JOURNAL OF VIROLOGY, June 1976, p. 1063-1070

Vol. 18, No. 3
Printed in U.S.A.

Replication of Human Cytomegalovirus DNA: Lack of Dependence on Cell DNA Synthesis

JEAN M. DEMARCHI AND ALBERT S. KAPLAN*

Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received for publication 29 December 1975

Human cytomegalovirus (CMV) DNA synthesis was studied in 5-fluorouracil (FU)-treated and untreated human embryonic lung cells, which differ greatly with respect to the number of cells in the culture synthesizing cellular DNA. CMV DNA synthesis proceeded at the same rate in FU-treated and in untreated cells. CMV infection also reversed the inhibitory effects of FU and activated cellular DNA synthesis in some of the cells in the FU-treated culture. Autoradiographic studies showed that more than 20% of the cells in the infected FU-treated culture synthesized viral DNA when less than 1% had synthesized cellular DNA, indicating that the synthesis of viral macromolecules proceeds in cells that do not synthesize cellular DNA from the time of infection, and that viral DNA synthesis proceeds independently of the host cell DNA synthesis. Combined autoradiographic and immunofluorescence studies of both the FU-treated and untreated infected cells showed that, whereas 20% of the cells in the cultures synthesize viral DNA and viral antigens, only about 3 to 6% of those cells that synthesize cellular DNA also synthesize viral antigen. Thus, productive infection was delayed or inhibited in those cells that were stimulated by CMV infection to synthesize cellular DNA.

Human cytomegalovirus (CMV) can infect a variety of cell types, but productive infection is limited to human fibroblasts (22). Both productive and nonproductive CMV infection can result in a stimulation of cellular DNA synthesis (7, 16, 17; J. DeMarchi and A. Kaplan, unpublished observations), and it has been proposed that a correlation exists between the synthesis of cellular DNA and that of CMV DNA (S. C. St. Jeor, R. Hutt, and F. Rapp, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, S153, p. 239).

The ability to stimulate cellular DNA after infection has been documented for oncogenic DNA viruses, in particular simian virus 40 and polyoma virus (5, 8). With these viruses, replication of viral DNA is related to specific events of the host cell cycle and occurs only after the cells have entered S phase (2, 3, 14, 19). A similar dependence on cellular functions would be unusual for CMV, since it contains a large genome ($\sim 10^8$ daltons) and can, in principle, code for all the proteins necessary for replication. However, another herpesvirus, equine abortion virus, which has a genome of approximately the same size, is dependent on the S phase when grown in certain cell lines (13).

The experiments described in this paper deal with the synthesis of CMV DNA in cells that have been treated with 5-fluorouracil (FU) and thymidine (TdR), a procedure that suppresses their ability to synthesize cellular DNA (12) but does not affect viral replication. The aim of these experiments was to determine whether CMV stimulates cellular DNA synthesis in FU-treated cells and whether CMV DNA synthesis occurs preferentially in the cells in which cellular DNA synthesis has been activated.

MATERIALS AND METHODS

Cell culture and virus. Primary cultures of human embryonic lung (HEL) cells were purchased from Flow Laboratories and, after reaching confluency, were passaged further according to Hayflick and Moorhead (9). The cells were grown in 90-mm glass petri dishes and maintained at 37 C in a humidified atmosphere of 5% CO_2. Growth medium consisted of Eagle minimal essential medium (MEM) containing 0.2% $NaHCO_3$, 100 U of penicillin, 100 μg of streptomycin, and 50 μg of chlortetracycline per ml, with 5% fetal bovine serum. For maintenance of cultures and for the experiments, the serum in the medium was reduced to 2%.

Stock cultures of the Davis strain of human CMV (20) were grown in HEL cells and stored in 10% glycerol at −70 C as infected cell suspensions. For inoculation onto HEL monolayers, the cell suspensions were first disrupted in a Raytheon sonic oscillator (1 min at maximum setting) and clarified by centrifugation. The supernatant was diluted appropriately to give the desired multiplicity of infection and was used as inoculum. After 1 h of adsorption at 37 C, the inoculum was removed and replaced with

medium. In all experiments, the multiplicity of infection used was approximately 0.1 to 0.2 PFU/cell. With the virus stocks used, these conditions of infection resulted in the best stimulation of cellular DNA synthesis we have observed.

Assay for infectious virus. The procedure of Wentworth and French (21) was used, except that adsorption was for 1 h at 37 C and the plates were overlayed with MEM containing 1% agarose (Sea-Kem). Plaques were counted 2 weeks later.

Chemicals and radiochemicals. Cesium chloride, optical grade, was purchased from the Harshaw Chemical Company; FU and TdR were purchased from Calbiochem; 5-iodo-2'-deoxyuridine (IUdR) was obtained from Schwarz Bioresearch. [*Methyl*-^{3}H]TdR (specific activity, 55 Ci/mmol), [2-^{14}C]TdR (specific activity, 57 mCi/mmol), and [5-^{3}H]deoxycytidine ([^{3}H]CdR; specific activity, 25 Ci/mmol) were purchased from Schwarz/Mann.

FU treatment of cell cultures. Cultures were incubated with FU (10 μg/ml) and TdR (1 μg/ml) (12) for a minimum of 16 h before inoculation. Control cultures were treated with TdR (1 μg/ml). FU and TdR were maintained in the treated cultures, and TdR was maintained in the control cultures, during the course of experiments. TdR was omitted when radiolabeled TdR was added.

Analysis of radiolabeled nucleotide incorporation into DNA. Cells were suspended in RSB (0.01 M Tris-hydrochloride [pH 7.4], 0.01 M KCl, and 0.0015 M $MgCl_2$) and lysed by the addition of sodium dodecyl sulfate (SDS) (final concentration, 1%). Portions were precipitated with 7% trichloroacetic acid at 0 C. The precipitates were collected on 0.45-μm pore size membrane filters (Millipore Corp.) and washed with cold 7% trichloroacetic acid. After drying, the filters were counted in a Packard liquid scintillation spectrometer. The amount of label incorporated into viral and cellular DNA was determined after isopycnic banding, as described previously (11). Alkali stability of labeled DNA was determined by treatment with 1 N NaOH for 16 h at 37 C, according to the method of Schmidt and Thannhauser (15). The samples were then neutralized and precipitated as described above.

Antisera. Human convalescent serum containing antibodies to CMV, human CMV-negative serum, and fluorescein isothiocyanate-conjugated rabbit antiserum to human globulin (immunoglobulin G) were supplied courtesy of Thomas D. Flanagan, Erie County Virology Laboratory, Buffalo, N.Y. The CMV-positive serum was reactive with early CMV antigen (as determined with cytosine arabinoside-treated, CMV-infected cells) at a dilution of 1:320. It reacted with late antigen (CMV-infected cells at 5 days postinfection) at a dilution of 1:5,120. It did not react with uninfected cells at a dilution of 1:10. In all experiments described in this paper, the serum was used at a dilution of 1:20.

Autoradiography and immunofluorescence. HEL cells grown on cover slips in 90-mm petri dishes were either infected or mock-infected and labeled with [^{3}H]TdR (1 μCi/ml). At the end of the labeling period, the cover slips were rinsed in phosphate-buffered saline (PBS) (4), fixed with cold acetone (3 min), air-dried, and mounted onto glass slides (cell side up). The slides were coated with Ilford Nuclear Research emulsion (type L-4) diluted with an equal amount of distilled water and stored in a desiccator under partial vacuum at 4 C. After an appropriate exposure time, the autoradiograms were developed with Kodak D-19 developer, stained with 0.03% aqueous methylene blue, and viewed by light microscopy.

When immunofluorescence was combined with autoradiography, the fixed cover slip cultures were first incubated (30 min, 37 C) with CMV antiserum diluted in PBS, then rinsed in PBS (10 min, room temperature), and incubated in the same manner with conjugated antiserum. After a final PBS rinse (30 min), the cover slips were immersed in water (2 min) and then in 95% ethanol (20 min) to fix the conjugate (18). The cover slips were then coated with emulsion. After an appropriate exposure time they were developed and mounted (cell side down) with a solution of 90% glycerol, 10% PBS. The preparations were viewed with a Zeiss microscope equipped with a dark field condenser, UV light source, and a 470-nm barrier filter. Fluorescence was observed with a BG-12 exciter filter, and autoradiographic grains were viewed with a combination of blue glass and NG-9 (neutral gray) filters.

RESULTS

Incorporation of [^{3}H]TdR into CMV-infected and mock-infected FU-treated and untreated cultures. Figure 1 shows the amount of [^{3}H]TdR that is incorporated into the DNA of infected and mock-infected untreated and FU-treated HEL cells. In untreated cells, a stimulation of incorporation of [^{3}H]TdR into DNA was detectable both at early times (2 days) and late times (4 and 5 days) postinfection (Fig. 1A). The stimulation of [^{3}H]TdR incorporation into DNA by CMV was much more marked in cells in which cellular DNA synthesis had been suppressed due to pretreatment with FU (Fig. 1B). By 4 and 5 days postinfection, FU-treated and untreated cells incorporated approximately the same amount of [^{3}H]TdR.

To determine the type of DNA (viral or cellular) synthesized by FU-treated and untreated cells, the DNA from the infected cultures that had been incubated with [^{3}H]TdR between 24 to 48 h and 72 to 96 h after infection was centrifuged in CsCl density gradients. (Human CMV DNA has a buoyant density of 1.716 g/cm^3 in CsCl [10] and can readily be separated by this method from cellular DNA, which has a density of 1.699 g/cm^3.) Figure 2 shows that most of the [^{3}H]TdR taken up by the cells from 24 to 48 h after infection in both FU-treated and untreated cells was incorporated into cellular DNA. Between 72 and 96 h after infection, however, [^{3}H]TdR was incorporated in large part into viral DNA.

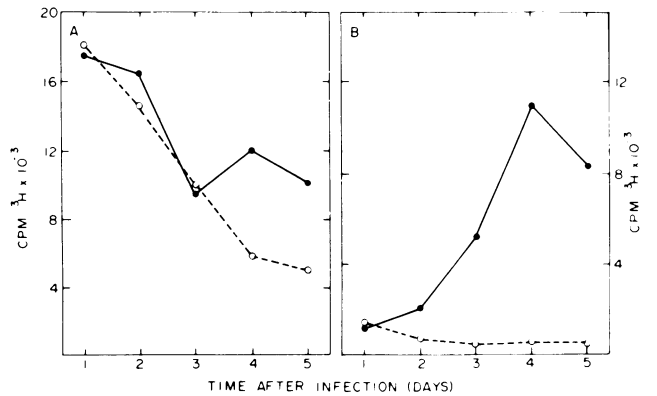

FIG. 1. *Incorporation of [³H]TdR into DNA of FU-treated and untreated cells. FU-treated and untreated cultures of HEL cells were either mock-infected or infected with CMV (approximately 0.1 PFU/cell). The cultures were incubated with [³H]TdR (10 μCi/ml) for sequential 24-h periods postinfection. At the end of each labeling period, the culture fluids were removed and the cells were harvested in RSB plus 1% SDS, as described in Materials and Methods. Each point in the figure represents the amount of radioactivity incorporated by the cells incubated with label for 24 h prior to the indicated day of harvest. Symbols: (●) CMV-infected cultures; (○) mock-infected cultures. (A) Untreated; (B) FU-treated.*

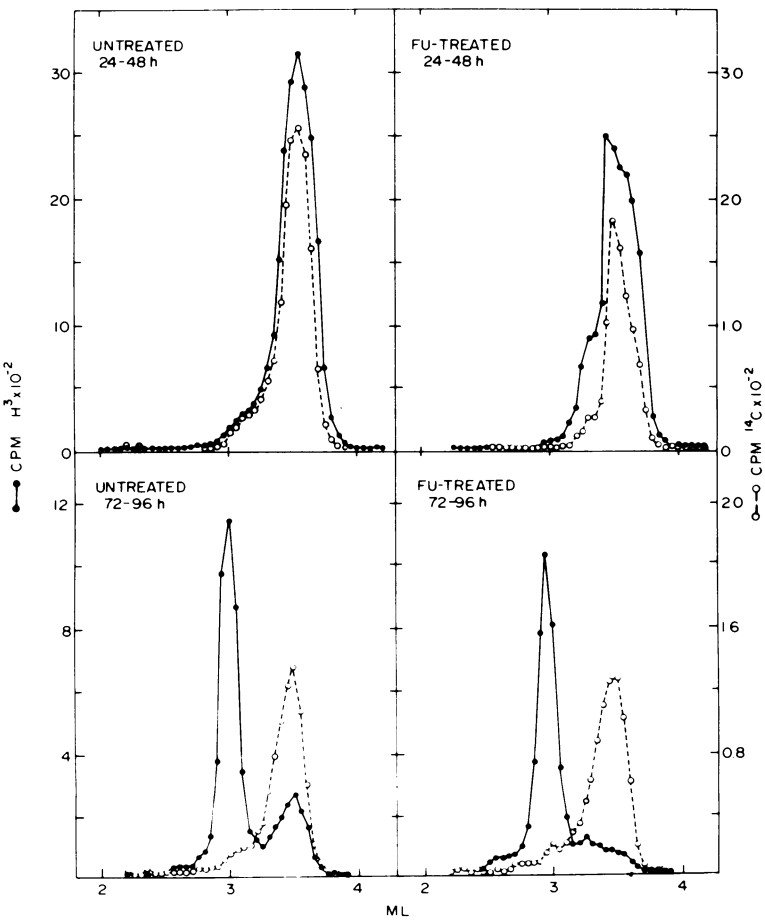

FIG. 2. *CsCl density gradient analysis of DNA synthesized after CMV infection in FU-treated and untreated cultures. Portions of CMV-infected, FU-treated and untreated cultures, harvested at 2 and 4 days after infection in the experiment presented in Fig. 1, were analyzed in CsCl buoyant density gradients. The samples analyzed in the gradients were not necessarily equal in volume. ¹⁴C-labeled cellular DNA was added as a marker.*

The amount of [^{3}H]TdR incorporated into viral and cellular DNA in FU-treated and untreated cells at various times after infection is summarized in Table 1. The following points emerge from these experiments. (i) Under the conditions used in these experiments, only a small amount of CMV DNA is synthesized before 48 h postinfection and is detectable only in FU-treated infected cultures. Labeled viral DNA was not detected at that time in untreated, infected cells, probably because of the high background of the cellular DNA synthesized. (ii) The amount of [^{3}H]TdR incorporated into viral DNA in FU-treated and untreated, infected cultures was approximately the same throughout infection. (iii) Infection with CMV partially reversed the FU-induced inhibition of TdR incorporation into cellular DNA. The amount of radioactivity incorporated into cellular DNA at 2, 3, and 4 days after inoculation was approximately threefold greater than the amount incorporated into the DNA of mock-infected cultures. (iv) Infection of untreated cells stimulated incorporation of TdR into cellular DNA at early stages of infection (24 to 48 h). This stimulation, though slight, is significant and was observed repeatedly.

Activation of cellular DNA synthesis by CMV infection in FU-treated cells. Table 1 shows that incorporation of [^{3}H]TdR into cellular DNA is stimulated after infection of FU-treated cells. That these results are reproducible is shown by the data in Table 2. In this experiment, infected and mock-infected FU-treated cultures were pulse-labeled at times when the background of label incorporated into cellular DNA of uninfected cells would be as low as possible, and when appreciable amounts of viral DNA would not yet be synthesized. The results (Table 2) show that, between 16 to 40 and 16 to 64 h after inoculation, nearly a threefold increase in the amount of labeled precursor incorporated into DNA in FU-treated cells could be detected after infection. An experiment similar to the one summarized in Table 2 showed that incorporation of [^{3}H]CdR into the DNA of infected FU-treated cells was also three times greater than that into the DNA of uninfected, FU-treated cells.

TABLE 1. *[^{3}H]TdR incorporation into viral and cellular DNA in CMV-infected cells*[a]

Labeling period (h)	Incorporation (counts/min)		
	Infected		Mock-infected
	Viral	Cellular	
FU-treated cells			
0-24	0	1,359[b]	1,413
24-48	114	1,962	691
48-72	3,501	1,666	555
72-96	9,215	1,768	635
Untreated cells			
0-24	0	17,467	17,983
24-48	0	16,819	14,629
48-72	2,972	6,436	10,036
72-96	8,908	3,162	5,924

[a] The experiment was performed as described for FU-treated and untreated cultures in the legend to Fig. 1. The amount of radioactivity incorporated into viral and cellular DNA, isolated by isopycnic centrifugation, was determined as described in Materials and Methods.

[b] Average of duplicate samples.

TABLE 2. *Incorporation of [^{3}H]TdR into cellular DNA of CMV-infected and mock-infected HEL cells treated with FU*[a]

Expt no.	Labeling period (h)	Incorporation (counts/min)		Infected/mock-infected
		Infected	Mock-infected	
1	16-40	1,070[b]	436	2.5
	16-64	1,837	619	3.0
2	16-40	957	338	2.8
	16-64	2,065	800	2.6

[a] FU-treated cultures were prepared as described in Materials and Methods, inoculated with CMV (approximately 0.1 PFU/cell), and incubated further for 16 h in the presence of FU. The cultures were then incubated with [^{3}H]TdR (10 μCi/ml) and harvested 24 and 48 h later. The DNA was banded in CsCl density gradients, viral DNA was separated from cellular DNA, and the amount of radioactivity associated with each DNA was determined. In all cases, more than 90% of the radioactivity was associated with cellular DNA.

[b] Average of duplicate samples.

That the increase in TdR incorporation into cellular DNA of infected cells is related to CMV infection was shown by two experiments. (i) Uninfected cell extracts did not stimulate cellular DNA synthesis. (ii) Antisera against CMV prevented the virus inoculum from stimulating cellular DNA synthesis (Table 3).

The increased incorporation of labeled deoxyribonucleosides into cellular DNA may reflect (i) enhanced utilization of TdR or CdR as a result of increased kinase activity, (ii) increased repair of cellular DNA, or (iii) enhanced synthesis of cellular DNA. The first possibility can be eliminated by the data presented below (Table 4), which show a threefold increase, after CMV infection, in the number of cells in FU-treated cultures in which cellular DNA synthesis occurred. To distinguish between the two last possibilities, the cells were labeled with [^{3}H]CdR in medium containing IUdR. If the increase in incorporation into cellular DNA in FU-treated infected cells were due to repair of preexisting cellular DNA, most of the label

TABLE 3. *Failure of serum-neutralized CMV to stimulate cellular DNA synthesis*[a]

HEL cells	Activity (counts/min) with antiserum:	
	CMV-negative	CMV-positive
Mock-infected	1,101[b]	992
CMV-infected	4,121	1,052

[a] Human CMV-positive and CMV-negative sera were incubated at a 1:10 dilution with CMV or with MEM (30 min, 37 C) prior to inoculation of FU-treated HEL cells. Between 24 and 48 h after inoculation, the cells were incubated with medium containing [^{3}H]TdR (10 μCi/ml). The cells were then lysed with SDS (1%), the DNA was banded in CsCl density gradients, and the radioactivity incorporated into cellular DNA was determined.

[b] Average of duplicate samples.

would be associated with a peak cosedimenting with marker ^{14}C-labeled cellular DNA in a CsCl density gradient. If, on the other hand, the increased incorporation is due to a stimulation of cellular DNA synthesis, the label should be associated with DNA banding at a position of higher buoyant density as a result of substitution of IUdR for TdR in one of the strands.

Figure 3 shows that practically all the label incorporated into DNA of both infected and mock-infected cells was found within the heavy peak of IUdR-substituted DNA. Figure 3C illustrates the results obtained when IUdR was omitted and serves as a control to show that, under the labeling conditions used, incorporation of CdR by the infected cells was mainly into cellular DNA. Thus, infection with CMV in FU-treated cells stimulates the synthesis of cellular DNA. These results confirm the findings of St. Jeor et al. (17), who showed that stimulation of cellular DNA synthesis occurs after CMV infection of permissive cultures in which DNA synthesis was suppressed by pretreatment with iododeoxyuridine.

Viral DNA synthesis is independent of cellular DNA synthesis. The experiments described below were undertaken to determine whether only those infected cells which had entered S phase after infection synthesize viral DNA. If this were the case, the number of cells in the infected cultures which synthesize viral DNA should not exceed the number of cells which synthesize cellular DNA. The cells that have synthesized cellular DNA and those that have synthesized viral DNA can be easily differentiated from each other. A cell that has synthesized cellular DNA during a 24-h labeling period incorporates much more [^{3}H]TdR than does a cell that has synthesized viral DNA. By autoradiography, one can therefore detect, after a short exposure time, only those cells in the culture which have synthesized cellular DNA. The nuclei of these cells are covered evenly with grains and look identical to the nuclei of cells that have synthesized DNA in uninfected cultures labeled under identical conditions. The cells that have synthesized viral DNA can be detected by autoradiography only after longer exposure times. These cells contain inclusion-like bodies covered sparsely with grains (after a time of exposure that completely blackens the emulsion over cells that have synthesized cellular DNA). These cells also contain viral antigens. The difference between the appearance of the cells that synthesized cellular DNA and those that synthesized viral DNA is illustrated in Fig. 4A.

Using the criteria described above, we detemined the number of cells synthesizing cellular or viral DNA at various times after infection. The results are summarized in Table 4. The following conclusions can be drawn from this experiment. (i) The number of cells synthesizing cellular DNA is increased after infection of both FU-treated and untreated cells. During four successive 24-h labeling periods, an average of 0.1% of the nuclei of uninfected FU-treated cells synthesized cellular DNA. This increased to an average of 0.5% in the infected cells. In untreated cells, approximately 26% of the infected cells, as opposed to 20% of the mock-infected cells, synthesized cellular DNA between 24 and 48 h postinfection. This increase, though small, is significant and has

TABLE 4. *Percentage of nuclei that incorporated [^{3}H]TdR into viral and cellular DNA in FU-treated and untreated cells*[a]

Labeling period (h)	% Nuclei incorporating [^{3}H]TdR in:		
	Infected		Mock-infected
	Viral	Cellular	
FU-treated cells			
0-24	0	0.2[b]	0.1
24-48	5.3	0.7	0.2
48-72	15.0	0.6	0.1
72-96	20.2	0.5	0.1
Untreated cells			
24-48	1.5	25.9	20.1
72-96	20.3	9.9	14.8

[a] FU-treated and untreated cells (see Materials and Methods) grown on cover slips were infected with CMV (approximately 0.2 PFU/cell). At various times after infection, the cultures were incubated in the presence of [^{3}H]TdR (1 μCi/ml). At the end of each labeling period, the cover slips were fixed and prepared for autoradiography, as described in Materials and Methods.

[b] Percentage values were calculated after counting at least 1,500 nuclei.

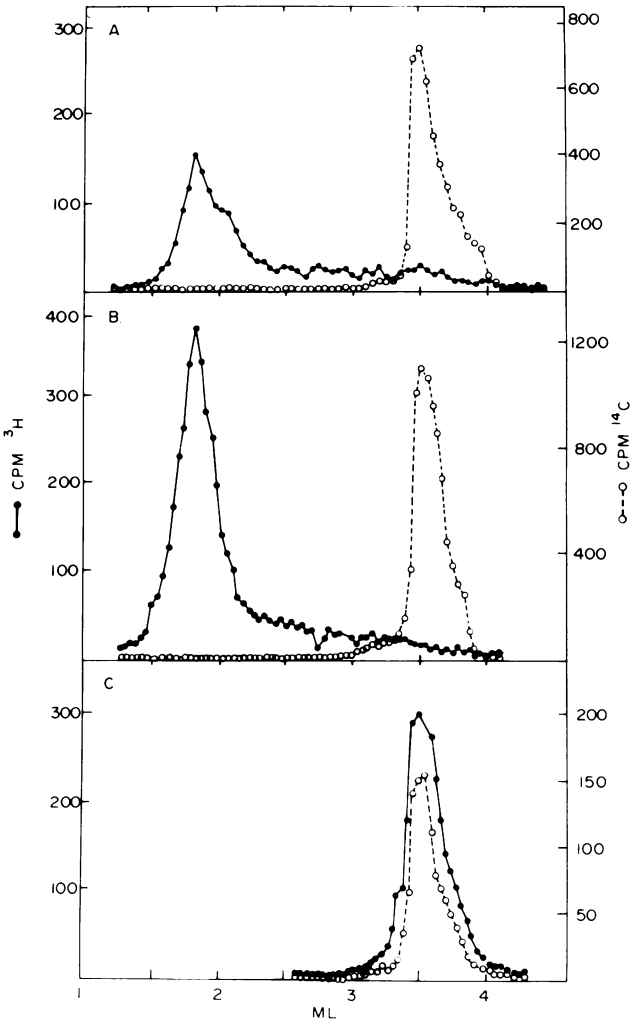

FIG. 3. *Cellular DNA synthesis in CMV-infected FU-treated cultures. FU-treated cultures were either mock-infected or infected with CMV (approximately 0.1 PFU/cell). Between 16 and 64 h after infection, the cultures were incubated with medium containing [^{3}H]CdR (10 μCi/ml) and IUdR (100 μg/ml). IUdR was omitted from a third culture of FU-treated infected cells. At 64 h, the cultures were harvested in RSB plus 1% SDS. Equal volumes from each of the cultures were analyzed in CsCl density gradients, with ^{14}C-labeled cellular DNA of normal density added as marker. (A) Mock-infected cells plus IUdR; (B) CMV-infected cells plus IUdR; (C) CMV-infected cells, no IUdR.*

been observed repeatedly. (ii) The number of cells synthesizing viral DNA is similar in FU-treated and untreated cultures, confirming the results obtained by CsCl density gradient analysis (see Table 1). The percentage of nuclei bearing grains associated with intranuclear inclusions in FU-treated cultures increased during the course of the experiment (Table 4). Whereas a small number of cells in CMV-infected, FU-treated cultures synthesized cellular DNA after infection, many more cells were able to synthesize viral DNA. Furthermore, the number of cells synthesizing viral DNA was the same in FU-treated and untreated infected cultures, despite a great difference in the number of cells synthesizing cellular DNA. We conclude, therefore, that viral DNA synthesis does not require prior synthesis of cellular DNA.

Whereas cellular DNA synthesis does not seem to be required for viral DNA synthesis, it seemed possible that viral growth might occur preferentially in those cells that synthesize cellular DNA. To ascertain whether this is the case, we used a technique described by Tennant et al. (18), by which the same cell can be exam-

ined by both autoradiography and immunofluorescence. By means of this technique, as illustrated in Fig. 4, we found that viral antigen synthesis is less likely to occur in the cells in which cellular DNA was synthesized after infection. Table 5 summarizes the results of this experiment. In the FU-treated infected monolayer, 0.7% of the cells incorporated [^{3}H]TdR into cellular DNA, and 23% synthesized viral antigen. However, only 0.02% of the cells (approximately 3% of those that had synthesized cellular DNA) synthesized both viral antigen and cellular DNA, indicating that cells synthesizing cellular DNA were less, rather than more, likely to synthesize viral antigen. Similarly, in untreated cultures, whereas 23% of the cells synthesized cellular DNA and 24% synthesized viral antigen, 1.4% of the cells (about 6% of those that had synthesized cellular DNA) also synthesized viral antigen. These findings support the conclusion, based on the experiments described above, that viral DNA synthesis is not dependent on the S phase of the cell growth cycle.

DISCUSSION

CMV infection results in an activation of cellular DNA synthesis in some of the cells in cultures in which DNA synthesis has been arrested by treatment with FU and TdR. This is reflected by a threefold increase in the amount of [^{3}H]TdR incorporated into cellular DNA, as determined by equilibrium centrifugation in CsCl, and a similar increase in the number of cells synthesizing cellular DNA after infection, as determined by autoradiography. A stimulation of cellular DNA synthesis after infection of untreated cells also was detected. This stimulation, though slight, was significant and was represented by an increase in the amount of TdR incorporated into cellular DNA, as well as by an increase in the number of cells synthesizing cellular DNA. Activation of cellular DNA synthesis was detectable in untreated cells only during the first 48 h postinfection.

Although cellular DNA synthesis is activated after infection, this activation is apparently not essential for viral DNA or viral antigen synthesis. Less than 1% of the cells in FU-treated cultures synthesized cellular DNA during the first 48 h after inoculation. However, a much greater number of cells synthesized viral DNA and viral antigens during this period of time (Tables 4 and 5). Furthermore, viral antigen synthesis appears to be delayed or inhibited in those cells that do synthesize cellular DNA after infection. In both FU-treated and untreated cultures, 23% of the total number of cells in the culture, but only 3 to 6% of those cells that had synthesized cellular DNA from

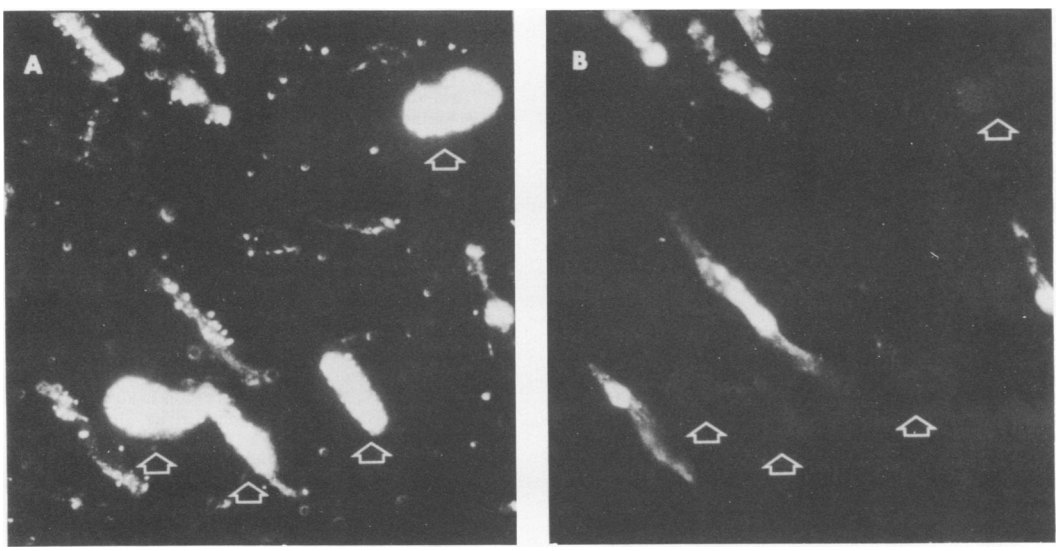

FIG. 4. *Absence of viral antigen production in cells which synthesize cellular DNA. FU-treated cultures of HEL cells on cover slips were labeled with [^{3}H]TdR (1 μCi/ml) after CMV infection. At 48 h, the cover slips were fixed in acetone, stained by indirect immunofluorescence, and coated with photographic emulsion. (A) Preparations viewed with neutral gray and blue glass filters. Autoradiographic grains are associated mainly with cellular chromatin (arrows). (B) Same cells viewed with a BG-12 exciter filter, which allows the visualization of the fluorescence. Note that the cells which had incorporated label into cellular DNA did not fluoresce.*

TABLE 5. *Percentage of nuclei that incorporated [^{3}H]TdR into cellular DNA and exhibited CMV-specific fluorescence after infection*[a]

Prepn	% Nuclei incorporating [^{3}H]TdR in:			
	Mock-infected	CMV-infected		
	Cellular DNA	Cellular DNA	Viral antigens	Cellular DNA and viral antigens
FU-treated	0.10[b]	0.71	23.42	0.02
Untreated	20.60	23.48	24.48	1.38

[a] FU-treated and untreated cells grown on cover slips were infected with CMV (approximately 0.2 PFU/cell) and incubated for 48 h with [^{3}H]TdR (1 μCi/ml). After acetone fixation, the cover slips were stained by indirect immunofluorescence and coated with photographic emulsion, and the percentage of cells in the culture which had synthesized cellular DNA, had synthesized viral antigens, or had synthesized both was determined.

[b] Percentage values were calculated after counting at least 7,000 nuclei.

the time of infection, synthesized viral antigens. Since the antiserum used in these experiments detects both early- and late-type antigens (see Materials and Methods), it is clear that most viral functions were less likely to be expressed in those cells that had synthesized cellular DNA after infection than in cells in which stimulation of cellular DNA synthesis was detected. Since the induction of cellular DNA synthesis is a direct result of infection with the virus (see Table 3), the interesting possibility arises that only a few specific viral functions are expressed in the cells that are stimulated to synthesize cellular DNA. This finding may have some bearing on the transforming potential of CMV (1). Therefore, the elucidation of the conditions that govern the activation of cellular DNA synthesis in certain cells and those that allow for the synthesis of viral antigens in others is of considerable interest.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-10947 from the National Institute of Allergy and Infectious Diseases. J. M. DeMarchi was supported by a fellowship from the National Institute of Allergy and Infectious Diseases (5 F02 AI 55816).

LITERATURE CITED

1. **Albrecht, T., and F. Rapp.** 1973. Malignant transformation of hamster embryo fibroblasts following exposure to ultraviolet-irradiated human cytomegalovirus. Virology 55:53-61.
2. **Ben-Porat, T., and A. S. Kaplan.** 1967. Correlation between replication and degradation of cellular DNA in polyoma virus-infected cells. Virology 32:457-464.
3. **Ben-Porat, T., A. S. Kaplan, and R. W. Tennant.** 1967. Effect of 5-fluorouracil on the multiplication of a virulent virus (pseudorabies) and an oncogenic virus (polyoma). Virology 32:445-456.
4. **Dulbecco, R., and M. Vogt.** 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. 99:167-182.
5. **Dulbecco, R., L. H. Hartwell, and M. Vogt.** 1965. Induction of cellular DNA synthesis by polyoma virus. Proc. Natl. Acad. Sci. U.S.A. 53:403-410.
6. **Fioretti, A., T. Furukawa, D. Santoli, and S. A. Plotkin.** 1973. Nonproductive infection of guinea pig cells with human cytomegalovirus. J. Virol. 11:998-1003.
7. **Furukawa, T., S. Tanaka, and S. A. Plotkin.** 1975. Stimulation of macromolecular synthesis in guinea pig cells by human CMV. Proc. Soc. Exp. Biol. Med. 148:211-214.
8. **Gershon, D., L. Sachs, and E. Winocour.** 1966. The induction of cellular DNA synthesis by simian virus 40 in contact-inhibited and X-irradiated cells. Proc. Natl. Acad. Sci. U.S.A. 56:918-925.
9. **Hayflick, L., and P. S. Moorhead.** 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25:585-621.
10. **Huang, E-S., S-T. Chen, and J. S. Pagano.** 1973. Human cytomegalovirus. I. Purification and characterization of viral DNA. J. Virol. 12:1473-1481.
11. **Kaplan, A. S.** 1969. Isopycnic banding of viral DNA in cesium chloride, p. 487-495. *In* K. Habel and N. P. Salzman (ed.), Fundamental techniques in virology. Academic Press Inc., New York.
12. **Kaplan, A. S., and T. Ben-Porat.** 1961. The action of 5-fluorouracil on the nucleic acid metabolism of pseudorabies virus-infected and noninfected rabbit kidney cells. Virology 13:78-92.
13. **Lawrence, W. C.** 1971. Evidence for a relationship between equine abortion (herpes) virus deoxyribonucleic acid synthesis and the S phase of the KB cell mitotic cycle. J. Virol. 7:736-748.
14. **Pages, J., S. Manteuil, D. Stehelin, M. Fiszman, M. Marx, and M. Girard.** 1973. Relationship between replication of simian virus 40 DNA and specific events of the host cell cycle. J. Virol. 12:99-107.
15. **Schmidt, G., and S. J. Thannhauser.** 1945. A method for the determination of desoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem. 161:83-89.
16. **St. Jeor, S., and F. Rapp.** 1973. Cytomegalovirus: conversion of nonpermissive cells to a permissive state for virus replication. Science 181:1060-1061.
17. **St. Jeor, S. C., T. B. Albrecht, F. D. Funk, and F. Rapp.** 1974. Stimulation of cellular DNA synthesis by human cytomegalovirus. J. Virol. 13:353-362.
18. **Tennant, R. W., K. R. Layman, and R. E. Hand.** 1969. Effect of cell physiological state on infection by rat virus. J. Virol. 4:872-878.
19. **Thorne, H. V.** 1973. Cyclic variation in susceptibility of Balb-c 3T3 cells to polyoma virus. J. Gen. Virol. 18:163-169.
20. **Weller, T. H., J. C. Macauley, J. M. Craig, and P. Wirth.** 1957. Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc. Soc. Exp. Biol. Med. 94:4-12.
21. **Wentworth, B. B., and L. French.** 1970. Plaque assay of cytomegalovirus strains of human origin. Proc. Soc. Exp. Biol. Med. 135:253-258.
22. **Wright, H. T.** 1973. Cytomegaloviruses, p. 353-388. *In* A. S. Kaplan (ed.), The herpesviruses. Academic Press Inc., New York.