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# Synthesis of Herpes Simplex Virus, Vaccinia Virus, and Adenovirus DNA in Isolated HeLa Cell Nuclei

## I. Effect of Viral-Specific Antisera and Phosphonoacetic Acid

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Purified nuclei, isolated from appropriately infected HeLa cells, are shown to synthesize large amounts of either herpes simplex virus (HSV) or vaccinia virus DNA in vitro. The rate of synthesis of DNA by nuclei from infected cells is up to 30 times higher than the synthesis of host DNA in vitro by nuclei isolated from uninfected HeLa cells. Thus HSV nuclei obtained from HSV-infected cells make DNA in vitro at a rate comparable to that seen in the intact, infected cell. Molecular hybridization studies showed that 80% of the DNA sequences synthesized in vitro by nuclei from herpesvirus-infected cells are herpesvirus specific. Vaccinia virus nuclei from vaccinia virus-infected cells, also produce comparable percentages of vaccinia virus-specific DNA sequences. Adenovirus nuclei from adenovirus 2-infected HeLa cells, which also synthesize viral DNA in vitro, have been included in this study. Synthesis of DNA by HSV or vaccinia virus nuclei is markedly inhibited by the corresponding viral-specific antisera. These antisera inhibit in a similar fashion the purified herpesvirus-induced or vaccinia virus-induced DNA polymerase isolated from infected cells. Phosphonoacetic acid, reported to be a specific inhibitor of herpesvirus formation and the herpesvirus-induced DNA polymerase, is equally effective as an inhibitor of HSV DNA synthesis in isolated nuclei in vitro. However, we also find phosphonoacetic acid to be an effective inhibitor of vaccinia virus nuclear DNA synthesis and the purified vaccinia virus-induced DNA polymerase. In addition, this compound shows significant inhibition of DNA synthesis in isolated nuclei obtained from adenovirus-infected or uninfected cells and is a potent inhibitor of HeLa cell DNA polymerase $\alpha$.

Isolated intact mammalian nuclei have been used by several groups of investigators for studying DNA synthesis in noninfected and certain viral-infected cells. Friedman and Mueller (9), Hershey et al. (11), and Bernard and Brent (4) have demonstrated the incorporation of [$^3$H]TTP into DNA of HeLa cell nuclei. This system required all four deoxynucleoside triphosphates, ATP, and $Mg^{2+}$ ions and was stimulated by a cytoplasmic fraction. Similar DNA synthesizing systems have been studied in other mammalian cells (17, 22, 26). A common feature of these isolated nuclei is the small amount of DNA made, which is usually 1% or less of the cell genome.

Nuclei isolated from viral-infected cells can also be shown to synthesize viral DNA in vitro in some cases. Thus, Winnacker et al. (35, 36) demonstrated the synthesis of polyoma DNA in isolated nuclei, and adenovirus DNA and simian virus 40 DNA synthesis have also been reported in isolated nuclei (23, 31). Radsak (24) examined DNA synthesis in mitochondria and nuclei isolated from HeLa cells infected with herpes simplex virus (HSV) type 1, but found that infected nuclei exhibit even lower DNA synthesis than normal nuclei. Bell (2) has reported that a monolayer of herpesvirus-infected BSC-1 cells, treated to remove the cytoplasm, will synthesize herpesvirus DNA in the residual nuclei-membrane monolayer. Kolber (15), Biswall and Murray (5), and Becker and Asher (1) have recently demonstrated that nuclei from HSV-infected cells synthesize HSV DNA in vitro, and LaColla and Weissbach (16) have shown synthesis of vaccinia virus DNA in isolated nuclei.

In this report we show that viral-specific antisera markedly inhibit the in vitro synthesis of DNA by nuclei isolated from viral-infected cells and compare this to the inhibition of the purified viral-induced DNA polymerases by these antisera. Similar experiments were carried out with phosphonoacetic acid (PPAA), which is a

known inhibitor of herpesvirus-induced DNA polymerase (18). It was found that PPAA is inhibitory to other DNA polymerases also. This compound can be demonstrated to partially inhibit DNA synthesis in nuclei obtained from uninfected cells or cells infected with adenovirus and to be a very potent inhibitor of nuclei from vaccinia- or herpesvirus-infected cells.

## MATERIALS AND METHODS

**Growth of cells and virus infection.** HeLa S3 cells were grown in suspension cultures in F-13 medium (Grand Island Biological Co.) supplemented with 5% fetal calf serum. They were synchronized in S-phase with fluorodeoxyuridine as described by Shatkin and Salzman (25). HeLa F cells, obtained from Flow Laboratories, were grown with 10% fetal calf serum in monolayer culture with F-11 medium (Grand Island Biological Co.). HeLa S3 cells were infected with the WR strain of vaccinia virus which had been grown in HeLa cells and purified according to Joklik (13). Logarithmically growing cells were infected with virus (10 PFU per cell) as previously described (7) and harvested 4 h postinfection. The procedure used for the infection of HeLa F cells with the Miyama strain of HSV type I (20) adapted to HeLa cells has been previously described (33). Cells were harvested 4 to 8 h after infection. Adenovirus type 2 was prepared by the procedure of Pettersson and Sambrook (21) and used to infect HeLa S3 cells at a multiplicity of infection of 10 PFU/cell. The cells were collected 18 h after infection.

**Preparation of nuclei and cytoplasm.** Cells were collected as previously reported (34), and nuclei were prepared by the procedure described by Berkowitz et al. (3). The resulting purified nuclei were suspended in 0.32 M sucrose, 0.002 M $MgCl_2$, and 0.001 M $KPO_4$, pH 7.0, frozen in a dry ice-acetone bath, and maintained at −70 C or in liquid nitrogen.

**Isolation of DNA.** The preparation of DNA from purified vaccinia virus or HeLa cell nuclei has been previously described by LaColla (16). HSV DNA was a generous gift of Robert Muller from this laboratory and was prepared from purified HSV by the method of Kieff et al. (14). Adenovirus and adenovirus DNA were purified as previously described (21).

**DNA-DNA hybridization.** Hybridizations were performed using modifications of procedures outlined previously by Denhardt (8). Single-stranded HeLa DNA (100 μg), vaccinia virus (5 μg), HSV (5 μg), and adenovirus type 2 (5 μg) DNA were immobilized on Sartorius nitrocellulose membrane filters (SM 1130) as described by Gillespie and Spiegelman (10). Input [$^3$H]DNA, after a 30-s treatment in an ultrasonic water bath (Heat Systems Ultrasonics, Inc.), was denatured in 0.16 M NaOH at room temperature and then neutralized with $NaH_2PO_4$. The hybridizations were performed in Denhardt solution for 36 to 42 h at 65 C. Filters were washed by soaking 5 min in 10 ml of 0.015 M NaCl-0.0015 M sodium citrate at 50 C and then finally washed on both sides with 50 ml of 0.3 M NaCl-0.03 M sodium citrate at 25 C. The radioactivity was determined in a Beckman scintillation counter.

**Nuclei incubation mix.** The standard assay (0.1 ml) contained: Tris-hydrochloride buffer (pH 8.3), 50 mM; $MgCl_2$, 4 mM; ATP, 2.5 mM; dithiothreitol, 2.5 mM; KCl, 20 mM; 0.14 enzyme units; dATP, dCTP, dGTP, and dTTP, each 0.2 mM; [$^3$H]TTP, final specific activity of 50 to 1,000 counts/min per pmol; and $0.05 \times 10^6$ to $5 \times 10^6$ nuclei. The assays were performed 10 min at 37 C and then stopped by chilling to 0 C, acid-precipitable material was collected and washed, and the radioactivity was determined as previously described (34).

**DNA polymerases.** Partially purified DNA polymerases α, β and γ (according to this nomenclature [32], DNA polymerase α is the large-molecular-weight DNA polymerase found in the cytoplasm and nucleus, whereas DNA polymerase β represents the small-molecular-weight DNA polymerase found only in the nucleus; DNA polymerase γ is characterized by its efficient copying of the oligomer-homopolymer $A_n \cdot dT_{15}$) from HeLa cells were isolated as previously described (28), in which the α-polymerase is listed as D-DNA polymerase II, the β-polymerase is listed as D-DNA polymerase I, and the γ-polymerase is listed as "R-DNA polymerase." The isolation of the HSV-induced and vaccinia virus-induced DNA polymerases has been reported (7, 33) and was utilized in these studies.

PPAA was the kind gift of Ed Heimer and Alan Cook from the Hoffmann-La Roche Research Laboratories.

**Antiserum.** Vaccinia antiserum was prepared as described by Citarella et al. (7). HSV antiserum was prepared in a similar manner using BHK-21 cells infected with 10 PFU of HSV per cell. After 18 h, the cells were collected, and the procedure described by Citarella et al. (7) was then followed. Adenovirus type 2 antiserum was a commercial product obtained from Microbiological Associates, Bethesda, Md. Antiserum to HeLa cell DNA polymerase α has been described by Spadari et al. (27). In each case the gamma globulin fraction was obtained by ammonium sulfate precipitation (50% saturation) of the crude serum.

**DNA polymerase assays.** The assay conditions used for the various DNA polymerases are described in the following references: HSV-induced DNA polymerase (33); vaccinia virus-induced DNA polymerase (7); and HeLa cell α-, β-, and γ-polymerase assays (28) (the α- and β-polymerase assays are under "D-DNA polymerase" conditions and the γ-polymerase is assayed as an "R-DNA polymerase"). A unit of enzyme activity is defined as that amount of enzyme catalyzing the incorporation of 1 nmol of nucleotide into DNA in 60 min.

## RESULTS

**Synthesis of DNA by nuclei from normal and infected cells.** Purified nuclei, isolated from fluorodeoxyuridine-synchronized, uninfected HeLa S3 cells by the detergent washing

procedure of Berkowitz et al. (3), demonstrate a capacity to synthesize DNA in vitro (Fig. 1). This activity is two to three times greater than that observed in nuclei isolated from nonsynchronized HeLa cells. DNA synthesis is linear for approximately 10 min and levels off after 30 min. Addition of more deoxyribonucleoside triphosphates after 10 min results in a 15 to 20% increase in incorporation of [$^3$H]TTP but does not cause restoration of DNA synthesis to the initial rate, indicating that the cause of the reduction in the rate of synthesis is probably not depletion or destruction of substrate. Addition of fresh nuclei, however, did result in further synthesis at the initial rate, suggesting that the limiting factor (or factors) resides in the nucleus. The DNA synthesis in isolated nuclei from uninfected, vaccinia virus-, or adenovirus-infected cells shows a linear dependence on nuclei concentration between $10^5$ and $5 \times 10^6$ nuclei per 0.1 ml of assay. Nuclei from herpesvirus-infected cells show a linear response in DNA synthesis at concentrations from $5 \times 10^4$ to $5 \times 10^5$ nuclei per assay. The incorporated label is more than 90% solubilized by DNase and is insensitive to RNase. The nuclei from uninfected, fluorodeoxyuridine-synchronized HeLa cells incorporated 8 to 15 pmol of [$^3$H]TTP into DNA/10 min per $10^6$ nuclei. Thus it can be calculated that the rate of DNA synthesis for the first 10 min by nuclei isolated from synchronized cells is about 5% of the rate known to occur in growing cells.

The synthesis of HSV DNA is known to occur in the nucleus of the cell (19), and recent studies in this laboratory have shown that significant synthesis of vaccinia virus DNA is associated with the nucleus. The evidence that vaccinia virus DNA synthesis in vivo takes place, in part, in the nucleus and that its presence there is not an artifact caused by cytoplasmic contamination has been discussed in a prior report (16). These in vivo findings are pertinent to the observed synthesis of DNA by isolated nuclei from either HSV- or vaccinia virus-infected cells. In the latter case, the nuclei, in vitro, synthesize DNA at approximately a fivefold higher rate than do nuclei from uninfected cells (Fig. 1). The stimulation of DNA synthesis in nuclei isolated from HSV-infected cells is even more dramatic. These nuclei synthesize DNA at a rate of 10 to 30 times that of nuclei from uninfected cells (Fig. 1). This increase in the rate of DNA synthesis in viral-infected cells agrees with the results of Bell (2) and Becker and Asher (1) using HSV-infected BSC-1 cells, the results of Kolber (15) using HEL cells, and the results of Biswal and Murray using rat embryo fibroblasts (5). Nuclei from adenovirus

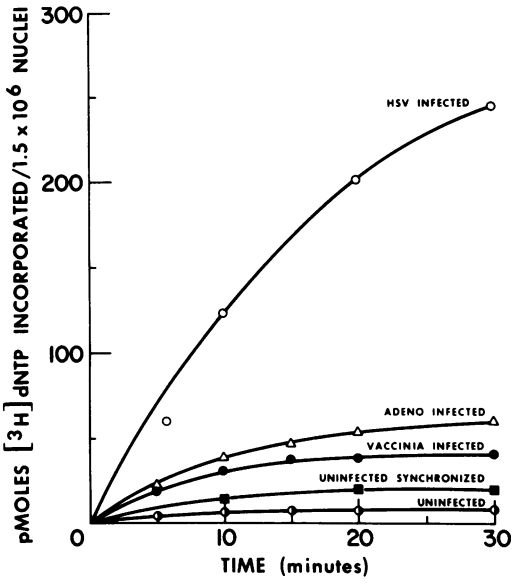


FIG. 1. *Kinetics of DNA synthesis in isolated nuclei. Nuclei were isolated and purified as described. Symbols: ◑, uninfected nuclei (3 × 10⁶); ■, uninfected synchronized nuclei (3 × 10⁶); ●, nuclei (1 × 10⁶) from 4-h vaccinia virus-infected cells; ○, nuclei (5 × 10⁴) from 4-h HSV-infected cells, △, nuclei from 18-h adenovirus-infected cells. Incubation conditions are as described, and incorporated acid-precipitable radioactivity is shown. The data is normalized to picomoles of total deoxynucleotide (dNTP) incorporated into an acid-insoluble form per 1.5 × 10⁶ nuclei.*

type 2-infected HeLa cells synthesize DNA in vitro at a rate slightly faster than vaccinia nuclei and five to six times faster than nuclei from uninfected cells.

**Nature of DNA synthesized in isolated nuclei.** An obvious possibility for the increase in the DNA synthetic ability of isolated nuclei from the virus-infected cells was that the isolated nuclei were synthesizing viral DNA. This was proven by DNA-DNA molecular hybridization studies (Table 1). Vaccinia virus, herpesvirus, or adenovirus DNA shows negligible cross-hydridization with HeLa DNA, as seen in the control studies. DNA hybridizations were carried out in aqueous solutions at 65 C, and control experiments have shown that the presence of a large amount of heterologous DNA does not interfere with the specific DNA-DNA hybridizations. Table 1 demonstrates that nuclei isolated from either vaccinia virus-, herpesvirus-, or adenovirus-infected cells show synthesis of both host and viral DNA. In this experiment, nuclei from vaccinia virus-infected cells synthesized DNA in vitro, the majority of which contained vaccinia virus DNA sequences. Host DNA sequences were also made in this system. The

TABLE 1. *Characterization of DNA synthesized in isolated nuclei*[a]

| [³H]DNA source | DNA input | | Hybridization (%) | | | |
|---|---|---|---|---|---|---|
| | Counts/min | μg | HeLa DNA filter | Vaccinia DNA filter | Herpes DNA filter | Adeno DNA filter |
| Vaccinia virus | 9,005 | 0.7 | 0 | 58.3 | | |
| Nuclei from vaccinia virus-infected cells | 1,273 | 0.5 | | 37.2 | | |
| | 7,784 | 3.1 | 2.7 | | | |
| HeLa cell nuclei | 16,816 | 6.0 | 15.6 | 0.22 | | |
| HeLa cell nuclei + exogenous vaccinia DNA | 619 | 2.4 | 17.6 | | | |
| | 1,117 | 4.3 | | 2.6 | | |
| | 4,615 | 17.7 | | 2.0 | | |
| HSV | 4,150 | 0.32 | 1.8 | | 48.3 | |
| Nuclei from HSV-1 infected cells | 4,994 | 0.7 | 5.1 | | 39.1 | |
| HeLa cell nuclei | 9,731 | 3.0 | 24.1 | | 0.27 | |
| Adenovirus 2 | 7,428 | 1.0 | 0.24 | | | 38.3 |
| HeLa cell nuclei | 183,000 | 1.8 | 15.8 | | | 0.16 |
| Nuclei from adenovirus-infected cells | 536 | 1.0 | 4.7 | | | 9.5 |
| | 1,267 | 2.2 | 3.5 | | | 6.4 |

[a] Nuclei were isolated and purified from uninfected, 4-h vaccinia virus-infected, 4-h HSV-infected, and 18-h adenovirus type 2-infected HeLa cells as described. Uninfected ($5 \times 10^7$), vaccinia virus-infected ($5 \times 10^7$), and HSV-infected ($5 \times 10^5$) HeLa nuclei were incubated for 10 min at 37 C in 1.2 ml of nuclei incubation mixes. Nuclei from adenovirus-infected cells ($6.4 \times 10^7$ nuclei) were incubated in 2 ml of the incubation mixture for 20 min at 37 C. As a control, $5 \times 10^7$ uninfected HeLa nuclei were also incubated in the presence of 5 μg of exogenous vaccinia DNA. The reactions were stopped by chilling to 0 C and centrifuged for 10 min at $400 \times g$. The nuclear pellets were suspended in 1.5 ml of 0.01 M Tris-hydrochloride buffer, pH 7.5, containing 0.005 M EDTA, and the DNA was isolated as described for HeLa DNA. DNA hybridizations were carried out as described in 0.45 M NaCl-0.045 M sodium citrate.

synthesis of vaccinia virus DNA in the nuclei of infected cells in vivo has been studied in this laboratory (16). Although it is known that extensive synthesis of vaccinia virus DNA occurs in the cytoplasm of the cell, the finding that isolated nuclei from infected cells also synthesize viral DNA supports the in vivo studies. The ability of the nuclei to synthesize vaccinia virus DNA is not due to casual contamination of nuclei by vaccinia virus DNA. The control experiment of Table 1 in which a relatively large amount of purified vaccinia virus DNA was incubated with nuclei from uninfected cells in the standard incorporation system showed that very little of the DNA synthesized in vitro contains vaccinia virus DNA sequences.

Isolated nuclei from HSV-infected cells are also capable of synthesizing large amounts of herpesvirus DNA in addition to host DNA (Table 1). The DNA synthesized in vitro by the nuclei obtained from HSV-infected cells seems to contain predominantly viral sequences, although host sequences were also detected. Since these isolated nuclei from HSV-1-infected cells also synthesize DNA at a high rate (Fig. 1), they offer an attractive in vitro system for the study of herpesvirus DNA formation. It should be pointed out that although the bulk of DNA synthesized by these isolated nuclei is HSV specific, the absolute amount of host DNA made per nucleus is greater than that synthesized by nuclei from uninfected cells. Nuclei isolated from cells 18 h after infection with adenovirus seem to make approximately equal amounts of both viral and host DNA. Relatively large amounts of DNA had to be used in these experiments (Table 1), and we were unable to obtain complete quantitation of our hybridization data.

**Requirements for DNA synthesis in isolated nuclei.** The necessary components for DNA synthesis in isolated HeLa cell nuclei are shown in Table 2 and are compared to nuclei obtained from HSV-, vaccinia virus-, or adenovirus 2-infected cells. The properties of isolated nuclear systems from HeLa S3, HeLa F, BSC-1, and BHK-21 cells, or vaccinia virus-, HSV-, or adenovirus-infected HeLa cells are similar under our assay conditions. $Mg^{2+}$ and a sulfhydryl source such as dithiothreitol seem necessary for maximal activity. DNA synthesis in uninfected nuclei, in the presence of ATP, is 10 times that found in the absence of ATP. Addition of the three other ribonucleoside triphosphates has no further effect, nor can they replace ATP. In marked contrast, nuclei from HSV- or vaccinia

TABLE 2. *Requirements for DNA synthesis in isolated nuclei*[a]

| Omissions | DNA synthesis (%) | | | |
|---|---|---|---|---|
| | Uninfected | HSV infected | Vaccinia infected | Adeno infected |
| None | 100 | 100 | 100 | 100 |
| $Mg^{2+}$ | 5 | 1 | 4 | 7 |
| ATP | 11 | 100 | 100 | 47 |
| Dithiothreitol | 63 | 5 | 74 | 51 |
| KCl | 75 | 100 | 87 | 93 |

[a] Nuclei were prepared from uninfected, 8-h HSV-infected, 4-h vaccinia virus-infected, and 18-h adenovirus type 2-infected HeLa cells. With the HSV-infected nuclear system, $2 \times 10^5$ nuclei were used. The uninfected and vaccinia virus-infected assays contained $2 \times 10^6$ nuclei, and incubations were carried out for 10 min at 37 C in the nuclei incubation mix as described. Adenovirus-infected incubations contained $6.4 \times 10^5$ nuclei. Acid-precipitable radioactivity incorporated was measured after the incubation period. The 100% values for the complete system in picomoles (and counts per minute) incorporated are as follows: uninfected HeLa nuclei, 8 (2,157); HSV-infected HeLa nuclei, 44 (1,992); vaccinia virus-infected HeLa nuclei, 54 (14,664); adenovirus-infected HeLa nuclei, 30 (5,247).

virus-infected cells show no dependence on added ATP, whereas nuclei from adenovirus-infected cells are stimulated twofold in the presence of ATP.

As discussed above, the DNA synthesized by nuclei from herpesvirus- or vaccinia virus-infected cells is largely viral specific. We therefore investigated the effect of antiserum prepared against herpesvirus- or vaccinia virus-infected cells, as described above, on DNA synthesis in isolated nuclei. DNA synthesis by isolated nuclei from vaccinia-infected (Fig. 2A) or HSV-infected (Fig. 2B) cells is strongly inhibited (70 to 75%) by the corresponding antiserum.

We have also, in a similar way, examined the effect of adenovirus type 2 antiserum on isolated adenovirus-infected nuclei, vaccinia virus-infected nuclei, and HSV-infected nuclei, and find no inhibition of DNA synthesis in the presence of up to 2.4 mg of gamma globulin per ml with any of these nuclei (data not shown). In addition, we tested antiserum prepared against HeLa cell DNA polymerase $\alpha$ and found it had no inhibitory effect against nuclei from uninfected or viral-infected cells. In fact, the inhibition shown by the viral antisera in these experiments is specific, i.e, the HSV antiserum will only inhibit DNA synthesis in nuclei from HSV-infected cells and will not inhibit any other nuclei tested. The vaccinia antiserum shows a similar specificity and inhibits exclusively nuclei from vaccinia virus-infected cells. This distinctive effect of each antiserum is partly illustrated in Fig. 2, where it can be seen that vaccinia virus antiserum has no effect on DNA synthesis by HSV nuclei and, conversely, HSV antiserum does not affect synthesis in vaccinia virus nuclei.

Under our conditions, it has not been possible to completely abolish DNA synthesis in either vaccinia- or herpesvirus-infected nuclei in vitro by the use of appropriate antiserum. We have examined the residual DNA synthesized by vaccinia-infected nuclei or herpesvirus-infected nuclei in the presence of vaccinia virus antiserum (1.26 mg of protein/ml) or HSV antiserum (1.7 mg of protein/ml) using molecular hybridization techniques. As shown in Fig. 2, this concen-

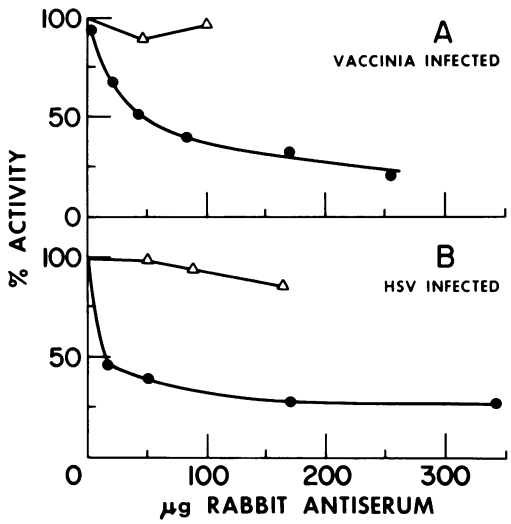


FIG. 2. *Effect of antisera on DNA synthesis in isolated nuclei. Nuclei from vaccinia virus-infected cells ($10^6$) and nuclei from HSV-infected cells ($6 \times 10^4$) were added to standard incubation mixtures which lacked ribo- and deoxyribonucleoside triphosphates. The nuclei were then preincubated, in a final volume of 90 μl, for 10 min at 20 C in the presence of partially purified antisera prepared against vaccinia virus- or HSV-infected cells. To start the reaction, deoxyribonucleoside triphosphates (10 μl) were added, and DNA synthesis was determined at 37 C as described. Over the same range of protein concentration, control normal rabbit serum gamma globulin, incubated with the nuclei as stated above, did not inhibit [$^3H$]TTP incorporation into DNA by any of the nuclei and was used as the control value. (A) Symbols: ●, nuclei from vaccinia virus-infected cells incubated with vaccinia antiserum; △, nuclei from vaccinia virus-infected cells incubated with HSV antiserum. The 100% value represents 34.7 pmol of deoxynucleotides incorporated into DNA. (B) Symbols: ●, nuclei from HSV-infected cells incubated with HSV antiserum; △, nuclei from HSV-infected cells incubated with vaccinia antiserum. The 100% value represents 12.6 pmol of deoxynucleotides incorporated into DNA.*

tration of viral antiserum will inhibit DNA synthesis in the appropriate isolated nuclei by approximately 75%. Though the data are not presented herein, we find that the ratio of viral-specific to host-specific DNA sequences synthesized under these conditions is similar to that found in nuclei incubated in the absence of the viral-specific antiserum (Table 1).

**Inhibition of purified DNA polymerases by antiserum.** The viral-directed antiserum used in the experiments shown in Fig. 2 will also inhibit purified viral-induced DNA polymerases in vitro. Figure 3 shows the inhibition of partially purified vaccinia virus-induced (Fig. 3A) and herpesvirus-induced (Fig. 3B) DNA polymerase by the corresponding antiserum. The vaccinia antiserum will inhibit the vaccinia DNA polymerase activity up to 85% (cf. Citarella [7]) but will not inhibit the purified herpesvirus-induced DNA polymerase. Similarly, the herpesvirus-directed antiserum inhibits the herpesvirus-induced DNA polymerase to greater than 90% but does not affect the vaccinia virus-induced DNA polymerase. Figure 3C shows the effect of antiserum prepared against purified HeLa DNA polymerase $\alpha$ on purified HeLa cell DNA polymerases $\alpha$ and $\beta$. As reported previously (27), antiserum against the $\alpha$-polymerase will not inhibit the $\beta$-polymerase. Although not shown in Fig. 3, we have found that this antiserum to the $\alpha$-polymerase will not inhibit the herpesvirus-induced DNA polymerase or the vaccinia virus-induced DNA polymerase. We have also tested adenovirus type 2 antiserum with the partially purified HeLa cell DNA polymerases $\alpha$, $\beta$, and $\gamma$ and the herpesvirus- or vaccinia virus-induced DNA polymerases. Adenovirus antiserum, at levels up to 1.2 mg of protein/ml, has no inhibitory effect toward any of these enzymes.

**Effect of PPAA on DNA synthesis in isolated nuclei.** PPAA has been shown to specifically inhibit the herpes viral-induced DNA polymerase in cell lysates and partially purified polymerase preparations, whereas the DNA polymerase from normal cells appeared to be unaffected (18). Experiments were performed to evaluate the effectiveness of PPAA in the inhibition of DNA synthesis employing: (i) isolated nuclei from uninfected cells; (ii) nuclei from vaccinia virus-, HSV-, and adenovirus-infected cells; and (iii) purified host and viral-induced DNA polymerases. DNA synthesis in nuclei from uninfected cells is somewhat sensitive to this compound, exhibiting 16, 34, and 45% inhibition at 10, 20, and 50 $\mu$g of PPAA per ml, respectively (Fig. 4). Nuclei from vaccinia- or HSV-infected cells are significantly more sensitive to PPAA.

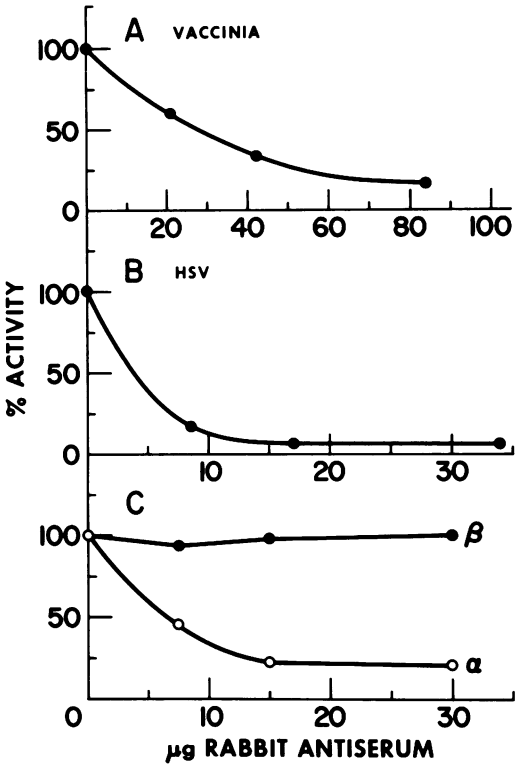


FIG. 3. *Inhibition of DNA polymerases by various antisera. Purified DNA polymerases and 50 $\mu$g of bovine serum albumin were preincubated for 10 min at 20 C with antiserum in a total volume of 30 $\mu$l. The appropriate DNA polymerase assay components were then added to a final volume of 200 $\mu$l, and the assays were performed at 37 C for 30 min. (A) Vaccinia virus-induced DNA polymerase (0.29 U) incubated with antiserum to vaccinia virus-infected cells; (B) herpesvirus-induced DNA polymerase (0.78 U) incubated with antiserum to HSV-infected cells; (C) HeLa cell DNA polymerase $\alpha$ (1.48 U) and polymerase $\beta$ (0.16 U) incubated with antiserum prepared against purified HeLa cell $\alpha$-polymerase. Controls, using preimmune rabbit serum, were run for each point shown, and acid-precipitable radioactivity was determined.*

DNA synthesis was inhibited 62, 72, and 82% at 10, 20, and 50 $\mu$g of PPAA per ml, respectively, in nuclei from vaccinia virus-infected cells, and 81, 87, and 95% in nuclei from HSV-infected cells. Nuclei from adenovirus-infected cells resemble the nuclei from uninfected cells in their response to PPAA, although the former are somewhat more inhibited at the lower concentrations (5 to 20 $\mu$g) of PPAA.

**Effect of PPAA on purified DNA polymerases in vitro.** The finding that PPAA is inhibitory to DNA synthesis in all the isolated nuclear systems described in this paper led us to

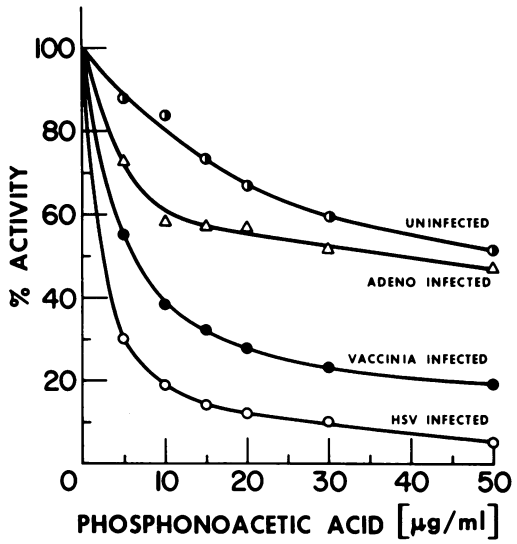


FIG. 4. *Effect of PPAA on DNA synthesis in isolated nuclei. Nuclei (2.7 × 10⁶) from uninfected cells (◑), nuclei (1 × 10⁶) from vaccinia virus-infected cells (●), nuclei (6.4 × 10⁵) from adenovirus-infected cells (△), and nuclei (1.2 × 10⁵) from HSV-infected cells (○) were incubated (10 min at 37 C) in the standard nuclei reaction mixture containing 0 to 50 μg of PPAA per ml. One hundred percent activity values (no PPAA) for the acid-precipitable radioactivity are as follows: nuclei from uninfected cells, 6.7 pmol; nuclei from vaccinia virus-infected cells, 29.1 pmol; nuclei from adenovirus-infected cells, 20.4 pmol; nuclei from HSV-infected cells, 26.6 pmol.*

examine the effect of this compound on a number of partially purified DNA polymerases. The enzymes used in these studies were HeLa cell DNA polymerases α, β, and γ and the herpesvirus- and vaccinia virus-induced DNA polymerases isolated from HeLa cells infected with these viruses. Table 3 summarizes the results. PPAA is almost equally effective in inhibiting HeLa cell DNA polymerase α, the HSV-induced DNA polymerase, and the vaccinia virus-induced DNA polymerase. It is completely ineffective as an inhibitor of the cellular β-polymerase and shows intermediate inhibition of the γ-polymerase. It is obvious that PPAA is not a specific inhibitor for the HSV-induced polymerase and is just as active against the vaccinia virus-induced DNA polymerase or the cellular α-polymerase. These results are consistent with the effects noted with the isolated nuclei.

## DISCUSSION

The synthesis of DNA in nuclei isolated from viral-infected cells proceeds at a higher rate and to a greater extent than does DNA synthesis in normal nuclei. This is found to be true for adenovirus, herpesvirus, and vaccinia virus nuclei as shown in this paper and previous reports (5, 15, 16, 31) and in nuclei from polyoma-infected cells (35, 36). Furthermore, our hybridization studies have shown that de novo synthesis of DNA by these isolated nuclei is predominantly that of viral-specific sequences which represent 50 to 80% of newly made DNA. Another important difference between the normal and viral-infected nuclei resides in their ATP requirements. Nuclei from uninfected cells require ATP for in vitro DNA synthesis, which drops 10-fold in the absence of ATP. By contrast, nuclei from herpesvirus- or vaccinia virus-infected cells show no requirement for ATP, and nuclei from adenovirus-infected cells still exhibit one-half of their activity in the absence of ATP.

Viral-infected nuclei and those from uninfected cells also vary in their response to the presence of antiserum. Viral-specific antiserum such as that made to a vaccinia virus-infected cell will inhibit DNA synthesis in nuclei from vaccinia virus-infected cells but not nuclei isolated from other cells. This is not unexpected since such antiserum probably contains antibodies to a number of the new vaccinia virus-induced proteins. For instance, as this paper shows, this antiserum is a powerful inhibitor in vitro of purified vaccinia virus-induced DNA polymerase, which is known to be found in the nucleus (7) and is presumably involved in vaccinia DNA synthesis. An analogous result is

TABLE 3. *Inhibition of DNA polymerases by PPAA*[a]

| PPAA (μg/ml) | Inhibition (%) of DNA polymerase | | | | |
|---|---|---|---|---|---|
| | α | β | γ | HSV induced | Vaccinia induced |
| 1 | 19 | | 0 | 22 | 20 |
| 5 | 48 | | 0 | 31 | 50 |
| 10 | 66 | | 11 | 61 | 66 |
| 20 | 73 | 9 | 16 | 84 | 87 |
| 50 | 82 | 0 | 43 | 87 | 90 |
| 100 | 90 | 5 | 63 | 95 | 98 |

[a] The assay conditions were as described except that the concentration of activated salmon sperm DNA in the incubation was 265 μg per ml. The units of DNA polymerase activity in 100 μl of incubation mixtures were: α-polymerase, 0.96; β-polymerase, 0.35; γ-polymerase, 0.39; HSV-induced DNA polymerase, 1.2; vaccinia virus-induced DNA polymerase, 0.56. The control values (no inhibition) of DNA synthesis were: α-polymerase, 480 pmol; β-polymerase, 178 pmol; γ-polymerase, 195 pmol; HSV-induced DNA polymerase, 600 pmol; and vaccinia virus-induced DNA polymerase, 280 pmol. Assays were carried out for 20 min.

found with the herpesvirus nuclei and antiserum prepared against herpesvirus-infected cells. The lack of inhibition of adenovirus nuclei by commercial adenovirus type 2 antiserum is dissimilar to that observed in the vaccinia virus or herpesvirus system and may reflect, in part, the different preparation procedure used for the commercial antiserum. It should be mentioned that the activities and distribution of DNA polymerases $\alpha$, $\beta$, and $\gamma$ in HeLa cells infected with adenovirus type 2-infected cells has been examined in the course of these studies. We found that the levels of these enzymes in the cell are unchanged at 22 h after infection with adenovirus type 2 and were unable to detect any new DNA polymerase activity in these adenovirus-infected cells. This failure to find a new DNA polymerase is consistent with the report of Ito et al. (12), who have reported the isolation of DNA polymerase $\gamma$ from an adenovirus type 2 DNA replication complex.

Nuclei from uninfected cells are not inhibited by any of the viral antisera nor by antiserum directed against HeLa cell DNA polymerase $\alpha$. The latter result was somewhat unexpected because of the postulated involvement of the $\alpha$-polymerase in DNA replication (R. W. Chiu and E. F. Baril, J. Biol. Chem., in press; 6, 29). The ineffectiveness of $\alpha$-polymerase antiserum to inhibit in vitro nuclear DNA synthesis can be explained if the antibody is unable to enter the nuclei, or if the $\alpha$-polymerase is located at a site inaccessible to the antibody molecule. A less-trivial explanation may be that the nuclear DNA synthesis as observed in vitro is unrelated to in vivo DNA replication, or that the $\alpha$-polymerase is not involved in vivo in replicative nuclear DNA synthesis. From the results obtained with the $\alpha$-polymerase antiserum and PPAA, we would favor the hypothesis that the DNA synthesis observed in isolated normal nuclei may indeed involve the $\beta$- or $\gamma$-polymerases. The apparent minor contribution of the $\alpha$-polymerase (in vitro) may be related to the observation that this enzyme is thought to be involved in the initiation of DNA synthesis (30). If the isolated nuclei are incapable of initiating new DNA chains and can merely extend preexisting, unfinished DNA molecules, then DNA polymerase $\alpha$ may not be required in the in vitro system. It should be mentioned that attempts to stimulate DNA synthesis in isolated nuclei from uninfected or viral-infected cells by addition of viral-induced or host cell DNA polymerases has been unsuccessful in our hands.

The inhibitory effects of PPAA seem to be more widespread than originally thought. PPAA prevents both herpesvirus and vaccinia virus formation (R. G. Duff and L. R. Overby, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, S161, p. 240), and this is reflected in the strong inhibitory action this compound shows toward DNA synthesis in nuclei isolated from such infected cells. However, we also find PPAA to partially inhibit uninfected nuclei or nuclei from adenovirus-infected cells. Furthermore, when PPAA was tested as an inhibitor of viral-induced and cellular DNA polymerase in our studies, it showed a similar interference toward the enzymatic activity of herpesvirus- and vaccinia virus-induced DNA polymerases and HeLa cell DNA polymerase $\alpha$.

Although the use of isolated nuclear systems to study DNA synthesis is still in a primitive stage, our studies show that isolated nuclei from viral-infected cells have different requirements and show different properties from normal, uninfected nuclei in their synthesis of DNA. Use of the parameters discussed above may provide further clues to both viral and host DNA synthesis.

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