Herpes Virus and Viral DNA Synthesis in Ultraviolet Light-Irradiated Cells

By J. COPPEY AND S. NOCENTINI

Foundation Curie, Institut du Radium, Section de Biologie, 26, rue d'Ulm, 75231 Paris 05, France

(Accepted 26 January 1976)

SUMMARY

The rate of virus DNA synthesis and the production of infectious virus are impaired in stationary monkey kidney CV-1 cells irradiated with u.v. before infection with herpes simplex virus (HSV). The inhibition of HSV multiplication is due to u.v.-induced damage in cell DNA.

CV-1 cells recover their capacity to support HSV growth during the 40 to 48 h after irradiation, and the final virus yield is enhanced by a factor of 10. The time course of the recovery is similar to that of the excision repair process occurring in u.v.-irradiated mammalian cells. Caffeine, hydroxyurea and cycloheximide inhibit the recovery. Fluorodeoxyuridine is without effect.

A small but significant amount of labelled dThd coming from irradiated cell DNA is incorporated into virus DNA.

HSV specified thymidine kinase seems to be more effective for virus DNA synthesis in irradiated than in control cells.

INTRODUCTION

The host cell functions involved in the regulation of herpes simplex virus type 1 (HSV) DNA synthesis are not fully understood (Ben Porat & Kaplan, 1973a). The synthesis of HSV DNA is independent of the growth cycle of infected cells (Cohen, Vaughan & Lawrence, 1971). To maintain a normal rate of virus DNA production, protein synthesis seems to be required (Roizman & Roanne, 1964).

In HSV infected cells, virus-specified enzymes involved in DNA metabolism are produced (Ben Porat & Kaplan, 1973b), namely thymidine kinase (Kit, Dubbs & Anken, 1967; Klemperer et al. 1967), deoxycytidine kinase (Perera & Morrison, 1970), deoxyribonuclease (Morrison & Keir, 1968) and DNA polymerase (Keir et al. 1966; Weissbach et al. 1973). The rate of synthesis of these virus enzymes is probably in part controlled by cellular mechanisms which are yet unknown. Moreover, the prospective role of these enzymes in virus DNA synthesis is not yet determined.

In the present study we have investigated the effects of u.v. irradiation of stationary cells prior to infection with HSV on the production of infectious virus, virus DNA and some virus enzymes. We have observed that the high sensitivities to u.v. of the cell capacity to support virus DNA synthesis and virus production is due to damage to the cell DNA, and that both capacities can be recovered during the 40 to 48 h after irradiation as the repair of cellular DNA progresses.

In addition, results are presented which suggest an active role of virus-specified thymidine
kinase for the HSV production in irradiated cells in which the de novo DNA synthesis is impaired by u.v.-induced damage. This is in agreement with recent genetical experiments (Jamieson, Gentry & Subak-Sharpe, 1974).

METHODS

Cells and viruses. We used an established line of green monkey kidney cells, CV-1, in which stocks of HSV type I (Shealey strain) and vesicular stomatitis virus (VSV) were prepared and titrated (Coppey, 1972).

Irradiation and infection. Stationary monolayers of CV-1 grown on 50 mm Greiner Petri dishes (4 to $5 \times 10^6$ cells/dish) were kept for 48 h before infection in a minimum essential medium (MEM) without serum and tryptose phosphate broth. The number of viable cells did not significantly vary during that time in control cultures, but it decreased by 7 to 11 and 26 to 33% (extreme values of three separate experiments) in cultures irradiated with 240 and 480 erg mm$^{-2}$, respectively. Different cultures were u.v.-irradiated (Coppey, 1972) at indicated time intervals before infection (= time 0). The removed medium was then added to the irradiated cultures.

Cells were infected at an input multiplicity of 5 p.f.u./cell and kept in an MEM medium supplemented with 1% heat-inactivated calf serum. At the end of the virus cycle, cells were scraped in their medium and the intracellular virus was released by a 60 s ultrasonic treatment (Mullard) at 0 to 4 °C. In some experiments the number of infectious centres was determined as previously described (Coppey, 1972).

Absorption of labelled virus. HSV was labelled with $^3$H-thymidine (dThd) and purified through 5 to 60% sucrose gradients (see below). The fractions containing the bulk of infectivity and corresponding to intact labelled virus DNA molecules (see Fig. 6) were diluted 1:10 in MEM medium supplemented with 1% calf serum. 0.2 ml/dish of HSV (= $8 \times 10^7$ p.f.u. or 14900 cts/min) was absorbed for 45 min at 37 °C. Cells were rinsed 5 times with phosphate-buffered saline (PBS) before incubation.

Inhibitors. Caffeine (Prolabo), hydroxyurea (Schuchardt, Germany), cycloheximide (Sigma) and fluorodeoxyuridine (Roche-Hoffman) were added at indicated concentrations to the maintenance medium during the 48 h before infection. Treated cultures were carefully rinsed with PBS before infection.

Determination of protein synthesizing activity. Immediately after the last irradiation (i.e. at time 0), cells were treated for 2 h with actinomycin D (Sigma; 5 μg/ml) and kept for 6 h in the presence of 0.1 μCi/ml of a 14C-amino acid mixture (140 mCi/mmol; CEA Saclay). The samples were treated with 1 N-KOH for 1 h to discharge labelled t-RNA, then neutralized with 1 N-HCl. The acid-precipitable material was centrifuged and re-dissolved with hyamine (1 ml/5 × 10$^6$ cells) and the radioactivity was counted using Aquasol (10 ml/sample; New England Nuclear).

Pyrimidine dimers. Cell DNA was uniformly labelled by growing cultures for 48 h in the presence of complete medium supplemented with 2-14C-dThd (50 mCi/mmol; CEA Saclay) at a final concentration of 4-5 μCi/ml. Cells were rinsed 3 times with PBS, irradiated with 240 erg/mm$^2$ of u.v. and incubated at 37 °C in complete medium. At indicated time intervals, cells were harvested and treated with 5% (w/v) trichloroacetic acid for 30 min at 4 °C. The amount of pyrimidine dimers was measured in the acid-soluble intracellular material by bidimensional chromatography according to the method of Setlow, Swenson & Carrier (1963).

Purification of intracellular HSV. $^3$H-dThd (26 Ci/mmol; CEA Saclay) was added to
HSV DNA synthesis in u.v.-irradiated cells

a final concentration of 10 μCi/ml from 1 to 18 h after infection. Infected cells were then scraped into a small volume (0.5 ml/2 × 10⁷ cells) of 0.01 M-MgCl₂, 0.01 M-tris-HCl, pH 7.4. The free DNA was digested with DNase I (Calbiochem; 162000 units/mg) at 500 μg/ml for 1 h at 4 °C. In these conditions about 95% of labelled DNA from control non-infected CV-1 was rendered acid soluble.

0.3 ml of each extract was centrifuged through linear gradients of 5 to 60% (w/v) sucrose in 0.1 M-NaCl, 0.001 M-ethylene diamine tetraacetic acid (EDTA), 0.01 M-tris-HCl, pH 7.4, at 18000 rev/min for 25 min at 2 °C in a SW 41 rotor. The 5% (w/v) trichloroacetic acid-precipitable radioactivity was measured on 50 μl samples of each fraction (about 30) and the infectivity was titrated in parallel on 3 to 4 pooled fractions as indicated. Usually 40 to 50% of the input infectivity was recovered in these gradients.

Sucrose sedimentation of native virus DNA. The 2 to 3 fractions containing the bulk of ³H-dThd-labelled infectious virus were pooled and treated with SDS at a final concentration of 1% (w/v). The preparations were dialysed against SSC (0.15 M-sodium chloride, 0.015 M-sodium citrate) containing 0.001 M-EDTA at 4 °C. 0.2 ml was then applied with 3 to 4 mm bore tips (Gilson) to the top of linear gradients of 5 to 20% (w/v) sucrose in 1 M-NaCl, 0.001 M-EDTA, 0.01 M-sodium phosphate, pH 7.5, and sedimented for 45 min at 45000 rev/min at 15 °C in a SW 501 rotor. Twenty-five fractions were collected dropwise on 3MM Whatman paper strips, washed 2 times with 5% (w/v) trichloroacetic acid and with 99% (v/v) ethyl alcohol. The radioactivity was counted using toluene based scintillant: 1 l toluene, 4 g PPO and 0.1 g POPOP (Hopkin & Williams) in a Packard liquid scintillation counter.

CsCl centrifugation of total DNA. ³H-dThd was added to infected cells as described above. Intracellular DNA was extracted 18 h after infection by the method of Crouch & Rapp (1972). Virus and cell DNA were separated in isopycnic caesium chloride gradients (density 1.71 g/ml) in 0.01 M-EDTA, 0.1 × SSC, run for 70 h at 33000 rev/min at 15 °C in an SW 50 angle fixed rotor. Forty-five to 50 fractions were collected and the radioactivity was counted as described above.

Extractions and assays of enzymes

DNA polymerase. Nine and 18 h after infection, extracts of infected cells were made and their enzyme content was measured as described (Hamada, Kamiya & Kaplan, 1966). Heat denatured (5 min at 100 °C) calf thymus DNA (Sigma) was present in the reaction mixtures at saturating concentration (0.6 mg/ml) with respect to enzyme activity. For each extract, at least 3 dilutions were tested over a range where the reaction was linear, i.e. at protein concentrations between 20 and 200 μg/ml. At higher concentrations, deoxyribonuclease (DNase) activity interfered with DNA polymerase activity.

DNase. Exonuclease activity was determined in cell extracts made as described above. The standard assays contained: 0.1 ml of extract + 0.1 ml of the following mixture: 2 μg of ³H-labelled CV-1 DNA (= 10000 ct/min), 0.01 M-MgCl₂, 0.05 M-tris-HCl, pH 7.4. The enzyme activities were taken as the protein contents of extracts which rendered acid soluble 50% of labelled DNA in 1 h at 37 °C. DNase activities in infected control and irradiated extracts gave similar results when tested on native or heat denatured CV-1 DNA.

Thymidine kinase. Cell extracts were made as described by Aron et al. (1973) at indicated times after infection. The enzyme activities were determined using the following standard reaction mixture: ³H-dThd, 5 × 10⁻⁵ M with a total radioactivity of 0.25 μCi, 5 mM-ATP, 5 mM-MgCl₂, 50 mM-tris-HCl, pH 8, and extract up to 0.25 ml. The reaction was incubated
Fig. 1. Virus yields from different cultures receiving a single u.v. dose of 240 erg/mm² at the indicated times before infection (= time 0). ●—●, 18 h HSV yields; ○—○, 18 h VSV yield; ▲—▲, number of HSV producing cells; ■—■, 6 h incorporation into acid-insoluble radioactivity of a mixture of 14C-amino acids in cultures irradiated and pre-treated with 5 μg/ml of actinomycin D for 2 h before labelling. The values corresponding to the mean of 3 Petri dishes are expressed as the % of non-irradiated controls.

RESULTS

HSV production by u.v.-irradiated cells

Exposure to a dose of 240 erg/mm² of u.v. light at different time intervals before infection (= time 0)

Fig. 1 shows: (a) in cultures irradiated at time 0 (u.v. 0 h cells), the virus yield corresponding to the first cycle (18 h) and the number of virus producing cells (infectious centres) are decreased by 93 and 94 %, respectively. This similar decrease could indicate that the residual virus yield in irradiated cultures is correlated to the survival of cells able to produce HSV. In contrast, the production of VSV, an RNA virus whose replication does not require transcription of the host cell genome, is decreased only by 30 % in irradiated cultures. Also, protein synthesis as measured by the incorporation of amino acids into...
acid-insoluble material in actinomycin D pre-treated cells, is reduced only by 25% after irradiation with 240 erg/mm²; (b) the irradiated cultures progressively recover their capacity to produce HSV when the time interval between irradiation and infection increases; for a 48 h interval (u.v.-48 h cells), the virus yield is enhanced by a factor of about 10 and the number of infectious centres by a factor of 7.

The growth curves of HSV were then established. They are slightly different in control and irradiated cultures, as seen in Fig. 2(a). The exponential phase of virus production is reached earlier in u.v.-48 h cultures and later in u.v. 0 h cultures than in the controls. The virus production after the first cycle continues to increase slowly up to 72 h after infection in u.v. 0 h cells (Fig. 2a). To measure the rate of absorption of infecting HSV, control and irradiated cultures were infected with a small stock of ³H-dThd-labelled and purified virus (see Methods) and the acid-insoluble radioactivity bound to cells was counted at different times after infection (see legend to Table 1). The rate of absorption of labelled HSV is the same in control and irradiated cultures (Table 1). The amount of labelled HSV DNA in cells is decreased 18 h after infection to the same extent in control and irradiated cultures, indicating that irradiated cultures do not exhibit a higher degradative activity towards infecting virus DNA than the control cultures during the virus cycle.

**Exposure to different doses of u.v. at 48 h and 0 h before infection**

The dose–response curve of HSV production obtained in u.v. 0 h cells is exponential and similar to that of cell division which was determined according to the method of
Table 1. Kinetics of absorption of $^3$H-dThd-labelled HSV in irradiated versus control cultures

<table>
<thead>
<tr>
<th>Hours after infection</th>
<th>u.v. irradiation (240 erg/mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>0.25</td>
<td>5780*</td>
</tr>
<tr>
<td>0.75</td>
<td>9460</td>
</tr>
<tr>
<td>3</td>
<td>9940</td>
</tr>
<tr>
<td>9</td>
<td>9120</td>
</tr>
<tr>
<td>18</td>
<td>6990</td>
</tr>
</tbody>
</table>

* Acid-insoluble radioactivity (ct/min) per culture: mean value of 3 separate Petri dishes.

Table 2. Effects of inhibitors on the recovery ratio of HSV production in irradiated cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Recovery ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>5 µg/ml</td>
<td>1.7</td>
</tr>
<tr>
<td>Caffeine</td>
<td>2 mM</td>
<td>2.2</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>1 mM</td>
<td>6.9</td>
</tr>
<tr>
<td>Fluorodeoxyuridine</td>
<td>0.01 mM</td>
<td>10.5</td>
</tr>
</tbody>
</table>

* Recovery ratio = virus yield in u.v. 48 h-treated cells / virus yield in u.v. 0 h-treated cells.

Puck, Marcus & Ciecura (1956) by D. Papadopoulo & J. Coppey (unpublished data and Fig. 2b). This indicates that both capacities have identical u.v. sensitivities. The dose-response curve obtained in u.v.-48 h cells has a shoulder at doses $\leq 120$ erg/mm$^2$ and is parallel at higher doses to that of u.v. 0 h cells (Fig. 2b), which demonstrates that the recovery ratio of HSV production is constant for doses of u.v. between 120 and 480 erg/mm$^2$. It can be noted that the absolute virus yield is significantly higher at low doses in u.v.-48 h than in control cells (Fig. 2b).

Effects of inhibitors on the recovery process of HSV production in irradiated cells

U.v.-48 h, u.v. 0 h and control cultures were kept for 48 h before infection in the presence of several inhibitors at concentrations which did not modify virus production in non-irradiated cultures. The results are shown in Table 2. The recovery process is strongly inhibited by cycloheximide and caffeine, slightly inhibited by hydroxyurea, but not affected by fluorodeoxyuridine.

HSV DNA synthesis

Fig. 3 shows the CsCl profiles of $^3$H-dThd-labelled DNA from infected cultures. The amount of radioactivity present in the peak of virus density DNA (1.725 g/ml) is smaller in u.v. 0 h cells than in controls and greater in u.v.-48 h than in u.v. 0 h cells. The relative amounts of virus DNA produced by irradiated versus non-irradiated cultures were estimated by graphically measuring the area delimited by corresponding peaks. Table 3 shows that the u.v.-48 h cultures produce 1.5, 3.1, 3.4 and 3.5 times more virus DNA than the corresponding u.v. 0 h cultures after u.v. irradiation with 120, 240, 360, and 480 erg/mm$^2$. The production of infectious virus and of virus DNA decrease in u.v.-48 h cells at similar rate with doses of u.v. higher than 120 erg/mm$^2$. 
HSV DNA synthesis in u.v.-irradiated cells

Fig. 3. CsCl gradient profiles of $^3$H-dThd-labelled DNA extracted from HSV infected cells 18 h after infection. (a) Control cells; (b) cells irradiated with a u.v. dose of 240 erg/mm$^2$ $\Delta-\Delta$, 48 h and $\circ-\circ$, 0 h before infection.

Table 3. Infectious virus (18 h) yields and $^3$H-dThd incorporation in u.v.-48 h (A) versus u.v. 0 h (B) cultures

<table>
<thead>
<tr>
<th>Dose of u.v. (erg/mm$^2$)</th>
<th>Infectious virus (%*)</th>
<th>ct/min in virus DNA (%*)</th>
<th>ct/min in cell DNA (%*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>120</td>
<td>180</td>
<td>30</td>
<td>65</td>
</tr>
<tr>
<td>240</td>
<td>65</td>
<td>7</td>
<td>49</td>
</tr>
<tr>
<td>360</td>
<td>16.5</td>
<td>1.3</td>
<td>17.8</td>
</tr>
<tr>
<td>480</td>
<td>5.5</td>
<td>0.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* The control values (100%) were: $8 \times 10^7$ p.f.u. for infectious virus; 844000 ct/min in virus DNA; 337000 ct/min in cell DNA.

On the other hand, virus DNA seems to be produced by u.v. 0 h cells in excess as compared to infectious virus. The production of this excess virus DNA is even more pronounced as the dose of u.v. increases.

Rate of HSV DNA synthesis

Infected cells were pulse labelled for 30 min with $^3$H-dThd at 9 h after infection, i.e. at a time corresponding to the exponential phase of virus DNA synthesis (Olshevsky, Levitt &
J. COPPEY AND S. NOCENTINI

Fig. 4. CsCl gradient profiles of DNA from HSV infected cells: tritiated dThd was added from 9 to 9.5 h after infection and chased for 6 h in the presence of 10 μg/ml of unlabelled dThd. (a) Control cells; (b) cells irradiated with 240 erg/mm² at 48 h or (c) 0 h before infection.

Becker, 1967) and of virus production (Fig. 2a). Cells were chased for 6 h with cold dThd and the total DNA was analysed in CsCl gradients. The radioactivity found in the DNA of virus density is about 7% in u.v. 0 h and 110% in u.v.-48 h cultures as compared to the controls (Fig. 4). These values can be taken as an estimate of virus DNA synthesis which is in agreement with that of infectious virus production (Fig. 2a). The amount of radioactivity found in cellular DNA is more important in comparison to that of virus DNA in u.v. 0 h cells. The same result was observed when DNA was labelled from 1 to 18 h after infection (see Table 3). This might indicate that the replicating HSV does not inhibit u.v.-induced repair synthesis as it does normal DNA synthesis (Ben Porat & Kaplan, 1973a).

Exchange of dThd-labelled material from cellular to virus DNA in irradiated cells

HSV was grown for 18 h on uniformly pre-labelled 2-14C-dThd cells (see Methods) which were exposed to a dose of 240 or 480 erg/mm² at time 0. The total DNA was centrifuged in CsCl gradients (density 1.71 g/ml). Radioactive material was observed banding near to the cellular DNA peak at a density corresponding to virus DNA. This suggests that labelled cellular material is incorporated into virus DNA in irradiated cells.

In order to obtain further evidence for such incorporation, HSV was grown for 18 h in the presence of bromodeoxyuridine (BrdUrd, Sigma) at 50 μCi/ml. The total DNA was analysed in CsCl gradients (density 1.80 g/ml). A peak of radioactivity was found at a density of 1.85 g/ml corresponding probably to substituted virus DNA, since such a peak was not seen in DNA from uninfected cells labelled in the same conditions (data not shown). HSV was grown for 18 h in the presence of BrdUrd (100 μg/ml) on uniformly pre-labelled cells (see Methods). These had been kept for 48 h before irradiation and infection in the presence of unlabelled dThd (10 μg/ml) to eliminate remaining free label. The total DNA was analysed in CsCl gradients (density 1.80 g/ml). With DNA from irradiated and infected cultures, a peak of radioactivity is found at a density of 1.85 g/ml, but not with the DNA from non-irradiated and infected cultures. The peak corresponds to about 0.1% and 0.02% of the starting material after a dose of u.v. of 240 and 480 erg/mm² respectively (Fig. 5b). Such a peak is not observed with DNA of irradiated but non-infected
HSV DNA synthesis in u.v.-irradiated cells

Fig. 5. Heavy region (density 1.83 to 1.87 g/ml) of CsCl gradients of DNA from cells uniformly 2-3HCl-pre-labelled, then irradiated and infected with HSV. Virus was grown in the presence of BrdUrd at a final concentration of 100 µg/ml. DNA was extracted 18 h after infection. The total radioactivity in each gradient was 2 to 2.5 x 10^6 cpm/min. (a) Non-infected controls: irradiated with: ■■■, 0 and ●●●, 240 erg/mm^2, 18 h before DNA extraction. (b) HSV infected cultures irradiated with ■■■, 0; ●●●, 240; and ▲▲, 480 erg/mm^2 0 h before infection.

cultures (Fig. 5a). These results clearly indicate a significant incorporation of dThd-labelled material from irradiated cellular DNA into virus DNA. However, since BrdUrd might modify to a certain extent normal virus DNA synthesis, possibly causing strand breakage following the substitution, it was impossible to evaluate from these data the amount of incorporation in our standard conditions.

In another experiment, the intracellular acid-soluble radioactive material from pre-labelled cells was found to contain about 4 % of pyrimidine dimers 12 and 24 h after u.v. irradiation with a dose of 240 erg/mm^2 (Table 4). To test whether there is some incorporation of pyrimidine dimers in the replicating HSV DNA, we grew HSV in the presence of BrdUrd (100 µg/ml) on pre-labelled and irradiated (240 erg/mm^2) cells. The radioactive fractions corresponding to the peak of virus DNA at a density of 1.85 g/ml were pooled, precipitated with cold 5 % (w/v) trichloroacetic acid and the precipitate analysed for the presence of pyrimidine dimers as described in Methods. After bidimensional chromato-
Table 4. Release of $^{14}$C-pyrimidine dimers ($\hat{X}T$) from labelled cultures exposed to a dose of 240 erg/mm$^2$

<table>
<thead>
<tr>
<th>Hours after u.v. dose</th>
<th>$^{14}$C-dThd*</th>
<th>$^{14}$C-$\hat{X}T$</th>
<th>Dimers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>3840</td>
<td>160</td>
<td>4.2</td>
</tr>
<tr>
<td>12</td>
<td>5790</td>
<td>240</td>
<td>4.1</td>
</tr>
<tr>
<td>24</td>
<td>9780</td>
<td>540</td>
<td>5.5</td>
</tr>
<tr>
<td>48</td>
<td>3680</td>
<td>250</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* Intracellular acid soluble radioactivity (ct/min/culture).

Table 5. Intracellular HSV enzyme activities in u.v.-48 h (A) and u.v. 0 h (B) cultures

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Time after infection (h)</th>
<th>0</th>
<th>120</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA polymerase</td>
<td></td>
<td></td>
<td>A (%)</td>
<td>B (%)</td>
</tr>
<tr>
<td>0</td>
<td>100 (0.037*)</td>
<td>94</td>
<td>89</td>
<td>82</td>
</tr>
<tr>
<td>9</td>
<td>100 (5.5)</td>
<td>87</td>
<td>93</td>
<td>78</td>
</tr>
<tr>
<td>18</td>
<td>100 (12.2)</td>
<td>90</td>
<td>96</td>
<td>70</td>
</tr>
<tr>
<td>Exonuclease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100 (750–900†)</td>
<td>95</td>
<td>85</td>
<td>73</td>
</tr>
<tr>
<td>9</td>
<td>100 (100–160)</td>
<td>95</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>18</td>
<td>100 (50–70)</td>
<td>95</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>Thymidine kinase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100 (5‡)</td>
<td>188</td>
<td>130</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>100 (5.9)</td>
<td>180</td>
<td>106</td>
<td>51</td>
</tr>
<tr>
<td>9</td>
<td>100 (12.3)</td>
<td>150</td>
<td>113</td>
<td>52</td>
</tr>
<tr>
<td>12</td>
<td>100 (19.6)</td>
<td>160</td>
<td>155</td>
<td>159</td>
</tr>
<tr>
<td>18</td>
<td>100 (52)</td>
<td>192</td>
<td>70</td>
<td>133</td>
</tr>
</tbody>
</table>

* pmol of thymidine-5'-triphosphate incorporated per mg protein in 2 h at 37 °C (mean values of 3 dilutions, see Methods).
† Concentration of protein (μg/ml) which renders acid-soluble 50% of denatured DNA in 1 h at 37 °C (extreme values of 3 determinations).
‡ pmol dThd phosphorylated per mg protein in 15 min at 37 °C (mean values of 3 dilutions, see Methods).

graphy, less than 1% of the total radioactivity was detected in the region of pyrimidine dimers. Thus there was no evidence of any substantial incorporation of pyrimidine dimers into virus DNA in our experimental conditions.

Properties of purified virus grown on irradiated cells

Extracts of infected cells were treated with DNase to digest free DNA prior to their analysis in 5 to 60% (w/v) neutral sucrose gradients.

Two peaks of radioactivity can be distinguished (Fig. 6, left panel) as already described (Aurelian & Wagner, 1966). The light peak from control and irradiated cultures contains the bulk of infectious virus (see legend to Fig. 6). The DNA extracted from that peak sediments in an almost homogeneous band in 5 to 20% (w/v) neutral sucrose gradient (Fig. 6, right panel), as seen for intact molecules of HSV DNA (Becker, Dym & Sarov, 1968). The sedimentation profiles are comparable for control and irradiated cultures. The amount of $^3$H-dThd incorporated per infectious particle can be calculated by dividing the total ct/min in the light peak by the number of infectious particles. The values obtained are 2-5 and 4 times higher in cells irradiated at time 0 with a u.v. dose of 240 and 480 erg/mm$^2$ than in the controls. The apparent excess of virus DNA produced in irradiated cells is simply due to an overestimation of the virus DNA synthesis as measured by the $^3$H-dThd incorporation (see Table 3).
HSV DNA synthesis in u.v.-irradiated cells

Fig. 6. Left panel: sedimentation patterns in 5 to 60% (w/v) sucrose gradients of cell extracts made 18 h after infection with HSV and treated with DNase I. HSV was grown either in the presence of 3H-dThd (●—○), or on 3H-dThd pre-labelled cells (■—■). (a) Control cells; (b) cells irradiated with a u.v. dose of 240 erg/mm² 48 h or (c) 0 h before infection. The infectious contents (p.f.u.) of pooled fractions were in (a) fractions 3 to 5 = 1.1 × 10⁷, 10 to 12 = 1.5 × 10⁸, 19 to 22 = 3 × 10⁸; (b) fractions 5 to 7 = 1 × 10⁷, 10 to 12 = 3.8 × 10⁸, 19 to 22 = 1.3 × 10⁸; (c) fractions 5 to 7 = 1.3 × 10⁸, 10 to 12 = 6 × 10⁸, 19 to 22 = 1.5 × 10⁸. Right panel: sedimentation patterns in 5 to 20% neutral sucrose gradients of HSV DNA extracted from corresponding fractions 19 to 22: (d) from (a), (e) from (b), (f) from (c). Total counts in (a) = 19 000, (b) = 6100, (c) = 1530.

The heavy peak contains a small proportion of infectious particles and labelled DNA of cellular density (1.700 g/ml) when HSV is grown on pre-labelled cells. This cellular DNA peak is slightly higher in u.v. 0 h cells than in u.v.-48 h or in control cells.

Levels of some virus-specified enzymes of DNA metabolism

In order to assess the prospective role of HSV-specified enzymes (DNA polymerase, DNase and thymidine kinase) in virus DNA synthesis in irradiated cultures, we measured
enzyme levels at 9 h and 18 h after infection, i.e. at times corresponding to the exponential phase of virus DNA synthesis and to the end of the virus cycle.

The level of DNA polymerase and of DNase decreases as a function of the u.v. dose in the same way in u.v.-48 h and in u.v. 0 h cultures at the two times chosen (Table 5). The residual enzyme levels seem not to be correlated to the concomitant residual synthesis of virus DNA in u.v. 0 h cultures (compare Tables 3 and 5).

In contrast, the level of thymidine kinase (TK) is higher throughout the virus cycle in u.v.-48 h than in u.v. 0 h cultures (Table 5). In addition the activity of the enzyme is constantly higher in uninfected, as in infected u.v.-48 h cells after exposure to a u.v. dose \( < 240 \text{ erg/mm}^2 \), than in the controls (Table 5). Finally, up to 12 h after infection, the TK level rises faster in u.v. 0 h cells irradiated with a dose of 120 erg/mm\(^2\) than in the controls (Table 5).

**DISCUSSION**

The rate of virus DNA synthesis and the production of infectious virus are impaired in stationary CV-1 cells u.v. irradiated before infection with HSV type I. We have the following reasons to believe that this must be due to the u.v.-induced damage in cellular DNA. The capacity of CV-1 to produce VSV, an RNA virus whose replication does not require transcription of cell DNA, and the protein synthesizing activity at a post-transcriptional level are about 10 times more resistant to u.v. than the capacity of HSV production. The latter capacity is as sensitive to u.v. as the capacity for cell division. The inhibition of cell division after u.v. irradiation depends mainly on photolesions induced in cellular DNA as concluded from physical and biochemical data (see review by Latarjet, 1972). Additional evidence comes from experiments with u.v. sensitive (Xeroderma Pigmentosum) human cell lines (Cleaver, 1970). In the same way, the loss of capacity of HSV plaque formation in irradiated potoroo cells was shown to be probably caused by u.v. damage to the cell DNA (Lytle & Benane, 1975).

CV-1 cells regain their ability to support virus DNA synthesis and virus production during a 40 to 48 h period after u.v. irradiation. A similar phenomenon can be observed in HeLa cells in the case of ultraviolet reactivation which consists of an enhancement of infectivity of u.v.-irradiated HSV in u.v.-irradiated cells (Lytle, Benane & Bockstahler, 1974). It remains to be elucidated that the increase of delayed ultraviolet reactivation and the recovery of HSV production depends on common pathways.

The recovery of HSV production seems to be bound to the excision repair process working on u.v.-induced lesions in eukaryotic cells, since the kinetics of this recovery is similar to those reported for the excision of pyrimidine dimers (Regan, Trosko & Carrier, 1968), for DNA strand breakages (Cleaver & Boyer, 1972; Cleaver, 1974), for repair replication (Cleaver & Boyer, 1972; Edenberg & Hanawalt, 1973) and for the disappearance of u.v. endonuclease susceptible sites from DNA (Patterson, Lohman & Sluyter, 1973). Moreover, the time course of u.v.-irradiated CV-1 cells to recover their capacity of transcribing pre-ribosomal 45S RNA appears to be similar (S. Nocentini, unpublished data). A recovery was also reported in the case of interferon mRNA transcription in u.v. irradiated CV-1 cells, which was under chromosomal control (Coppey, 1971).

Caffeine inhibits the recovery of HSV production as it does the repair of DNA for cell division (Domon & Rauth, 1969). The inhibition by cycloheximide might indicate that the recovery process depends on the synthesis of repair enzyme not present at the time of irradiation. However, the half-life of preexisting enzymes for repair replication is reported to be at least 30 h (Gautschi, Young & Cleaver, 1973). But if repair replication reflects
HSV DNA synthesis in u.v.-irradiated cells

a biological functional process (Painter, Umber & Young, 1970) it does not necessarily comprise the whole biological repair. The partial inhibitory effect of hydroxyurea (HU) on the recovery of HSV production may be explained in this way since HU does not inhibit repair replication (Cleaver, 1969), but inhibits the rejoining of DNA strand breaks occurring during the repair period (Ben Hur & Ben Ishai, 1971), a process linked to the biological repair (Buhl, Setlow & Regan, 1972). Fluorodeoxyuridine does not affect this recovery which seems to be independent of normal DNA synthesis.

The mechanisms leading to the reduction of the rate of virus DNA synthesis in u.v.-irradiated cells are not yet clear. Degradation of cell DNA is known to occur for several hours after u.v. irradiation (Cleaver & Trosko, 1969). The degradation products contain fragments of DNA and short acid soluble oligonucleotides (Cleaver & Boyer, 1972). A similar process seems to occur in irradiated and HSV infected CV-1 cells as reflected by the incorporation of a small amount of dThd labelled cellular material into virus DNA. However, we did not observe an extensive or abnormal degradation of virus DNA throughout the virus cycle in irradiated cells. The degradation of parental virus DNA did not differ in irradiated cultures from that of the controls. The level of virus-induced DNase activity was lower in irradiated cultures than in the controls.

Genetical experiments show that HSV requires virus-specified pyrimidine kinase activity under conditions in which the host cell’s de novo metabolism is low (Jamieson et al. 1974). The de novo metabolism is impaired in u.v.-irradiated mammalian cells in which the overall process of DNA synthesis is depressed, for several hours (Powell, 1962; Cleaver, 1965; Domon & Rauth, 1968). Similarly, HSV seems to require thymidine kinase (TK) activity in u.v.-irradiated cells, since the corresponding (exogenous) pathways of thymidilate synthesis appear to be more effective for virus DNA production in these cells than in controls. This is shown by three results: (a) the amount of labelled exogenous dThd incorporated in purified virus DNA per infectious particle is greater in irradiated than in control cells and the difference was more pronounced as the u.v. dose was increased at higher doses of u.v.; (b) when HSV was grown in the presence of fluorodeoxyuridine or cytosine arabinoside, inhibitors of the endogenous pathways of deoxythymidilate (Hartman & Heidelberg, 1962) and of deoxycytidilate (Chu & Fischer, 1962) respectively, the production of infectious virus is much more inhibited in control than in irradiated cultures (J. Coppey, manuscript in preparation); (c) during the exponential phase of virus DNA synthesis, i.e. from 6 to 12 h after infection, the virus TK level increases faster in cells irradiated at time 0 with 120 erg/mm² than in the controls.

In contrast to the virus DNA polymerase and DNase, the level of virus TK is constantly higher throughout the virus cycle in repaired than in non-repaired cells. This might indicate that in repaired cells in which the de novo metabolism of DNA is restored, both exogenous and endogenous pathways of thymidilate synthesis contribute to HSV DNA synthesis.

This work was supported by an INSERM contract no. 72.4.004.1, and by a grant from the Commissariat à l’Energie Atomique to Dr R. Latarjet. We thank Dr D. Averbeck and Dr R. Latarjet for suggestions and help in the preparation of the manuscript.
REFERENCES


HSV DNA synthesis in u.v.-irradiated cells


(Received 16 June 1975)