Mild Acidic pH Inhibition of the Major Pathway of Herpes Simplex Virus Entry into HEp-2 Cells

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SUMMARY

Penetration of the KOS strain of herpes simplex virus type 1 (HSV-1) and the MS and 333 strains of herpes simplex virus type 2 (HSV-2) into HEp-2 cells at pH 6.3 was at least 100-fold less efficient than at pH 7.4. Penetration of two low passage clinical isolates was completely blocked at pH 6.3. The syncytium-forming HSV-1 strains GC and MP were less sensitive than KOS to the mild acidic conditions. The inhibition was completely reversed upon neutralization of the medium. Penetration was assayed by plaque production following protection from acid inactivation upon virus entry. Penetration of HSV-1 KOS into Vero and HEL diploid fibroblast cells was similarly inhibited. HSV-1 KOS grown in 2-deoxy-D-glucose and monensin was also extensively inhibited at pH 6.3 but virus grown in 2-deoxy-D-glucose penetrated more slowly than normal virus at pH 7.4. Electron microscopy of HSV-1 KOS infection indicated that fusion and endocytosis occur at both pH 7.4 and 6.3 but that fusion predominates at pH 7.4 and endocytosis predominates at pH 6.3. These results indicate that fusion at the plasma membrane is the major route of productive entry for HSV, that strains of HSV can differ in their pH dependence for penetration and this may determine whether virus infection can occur following endocytic uptake.

INTRODUCTION

Penetration of an enveloped virus into the cell occurs by the fusion of the viral envelope with a cellular membrane (Hsu et al., 1982; Lenard & Miller, 1982; White et al., 1983). These processes require both viral and cellular structures and functions. The site of fusion appears to be determined by the pH optimum of a viral fusion protein (Lenard & Miller, 1982). Sendai virus is the prototype of viruses that enter cells by direct fusion of the viral envelope with the plasma membrane at neutral or slightly basic pH (Nagai et al., 1983; Hsu et al., 1982). Viruses such as Semliki Forest virus, vesicular somatitis virus and influenza A virus, with mild acidic pH optima for fusion, enter by a receptor-mediated endocytic pathway before their fusion to endosomal membranes (White et al., 1981, 1983; Lenard & Miller, 1982).

The cell type can also play a role in virus penetration. The mode of entry of Epstein–Barr virus (EBV), another herpesvirus, depends heavily on the target cell type (Nemerow & Cooper, 1984). The predominant mode of entry of EBV into peripheral blood lymphocytes is receptor-mediated endocytosis whereas fusion at the plasma membrane predominates for lymphoblastoid and lymphoma cells.

HSV penetration into the target cell has been observed by both fusion at the surface of the cell (Morgan et al., 1968; Smith & deHarven, 1974; Epstein et al., 1964) and endocytosis (Hummeler et al., 1969; Smith & deHarven, 1974; Dales & Silverberg, 1969). However, several studies have suggested that plasma membrane fusion is the preferred mode of entry for HSV. These include: expression of viral glycoproteins on the cell membrane immediately following infection, in the...
absence of viral protein synthesis (Para et al., 1980); HSV immobilized to Staphylococcus aureus through antibody linkages binds and infects cells leaving the bacteria bound to the cell surface (Hodnichak et al., 1984); fluorescence energy transfer from fluorescein–octyldodeylamine-labelled virus to rhodamine–octyldodeylamine-labelled cell membranes follows infection (Keller et al., 1977); HSV glycoprotein-containing virosomes can fuse with cell membranes (Johnson et al., 1984). HSV infection can also induce syncytium formation which requires expression of a fully glycosylated gB glycoprotein (Bzik et al., 1982; Manservigi et al., 1977) and occurs at neutral pH but not mildly acidic pH (Lancz & Bradstreet, 1976). However, these studies were done with relatively high multiplicities of infection and do not discriminate individual viral events. In this study we show that the predominant pathway for productive entry of HSV is fusion at the cell membrane, and it is inhibited at slightly acidic conditions. Strains of virus can differ in their pH dependence for penetration and this is likely to determine whether any virus can enter cells from endocytic vesicles.

METHODS

Cells and virus. HEp-2 and Vero cells were maintained in Eagle’s minimal essential autoclavable medium (Gibco) supplemented with foetal calf serum (10%), 50 μg/ml gentamycin and 29 μg/ml L-glutamine. Maintenance medium was supplemented with 2% newborn calf serum instead of foetal calf serum. Tris-buffered saline (TBS) consisted of 10 mM-Tris-HCl pH 7.4 and 150 mM-NaCl; phosphate-buffered saline (PBS) was Dulbecco’s PBS at pH 7.4, unless otherwise noted.

HSV-1 KOS was provided by Deborah Parris, Ohio State University Medical School. MP-2, GC, EKN and HTZ were provided by Richard Dix, Bascom-Palmer Eye Institute, University of Miami Medical School. EKN and HTZ are low passage clinical isolates (EKN passage < 5, HTZ passage < 15). Virus stocks were prepared by low multiplicity infection of HEp-2 cells and infectious units were quantified by plaque assay on Vero cells using a 1.5 μl methyl cellulose overlay (Dreesman & Benyesh-Melnick, 1967). Virus was obtained from cells treated with 15 μg/ml monensin (Johnson & Spear, 1982) or 6 mM-2-deoxy-D-glucose (2-DG) (Spivack et al., 1982) by freeze-thaw lysis of the infected, treated cells 24 h post-infection.

Penetration assay. Penetration of HSV into HEp-2 cells was assayed as described earlier (Rosenthal et al., 1985). A confluent layer of HEp-2 cells was grown in 24-well tissue culture plates and incubated for 1 or 4 h at 4 °C with approximately 10^5 p.f.u. to allow attachment, but not penetration, of 10 to 15% of the virus. The unbound virus was washed away, the cells were incubated with PBS of the appropriate pH at 4 °C and then shifted rapidly in the same buffer to 37 °C. Extracellular virus was inactivated by a 1 min treatment with PBS at pH 3.0 at appropriate times. Following several washes in medium to neutralize the acid, intracellular virus was quantified by plaque assay in methyl cellulose-containing medium. Acid inactivation of a cell monolayer at the time of the temperature shift was performed to ensure that virus penetration did not occur prior to the experiment. In the experiments described in Fig. 4, cells were incubated with serial dilutions of the cell lysates from infections with normal viruses or those grown in 6 mM-2-DG or 15 μg/ml monensin. Penetration of virus at pH 6.3 required a 100-fold higher m.o.i. to increase the sensitivity of the assay. The reversibility of mild acidic pH inhibition was investigated by neutralization of the extracellular buffer after 20 min, and incubation of the cells for an additional 20 min in PBS at pH 7.4, prior to acid inactivation of extracellular virus. Variability in plaque count for identically treated monolayers was less than 10%. Data presented are representative of at least three studies with similar results.

Several plaque-purified clones of HSV-1 KOS were tested for their ability to penetrate HEp-2 cells at pH 6.3 and 7.4. Replicate cultures of HEp-2 cells grown in 24-well tissue culture plates were incubated with 16 individual viral clones at 4 °C for 4 h; the cells were washed, incubated at pH 6.3 or 7.4 for 15 min and then incubated at the higher temperature of 37 °C for 30 min. Extracellular virus was then inactivated and intracellular virus quantified by plaque assay.

Electron microscopy. HEp-2 cells grown on lined 60 mm tissue culture plates (Falcon) were rinsed, cut into notched rectangles and infected with approximately 10^4 p.f.u. HSV-1 KOS at an m.o.i. of 10^2 to 10^3 for 4 h at 4 °C. The unbound virus was removed, the cells were incubated with PBS at pH 6.3 or 7.4 for 5 min at 4 °C and then the temperature was shifted rapidly to 37 °C. After 2, 5, 10 and 30 min, the cells were rinsed with warmed TBS, fixed with 1% glutaraldehyde (Fisher), 1% paraformaldehyde (Fisher), 2 mM-CaCl₂, 2 mM-MgCl₂ in 0.15 M-HEPES (Sigma) pH 7.4. They were then post-fixed with 1% (w/v) OsO₄ in water (Stevens Metallurgical Company), dehydrated through a graded series of ethanol and then propylene oxide and embedded in EMBED 812 (E.M. Sciences) (Chang, 1971). Thin sections were prepared and stained with 2% aqueous uranyl acetate (Fisher) and Reynolds’s lead citrate. They were viewed with a JEOL JEM100S transmission electron microscope at 100 kV.

A data coding system was developed to allow statistical analysis of the viral entry pathway, observed by electron
microscopy. Examples of the virus entry events with the assigned numerical code are presented in the micrographs shown in Fig. 5. Each sample was examined at x 50000 until 15 non-zero penetration events were recorded. A two-way analysis of variance was then performed to determine significant differences between penetration events at the two pH conditions for each time point and for the entire period.

SDS-polyacrylamide gel electrophoresis. Glycoproteins of HSV-1-infected cells grown under normal conditions and of those grown in the presence of 15 μg/ml monensin or 6 mM-2-DG were radiolabelled with [3H]glucosamine 4 h after infection. The infected cells were solubilized in 150 mM-NaCl, 10 mM-Tris-HCl pH 7-4, 1 mM-MgCl₂, 0-1% Triton X-100, 1% sodium deoxycholate, 0-1% SDS and 1% aprotinin. The cell extracts were boiled in sample buffer (Laemmli, 1970) and electrophoresed on 8.7% polyacrylamide gels cross-linked with NN'-diallyltartardiamide (DATD) (Heine et al., 1974). The glycopeptides were visualized by fluorography using Resolution (EM Corp).

RESULTS

pH dependence of HSV-1 KOS penetration

Penetration of HSV-1 KOS into HEp-2 cells was measured by protection from acid inactivation. This assay measures only productive infections involving individual virus–cell interactions. Other techniques which may identify possible modes of entry, e.g. electron microscopy or fluorescence probe transfer (Keller et al., 1977; Hoekstra et al., 1984) cannot distinguish between productive and non-productive routes of infection. Inactivation of the virus remaining on the cell surface with acid was a preferred method to neutralization by antibody or light inactivation of photosensitized virus (Spivack et al., 1982; DeLuca et al., 1981) because it requires a minimum amount of time for handling, allows better evaluation of the kinetics of virus uptake and deals with only those events which are occurring at the cell surface. Penetration of the virus was synchronized by incubation with the cells at 4 °C, followed by removal of unbound virus prior to the temperature shift to 37 °C (Huang & Wagner, 1964; Farnham & Newton, 1959).

At physiological pH (7-4), HSV penetration occurred following a 5 to 7 min lag period. The lag period was also observed with 10²- to 10⁴-fold higher multiplicities of infection and when the virus was added to the cells at 37 °C (Rosenthal et al., 1984); it was not an artefact of the temperature shift or the sensitivity of the assay. Following the lag period, penetration of the virus proceeded rapidly for 10 to 20 min with most of the virus (approx. 90%) of the cell-bound inoculum) taken up within 20 min (Fig. 1). Following the initial 20 min, penetration continued at a much slower rate.

As a result of the incubation of the cells in PBS buffer < pH 7-0, the lag period prior to detection of penetration became longer and the rate and extent of virus penetration was reduced. At pH 8-0 and 9-0 (data not shown), the penetration of virus proceeded at the same initial rate as at pH 7-4.

The inhibition of HSV-1 penetration at the lower pH was completely reversible. Following an initial 20 min treatment at the different pH conditions, the cells were incubated for an additional 20 min at pH 7-4 to allow uptake of all cell-bound virus. No significant difference could be observed between the extent of virus penetration for the infected cells incubated initially at pH 7-4 and that at the lower pH conditions (as determined by a Student's t-test). No loss of viral infectivity occurred following incubations of 2 or more h at pH 6-3. Virus became labile at pH values lower than 6-0 (data not shown). The complete recovery of infectious virus after restoration of neutral pH indicates that the mild acidic pH treatment was not toxic to the cells and that the virus was not eluted or inactivated by the treatment. The mild acidic pH conditions must therefore be inhibiting the penetration of the virus into the cell rather than an earlier or later step in virus replication.

The mild acidic pH inhibition of HSV penetration is best illustrated at pH 6-3. Uptake of virus was undetectable until 20 min after the temperature shift except when a 100-fold higher m.o.i. was used. The penetration of virus at the higher m.o.i. showed the same 5 min lag period as that at pH 7-4 and proceeded at a slower rate for at least 2 h. The inhibition of penetration at pH 6-3 was also observed for HSV-1 KOS infection of Vero cells (Rosenthal et al., 1988) and HEL 299 human diploid fibroblasts (data not shown).
Penetration of plaque-purified clones of HSV-1 KOS at pH 6.3 was compared. These clones were picked randomly and were not selected for any special characteristic. The extent of penetration for each of the clones was assayed at 30 min after the shift in temperature. Penetration of all clones was less efficient at pH 6.3. Of the 16 clones, penetration of eight was inhibited by more than 90%, seven were inhibited by 80 to 90%, and one clone was inhibited by less than 80% at pH 6.3. These results indicate that small differences in the pH sensitivity of penetration exist between different clones of HSV-1 KOS and that the ability to penetrate at pH 6.3 is not a phenotype of an unselected substrain of the HSV-1 KOS isolate used in these studies.

Efficiency of penetration of other strains of HSV-1 and the MS and 333 strains of HSV-2

Penetration efficiency at pH 7.4 and 6.3 of two low-passage clinical isolates, HTZ and EKN, and two syncytium-forming strains, of HSV-1, MP-2 and GC, were compared (Fig. 2). Rapid uptake of MP-2 and GC into HEp-2 cells at pH 7.4 was observed after 5 min with approximately 90% of virus penetration occurring within the first 30 min, similar to that observed for KOS. However, penetration was only inhibited by three- to five-fold at pH 6.3. In contrast, the penetration of the HTZ and EKN strains was totally inhibited at pH 6.3 and no penetration could be observed even after 1 h. Penetration of the HTZ strain exhibited kinetics similar to that of KOS, with rapid penetration following an initial 5 min lag period at pH 7.4. Penetration of the lower passage EKN isolate (< 5 passages) also followed a 5 min lag period but proceeded at a slower rate. The inhibition of penetration was completely reversible upon neutralization of the buffer medium. Penetration of another low passage clinical isolate, H129, was also highly sensitive to mild acidic pH (data not shown). The inhibition of penetration at pH 6.3 was reversible upon neutralization of the extracellular buffer for all of the viruses.

The pH sensitivity of the MS and 333 laboratory strains of HSV-2 was also determined. As for HSV-1, penetration of both strains of HSV-2 commenced after a 5 min lag period, followed by rapid uptake of virus for the next 15 min at pH 7.4. Virus penetration at pH 6.3 was undetectable for up to 60 min. The inhibition was completely reversed upon neutralization of the medium and a 30 min incubation period.
HSV entry inhibited by mild acidic pH

Fig. 2. pH dependence of penetration of HSV-1 strains GC, MP-2, HTZ and EKN. Virus penetration was evaluated as described for Fig. 1. (a) GC (●, pH 7.4; ○, pH 6.3) and MP-2 (■, pH 7.4; □, pH 6.3) are highly passaged syncytium-forming strains. (b) EKN (●, pH 7.4; ○, pH 6.3) is a low passage (<5) and HTZ (■, pH 7.4; □, pH 6.3) is a higher passage (<15) clinical isolate.

Fig. 3. Glycoprotein profile of HSV-1 KOS grown under normal conditions or in the presence of monensin or 2-DG. HEp-2 cells were either untreated or treated with 15 μg/ml monensin or 6 mM-2-DG 1 h after infection with HSV-1 KOS. The infected cells were radiolabelled with [3H]glucosamine at 4 h post-infection. Infected cell lysates were electrophoresed on SDS–polyacrylamide gels cross-linked by DATD and the glycopeptides visualized by fluorography: normal virus (lane 1); virus grown in monensin (lane 2); virus grown in 2-DG (lane 3).
The extent of protein glycosylation and pH dependence of penetration

Differences in glycosylation of the viral glycoproteins grown with or without 2-DG or monensin (Fig. 3) are indicated by differences in their mobility on SDS–PAGE. The effects of 2-DG were more apparent than those of monensin and for 2-DG they could be seen as the loss of the mature forms of gB and gC. 2-DG blocks incorporation of glucose and mannose (Blough & Ray, 1980) whereas monensin blocks the translocation of virions and viral glycoproteins from the medial Golgi apparatus lamellae and final processing of the viral glycoproteins (Johnson & Spear, 1982). Inhibition of protein glycosylation with 2-DG or monensin causes the inhibition of HSV-1-induced cell–cell fusion (Bzik et al., 1982) but virions obtained from cell lysates are still infectious (Johnson & Spear, 1982; Spivack et al., 1982).

Virus produced under normal conditions (Fig. 4a) or in the presence of monensin exhibited similar kinetics of penetration at pH 7-4 (Fig. 4b). Total virus penetration was determined after a 1 h incubation at pH 7-4 or after a 30 min incubation at pH 6-3 and an additional 30 min incubation at pH 7-4. Penetration of HSV-1 grown in 2-DG occurred at a slower initial rate (Fig. 4c). At 20 min, only 37% penetration occurred for virus grown in 2-DG as compared to 53% for virus grown in monensin and 70 to 90% for normal virus. The 100-fold higher m.o.i. enabled the detection of virus penetration at pH 6-3 after the 5 min lag period. The rate of penetration of virus grown in monensin was reduced to a similar extent as that of normal virus at pH 6-3. Penetration of virus grown in 2-DG was inhibited only 25-fold at pH 6-3.

**Electron microscopic evaluation of HSV-1 uptake**

HSV-1 penetration at pH 7-4 and 6-3 was examined by electron microscopy to identify the pathways of viral uptake under the different pH conditions. HEp-2 cells were incubated with...
HSV-1 KOS at 4 °C for 4 h, washed, shifted to incubation at 37 °C and then fixed with glutaraldehyde at various times after the temperature shift. In contrast to the penetration plaque assays, a high m.o.i. was necessary to allow visualization of the cell-bound virus. Approximately 100 to 200 cell-associated virions were examined at high magnification in order to identify 15 events which are characteristic of virus uptake (Fig. 5). The events were scored on an arbitrary scale to classify the different modes of uptake. This analysis allows comparison of the modes of virus uptake under different pH conditions but does not provide the total number of events occurring for each sample (Table 1). Virions in the process of penetration but still associated with the cell membrane were weighted less because their evaluation was more subjective and less definitive regarding the final means of uptake. Virions bound to the cell surface but not in the process of penetration were assigned a zero value and not included in the statistical evaluation.

At pH 7.4, naked capsids could be identified in the cytoplasm within 5 min of the temperature shift. Virus could be observed at the cell surface in the early stages of both modes of penetration. At 10 min after the temperature shift, the majority of virus in the process of entry appeared to be fusing with the cell membrane; however, a few enveloped viruses in vesicles or in membrane invaginations were also observed at this time suggesting endocytic uptake of the virus. Within 30 min of the temperature shift, naked capsids could be seen in the cytoplasm with a small proportion of enveloped virus in vesicles or membrane invaginations. The predominant pathway for entry noted at this pH was indicative of fusion, with a fraction of the virus population being taken up by endocytosis.

Initially, the virus penetration events at pH 6.3 were indistinguishable from those observed at neutral pH. A difference was observed after 10 min when at least half of the virions in the
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Table 1. Electron microscopic evaluation of HSV-1 KOS penetration

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* Penetration of HSV-1 KOS into HEp-2 cells was stopped at various times after temperature shift, by fixation. At each time point, 15 viral penetration events were evaluated by the coding system described in Fig. 5.

The predominant pathway of HSV-1 KOS uptake into HEp-2 cells under different pH conditions was inferred from the results of a two-way analysis of variance of the coded values for the observed penetration events. Both modes of uptake were observed under both conditions but a statistically significant difference in preference could not be calculated for any individual time point. A statistically significant difference in the preferred mode of penetration was obtained to the 99% level of confidence over the full time course. At pH 7.4, the preferred mode of uptake was fusion, while at pH 6.3 endocytosis was the preferred pathway. Although the endocytic route appears to be the predominant mode of uptake at pH 6.3, the actual fate of these viruses cannot be definitely determined from the electron micrographs and these may be non-productive routes of uptake. Since only viruses observed in the process of penetration were included in the electron microscopic evaluation, a decrease in the rate of fusion at the plasma membrane would be seen as an increase in the ratio of viruses taken up into vesicles.

**DISCUSSION**

The earliest steps in HSV replication are viral attachment and penetration. The rate and efficiency of these activities are determined by the nature of the interaction of the viral glycoproteins and their cell-surface receptors, and the environment in which this interaction takes place. The site of fusion of enveloped viruses and therefore the means of penetration into the cell is dependent upon the pH optimum for the fusion process. For fusion, a requirement for acidic conditions would not allow plasma membrane fusion at physiological pH, and similarly a requirement for neutral or basic pH would prevent fusion in endocytic environments (Lenard & Miller, 1982). Viruses that do not have an absolute pH requirement for fusion would be able to enter cells by either means.

Penetration of HSV-1 follows a 5 min lag period, coincident with a limitation of all cell surface protein mobility (Rosenthal et al., 1984). During this period, HSV could be observed by electron microscopy to be attached and in the early phases of envelope–plasma membrane fusion. Rapid uptake of the virus followed the 5 min lag period, with penetration of 80 to 90% of the cell-bound virus occurring by 20 min. These kinetics are coincident with a significant disruption of membrane structure which can be blocked by inhibitors of HSV penetration, including mild acidic pH (Rosenthal et al., 1984, 1988). Penetration of the remainder of the virus proceeded at a slower rate and continued for up to 1 or 2 h. Electron microscopic observation indicated that the majority of HSV-1 KOS enters by fusion at the plasma membrane but some of the inoculum is
taken into the cell by endocytosis. Interestingly, both modes of virus uptake could be observed on the same cell.

The efficiency of productive penetration for all of the HSV-1 and HSV-2 strains tested was reduced, but to different extents, at mild acidic pH. The most sensitive group of viruses were the low passage clinical isolates which were incapable of penetration at pH 6-3. Recovery of the virus infectivity upon neutralization of the extracellular buffer indicates that penetration through the plasma membrane is their only productive means of uptake.

Penetration of HSV-1 KOS was at least 100-fold less likely to occur at pH 6-3 than at pH 7-4. The pH dependence of HSV-1 KOS penetration into HEp-2 cells at 20 min resembled a sigmoidal pH titration curve. This was true for penetration into HEp-2, Vero cells (Rosenthal et al., 1988) and HEL 299 cells, non-transformed diploid fibroblasts (data not shown). Uptake of the MS and 333 strains of HSV-2 was at least as sensitive as HSV-1 KOS to mild acidic pH. The strains that caused syncytia that were tested were less sensitive to the mild acidic pH than those which did not.

Virus was taken into endocytic vesicles at both pH 6-3 and 7-4 but whether this is a productive route of entry cannot be distinguished from electron micrographs. Accumulation of virus into vesicles without infection has been observed in cells stably transformed and expressing the gD gene of HSV-1 (Campadelli-Fiume et al., 1988). Expression of gD in these cells may have inhibited fusion processes. Once inside an endocytic vesicle at pH 6-3 or a lower pH, the potential for productive entry would depend on the pH dependence for fusion of the virus strain. Penetration of the syncytium-forming strains, MP or GC, would have more potential for entering the cell by both endocytosis and fusion because penetration of these strains was least sensitive to mild acidic pH.

HSV-1 glycoproteins gB, gD and gH are required for HSV-1 penetration although their individual roles are not known (Highlander et al., 1988; Gompels & Minson, 1986; Ligas & Johnson, 1988; Sarmiento et al., 1979). Differences in either the protein or the carbohydrate structure of the glycoproteins can influence the pH sensitivity and kinetics of virus uptake. Strain differences in the penetration rate at pH 7-4 have been observed by protection from photoinactivation of fluorescein isothiocyanate-modified virus (DeLuca et al., 1982) and attributed to genetic differences in gB. Strain differences were also observed in the sensitivity of penetration to pH. This can be seen in the differences between HSV-1 KOS, MP and GC but is best demonstrated by the differences of the individual clones of KOS. The carbohydrate structure of the glycoproteins may also be a factor in the differences in penetration efficiency. HSV-1 KOS grown in 2-DG, containing underglycosylated glycoproteins, penetrated more slowly than normal virus but their penetration was less sensitive to mild acidic conditions. Differences in the penetration capacity and kinetics between normal virus and virus grown in 2-DG have been detected by resistance to elution by a heparin-EDTA-NaCl wash (Spivack et al., 1982). Low passage isolates like EKN generally have underglycosylated glycoproteins as indicated by SDS-PAGE mobility (Costanzo et al., 1986 and preliminary studies). However, the difference in penetration sensitivity to mild acidic pH between the 2-DG-grown virus and the low passage isolates indicates that the extent of glycosylation is not likely to be the determinant for pH sensitivity of penetration for these isolates.

Mild acidic pH conditions also inhibit HSV-induced cell–cell fusion and syncytium formation (Lancz & Bradstreet, 1976). Inhibition of both virus penetration and syncytium formation inhibits the two predominant means of HSV-1 spread within the host. Conditions that promote an acidic environment, such as the acid produced during inflammation (Menkin, 1934), may limit the spread of the virus but conditions that promote an increase in pH may also promote the spread of the virus.

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HSV entry inhibited by mild acidic pH

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