▲▲; density: ■■■■.

The total amount of mitochondrial DNA in NIL-8 chick and (tk−) 3T3 cells

The stimulation of the rate of ³H-dThd incorporation into the mitochondrial DNA is not sufficient evidence to conclude that there is a stimulation of the mitochondrial DNA synthesis in the cells, since the rate of uptake, the thymidine kinase activity, or the pool sizes of thymidine in infected and uninfected cells may be different.

The total amount of the mitochondrial DNA obtained after sucrose gradient sedimentation was therefore measured by using the EBr-DNA fluorimetric method (Le Pecq & Paoletti, 1966). Results are summarized in Table 4. The amount of mitochondrial DNA in infected cells was found to be twice that of mock infected cells.
Table 4. Amount of mitochondrial DNA (μg) in cells infected by heat inactivated MCMV*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells</th>
<th>Mock infection</th>
<th>Virus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIL-8 (1 × 10^7 cells)</td>
<td>0.62</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Chick (2 × 10^7 cells)</td>
<td>0.82</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>(tk−) 3T3 (5 × 10^6 cells)</td>
<td>0.26</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* Mitochondrial DNA was estimated by the EBV-DNA fluorometric method; standard curve with polyoma DNA.

Fig. 4. Early antigens in heat-inactivated MCMV-infected NIL-8 cells 24 h post infection. Cells were grown on coverslips and then arrested and infected as described in the legend of Fig. 1. Twenty-four h after infection the cells were fixed and stained by the indirect immunofluorescent technique.

Early antigens in NIL-8 and chick cells after infection with heat-inactivated MCMV

The indirect immunofluorescent technique was used to study the presence of MCMV specific antigens in the NIL-8 and chick cells. While we were unable to detect antigens at 5 h after infection, diffuse nuclear antigens could be demonstrated in both kinds of cell 24 h later. Since there was no virus DNA synthesis in the cells, these antigens can be considered as early antigens, coded for by the parental virus DNA. Fig. 4 shows the diffuse nuclear fluorescence in NIL-8 cells at 24 h after infection.

DISCUSSION

The stimulation of host DNA synthesis by the small oncogenic DNA viruses (Dulbecco et al. 1965, Gershon et al. 1966) and oncogenic adenoviruses (Takahashi et al. 1966, McAllister & Macpherson, 1968; Takahashi et al. 1969) has been documented and recently an increasing quantity of experimental evidence has suggested that in some circumstances herpes viruses stimulate host DNA synthesis (Gerber & Hoyer, 1971; Lee, 1972; St. Jeor et al. 1974; Marcon & Kucera, 1976; Moon et al. 1976; Waner & Budnick, 1977). Since one of the main characteristics of herpes viruses is their ability to persist in the host for long periods, if not for the whole life, after the primary infection, the conditions necessary for the virus stimulation of host DNA synthesis may also exist in vivo.
Contradictory data have been reported for the effect on host DNA synthesis of active, u.v.-irradiated and heat-inactivated HCMV and of the similarly treated MCMV (St. Jeor et al. 1974; Moon et al. 1976; J. Boldogh et al. unpublished data). Active simian cytomegalovirus was also found to stimulate host DNA synthesis (Waner & Budnick, 1977).

It has been claimed (St. Jeor et al. 1974; J. Boldogh et al. unpublished data) that the semi-conservative DNA synthesis of the HCMV-infected cells is stimulated. Furukawa et al. (1976) found, however, that much of the early stimulation of DNA synthesis is due to an increased rate of mitochondrial DNA synthesis. Studies on the nature of the stimulated host DNA in cells infected with MCMV and simian CMV have not been published.

We have shown that active and u.v. irradiated MCMV does not stimulate the DNA synthesis of infected WME, NMG, 3T3, Wg1A, chick and NIL-8 cells but heat-inactivated MCMV does stimulate synthesis in these cells (Table 1). In the experiments summarized in Table 1 we did not characterize the stimulated DNA. We suggest, however, that the increased rate in the incorporation of 3H-dThd into the heat-inactivated virus-infected cells means an increased incorporation into cell DNA and not virus DNA synthesis, since this effect cannot be observed in the active-virus infected cells. Of the cells tested, the NIL-8 and chick cells were the least permissive for virus replication; the number of infectious particles in the media of the cultures on the fourth day after infection was so low that they could even have come solely from the inoculum (Table 2). The newly synthesized DNA of the NIL-8 and chick cells was further analysed. The results indicate that the synthesis of both nuclear and mitochondrial DNA was stimulated in these cells after infection with heat-inactivated MCMV.

The degree and timing of the stimulation of nuclear and mitochondrial DNA synthesis varied between experiments, but it could usually be demonstrated on the second, or sometimes the first day after infection. The degree of stimulation from 1·5 to 9·5 times increase in the nuclear and from 1·5 to 3 times increase in the mitochondrial DNA also depended on how well the cellular DNA synthesis was blocked before the infection and how low the background (the incorporation into the DNA of mock infected cells) was.

Our data on the heat-inactivated MCMV-infected (tk-) 3T3 cells confirm the existence of a separate thymidine kinase for mitochondrial DNA (Kit et al. 1974) and also show that it is not only its enzymic activity which is increased after the infection but the synthesis of mitochondrial DNA is stimulated as well (Table 4).

It can be hypothesized that in heat-inactivated MCMV a suppressor protein(s) (which would have to be one or more of the virus structural proteins) has been inactivated by the heat, thus permitting the virus genome to stimulate host DNA synthesis. This could be tested using purified MCMV-DNA to infect the cells.

SV40 and polyoma virus stimulate mitochondrial DNA synthesis concomitantly with virus-directed induction of nuclear DNA synthesis (Levine, 1971; Vesco & Basilico, 1971). In HSV type-i infected cells nuclear DNA synthesis is inhibited in the first 5 h after infection, whereas mitochondrial DNA synthesis is stimulated (Radsak & Freise, 1972). In adenovirus type-2 infected HeLa cells the virus infection turned off the host chromosomal DNA synthesis, but the mitochondrial DNA synthesis was not inhibited (Fisher & Horwitz, 1977). The stimulation of mitochondrial DNA synthesis induced by HCMV was detected early in the infection, the stimulation of chromosomal DNA synthesis later, but still before or without virus DNA synthesis (St. Jeor et al. 1974; Albrecht et al. 1976; Furukawa et al. 1976; J. Boldogh et al. unpublished data). Our experiments on NIL-8 and chick cell indicate a concomitant effect of heat-inactivated MCMV on the chromosomal and mitochondrial DNA synthesis.
Host DNA synthesis in MCMV-infected cells

The data on the (tk-) 3T3 cells are not sufficient to conclude that the stimulation of mitochondrial DNA is not mediated through a stimulation of nuclear DNA, since the chromosomal DNA synthesis was measured only by the incorporation of labelled thymidine. This was obviously not taken up into the chromosomal DNA by those cells which specifically lack the thymidine kinase necessary for chromosomal DNA synthesis.

Our studies with the immunofluorescent technique suggested there are early proteins in the heat-inactivated MCMV infected cells which may be involved in the stimulation of host DNA synthesis.

Dr Beverly Griffin aided the authors greatly by providing the 32P-labelled and unlabelled polyoma DNA and also by useful criticism and assistance during the course of this research. We are also grateful to Dr Valli Egilsson for valuable discussions, to Drs John Wyke and Peter Frearson for critical reading and Mrs Joyce Newton for help in the preparation of this paper.

REFERENCES


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