Cell-Dependent Differences in the Production of Infectious Herpes Simplex Virus at a Supraoptimal Temperature

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The replication of herpes simplex virus (HSV) was compared in rabbit and hamster cells at optimal and supraoptimal temperatures. Replication occurred in cells of either species at 33 C, but the total infectious virus yield was routinely about 10-fold greater in rabbit cells than in hamster cells. At 39 C, this difference was exaggerated to greater than 100,000-fold. Whereas infectious virus was produced and plaques formed in rabbit kidney cell monolayers at the higher temperature, neither developed in those derived from hamster embryos. Elevating the temperature from 33 C to 39 C at various time intervals after exposure of the cultures to virus revealed that production of infectious virus in hamster cells was completely heat-sensitive up to 6 hr after infection. Specific viral antigens and viral deoxyribonucleic acid (DNA) were synthesized in both rabbit and hamster cell cultures. In addition, cellular DNA synthesis was depressed and cytopathic effects occurred in both cell systems. These cytopathic effects were not observed in cell cultures treated with HSV previously inactivated with ultraviolet light. Compared with parallel cultures at 33 C, the amount of viral DNA synthesized at 39 C was greatly reduced in both systems. In hamster cells, the reduction was twofold greater than in rabbit cells. This cell-dependent thermal inhibition of HSV replication in hamster cells did not occur with vaccinia virus.

Previous reports based mainly on seroepidemiological data (12, 14, 16) have suggested that herpes simplex viruses (HSV) may be oncogenic. Recently, this possibility was further substantiated by the observation (2, 3) that foci of transformed cells develop after exposure of hamster cell cultures to inactivated HSV type 2 (HSV-2); these cells were readily oncogenic when injected into experimental animals. In this work, the virus preparation examined was modified by ultraviolet (UV) irradiation prior to its interaction with hamster embryo cell cultures. This was necessary because HSV characteristically causes a productive and cytotoxic infection in cell cultures derived from a wide variety of animals. These infections are characterized by synthesis of infectious virus, depression of cell macromolecular synthesis (1, 18, 20), severe chromosome damage (17), and other changes ultimately resulting in cell death. Since cytotoxic effects obviously preclude observations of oncogenic transformation, it is imperative either that the virus itself be modified or that conditions nonpermissive for viral replication be employed to prevent cell destruction.

In an attempt to find such nonpermissive conditions, we investigated the effect of altering the incubation temperature on viral replication. Elevating the temperature has been reported to inhibit productive HSV infection in various cell cultures (4, 6, 10, 19, 21, 22). Therefore, we examined the effect of incubating HSV-infected hamster and rabbit cells at 39 C. As a result, we observed a dramatic host-dependent difference in the thermal sensitivity of HSV replication in these two cell systems. Under identical culture conditions, infectious virus was produced in rabbit kidney cells, but none could be detected in those derived from hamster embryos. In this report, we compare the replication of HSV in these two cell systems under permissive and nonpermissive conditions.

MATERIALS AND METHODS

Cell cultures. Primary cell cultures were prepared from the kidneys of 3-week-old New Zealand white rabbits (RK) and from decapitated 12-day-old LSH strain hamster embryos (HEF) as previously described (11). The cells were grown at 37 C in 8-oz (237-ml) glass prescription bottles in Eagle's medium supple-

mented with 10% fetal calf serum (FCS), 10% Tryptose phosphate broth (TPB), 0.075% sodium bicarbonate, 100 units of penicillin/ml, 100 μ g of streptomycin/ml, and 100 μ g of kanamycin/ml. Unsealed vessels were incubated in a humidified atmosphere containing 5% CO₂ in air, and the amount of sodium bicarbonate buffer was increased to 0.23%. At confluency, and after virus inoculation, the cultures were renourished with a maintenance medium consisting of Eagle's medium with 2.5% FCS, 10% TPB, 0.23% sodium bicarbonate, and the usual concentrations of antibiotics.

Viruses. HSV-2 (strain 307) was obtained from William Rawls, Baylor College of Medicine, Houston, Tex. Virus stocks were prepared by inoculating primary RK monolayers in 8-oz prescription bottles with approximately 5×10^8 plaque-forming units (PFU) of HSV-2 in 0.5 ml. After virus adsorption at room temperature for 2 hr, 5 ml of maintenance medium was added, and the cultures were incubated at 33 C. At 36 to 48 hr after infection, the cultures were frozen and thawed once, treated for 1 min in a Branson Sonifier (Cole-Parmer Instrument & Equipment Co.), and clarified by centrifugation at $600 \times g$ for 5 min. The virus-containing supernatant fluids were stored at -65 C and had titers of greater than 10^8 PFU/ml.

The WR strain of vaccinia virus was also obtained from Dr. Rawls. Stocks were prepared in the same manner as described for HSV, except that incubation was at 37 C rather than at 33 C.

Virus infection. Virus-cell ratios of greater than 10 PFU/cell were used in all experiments (excluding plaque assays) to initially infect greater than 90% of the cells in each culture and to insure single virus replicative cycles at 33 C and at 39 C. The number of HEF and RK cells per culture was kept similar for comparative purposes and never varied more than 20%. Virus was adsorbed at room temperature for 2 hr before incubation at the test temperatures to avoid possible virus multiplicity discrepancies resulting from different rates of thermal inactivation of virus at 33 C and at 39 C.

Infectious virus assay. A previously described plaque technique (7) was used to quantitate infectious virus. Replicate infected cultures to be assayed were frozen and thawed, pooled, sonically treated, and diluted serially 10-fold in 0.025 M tris(hydroxymethyl)-aminoethane (Tris)-saline (pH 7.4), containing 2.5% bovine serum. Confluent monolayers of primary RK cells in petri dishes (60 by 15 mm; Falcon Plastics) were then inoculated with 0.2 ml of the diluted culture material. After virus adsorption, each monolayer was overlaid with 4 ml of Eagle medium containing 0.5% methylcellulose (Matheson, Coleman & Bell), 5% bovine serum, 0.23% sodium bicarbonate, and the usual concentrations of antibiotics. Assays for HSV were incubated at 33 C and those for vaccinia virus at 37 C. Four days after inoculation, the cultures were stained with a 1/7,500 solution of neutral red in Tris-saline, and the plaques were counted 1 to 2 hr later.

Immunofluorescence tests. Confluent monolayers of HEF and RK cells on 18-mm circular glass cover slips were placed into dry plastic petri dishes (35 by 10 mm) and inoculated with 0.1 ml of HSV (10 PFU/cell). Controls received 0.1 ml of medium. The virus was

adsorbed, 2 ml of maintenance Eagle medium was added, and the cultures were incubated at 33 or 39 C. At various times after infection, replicate cultures were rinsed in Tris-saline, air-dried, and fixed in acetone for 5 min. The fixed monolayers were flooded with specific rabbit anti-HSV serum or normal rabbit serum and allowed to react at room temperature for 30 min. The cells were then washed in Tris-saline and flooded with horse anti-rabbit gamma globulin conjugated to fluorescein isothiocyanate. After exposure at room temperature for 30 min, the cultures were again rinsed in Tris-saline. Finally, the cover slips were mounted on glass slides in Tris-buffered glycerol (pH 7.3) and observed for specific, intracellular immunofluorescence by use of a Zeiss fluorescence microscope.

DNA analysis. Confluent HEF and RK monolayers in 1-oz (ca. 30 ml) glass prescription bottles were inoculated with 0.1 ml of HSV (10 PFU/cell). Controls received 0.1 ml of medium. After virus adsorption, virus-treated and control monolayers were rinsed twice with 5 ml of Tris-saline and then covered with 1 ml of maintenance Eagle medium containing 10 μ Ci of thymidine-methyl-³H (³H-TdR; specific activity, 20 Ci/mmol; New England Nuclear Corp.). One group of HSV-infected and control cultures was incubated at 33 C, and another group at 39 C. Replicate isotope-labeled cultures were analyzed 22 hr later for the amount of ³H-TdR incorporated into acid-insoluble deoxyribonucleic acid (DNA). The cultures were quickly frozen in dry ice and thawed, and the replicate samples were carefully pooled quantitatively into glass tubes. Each pooled sample was digested for 2 to 3 hr at 37 C with 0.1% Sarkosyl NL 30 (Geigy Chemical Corp.), 0.02 M ethylenediaminetetraacetic acid, and 0.1% heat-inactivated Pronase (Calbiochem, B grade). The Sarkosyl and Pronase were prepared in 0.015 M sodium chloride and 0.0015 M sodium citrate (0.1 $\times$ SSC, pH 7.3). After digestion, 0.2 ml of sample was mixed with 3.8 ml of 0.1 $\times$ SSC-buffered cesium chloride (Harshaw Chemical Co., $\rho = 1.745$ g/cc) and centrifuged at 30,000 rev/min for 60 hr at 17 C in a Beckman L2-65B ultracentrifuge with a 40.3 fixed-angle rotor. Ten-drop fractions were collected from the bottom of each gradient onto filter-paper discs (Whatman, 3 mm), and acid-insoluble material was precipitated by treatment with 5% trichloroacetic acid at room temperature. The filters were then dehydrated in ethyl alcohol and ether, air-dried, and placed in 10 ml of Omnifluor (New England Nuclear Corp.); the ³H-TdR was measured in a Beckman LS-250 liquid scintillation counter. Refractive indexes of periodic fractions were determined, by use of a Bausch & Lomb refractometer, to obtain the gradient densities.

RESULTS

The effect of elevated temperature on plaque formation by HSV in RK and HEF cell cultures was first investigated. Confluent cultures were inoculated with serially diluted virus according to the procedure described above for infectious virus assay. After virus adsorption and the addition of overlay medium, the cultures were divided into two groups and incubated at 33 C or at 39 C. Four

days later, the cell monolayers were examined for the presence of discrete virus plaques.

The results of a typical experiment are presented in Table 1. About the same number of plaques of approximately the same size (2 to 3 mm) developed in both RK and HEF cultures at 33 C. In contrast, a marked difference was observed at 39 C. Although the number was always 25 to 85% less than at 33 C, plaques 1.5 to 2.5 mm in size routinely formed in RK monolayers. No plaques, however, were detected in HEF cell cultures. Even when the inoculum contained 1,000 times more infectious units, no focal spread of virus occurred in HEF monolayers. These monolayers were grossly affected only at HSV concentrations sufficient to infect most of the cells. Under such conditions, cells throughout the culture became rounded, indicating widespread cytopathic effects (CPE). When the plaque-forming potential of vaccinia virus was similarly examined, this extreme thermal inhibition in HEF cells did not occur. Although 40 to 60% fewer plaques formed at the elevated temperature, no significant variation was found between RK and HEF monolayers (Table 1).

In conjunction with plaque formation, RK and HEF cultures were examined for total infectious virus production at 33 C and at 39 C. Confluent monolayers in tube cultures were treated with 0.1 ml of HSV (20 to 40 PFU/cell). After virus adsorption, each culture was washed twice with Tris-saline and replenished with 1 ml of maintenance medium. The cultures were then divided into two groups and incubated at 33 C or at 39 C. At various times after infection, replicate cultures were selected, quick-frozen, and assayed for total infectious virus as described in Materials and Methods.

Table 2 presents the total infectious virus yields 24 hr after infection. At 33 C, titers were always at least 10-fold higher in RK than in HEF cell cultures. At 39 C, the virus yield was reduced in both systems. In HEF cultures, this reduction was much more pronounced. Whereas greater than 10^5 PFU of HSV were still found in RK cultures at 39 C, none could be detected even in undiluted HEF culture material. Although this yield in RK cells at 39 C was less than 1 PFU/cell (based on 3×10^5 to 4×10^5 cells/culture), it did represent progeny HSV. At 6 hr after infection, both RK and HEF cultures contained only 10^2 to 10^3 PFU of residual input virus (Crouch, *unpublished observations*). In comparison, production of infectious vaccinia virus was not significantly reduced in either cell system at the elevated temperature.

Microscopic examination of the HSV-inoculated cell cultures 24 hr after infection revealed

TABLE 1. *Effect of incubation temperature on virus plaque formation*

Virus ^a	Cell mono-layer ^b	Incubation temp (C)	Virus dilution ^c	No. of plaques ^d	Plaque ratio 39/33 C (%)
Herpes simplex	RK	33	10^{-5}	43	19
		39	10^{-4}	83	
	HEF	33	10^{-5}	42	
		39	10^{-2}	0	
Vaccinia	RK	33	10^{-6}	102	<0.002
		39	10^{-6}	64	
	HEF	33	10^{-6}	97	
		39	10^{-6}	54	

^a A 0.2-ml amount adsorbed at room temperature for 2 hr.

^b RK, secondary rabbit kidney; HEF, secondary hamster embryo fibroblast cultures.

^c Dilution at which plaques were enumerated.

^d Average of replicate monolayers 4 days after infection.

TABLE 2. *Effect of incubation temperature on infectious virus production*

Virus ^a	Cells ^b	Incubation temp (C)	Infectious virus yield ^c (PFU/culture)
Herpes simplex	RK	33	4.8×10^7
		39	1.9×10^5
	HEF	33	9.8×10^5
		39	$<7.5 \times 10^{0d}$
Vaccinia	RK	37	9.7×10^6
		39	4.7×10^6
	HEF	37	9.6×10^6
		39	7.3×10^6

^a Amounts of 20 to 40 PFU/cell adsorbed at room temperature for 2 hr.

^b RK, secondary rabbit kidney; HEF, secondary hamster embryo fibroblast cultures.

^c Total PFU 24 hr after infection.

^d No infectious virus present even in undiluted culture material.

CPE in greater than 90% of each monolayer, regardless of the cell system or incubation temperature. Essentially all of the cells were rounded and many were detached from the glass surface.

To ascertain how long after infection HSV production remained temperature-sensitive, temporal studies were conducted. The experimental procedure was identical to that described above for assaying total infectious virus yields, except that all of the cultures were initially incubated at 33 C and then replicate cultures were transferred to 39 C at various intervals after infection. At 24

hr after the initial infection, all cultures were assayed for total infectious HSV.

As shown in Table 3, the temperature shift affected the virus yield in both RK and HEF cultures, but the effect in HEF cells was much more dramatic. No infectious HSV could be detected in HEF cultures shifted to 39 C up to 4 hr after infection. Even at 8 hr, elevating the temperature partially reduced the virus yield. In other experiments, no infectious HSV was produced in HEF cultures shifted to 39 C at 6 hr after infection. In contrast, although less infectious virus was present in RK cultures shifted to 39 C early after infection, they still contained significant quantities of virus.

Virus cytopathology and specific viral antigen formation. Experiments were conducted to determine whether the extensive CPE observed at 39 C in HEF cultures treated with virus at high multiplicities resulted from initial virus-directed events or from cytotoxicity of the undiluted inoculum. Tube cultures identical to those described in the previous section were treated as before with HSV. In addition, parallel cultures were treated with the same inoculum after eliminating infectivity by exposure to UV light. At various times after virus inoculation, unfixed, unstained cultures were examined microscopically for gross CPE.

Under permissive conditions, strain 307 caused cytopathology characterized by cell rounding and eventual detachment from the glass surface. These effects occurred in both HEF and RK cell cultures at 33 C, but appeared to occur more rapidly in the former. At 39 C, typical HSV cytopathology also occurred in both systems (Table 4), but only in cultures treated with active virus. The morphology of UV-inactivated HSV-inoculated cultures was indistinguishable from that of the normal controls.

To determine whether replicative events including specific HSV antigen synthesis could occur in HEF as well as RK cells at 39 C, virus-treated cells were examined qualitatively by use of the immunofluorescence technique, as described in Materials and Methods. Representative data are given in Table 4. Greater than 90% (essentially all) of both HEF and RK cells treated with active HSV contained specific antigens 12 hr after infection at 39 C. No qualitative differences could be ascertained between the cell systems. Specific antigens were not found in cells treated with UV-inactivated virus.

DNA synthesis. Viral and cellular DNA synthesis in HSV-infected HEF and RK cells was measured by the continuous incorporation of ³H-TdR into acid-insoluble material 22 hr after infection. Parallel virus-treated cell cultures not

TABLE 3. *Effect of transferring rabbit kidney (RK) and hamster embryo (HEF) cell cultures from 33 C to 39 C at various times after herpes simplex virus (HSV) infection^a*

Time of transfer of cultures from 33 to 39 C (hr post-inoculation)	Time at 39 C (hr)	Infectious virus yield ^b (PFU/culture)	
		RK cells	HEF cells
3	21	1.9×10^5	$<7.5 \times 10^0$ ^c
4	20	2.7×10^5	$<7.5 \times 10^0$ ^c
8	16	7.1×10^4	3.5×10^3
12	12	2.3×10^6	1.4×10^5
16	8	9.8×10^6	4.1×10^5
20	4	2.1×10^7	6.6×10^5
24	0	4.8×10^7	9.8×10^5

^a Amounts of 20 to 40 PFU/cell adsorbed at room temperature for 2 hr.

^b Total PFU 24 hr after infection.

^c No detectable PFU in undiluted culture material.

TABLE 4. *Effect of ultraviolet irradiation of HSV on ability to induce cytopathic effects and virus-specific antigens at 39 C*

Cells ^a	Inoculum	Cytopathic effect ^b	HSV-specific antigen ^c (per cent positive cells)
RK	Medium	0	0
	HSV ^d	4+	>90
HEF	UV-HSV ^e	0	0
	Medium	0	0
	HSV ^d	4+	>90
	UV-HSV ^e	0	0

^a HEF, secondary hamster embryo fibroblasts; RK, secondary rabbit kidney cell cultures.

^b Gross observation 24 hr after infection; 4+, essentially all cells affected.

^c Immunofluorescent antibody assay 12 hr after infection.

^d Inoculum of 10 PFU/cell adsorbed at room temperature for 2 hr.

^e Identical to nonirradiated virus inoculum except for UV exposure to abolish infectivity.

labeled with isotope were assayed for CPE and total infectious virus production.

A typical analysis of DNA in HSV-infected HEF cells is presented in Fig. 1. Cellular DNA found in both infected and noninfected cultures had a density of 1.696 g/cc. This value was similar to other reports for mammalian cell DNA (1, 13) and was the same as that found in RK cells. A second DNA peak, absent from the uninfected

FIG. 1. Analysis of DNA isolated from HSV-infected and noninfected HEF cells by isopycnic centrifugation in CsCl. Confluent cell monolayers were infected with 10 HSV PFU/cell. Tritium-labeled thymidine was added at 2 hr and remained continuously until DNA analysis at 24 hr postinfection. (A) DNA extracted from virus-inoculated cells at 33 C. (B) DNA extracted from noninfected cells at 33 C. (C) DNA extracted from virus-inoculated cells at 39 C. (D) DNA extracted from noninfected cells at 39 C.

cells, had a density of 1.724 g/cc and was considered to be HSV-specific. Although this density was slightly lower than that previously reported for HSV-2 (9, 13), the discrepancy is most likely because the DNA preparations analyzed in our investigations were not phenol-extracted.

The HSV infection of HEF cells resulted in viral DNA synthesis at both incubation temperatures. At 39 C, however, the amount was only 12% of that found at 33 C. In addition, cellular DNA synthesis was affected as described previously for herpesvirus infections (1, 8). Compared with the uninfected control cells, cellular DNA synthesis was depressed 77% by infection at 33 C and 48% at 39 C. It was also observed that elevating the incubation temperature decreased the synthesis of cellular DNA in normal control cells by 31%. Examining the cultures for viral CPE and total virus yield revealed the usual results. Essentially every cell manifested virus CPE at either temperature, and the uninfected control monolayers appeared normal. At 39 C, no infectious HSV could be detected, whereas greater than 10^6 PFU/culture were produced at 33 C.

The decreased amount of HSV DNA in HEF cells at 39 C suggested that the dramatic difference in infectious virus production in HEF and RK cells at this temperature might be mediated by a discrepancy in viral DNA synthesis. Experiments were conducted comparing the relative amounts of HSV DNA synthesized in the two cell systems. The procedure employed was the same as described above.

In the absence of detectable infectious virus formation, HSV DNA in HEF cells at 39 C was about one-ninth of that present at 33 C (Table 5). Although greater than 10^5 PFU of HSV were produced per culture, the amount of HSV DNA in RK cells was also reduced by elevating the incu-

bation temperature. The amount present at 39 C was about one-fourth of that found at 33 C. A similar decrease was observed in other experiments. Upon comparing the relative levels of HSV DNA synthesized in HEF and RK cells at 33 C, the amount formed in RK cells was almost twice that present in HEF cells. This probably reflects variation in the number of cells per culture as well as differences in the ability of these two cell types to support viral DNA synthesis. Assuming that this same relative difference exists at 39 C, the level of HSV DNA synthesis in HEF cells was about twofold less than in RK cells.

DISCUSSION

The results reported in this communication are in general agreement with those of others concerning the effect of temperature of incubation on the overall production of infectious HSV. Previous studies demonstrated that maximal HSV yields are obtained at temperatures below 35 C (4, 6) and that temperatures of 39 C or greater inhibit the formation of infectious virions (4, 6, 10, 19, 21, 22). In addition, however, we have described an *in vitro* system in which the effect of supraoptimal temperature on HSV production is clearly cell-dependent. At 39 C, greater than 100,000-fold more infectious HSV was formed in RK cell cultures than in HEF cell cultures. Whereas RK cells were still permissive at this temperature, although the total virus yield was significantly reduced, HEF cells were nonpermissive and no infectious virus was detectable.

In comparing HEF and RK cell cultures we found that HSV adsorption was similar in both. Essentially the same amounts of unadsorbed virus were found in cultures of either type after the adsorption period (Crouch, *unpublished observations*). Inoculating diluted virus onto monolayers of either cell type resulted in the same number of plaques at 33 C. The inoculum routinely used for comparative experiments at 39 C, excluding plaque formation, was sufficient to permit adsorption of at least 1 PFU per HEF or RK cell, as indicated by CPE and intracellular viral protein in greater than 90% of the cells at 12 hr after infection. Consequently, the dramatic difference observed in virus production at 39 C in these two systems does not reflect differences in the number of cells initially infected.

The possibility that HSV is thermally inactivated or enzymatically degraded more readily in HEF cells than in RK cells is unlikely. Since specific viral antigens appeared in HEF cells at 39 C, the input virus was not inactivated or degraded before the expression of genetic information. Enzymatic degradation of progeny virus could

TABLE 5. *Synthesis of herpes simplex virus (HSV)^a DNA at different temperatures*

Cells ^b	Incubation temp (C)	Viral DNA ^c (precipitable counts/min)	39 C/33 C ratio
HEF	33	76,087	1/9.1
	39	8,276	
RK	33	131,382	1/4.4
	39	29,955	

^a Inoculum of 10 PFU/cell adsorbed at room temperature for 2 hr.

^b HEF, secondary hamster embryo fibroblasts; RK, secondary rabbit kidney cell cultures.

^c Density of 1.724 g/cc; continuous ³H-thymidine incorporation during infection for 24 hr.

presumably result from lysozomal enzyme activity, and cannot be excluded by our experiments. The release of these nonspecific agents should, however, also degrade other viruses in HEF cells at 39 C. Vaccinia virus was not degraded under these conditions, suggesting either that lysozomal enzymes are not involved or that vaccinia virus is less sensitive to these enzymes. In considering greater thermal inactivation of progeny HSV in the HEF cells, the thermal inactivation rate of free HSV at 39 C (4, 6) is not rapid enough to account for the 100,000-fold difference in infectious virus yield in HEF versus RK cells 24 hr after infection. In addition, the thermal inactivation in RK cells should be the same as in HEF cells. Although the virions produced in HEF at 39 C could be more thermally sensitive, there is no evidence to support this theory.

The different virus productivity of HEF and RK cells at 39 C apparently involves temperature-sensitive cell factors that control HSV replication. Gharpure (5) demonstrated that replication of DNA viruses, including HSV and vaccinia virus, was inhibited in cell cultures preincubated at supranormal temperature prior to virus inoculation, and he concluded that some heat-sensitive cell function required for DNA virus replication was destroyed by the preheating. We considered the possibility that similar cell functions may be sensitive at 39 C in HEF cells. Preliminary experiments, however, did not confirm this. In HEF and RK monolayers preincubated at 39 C for 24 hr before inoculation with serially diluted HSV, the same number of virus plaques formed at 33 C as in monolayers not preheated (Crouch, *unpublished observations*).

According to our data, the event(s) that is more sensitive in HEF than in RK cells occurs before 8 hr after HSV infection. Elevation of the temperature after this time no longer completely blocked HSV production in the HEF cells. In other permissive cell systems (15), this is the time when HSV DNA synthesis is completed. Although HSV DNA synthesis was found to be depressed in both HEF and RK cells at 39 C, the depression was greater in HEF, suggesting that viral DNA synthesis may be more sensitive to temperature in HEF cells. However, it is difficult to conceive that the twofold discrepancy in viral DNA synthesis observed between HEF and RK cells could account for the 100,000-fold difference observed in HSV PFU. Perhaps effects on viral DNA synthesis, as well as on functions related to the synthesis of critical viral proteins, are involved.

Although the data presented do not reveal the mechanism responsible for the marked difference between HEF and RK cells with respect to HSV

production at 39 C, the comparative system described in this communication provides an experimental model that should prove useful for further investigation of two important aspects of HSV-cell interaction: (i) the influence of the host cell in controlling productive infection and (ii) the possible expression of HSV oncogenic potential in HEF cells under nonpermissive conditions. The latter must include additional steps to overcome the problem of CPE. These ideas are currently being investigated.

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