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Effect of Herpes Simplex Virus Type 1 Infection on the Cellular DNA Polymerase Activities of Mouse Cell Cultures

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SUMMARY

Thymidine kinase-deficient mouse cell cultures infected with herpes simplex virus type 1 exhibited a maximum of virus DNA synthesis around 8 h post-infection as determined by pulse labelling with ^{3}H-thymidine. Cellular DNA synthesis was progressively inhibited, but still appreciable until 8 h post-infection and not completely abolished at any time during the infectious cycle. Phosphonoacetic acid was found to be a potent and selective inhibitor of virus DNA synthesis only when added to infected cultures before the onset of virus DNA synthesis. During the interval of increasing virus DNA synthesis the activity of cellular α polymerase decreased rapidly, whereas the β polymerase activity increased significantly; a slight increase was observed for the γ polymerase activity. When infected cells were kept in the presence of phosphonoacetic acid following virus adsorption the effect on cellular DNA polymerases was less pronounced.

INTRODUCTION

Infection of cell cultures with herpes viruses results in the induction of a virus-specific and presumably virus-coded DNA polymerase (Keir *et al.* 1966; Weissbach *et al.* 1973). The new enzyme can be distinguished readily from the cellular DNA polymerases α, β and γ by its salt dependence and selective sensitivity to phosphonoacetic acid (Weissbach *et al.* 1973; Overby *et al.* 1974; Bolden *et al.* 1975). Its function appears to be indispensable for the synthesis of virus DNA (Mao *et al.* 1975) which is increasingly produced by infected cells in the beginning of the infectious cycle whereas synthesis of cellular DNA is progressively inhibited (Ben-Porat & Kaplan, 1973). However, depending on the type of cells used for infection there are still significant amounts of cellular DNA synthesized even late in the infectious cycle (Jacob & Roizman, 1977). It has remained unclear in this context whether some cellular DNA synthesis is necessary for virus DNA synthesis. Since selective inhibitors for cellular DNA synthesis are not available, this problem cannot be solved by a direct approach. Positive evidence could, however, be provided by an analysis of the cellular DNA polymerase activities during the infectious cycle. Although indirect, this approach could present the additional advantage that it may point to the type of cellular DNA synthesis (replication or repair) present in infected cells. For these reasons the activities of the cellular DNA polymerases α, β and γ were determined at various times post-infection in thymidine kinase deficient mouse cell cultures infected with herpes simplex type 1. It will be shown that an increase in total phosphonoacetic acid-resistant DNA polymerase activity in infected cells is paralleled most clearly by the β polymerase activity.

The α polymerase activity, on the other hand, decreases in the course of the infection. The activity of the γ polymerases was found to be slightly elevated.

METHODS

Cells and virus. C1-1D cells, a permanent mouse cell line (Clayton & Teplitz, 1972) which is deficient in the major cellular thymidine kinase activity, but not deficient in the mitochondrial thymidine kinase, were used for all experiments. The monolayer cultures were propagated in F-16 medium (GIBCO) supplemented with 5% (v/v) calf serum, 200 units penicillin/ml and 10 μg 5-bromodeoxyuridine/ml. This cell line incorporates exogenous thymidine exclusively into mitochondrial DNA and cannot replicate in medium containing hypoxanthine (15 μg/ml), aminopterin (20 μg/ml) and thymidine (4 μg/ml). Routine examinations for mycoplasma contamination were found to be negative.

Herpes simplex virus type 1 (strain KOS) was routinely propagated in C1-1D cells cultivated in medium without 5-bromodeoxyuridine for at least two passages prior to virus infection by adsorption to the monolayers at a multiplicity of 1 for 1 h at 35 °C. After 24 h in serum-free medium, cells were harvested by scraping and subjected to two cycles of quick freezing and thawing in the same medium. Following sedimentation of the cell debris at 2000 *g* for 20 min the supernatant was separated from the pellet and frozen at −80 °C either directly or after filtration through 0·45 μm Millipore filters to remove microsomes. The infectious supernatant contained on average 10^8 infectious units/ml as determined by titration on C1-1D cells (Radsak & Freise, 1973). For experimental infections the virus was propagated one more time in C1-1D cells and the freshly prepared virus-containing supernatant fluid adsorbed to the cells at a multiplicity of 10 infectious units/cell for 1 h. Mock-infection was carried out either with serumless used medium or a supernatant fluid from uninfected C1-1D cells prepared identically to that from virus-infected cells.

Labelling and analysis of cellular and virus DNA by isopycnic centrifugation in CsCl. Pulse labelling was performed by addition of ^{3}H-thymidine to the culture medium at a concentration of 5 μCi/ml. Subsequently the cell monolayers were washed with balanced salt solution, harvested by scraping and resuspended in 10 mM-tris-HCl, pH 7·2, 100 mM-NaCl and 2 mM-EDTA at 2×10^6 cells/ml. Cells were then lysed by addition of sarkosyl to a final concentration of 1% for several hours at 37 °C followed by addition of pronase (2·5 mg/ml sample vol.) and further incubation overnight at 37 °C. Samples of 200 μl of the various lysates corresponding to approx. 12 to 14 μg DNA were mixed with 10 ml of a CsCl solution of a mean density of 1·7 g/ml and centrifuged for 65 h in the 50Ti fixed-angle rotor of a Beckman centrifuge. Gradients were fractionated by puncturing the bottom of the tube and collecting dropwise between 45 and 50 fractions per gradient before assay of acid-insoluble radioactivity.

Cell fractionation, preparation of cellular extracts and partial purification of DNA polymerases. The methods of cell fractionation were as described before (Radsak & Albring-1974). After homogenization of the cells (1·5 to $2{\cdot}0 \times 10^7$ cells/ml) in isotonic homogenization medium (0·3 M-sucrose, 10 mM-tris-HCl, pH 7·4, 1 mM-EDTA) by means of a Dounce type glass homogenizer the nuclei were sedimented at 1500 *g* for 15 min and washed once in homogenizing medium. Mitochondria were separated from the cytoplasmic fraction by sedimentation at 10000 *g* for 10 min and washed once with homogenizing medium. This partially purified mitochondrial preparation was free of whole cells and nuclei when examined in the light microscope. The supernatant represented the post-mitochondrial fraction.

For the solubilization of the DNA polymerases, suspensions of cells in homogenizing medium or the cell fractions were made in 0·4 M-potassium phosphate, pH 7·5, 0·5 mM-dithiothreitol and 0·2% Triton X-100, subjected twice to ultrasonic vibrations for 10 s (sonifier TG 125, Schoeller & Schall, Frankfurt) at maximum setting and left for 30 min on ice. After removal of insoluble material by centrifugation at 100000 *g* for 30 min the supernatant was freed of contaminating DNA by passage through DEAE cellulose columns at an ionic strength of 0·4 M-potassium phosphate (Spadari & Weissbach, 1974). The eluates were dialysed overnight against 0·02 M-potassium phosphate, pH 7·5, 0·5 mM-dithiothreitol, 0·2% (v/v) Triton X-100 and 20% (v/v) glycerol. For separation of the DNA polymerases the dialysed cytoplasm was readsorbed to DEAE cellulose columns (approx. 1 mg protein/ml column vol.) and the enzyme activities eluted with gradients of 0·02 M- to 0·5 M-potassium phosphate, pH 7·5, 0·5 mM-dithiothreitol, 0·2% (v/v) Triton X-100 and 20% (v/v) glycerol (Pedrali-Noy & Weissbach, 1977). In the case of nuclear and mitochondrial dialysed extracts, adsorption to DEAE cellulose columns was followed by stepwise elution with 1 and 3 column vol. of 0·02 M- and 0·25 M-potassium phosphate, pH 7·5, respectively. The 0·02 M- flow-through and eluate of nuclear extracts was subsequently adsorbed to phosphocellulose columns and the β polymerase activity eluted with gradient of 0·02 M- to 0·5 M-potassium phosphate, pH 8·0, 0·5 mM-dithiothreitol, 0·2% (v/v) Triton X-100 and 20% (v/v) glycerol. The 0·25 M-DEAE cellulose eluates were subjected to identical chromatography on phosphocellulose for analysis of the γ polymerases after dialysis for at least 5 h against 0·02 M-potassium phosphate, pH 8·0, 0·5 mM-dithiothreitol, 0·2% (v/v) Triton X-100 and 20% glycerol. Peak activity for the β polymerase appeared at about 0·25 M and for the γ polymerase around 0·3 M-potassium phosphate (Weissbach, 1977).

Assay conditions for the various DNA polymerase activities. The basic assay (Pedrali-Noy & Weissbach, 1977) for the determination of DNA polymerase activities (which represents the optimal conditions for the α polymerase) contained in 200 μl: 90 μg of bovine serum albumin, 50 mM-tris-HCl, pH 8·0, 10 mM-$MgCl_2$, 0·5 mM-dithiothreitol, 100 μg of activated (Weissbach *et al.* 1973) calf thymus DNA and 0·1 mmol of all four deoxyribonucleoside triphosphates with ^{3}H-thymidine triphosphate as labelled substrate (sp. act. 100 ct/min/pmol). For the determination of β polymerase activity the assay was supplemented with 50 mM-KCl, for herpes simplex virus-induced DNA polymerase with 100 mM-KCl (Powell & Purifoy, 1977); 250 mM-KCl were found to be strongly inhibitory for the virus enzyme (Weissbach *et al.* 1973).

The γ polymerase activity was determined (Knopf *et al.* 1976) in 200 μl assays containing: 90 μg of bovine serum albumin, 50 mM-tris-HCl, pH 8·0, 0·5 mM-$MnCl_2$, 150 mM-KCl, 2·5 mM-dithiothreitol, 5 μg $A_n.dT_{12}$ and 0·1 mM-^{3}H-thymidine triphosphate (sp. act. 400 ct/min/pmol). Twenty to 50 μl of enzyme solution were employed per assay. If phosphonoacetic acid was present it was included at a final concentration of 10 μg per assay.

Incubations were usually performed in duplicate in two separate experiments for 30 min at 37 °C. The reaction was terminated by addition of 1 ml cold 10% (w/v) trichloroacetic acid containing 10 mM-sodium pyrophosphate prior to processing for acid insoluble radioactivity.

Determination of radioactivity and protein. Acid-insoluble radioactively-labelled material was collected under suction on nitrocellulose membrane filters. After thorough washing with 5% (w/v) cold trichloroacetic acid the filters were dried at 60 °C and radioactivity determined in a Beckman liquid scintillation counter (LS 230) using a toluene based counting cocktail (Radsak *et al.* 1971).

Protein was determined according to the method of Lowry *et al.* (1951).

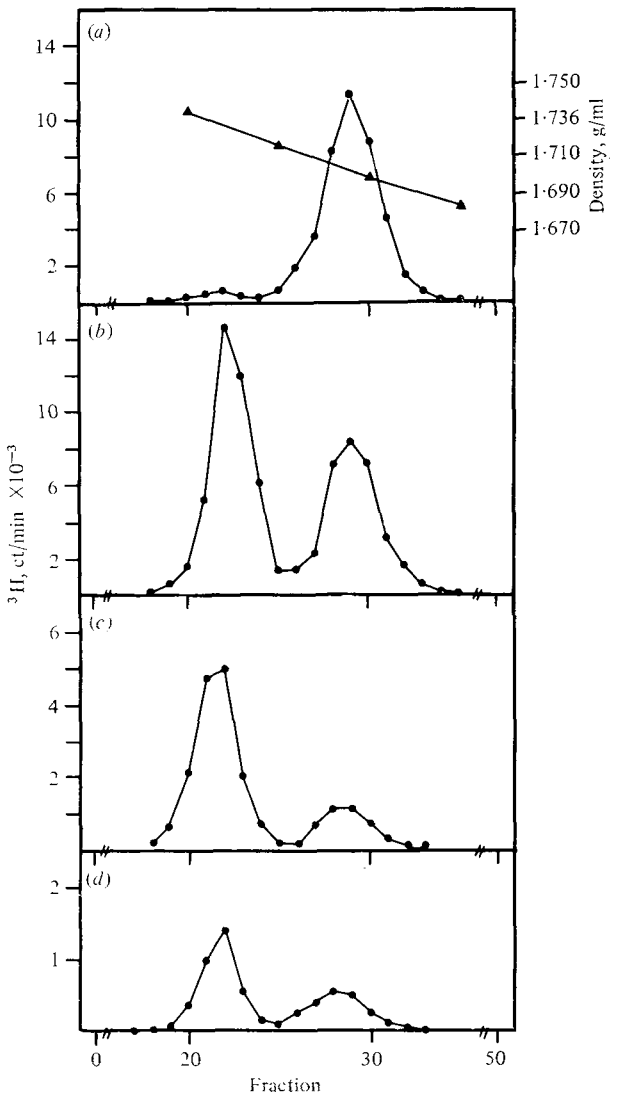

Fig. 1. Isopycnic sedimentation in CsCl (mean density 1·7 g/ml) of approx. 12 μg DNA extracted from virus-infected thymidine kinase-deficient mouse cells exposed to 2 h pulses with ^{3}H-thymidine (5 μCi/ml culture medium; sp. act. 62 Ci/mmol) at various times post-infection. (*a*) Pulse interval from 1 to 3 h; (*b*) from 7 to 9 h; (*c*) from 13 to 15 h and (*d*) from 23 to 25 h p.i. Bottom of the gradients is to the left. The heavy peaks (1·725 g/ml) represent viral and the light peaks (1·7 g/ml) cellular DNA. Sedimentation conditions: 36000 rev/min, 65 h, 20 °C in a 50Ti fixed-angle rotor of a Beckman centrifuge.

Chemicals. ^{3}H-thymidine (sp. act. 62 Ci/mmol) and ^{3}H-thymidine triphosphate (sp. act. 21 Ci/mmol) was purchased from Amersham Buchler, Braunschweig, F.R.G.; cold deoxyribonucleoside triphosphates and $A_n.dT_{12}$ from Böhringer, Mannheim, F.R.G.; nitrocellulose membrane filters from Sartorius, Göttingen, F.R.G.; DEAE- (DE-52) and phosphocellulose (P-11) from Whatman Biochemicals, Maidstone, U.K.; phosphonoacetic acid from ICN Lab., Plainview, U.S.A.; all other chemicals from Merck, Darmstadt, F.R.G.

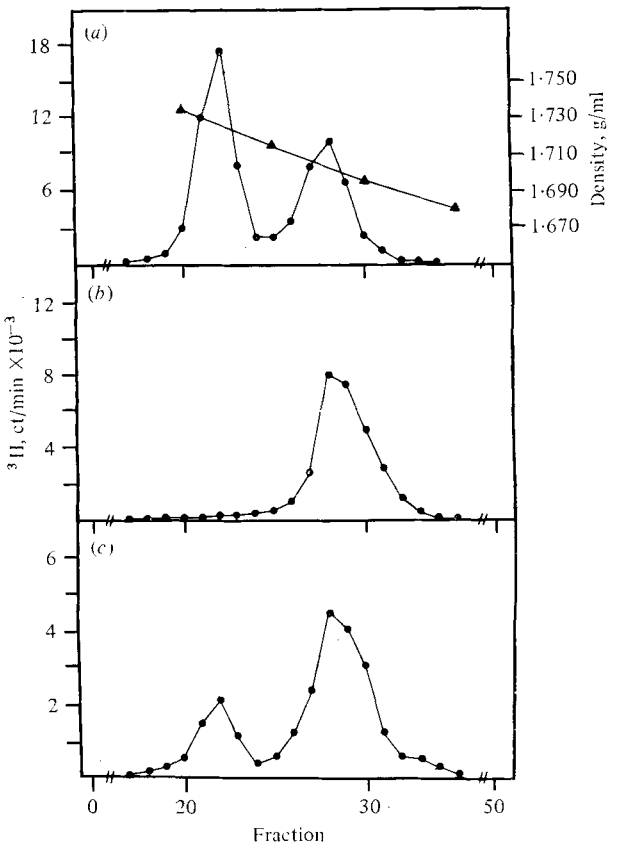

Fig. 2. Isopycnic sedimentation in CsCl (mean density 1·7 g/ml) of approx. 12 μg DNA extracted from virus-infected thymidine kinase-deficient mouse cell cultures exposed to 2 h pulses with ^{3}H-thymidine (5 μCi/ml culture medium; sp. act. 62 Ci/mmol) from 7 to 9 h p.i. (*a*) Untreated virus-infected cells corresponding to Fig. 1(*b*); (*b*) virus-infected cells were exposed to phosphonoacetic acid (200 μg/ml culture medium) starting 1 h p.i.; (*c*) virus-infected cells were exposed to phosphonoacetic acid (200 μg/ml culture medium) starting 6 h post-infection. The heavy peaks (1·725 g/ml) represent viral and the light peaks (1·7 g/ml) cellular DNA. Sedimentation conditions: 36000 rev/min, 65 h, 20 °C in a 50Ti fixed-angle rotor of a Beckman centrifuge.

RESULTS

Virus and cellular DNA synthesis in the course of the infectious cycle

It has been reported that the amount of cellular DNA synthesized during the infectious cycle appears to depend on the type of cell cultures used for infection (Jacob & Roizman, 1977). In order to define the situation for the cells used, parallel cultures of 4×10^6 Ci-1D cells infected with a multiplicity of approx. 10 were given 2 h pulses with ^{3}H-thymidine (5 μCi/ml) at various times post-infection (p.i.). After lysis of the cells subsequently to the pulse, the labelled DNA was analysed by isopycnic centrifugation in CsCl. The amounts of radioactivity banding in the position of virus (1·725 g/ml) and cellular DNA (1·7 g/ml) were considered representative for virus and cellular DNA synthesis, respectively.

Thymidine kinase-deficient cells only incorporate exogenous thymidine, in this case, after infection with herpes simplex virus, so that there is no background of precursor incorporation into uninfected cells. Furthermore, the high multiplicity used for infection ensured that

Table 1. *DNA polymerase activity in total cellular extracts from control- and virus-infected cell cultures*

Time after mock- or virus-infection	Units* of DNA polymerase activity as determined		
	Without salt	With 100 mM-KCl	With phosphonoacetic acid†
1 h mock	7·6	5·3	3·6
1 h virus	8·0	6·2	4·1
5 h mock	7·7	4·2	3·5
5 h virus	26·6	34·7	4·5
5 h virus‡	30·0	39·2	4·6
9 h virus	61·6	80·0	4·1
9 h virus‡	39·8	53·0	3·6

* Units per 4×10^7 cells; one unit of DNA polymerase is defined as the activity that catalyses the incorporation of 1 nmol ^{3}H-TTP into an acid-insoluble form in 30 min.

† Assays were performed in the presence of 10 μg phosphonoacetic acid.

‡ Cells were treated with 200 μg phosphonoacetic acid/ml culture medium starting at 1 h p.i.

more than 95% of the cells were infected as judged by the cytopathic effect which was evident by 5 to 7 h p.i.

Fig. 1 shows that virus DNA synthesis is at its maximum in the pulse interval 7 to 9 h p.i. and decreases in the subsequent pulses. In contrast, although appreciable incorporation occurs into cellular DNA throughout the infectious cycle its synthesis progressively decreases, particularly in the late phase, when virus DNA synthesis is also already declining. When pulse labelling was performed on infected cells treated with phosphonoacetic acid, precursor incorporation occurred exclusively into DNA with the density of cellular DNA if the drug (200 μg/ml) was added immediately after virus adsorption at 1 h p.i. (Overby *et al.* 1974; Mao *et al.* 1975; Fig. 2*b*). Addition of the drug at 6 h p.i., however, with pulse-labelling 1 h later, revealed that virus DNA synthesis could not be suppressed completely under these conditions although the concentration of the drug used was four times that which effectively inhibited the activity of the partially-purified virus enzyme *in vitro*.

Activity of the cellular DNA polymerases in the course of the infectious cycle

DNA polymerase activity in total cellular extracts

In order to examine cellular DNA polymerase activity during the interval of the infectious cycle when virus DNA synthesis was increasing and cellular DNA synthesis still very prominent, parallel cultures of 4×10^7 cells were infected with a multiplicity of 10 and total cellular extracts prepared from one of the cultures at different times post-infection. In some of the experiments the cells were treated with phosphonoacetic acid (200 μg/ml) subsequent to virus adsorption.

Table 1 demonstrates that there is a several-fold increase in total salt-dependent DNA polymerase activity after infection which is not as pronounced at 9 h post-infection for cells kept in phosphonoacetic acid. Taking advantage of the selective inhibitory effect of phosphonoacetic acid *in vitro* on the virus-induced DNA polymerase, it was found that the phosphonoacetic acid-resistant DNA polymerase activity rose in the extracts from infected over that from control cells (Table 1). In extracts from cells treated with phosphonoacetic acid this effect could only be observed at 5 h p.i. Of course, the cell α polymerase is also sensitive to phosphonoacetic acid, but to a lesser extent (Bolden *et al.* 1975).

Table 2. *DNA polymerase activities in cellular fractions from control- and virus-infected cell cultures*

	Units* of DNA polymerase activity as determined without (−) and in the presence of phosphono-acetic acid (+)†					
			Nuclear fractions§			
	Cytoplasm		0·02 M-eluate		0·25 M-eluate	
Time after mock- or virus-infection	−	+	−	+	−	+
1 h mock	10·2	2·3	1·7	1·4	2·8	1·96
1 h virus	11·9	1·53	1·75	1·28	2·6	1·87
5 h mock‡	6·8	2·8	1·7	1·62	2·7	2·0
5 h virus	24·8	0·75	4·05	3·09	9·8	2·33
5 h virus‡	23·9	2·0	3·92	2·66	16·2	3·4
9 h mock‡	7·2	1·84	1·6	1·38	3·7	2·7
9 h virus	45·3	0·56	4·48	3·2	37·3	2·52
9 h virus‡	43·2	2·2	4·06	2·82	17·6	2·05

* Units per 8×10^7 cells; one unit of DNA polymerase is defined as the activity that catalyses the incorporation of 1 nmol ^{3}H-TTP into an acid-insoluble form in 30 min.

† Assays were performed in the presence of 10 μg phosphonoacetic acid.

‡ Cells were treated with 200 μg phosphonoacetic acid/ml culture medium starting at 1 h p.i.

§ DNA polymerase activity of DNA-free dialysed nuclear extracts was adsorbed to DEAE cellulose columns and eluted stepwise with 0·02 M- and 0·25 M-potassium phosphate buffer, pH 7·5, as described in Methods.

DNA polymerase activity in extracts from cell fractions

In order to relate these moderate variations in total cellular extracts to the cellular DNA polymerases α, β and γ, parallel cultures of 8×10^7 cells were infected at a multiplicity of 10, harvested at different times post-infection and fractionated as described in Methods into nuclei and cytoplasm. In several experiments the cytoplasm was further fractionated into a mitochondrial and post-mitochondrial fraction. After preparation of extracts from these cell fractions the nuclear extract was further fractionated by means of adsorption to DEAE cellulose and step-wise elution as described above. This procedure resulted in a reproducible distribution of the DNA polymerases in the extracts obtained: most of the α polymerase activity will be found in the post-mitochondrial extract. Although this observation is largely an artefact of cell fractionation in aqueous media (Pedrali-Noy & Weissbach, 1977), it is not of importance in this context where only the pattern of the total activity of a given cellular enzyme was determined. In the case of infected cells the post-mitochondrial fraction also contains large amounts of the virus-induced enzyme. Relatively small amounts of a γ polymerase are also present. The two fractions of the nuclear extract contain the β polymerase, on one hand, and the γ polymerase, on the other hand, as well as the virus enzyme if the cells are infected. The mitochondrial γ polymerase is restricted to the mitochondrial extract.

Table 2 shows the distribution of the roughly separated DNA polymerase activities, as determined without and in the presence of phosphonoacetic acid. It is apparent that both the cytoplasmic extract and the 0·25 M-eluate of the nuclear extract contain virus-induced enzyme. The phosphonoacetic acid-resistant activities of these extracts behave differently however. The progressively decreasing activity in the cytoplasm suggests that α polymerase activity is quickly and progressively lost in the course of the infectious cycle, whereas the fraction containing the nuclear γ polymerase (0·25 M-eluate) maintains its activity and even

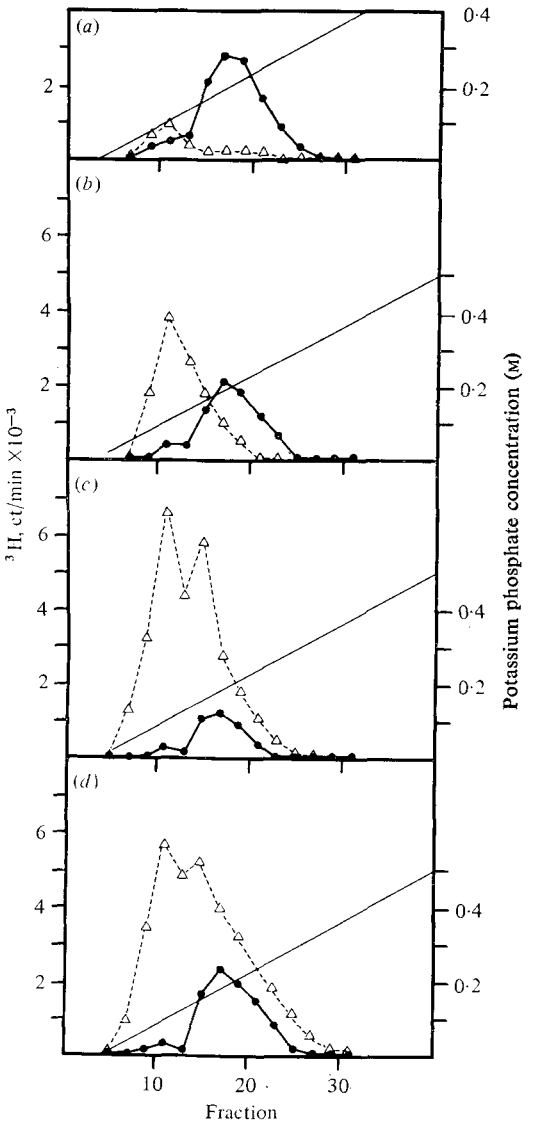

Fig. 3. DEAE cellulose chromatograms of the DNA polymerase activity of post-mitochondrial supernatants from virus-infected cells harvested (*a*) 1 h, (*b*) 5 h, (*c*) 9 h p.i. and of post-mitochondrial supernatant from infected cells treated with 200 μg phosphonoacetic acid/ml culture medium from 1 to 9 h p.i. DNA polymerase activity was solubilized and freed of contaminating DNA as described in Methods. The dialysates were adsorbed to DEAE cellulose columns (approx. 1 mg of protein/ml column vol.) and eluted with a gradient of 0·02 M- to 0·5 M-potassium phosphate, pH 7·5, 0·5 mM-dithiothreitol, 0·2 % Triton X-100 and 20 % glycerol. Between 35 and 40 fractions were collected. DNA polymerase activity was determined with 20 μl fractional vol. using activated DNA in the presence of 100 mM-KCl (△---△) and without salt in the presence of 10 μg phosphonoacetic acid/assay (●—●). Alpha polymerase activity is inhibited by 75 % at this drug concentration, virus DNA polymerase activity is completely abolished. The diagram for mock-infected cells which was identical to that 1 h post-infection is not shown. Incubations were for 30 min prior to determination of acid-insoluble radioactivity.

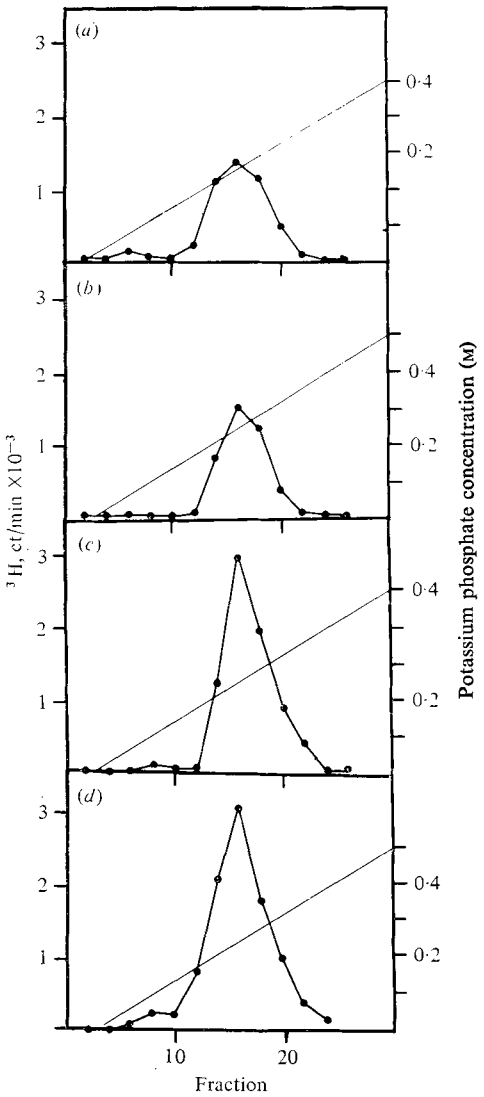

Fig. 4. Phosphocellulose chromatograms of the β polymerase activity from nuclei of (*a*) mock-infected cells harvested (*b*) 1 h, (*c*) 5 h, and (*d*) 9 h p.i. DNA polymerase activity was solubilized and freed of contaminating DNA as described in Methods. The dialysates were adsorbed to DEAE cellulose columns (approx. 1 mg of protein/ml column vol.) and washed with one column vol. of 0·02 M-potassium phosphate. This eluate was subsequently adsorbed to phosphocellulose columns (2 ml volume) and the DNA polymerase activity eluted with a gradient of 0·02 to 0·5 M-potassium phosphate, pH 8·0, 0·5 mM-dithiothreitol, 0·2 % Triton X-100 and 20 % glycerol. Between 30 and 35 fractions were collected. DNA polymerase activity was determined with 50 μl fractional volume under β polymerase conditions (see Methods) in the presence of 10 μg phosphonoacetic acid. Incubations were for 30 min prior to determination of acid-insoluble radioactivity.

shows a slight increase. The phosphonoacetic acid-resistant activity in the mitochondria exhibited a similar pattern (not shown).

The only significant increase of phosphonoacetic acid-resistant activity in the course of the infection was consistently observed in the fraction representing the β polymerase activity (0·02 M-eluate). Different results were obtained for the activities of extracts from

Table 3. *Purification of nuclear γ polymerase from control- and virus-infected cell cultures*

Purification step	Total protein of control- and virus-infected cells (mg) Time p.i. (h)				Total DNA polymerase units* of control- and virus-infected cells Time p.i. (h)			
	C†	1	5	9	C†	1	5	9
Cell homogenate	12·5	11·5	12·5	13·5	—	—	—	—
Isolated nuclei	6·36	5·57	5·96	6·82	—	—	—	—
Nuclear dialysate	3·49	3·23	3·56	3·78	1·28	1·2	1·5	1·3
0·25 M-DEAE cellulose eluate	1·27	1·09	1·25	1·17	0·97	0·92	1·26	1·03
Phosphocellulose	0·9	0·83	0·9	0·82	0·45	0·39	0·56	0·50

* Units per 8×10^7 cells; one unit of DNA polymerase is defined as the activity that catalyses the incorporation of 1 nmol ^{3}H-TTP into an acid-insoluble form in 30 min.
† Mock-infected control cells.

phosphonoacetic acid-treated infected cells. The effect of the virus infection on the activities of the cellular DNA polymerases was less pronounced, the inhibition of phosphonoacetic acid-resistant DNA polymerase activity in the cytoplasmic fraction was hardly evident at 9 h post-infection. The activity patterns of the cellular DNA polymerases in the nuclear extracts, on the other hand, were comparable to those of untreated infected cells.

Chromatographic analysis of the cellular DNA polymerase activities

In order to verify these observations on more purified enzyme preparations the extracts from the cellular fractions were subjected to ion exchange chromatography.

Fig. 3 shows representative patterns for the elution from DEAE cellulose of the DNA polymerase activities as determined under various conditions from the post-mitochondrial supernatants of different sets of cells harvested at 1, 5 and 9 h post-infection. As expected, the residual phosphonoacetic acid-resistant α polymerase activity (which is inhibited 75% at 10 μg phosphonoacetic acid/assay) eluting at 0·18 M-potassium phosphate (Weissbach *et al.* 1971, 1973) was clearly decreasing in the course of the infection, whereas the effect of virus infection in phosphonoacetic acid-treated cells was less pronounced as already suggested by the results with crude extracts (Table 2).

Fig. 4 demonstrates phosphocellulose chromatography of the β polymerase preparations isolated from infected cells at different intervals post-infection as determined in the presence of phosphonoacetic acid. This result is consistent with a stimulation of the β polymerase activity in infected cells which is in accordance with the analysis in Table 2.

Table 3 presents the results for the nuclear γ polymerase. Compared to the data given in Table 2 the chromatographic analysis revealed slightly higher γ activities in the samples originating from cells which had been infected for 5 and 9 h, respectively.

The mitochondrial γ polymerase which represents approximately one half of the total cellular γ polymerase activity in the cells used (Bertazzoni *et al.* 1977; Bolden *et al.* 1977) showed slight variations in activity during infection (data not shown) which were directly comparable to that of the nuclear γ polymerase.

DISCUSSION

Unlike other herpes viruses which stimulate cellular DNA synthesis (Gerber & Hoyer, 1971; St Jeor *et al.* 1974) herpes simplex virus has been shown progressively to inhibit cellular DNA synthesis (Ben-Porat & Kaplan, 1973). However, the progression of inhibition depends on the type of cells used for experimental infection (Jacob & Roizman, 1977). Furthermore, it has been reported that herpes simplex virus replication is sensitive, although less so than cytomegalovirus, to a pre-treatment of the host cells by u.v.-light, suggesting that herpes simplex virus is also dependent on u.v.-sensitive host cell functions (Furukawa *et al.* 1975). More rigorous analysis revealed that the kinetics of cellular recovery from u.v.-irradiation to support virus DNA synthesis and virus production appears to be similar to the time course of the recovery of their capacity to transcribe 45S RNA (Coppey & Nocentini, 1976) and to divide. In addition, it has been shown that the cellular RNA polymerases maintain appreciable levels of activity until several hours post-infection (Preston & Newton, 1976). These observations are compatible with the view that an intact cellular genome for the transcription of some cellular gene products is essential for the replication of virus DNA. This would require an unimpaired repair enzyme system in infected cells for which herpes virus-induced nucleases might produce additional substrates (Weissbach *et al.* 1973; Kolber, 1975) as has been recently suggested.

Our results demonstrating an increasing activity after infection of the β polymerase, which has been shown by several laboratories to double in activity only in the G 2 phase and thus to be involved in DNA repair (Spadari & Weissbach, 1974; Chiu & Baril, 1975), are consistent with the view that cellular DNA repair enzymes are induced in infected cells for the repair of some essential cellular DNA and possibly virus DNA (Morse *et al.* 1977). The latter seems particularly likely since a pulse with phosphonoacetic acid during the interval of maximum virus DNA synthesis reduced but did not abolish virus DNA synthesis. A several-fold lower concentration of this drug stops the action of the herpes simplex virus-induced DNA polymerase *in vitro* immediately and also inhibits the activity of the cellular α polymerase (Bolden *et al.* 1975), the presumed cellular replication enzyme (Spadari & Weissbach, 1974), to a much higher extent than the β and γ polymerases.

Cellular protein synthesis is efficiently suppressed following infection with herpes simplex virus (Ben-Porat & Kaplan, 1973) and there appear to be specific changes in the cellular DNA polymerase activities after the drug-mediated suppression of protein synthesis (Pedrali-Noy & Weissbach, 1977). Within 7 h of cycloheximide treatment of HeLa cells the activity of the β polymerase is diminished by two thirds, whereas that of the nuclear γ polymerase slightly increases and the total activity for α and α_1 remains unchanged. These observations are in obvious contrast to the present results for the α and β polymerase activities in infected cells. The kinetics of the γ polymerase after infection seem to be similar to that after cycloheximide treatment. On the other hand, analysis of the RNA polymerase activities in herpes simplex virus infected cells (Preston & Newton, 1976) has shown that the effect of the virus infection and that of cycloheximide are apparently not directly comparable. It might well be that the increase in activity of the β polymerase after infection is not due to an induction of the enzyme but to an activation the mechanism of which remains to be clarified. Suggestive evidence for such an alteration has been recently reported for the α-amanitin-sensitive RNA polymerase in herpes simplex virus-infected cells (Preston & Newton, 1976).

Analysis of the DNA polymerase activities from herpes simplex virus-infected cells has to take into account that this virus leads to the induction of specific nucleases (Weissbach

et al. 1973; Powell & Purifoy, 1977). DNA polymerase activities cannot be determined exactly in the presence of large amounts of nuclease (Powell & Purifoy, 1977). Thus our results, in particular for the α polymerase activity in crude extracts, might suffer from this fact. However, the chromatographic behaviour of the virus nuclease (Powell & Purifoy, 1977) makes it unlikely that there is any interference with the activities of the determination of the cellular DNA polymerases during chromatographic separation.

Our experiments showed that there are some differences in the DNA polymerase activity patterns in cells which had been exposed to phosphonoacetic acid after virus infection. In general, the effect of the virus infection was less significant, the virus-induced enzyme did not reach as high an activity as in untreated cells, the α polymerase activity was hardly changed, whereas β and γ polymerase activities behaved similarly to those in untreated cells. The lack of a further linear increase of virus DNA polymerase activity beyond 5 h post-infection could result from the lower amount of virus genome information in drug-treated cells. It is not clear at present why the activity of the α polymerase should behave differently than that in untreated infected cultures.

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This paper is dedicated to Professor Dr P. Karlson on the occasion of his 60th birthday.

Note added in proof: While this manuscript was under consideration a report was published by Müller, W. E. G., Zahn, R. K. & Falke, D. (*Virology* **84**, 320–330, 1978) describing variations for the cellular DNA polymerases α and β in rabbit kidney cells following infection with herpes simplex virus type 1. The observation of these authors is directly comparable with the results described here.

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