Simian Virus 40 – Chinese Hamster Kidney Cell Interaction. IV. Enhanced Virus Replication in Infected Cells upon Treatment with Mitomycin C

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SUMMARY

Chinese hamster kidney cells are semi-permissive to simian virus 40 (SV40). Exposure to mitomycin C (MC) of Chinese hamster kidney cells infected with SV40 DNA enhanced the yield of infectious virus 10- to 100-fold. This stimulation occurred whether the treatment was performed before or after infection. A simultaneous increase in the number of V antigen-synthesizing cells and virus-producing cells, as well as the virus burst size, was observed upon MC pretreatment, whereas the proportion of T antigen-synthesizing cells remained unchanged. MC pretreatment clearly stimulated virus DNA replication in SV40 virus-infected cells. Cells treated with MC exhibited an unbalanced growth pattern, with continuing protein synthesis in the absence of cell division and a markedly reduced ability to replicate the cellular DNA. These results suggest that MC enhances the permissiveness of Chinese hamster kidney cells by inducing the synthesis of a specific cellular factor(s) required for SV40 replication in these cells. Exposure to ultraviolet light also enhanced infectious virus production in Chinese hamster kidney cells.

INTRODUCTION

Several chemical and physical agents are inducers of virus production in simian virus 40 (SV40)- and polyoma virus-transformed cells, the efficiency of induction for a given agent depending on the transformed cell type considered (Gerber, 1964; Burns & Black, 1968, 1969; Fogel & Sachs, 1970; Margalith et al. 1970; Rothschild & Black, 1970; Fogel, 1972, 1973, 1975; Kaplan, Wilbert & Black, 1972; Kaplan et al. 1975; Morris et al. 1975, 1977). The precise mechanisms underlying the induction event are not known at present. Recent experimental data obtained by Rakusanova et al. (1976) suggest that mitomycin C (MC) and γ-irradiation promote excision of the SV40 genome from the cellular DNA of SV40-transformed Syrian hamster cells.

In the accompanying paper (Morris et al. 1977) we have shown that in virus-producer SV40-transformed Chinese hamster kidney cells, infectious virus synthesis can be activated by treatment with different chemical or physical agents, the most powerful being MC. The semi-permissive character of Chinese hamster kidney cells (Lavialle et al. 1976) might favour virus production in high yields upon treatment.

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In the present work, we have tried to determine whether an inducing agent would be capable of modifying the permissive state of cells in such a way as to amplify virus multiplication. We have shown that MC is effective in enhancing SV40 replication in Chinese hamster kidney cells infected with virus or virus DNA, and have analysed some characteristics of this phenomenon. The effect of ultraviolet (u.v.) light is also examined.

METHODS

 CELLS. CV1 is an established line of African green monkey kidney cells (Jensen et al. 1964). CHK Cl.10 is a clone derived from an established line of Chinese hamster kidney cells (Lavialle et al. 1975). Cell culture techniques have been described previously (Lavialle et al. 1975).

 SV40 virus and virus DNA. The stock of SV40 virus (strain SVLP; Suárez et al. 1974) used in this work was prepared from plaque-purified virus and assayed by plaque titration on CV1 cell monolayers (Tournier et al. 1967). The infectious titre was $2 \times 10^8$ p.f.u./ml.

 Hirt supernatants (Hirt, 1967), obtained from SV40-infected CV1 cell cultures as described previously (Lavialle et al. 1976), were used as a source of infectious virus DNA after extensive dialysis against saline sodium citrate (SSC: 0.15 M-NaCl, 0.015 M-sodium citrate, pH 7.0). The infectious titre of the SV40 DNA stocks was $5 \times 10^6$ p.f.u./ml, as determined by plaque assay on CV1 cell monolayers (Kit et al. 1968).

 Mitomycin C treatment. Mitomycin C (MC; Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved to 1 mg/ml in phosphate-buffered saline (PBS), sterilized by filtration and stored at $-20^\circ C$. The drug was further diluted to the desired concentration with culture medium just before use. Cultures treated with MC were handled under subdued light.

 Treatment of CHK Cl.10 cells was started at the times indicated in infection with SV40 virus or virus DNA (see below) by directly adding an appropriate amount of the drug to the culture medium. After 3 h incubation at $37^\circ C$, the supplemented medium was removed, the cultures rinsed and fresh medium added. Control cultures were handled similarly but without addition of MC.

 U.v.-irradiation. Either before or after infection with SV40 DNA (see below), CHK Cl.10 cell cultures in 60 mm Falcon plastic dishes were mock-irradiated or u.v.-irradiated as already described (Morris et al. 1977).

 Synthesis of infectious SV40 virus. CHK Cl.10 cell cultures in 25 cm² flasks or 60 mm dishes (Falcon plastics) were exposed to MC or u.v. light as described above, either before or after infection with SV40 DNA, at the respective times indicated in the Results section. Infection with SV40 DNA was performed as follows: 24 h after seeding (10⁶ cells/culture), control and experimental cell cultures were rinsed with tris-buffered saline (TBS) and infected with 0.4 ml SV40 DNA in TBS containing 0.5 mg/ml diethylaminoethyl (DEAE)-dextran (Pharmacia, Uppsala, Sweden) at a m.o.i. of 1 p.f.u./cell. After incubation at room temperature for 30 min, the inoculum was removed and cell cultures were rinsed and covered with fresh medium. At designated times after infection, control and treated cell cultures were harvested by freezing the flasks at $-20^\circ C$. In the case of u.v.-irradiation, for which dishes were used, the cells were scraped with a rubber policeman and the suspensions were transferred to flasks before freezing. Cell lysates were prepared by three cycles of freezing and thawing followed by sonication, and virus yields were determined by plaque titration on CV1 monolayers (Tournier et al. 1967).

 Infectious centres. Control and MC-treated CHK Cl.10 cell cultures in 60 mm dishes were
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Infected with SV40 DNA as described above. Two hours after infection, cells were trypsinized and plated with CV1 cells for infectious centre determination (Morris et al. 1977).

In order to determine the virus burst per cell, fractions of 10⁶ CHK Cl.1o cells from the same suspensions as those used for the infectious centre assay were seeded in 25 cm² flasks and the kinetics of virus production was followed over a period of 7 days. Care was taken for the culture conditions (serum concentration, pH of the medium) to be as similar as possible in the infectious centre assay and in the kinetic experiment (see Morris et al. 1977). The burst per cell was estimated by the ratio of the maximum virus yield per 10⁶ seeded cells, determined from SV40 growth curves, to the number of virus-producing cells per 10⁶ seeded cells, obtained from the infectious centre assay.

SV40 T and V antigen synthesis. Samples of the same CHK Cl.10 cell suspensions as used for the infectious centre assay were seeded on to coverslips. At various times thereafter, coverslips were fixed in cold acetone and stained for T and V antigen by an indirect immunofluorescence technique (Wicker & Avrameas, 1969).

SV40 DNA replication. Control and MC-treated CHK Cl.10 cell cultures in 60 mm dishes (10⁶ cells/dish) were rinsed with serum-free medium and inoculated with 0.5 ml SV40 virus (m.o.i. of 100 p.f.u./cell). After 2 h adsorption, the inoculum was removed, the cultures were rinsed twice and fresh medium was added. Fourteen hours after infection, cell cultures were labelled for 10 h with ³H-thymidine (20 µCi/ml, 25 Ci/mM), washed with PBS and the low molecular weight DNA was extracted by the selective Hirt procedure (Hirt, 1967). Labelled DNA in the Hirt supernatants was analysed by CsCl-ethidium bromide (EtBr) density gradient centrifugation and fractions containing supercoiled DNA were pooled and further centrifuged through neutral 5 to 20% sucrose gradients to separate SV40 DNA from mitochondrial DNA, as described in the preceding paper (Morris et al. 1977).

Incorporation of radiolabelled precursors of DNA, RNA and proteins in MC-treated cells. Twenty-four hours after seeding, CHK Cl.10 cell cultures in 60 mm dishes were either treated with MC or mock-treated. Twenty-four hours after MC addition, control and treated cultures were pulse-labelled for 2 h with ³H-thymidine, ³H-uridine or ³H-leucine (25, 28 and 50 Ci/mM respectively). ³H-leucine was added to medium without leucine supplemented with calf serum dialysed against three changes of PBS. After three rinses with cold PBS, the cells were scraped with a rubber policeman and incorporation of radioactive precursors was measured (Morris et al. 1977).

Effect of caffeine on SV40 virus synthesis. Control and MC-treated CHK Cl.10 cell cultures were infected with SV40 DNA as described above. Comparison was made between the virus yields observed either in the absence or in the presence of 1 mM-caffeine (Sigma, St Louis, Missouri), added to the culture medium after infection.

Effect of caffeine on the plating efficiency of MC-treated cells. The procedure has been described by Morris et al. (1977).

Isotopes. All isotopes were obtained from the Commissariat à l'Energie Atomique, France.

RESULTS

Stimulation of SV40 production by MC treatment of CHK Cl.10 cells infected with SV40 DNA

Upon infection with SV40 virus or virus DNA, Chinese hamster kidney cells supported virus DNA and virus synthesis at a low level and this cell system was defined as semi-permissive (Lavialle et al. 1976). To demonstrate infectious SV40 virus synthesis, these cells
Fig. 1. Effect of MC on infectious virus production in CHK Cl.10 cells infected with SV40 DNA. Two hours after infection, cell cultures were treated for 3 h with MC at the indicated concentrations. Virus yields from cultures harvested 3 days post infection (p.i.) were determined as described in Methods. ○—○, Experiment 1; □—□, experiment 2.

Table 1. Synthesis of infectious virus in CHK Cl.10 cells infected with SV40 DNA and treated with MC at various times after infection

<table>
<thead>
<tr>
<th>Time of MC addition after infection with SV40 DNA (h)</th>
<th>Virus yield (p.f.u./culture) 3 days after MC treatment</th>
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<tbody>
<tr>
<td></td>
<td>Control (2 μg/ml for 3 h)</td>
</tr>
<tr>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>48</td>
<td>31</td>
</tr>
<tr>
<td>72</td>
<td>69</td>
</tr>
</tbody>
</table>

had to be infected with virus DNA in order to overcome their limited susceptibility to the virus particles and to avoid masking of the virus progeny by the input virus inoculum (Lavialle et al. 1976). CHK Cl.10 cells were chosen for the present work because they produced less infectious virus following infection with SV40 DNA, than the other clones tested.

Fig. 1 shows that treatment of these cells with MC 2 h after infection with virus DNA, significantly enhanced the yield of virus. Maximum stimulation occurred upon a 3 h exposure of the infected cells to 2 μg/ml of MC. Compared to untreated cultures, the
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Fig. 2. Kinetics of infectious virus production in CHK Cl.10 cells treated with MC either before or after infection with SV40 DNA. (a) MC treatment (2 μg/ml for 3 h) was started 2 h after the end of SV40 DNA infection. (b) Cell cultures were first treated with MC (2 μg/ml for 3 h); they were then rinsed and incubated in drug-free medium for 2 h before inoculating the virus DNA. Cell cultures were then processed as described in Methods. ○—○, Control; ●—●, MC-treated cells.

increase in virus production following MC treatment at the optimum dose varied from 10- to 100-fold depending on the experiments. Table 1 shows that MC was also active in enhancing the yield of virus when the treatment was performed 24, 48 and 72 h after infection with SV40 DNA.

Kinetic studies demonstrated a sharp rise in virus titres 2 to 3 days after infection and treatment with MC (Fig. 2). Thereafter, most of the cells detached from the substratum due to the high toxicity of MC and a progressive decline in virus yield was observed, probably due to virus degradation.

It is interesting that treatment with MC was equally efficient at increasing virus production whether carried out before or after the infection of CHK Cl.10 cells with SV40 DNA (compare Fig. 2a and b). This result was confirmed in several experiments and it was found that such a stimulation occurred when MC was added up to 12 h before infection (data not shown). However, pretreatment of the cells 24 h before infection had no effect on virus production; this negative result was probably due to cell damage at the time of inoculation with virus DNA, as a consequence of the cytotoxic effect of MC.

In order to minimize a possible direct interaction between active MC metabolites and the infecting virus genomes, all the following experiments were done by treating the cells with...
Table 2. Effect of MC on the SV40 burst size and number of virus-producing cells*

<table>
<thead>
<tr>
<th></th>
<th>Maximum virus yield (p.f.u./10^6 cells)</th>
<th>Infectious centres per 10^6 cells</th>
<th>Virus burst size (p.f.u./cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>75</td>
<td>58</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>MC-treated</strong></td>
<td>3550</td>
<td>175</td>
<td>20.4</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>190</td>
<td>36</td>
<td>5.3</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>1750</td>
<td>173</td>
<td>10.1</td>
</tr>
<tr>
<td><strong>MC-treated</strong></td>
<td></td>
<td></td>
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</tr>
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</table>

* MC was added to 24 h-old CHK Cl.10 cell cultures at a final concentration of 2 μg/ml. After 3 h incubation at 37 °C, the cultures were rinsed and further incubated for 2 h in drug-free medium before infection with SV40 DNA. Control cultures were handled similarly but without addition of the drug. Control and MC-treated cell cultures were trypsinized and the cell suspensions used for the determination of virus yields and infectious centres as described in Methods.

Table 3. Effect of MC pretreatment on SV40 T and V antigen synthesis in SV40 DNA-infected CHK Cl.10 cells*

<table>
<thead>
<tr>
<th></th>
<th>% of cells positive for:</th>
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<th>% of cells positive for:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T antigen at hours p.i.</td>
<td>V antigen at hours p.i.</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>24</td>
<td>48</td>
<td>72</td>
</tr>
<tr>
<td><strong>MC-treated</strong></td>
<td>6.7</td>
<td>8.8</td>
<td>7.8</td>
</tr>
</tbody>
</table>

* Samples of the same CHK Cl.10 cell suspensions as those used in experiment 2 (Table 2) were seeded on to coverslips. Coverslip cultures were fixed at the times indicated and stained for SV40 T and V antigens as described in Methods. The numbers were obtained by counting 1000 cells per coverslip. Only definitely positive cells were counted.

The drug before infection: 24 h-old cell cultures were routinely exposed for 3 h to MC at a final concentration of 2 μg/ml followed by a 2 h incubation in drug-free medium before inoculation of virus DNA or virus.

Effect of MC on the SV40 burst size and number of virus-producing cells

To further analyse the nature of the MC-promoted activation of SV40 synthesis in CHK Cl.10 cells infected with SV40 DNA, infectious centre experiments were performed. Table 2 shows that MC treatment increased the number of virus-producing cells by a factor of three to five. However, this increase did not fully account for the enhancement of the total virus yield (10- to 50-fold) and indicated that there was an increase in the virus burst size which could be estimated from the ratio of the final virus yields to the respective number of virus-producing cells.

Effect of MC on SV40 T and V antigen synthesis

As shown in Table 3, pretreatment with MC of CHK Cl.10 cells did not significantly change the proportion of T antigen positive cells up to 3 days following infection with virus DNA, indicating that MC did not stimulate the expression of the early viral functions preceding SV40 DNA replication. However, after exposure to MC, there was a three- to fivefold increase in the proportion of cells synthesizing V antigen, which is in good agreement with the increase in the number of infectious centres (see above).

In MC-treated cultures, the fluorescence labelling for V antigen was often associated with
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Fig. 3. Effect of MC pretreatment on form I SV40 DNA synthesis in CHK Cl.10 cells infected with virus. Twenty-four hour-old cell cultures were (a) mock-treated or (b) treated with MC (2 μg/ml for 3 h), rinsed and incubated in drug-free medium for 2 h before inoculation of SV40 virus. Control and treated cell cultures were then labelled with 3H-thymidine; supercoiled (form I) DNA was isolated and analysed by centrifugation through 5 to 20 % neutral sucrose gradients. The arrow indicates the position of form I SV40 DNA marker (21S) run on a parallel gradient.

multinucleated giant cells. Such cells, containing several V antigen positive nuclei were counted as one individual positive cell in order to avoid any overestimation.

Effect of MC on SV40 DNA synthesis in CHK Cl.10 cells infected with SV40 virus

Although enhanced V antigen synthesis and infectious virus production strongly suggested that MC treatment of CHK Cl.10 cells activated virus DNA replication, this was tested directly by 3H-thymidine incorporation into SV40 supercoiled DNA. Control and MC-treated CHK Cl.10 cell cultures were infected with SV40 virus, labelled with 3H-thymidine, harvested by the selective Hirt procedure 24 h post-infection (31 h after MC addition) and the Hirt supernatant was subjected to CsCl-EtBr density gradient centrifugation as described in Methods. Twice as many counts were recovered in form I (supercoiled) DNA from MC-treated cultures compared with mock-treated cultures. Fractions containing form I DNA were pooled and further analysed through neutral sucrose gradients. Only mitochondrial DNA could be demonstrated in CHK Cl.10 cells infected with SV40 (Fig. 3a). MC treatment stimulated 3H-thymidine incorporation into SV40 supercoiled
Table 4. Incorporation of $^3$H-thymidine, $^3$H-uridine and $^3$H-leucine in MC-treated CHK Cl.10 cells

<table>
<thead>
<tr>
<th>TCA-precipitable radioactivity* (ct/min/10^6 cells × 10^-6)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^3$H-thymidine</td>
<td>$^3$H-uridine</td>
</tr>
<tr>
<td>Control</td>
<td>33·3</td>
<td>69·4</td>
</tr>
<tr>
<td>MC-treated</td>
<td>5·6</td>
<td>56·3</td>
</tr>
</tbody>
</table>

* Each number represents the mean of values from duplicate cultures, pulse-labelled from 22 to 24 h after mock-treatment or MC treatment (2 μg/ml for 3 h).

DNA (21S), but inhibited incorporation into mitochondrial DNA by 50% (Fig. 3b). It should be stressed that in this experiment MC inhibited $^3$H-thymidine incorporation into cellular DNA by more than 80% as measured from TCA-insoluble radioactivity in the Hirt pellets (data not shown).

Effects of MC on cellular DNA, RNA and protein synthesis

Treatment of CHK Cl.10 cells with MC, under optimum conditions for stimulation of SV40 replication, completely inhibited cell multiplication. However, examination under the light microscope revealed a significant enlargement of the cells 24 to 48 h after the treatment, suggesting an unbalanced growth of MC-treated cells. In order to analyse this possibility, the incorporation of labelled precursors into DNA, RNA and proteins was measured in CHK Cl.10 cells treated or untreated with MC. Table 4 shows that although MC drastically inhibited $^3$H-thymidine incorporation, compared with exponentially growing control cells, $^3$H-uridine was incorporated at an almost normal rate, and $^3$H-leucine incorporation appeared to be stimulated in cells exposed to MC.

Effect of caffeine on SV40 replication in CHK Cl.10 cells treated with MC and infected with SV40 DNA

As shown in the accompanying paper (Morris et al. 1977), caffeine significantly inhibited virus induction by MC in SV40-transformed Chinese hamster kidney cells. Thus, it was of interest to test its effect in the present system. SV40 virus production was inhibited by 80 to 90% when CHK Cl.10 cells pretreated with MC and infected with virus DNA were incubated continuously in the presence of 1 mM-caffeine (Fig. 4b). However, a similar inhibition of virus synthesis was also observed when untreated cultures were maintained in mM-caffeine after SV40 DNA infection (Fig. 4a).

When tested by plating efficiency, caffeine exhibited a synergistic effect on the cell killing induced by MC (Fig. 5), although it had no significant effect on the number of colonies formed by CHK Cl.10 cells not treated with MC. As with SV40-transformed cells (Morris et al. 1977), caffeine decreased the incorporation of $^3$H-leucine into proteins in CHK Cl.10 cells treated with MC, but not in mock-treated cells (data not shown).

Effect of u.v.-irradiation on SV40 production in CHK Cl.10 cells infected with virus DNA

Having demonstrated the capacity of MC to activate SV40 replication in infected CHK Cl.10 cells, it was of interest to analyse the effect of a physical agent such as u.v. light which
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Fig. 4. Effect of caffeine on infectious virus production in CHK Cl.10 cells not treated (a) or treated with MC (b) before infection with SV40 DNA. Twenty-four hour-old cell cultures were treated with MC and infected with SV40 DNA as quoted in the legend of Fig. 2(b). Control and MC-treated cultures were then incubated either in the absence (○—○), or in the presence (●—●) of 1 mM-caffeine and further processed as described in Methods for the determination of virus yields. Note the difference in the scale between (a) and (b).

is also an efficient virus inducer in SV40-transformed cells (Kaplan et al. 1975; Morris et al. 1977).

Fig. 6 shows that u.v.-irradiation of CHK Cl.10 cells 24 h after infection with SV40 DNA enhanced the yield of virus detected 3 days after treatment compared with control cultures. This enhancement was observed reproducibly when u.v.-irradiation was performed at various times (0 to 24 h) after infection. However, the degree of stimulation of SV40 production did not exceed 10-fold in 4 independent experiments (not shown), which is significantly less than that usually observed upon MC treatment; this difference is in agreement with the relative inducing efficiency of these two agents on SV40-transformed Chinese hamster cells (Morris et al. 1977).

In two independent experiments, CHK Cl.10 cells were irradiated 6 or 2 h before inoculation of virus DNA. No clear stimulation of virus growth was observed at u.v. doses significantly efficient at increasing the yield of SV40 in parallel cultures irradiated 2 h or 24 h after infection. Since MC was equally active upon pretreatment as well as post-treatment, this result would suggest that these two agents stimulate virus growth by different mechanisms. However, it could also be explained by the fact that MC is a chemical drug
Fig. 5. Effect of caffeine on the plating efficiency of CHK Cl.10 cells treated with various doses of MC. The cells were seeded in Petri dishes (250 cells per dish) and 24 h later, the cultures were either mock-treated or treated with MC for 3 h. Cell colonies were grown either in the absence (○—○) or in the presence (●—●) of 1 mM-caffeine. Plating efficiency is expressed as percent of colonies formed in the absence of MC treatment. Each point represents the mean of values from triplicate dishes. In this experiment, caffeine reduced the plating efficiency of untreated cells by 5%.

Fig. 6. Effect of u.v.-irradiation on infectious virus production in CHK Cl.10 cells infected with SV40 DNA. Twenty-four hours after infection, cell cultures were irradiated at the various u.v. doses indicated and virus yields were determined from cultures harvested 3 days later as described in Methods.

which may be retained in the cells for some time with a consequent less transient effect than u.v.-irradiation.

DISCUSSION

A possible explanation for the induction of oncogenic DNA viruses by treatment of transformed cells with chemical or physical agents is that damage to cellular DNA and activation of DNA repair mechanisms trigger the excision of the virus genome from its integrated state (Rakusanova et al. 1976). The free virus DNA molecules thus rescued would be available for production of infectious virus, depending on the intrinsic level of permissiveness of the host cell.

In the present work, we have shown that MC, one of the most powerful inducers of polyoma and SV40 in cells transformed by these viruses (Fogel, 1972; Kaplan et al. 1975; Morris et al. 1977) is also capable of stimulating virus multiplication in Chinese hamster kidney cells infected with SV40. A 10- to 100-fold increase in infectious virus production was reproducibly observed upon MC treatment of CHK Cl.10 cells infected with SV40 DNA.
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This stimulation occurred when the cells were exposed to MC as late as 72 h after inoculation with virus DNA, indicating that the infecting virus genomes kept their integrity at relatively late times after entrance into the cells. Results published by Hirai, Lehman & Defendi (1971) indicate that integration of SV40 DNA into the host cell genome occurs within 30 h following infection of Chinese hamster embryo cells with SV40 virus. Thus, one has to consider the possibility that, as in the case of SV40-transformed cells, MC could promote the excision of the SV40 genome from the DNA of infected cells before initiation of virus DNA replication takes place.

However, this is unlikely to be the only reason for the enhancing effect of MC on SV40 replication because treatment of CHK Cl.10 cells with MC, before infection with virus DNA, was as efficient as post-treatment. Moreover, this suggests that the stimulation of SV40 production is not the consequence of a specific chemical interaction between the drug and the virus genome or a virus gene product, but rather indicates that the factor(s) involved in the enhancement of virus growth are of cellular origin. Relevant to this possibility is the observation that MC treatment, although markedly altering 3H-thymidine incorporation into cellular DNA and totally arresting cell division, did not impede 3H-uridine incorporation into RNA and even stimulated 3H-leucine incorporation into proteins compared with mock-treated cultures. Assuming that the pool of precursors of the cellular macromolecules is not drastically altered, such an unbalanced growth of MC-treated cells allows us to speculate on the mechanisms of the MC-enhancing effect on SV40 replication in this cell system. It is possible that larger amounts of cellular enzymes implicated in DNA metabolism are synthesized in MC-treated cells and, as they cannot be used by the cells due to the block of the cellular DNA machinery, they are fully available for the replication of the infecting virus DNA. This hypothesis is further supported by the fact that SV40-infected CHK Cl.10 cells, pretreated with MC, were actively synthesizing SV40 DNA form 1 24 h after infection, whilst no incorporation of 3H-thymidine into virus DNA was detected in mock-treated cultures. Clearly, there is an inverse relationship between cellular and virus DNA synthesis when MC-treated and control cells are compared.

The simultaneous increase in the number of V antigen-synthesizing and virus-producing cells and in the burst per cell indicates that MC treatment enhances the mean level of permissiveness of the cell population, probably by stimulating the synthesis of a specific factor(s) required for SV40 replication. Thus, CHK Cl.10 cells already showing some permissiveness would accumulate a greater amount of this factor(s), while other cells would start to produce it (them) above the threshold necessary for the formation of detectable infectious virus.

Continuous incubation in the presence of caffeine significantly inhibited infectious virus production following SV40 DNA infection of MC-treated as well as control CHK Cl.10 cells. This suggests that caffeine interferes with some step of the virus growth cycle in Chinese hamster cells. We have shown in the preceding work (Morris et al. 1977) that caffeine also inhibits spontaneous virus production and MC induction in SV40-transformed cells. The results obtained in the present work using infected cells, suggest that caffeine may not impede excision of the virus genome from its integrated state, but rather interferes with the replication of the SV40 DNA. Possible connections between this effect and the synergistic effect of caffeine on the MC-induced cell killing, as a consequence of post-replication repair inhibition, are discussed in the accompanying paper (Morris et al. 1977).

The proportion of SV40 DNA-infected cells synthesizing T antigen was not significantly changed by pretreatment with MC. This observation should be compared with the results obtained using 5-iodo-2'-deoxyuridine (IdUrd) which enhances the growth of adenovirus
type 7 and SV40 upon pretreatment of cells semi-permissive for these viruses (Staal & Rowe, 1975; Suárez et al. 1976). In these two cases, it had been found that IdUrd pretreatment stimulated the percentage of T antigen-synthesizing cells to about the same extent as it enhanced virus production, suggesting that this drug acted at a step preceding the expression of early viral functions. It would appear that the MC enhancement of cell permissiveness occurs at a later step, intermediate between T antigen synthesis and SV40 DNA replication, suggesting a different mode of action for the two agents. Possibly, IdUrd could stimulate the synthesis of a virus-coded initiator of SV40 DNA replication [evidence is accumulating that SV40 T antigen is the carrier of such a regulatory function (Graessmann, Graessmann & Mueller, 1976)], whereas MC would trigger the production of a cellular factor(s) as proposed above.

The data of Rakusanova et al. (1976), combined with the results reported in this work, suggest that SV40 induction by MC in transformed cells is the consequence of two consecutive events: (1) excision of the virus genome from the host cell DNA, (2) enhancement of the cell permissiveness to SV40 leading to a more efficient replication of the rescued virus genome and to the synthesis of larger amounts of infectious virus. However, the fact that IdUrd appears to stimulate virus production by a different mechanism compared to MC and the observation that u.v.-irradiation, unlike MC, was not effective before infection, raises the question of whether SV40 induction proceeds through different pathways depending on the inducing agent employed. Further investigation is required in order to compare the effects of various inducing agents such as u.v.-, γ- and X-irradiation, N-methyl-N'-nitro-N-nitrosoguanidine, cycloheximide etc., on both provirus excision and virus replication. This approach should allow definition of the restrictions imposed by semi-permissive or non-permissive cells to virus growth and, thus, to define better the concept of cell permissiveness to SV40.

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REFERENCES


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