Thermal-pH Inactivation of Herpes Simplex Virus: 
Interdependence of the Medium Composition and 
the pH on the Rate of Virus Inactivation 

Brief Report

By

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With 2 Figures

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Summary

The composition and pH of the suspending medium were shown to be interdependent effectors of the \textit{in vitro} thermal inactivation of herpes simplex virus (HSV). In addition, enhanced thermal sensitivity of HSV at alkaline pH was a medium dependent effect and, therefore, is not an inherent property of the virus.

A differential in thermal lability has been described as a biologic marker distinguishing herpes simplex virus (HSV) type 1 from type 2 (2, 7), HSV-2 being more thermolabile. However, this difference in thermal lability is not always observed (1). More recently studies have shown that the kinetics of HSV inactivation in the range of $4^\circ$ to $40^\circ$ C is markedly accelerated when the pH of the tissue culture medium in which the virus is suspended is adjusted to an alkaline value, e.g., pH 7.8. Under these conditions, the thermal sensitivity of laboratory strains and recent clinical isolates of both HSV types 1 and 2 are equivalent (5, 6).

A limited number of studies have examined the relationship between the thermal inactivation of HSV and the composition of the suspending medium. WALLIS and MELNICK (10) have shown that the amount of HSV inactivated at $50^\circ$ C is dramatically influenced by the presence of particular cations and anions. The thermolability of HSV is also enhanced when the virus is suspended in a salt solution that is isosmotic for mammalian cells (10, 11).
However, evidence to the contrary also has been presented (9). In addition, a 10⁻² dilution of HSV into water with an inherent reduction in salt ions, rendered the virus relatively resistant to thermal inactivation (10, 11).

Considering these observations, a study was initiated to examine the thermal inactivation of HSV with specific regard to the following questions. Is the enhanced thermolability at alkaline pH an inherent property of HSV? To what extent does the relative purity of the HSV preparation influence the rate of inactivation? To what degree does the composition of the suspending medium affect the rate of HSV inactivation? Two HSV pools prepared by different protocols, were suspended in media of different composition and monitored for thermal-pH inactivation. The results indicate that the heightened thermal sensitivity of the highly purified HSV was due to the absence of protein and other macromolecules that stabilize the virus to thermal inactivation. In addition, the alkaline pH related enhancement of the rate of thermal inactivation of the highly purified virus was itself dependent upon the particular medium in which the virus was suspended.

Polyethylene glycol (PEG) precipitated stocks of the VR₃ strain of HSV-1 (Dr. Nahmias, Emory University, Atlanta) are prepared as described (3). The highly purified VR₃ stocks of HSV were prepared from infected rabbit skin (RS) cell cultures. After the removal of large particulate material by centrifugation (1000 × g, 15 minutes, 4 °C), the supernatant fluid was layered over a 3 ml cushion of sucrose (50 percent w/w) containing 10 mM HEPES, pH 7.0 and centrifuged (83,000 × g av, 30 minutes, 2 °C). The HSV was collected from the bottom 1—1.5 ml of the cushion and was layered on a 30—70 percent (w/v) linear sucrose gradient (10 mM HEPES, pH 7.0) and centrifuged to equilibrium (83,000 × g av, 16 hours, 2 °C) as modified from YANAGI (12). One ml fractions were collected by tube puncture and assayed for virus plaque forming units (PFU) as described (4). The sucrose was removed from the HSV preparation by dialysis overnight against a minimum of 1000 volumes of phosphate buffered saline (PBS), pH 6.3 at 4 °C, immediately prior to use.

Each of the HSV stock preparations was diluted 10⁻² into Hanks’ balanced salt solution (HBSS), Eagles’ medium (EMEM) or serum containing complete medium (8; CM) that had been pre-adjusted to pH 6.3 or pH 7.8. This did not alter the pH of the solution. At the times indicated, an aliquot was removed and immediately assayed for residual HSV PFU (4). The data from representative experiments (Table 1) indicated the following: First, the level of inactivation of each of the stock virus preparations was comparable when serum containing medium was employed. Second, the inactivation of the highly purified HSV was accelerated when the virus was suspended in medium lacking serum. Thus, the thermostabilizing effects of serum are readily evident. Third, except for the sucrose gradient purified HSV diluted into EMEM, the level of HSV inactivation at pH 7.8 was
Table 1. *Effect of medium composition and pH on the thermal inactivation of HSV that had been prepared by different protocols*.

<table>
<thead>
<tr>
<th>Protocol for preparation of HSV stock</th>
<th>Suspending medium</th>
<th>pH</th>
<th>Surviving fraction after 37°C incubation for 2 hours</th>
<th>4 hours</th>
<th>12 hours</th>
<th>24 hours</th>
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<tbody>
<tr>
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<td></td>
<td>CM</td>
<td>EMEM</td>
<td>HBSS</td>
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<td>6.3 ND ND</td>
<td>ND ND</td>
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<td>2.5 x 10^-1</td>
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<td>PEG precipitation</td>
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<td>7.8 ND ND</td>
<td>ND ND</td>
<td>ND ND</td>
<td>2.5 x 10^-2</td>
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<td>6.3 ND ND</td>
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<td>7.8 ND ND</td>
<td>ND ND</td>
<td>ND ND</td>
<td>2.5 x 10^-2</td>
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<tr>
<td>Sucrose gradient purification</td>
<td></td>
<td></td>
<td>CM</td>
<td>EMEM</td>
<td>HBSS</td>
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<td>6.3 1 x 10^-1</td>
<td>1.5 x 10^-2</td>
<td>3 x 10^-3</td>
<td>&gt;1 x 10^-5</td>
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<td></td>
<td></td>
<td></td>
<td>7.8 2 x 10^-1</td>
<td>1 x 10^-2</td>
<td>2.5 x 10^-3</td>
<td>&gt;1 x 10^-5</td>
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<td>&gt;1 x 10^-3</td>
<td>&gt;1 x 10^-5</td>
<td>&gt;1 x 10^-5</td>
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</table>

* Stock preparations of HSV were obtained by PEG precipitation (3) and sucrose gradient equilibrium centrifugation as described in the text. Each was diluted 10^-2 into the medium indicated and incubated at 37°C for the times shown. The residual virus titers (PFU/ml) were determined and expressed as a ratio of the 0 time titer (surviving fraction). In all experiments, the 0 time virus titers ranged from 2-5 x 10^5 PFU/ml.

* CM — complete medium; Eagle's minimum essential medium supplemented with 5 percent calf serum and antibiotics (8)

* EMEM — Eagle's minimum essential medium; this is CM without serum

* HBSS — Hanks' balanced salt solution

* ND — not determined

greater than that observed at pH 6.3. Fourth, only the highly purified HSV preparation displayed maximal sensitivity to thermal inactivation when suspended in an isotonic balanced salt solution.

The third point above suggested that the inactivation of highly purified HSV, when suspended in EMEM, was not influenced by the pH. This possibility was examined further by titrating the level of virus inactivation against the pH of the medium. The HSV surviving fraction after a 90 minute incubation at 37°C was the same from pH 6.0 through pH 8.0 (Fig. 1) and was comparable to that observed at pH 6.3 and 7.8 (Table 1). It was subsequently shown that the inactivation rate of the highly purified HSV also was the same at pH 6.3 and 7.8 when the virus was suspended in 1 m sodium sulfate (Fig. 2). One molar sodium sulfate is known to stabilize HSV to thermal inactivation (10). This indicated that thermal inactivation of
HSV in EMEM and 1 m Na$_2$SO$_4$ is independent of the pH in the range of 6.0—8.0 and 6.3 and 7.8 respectively. Therefore, the enhanced rate of HSV inactivation observed in CM and HBSS at pH 7.8 was not due simply to the inherent lability of the virus at this alkaline pH.

The fourth point suggested by the data in Table 1 is that the PEG prepared HSV, compared to the highly purified HSV preparation, is considerably more resistant to thermal inactivation when each is suspended in HBSS. The possibility was tested that this could be due to a change in the composition of the suspending medium because of macromolecules that co-precipitated with the virus (3). The effect of PEG precipitated material

Fig. 1. Effect of pH on thermal inactivation of highly purified HSV suspended in EMEM. Highly purified HSV was diluted $10^{-2}$ into EMEM that had been adjusted to the pH indicated. The surviving virus fraction (PFU) was determined after 90 minutes incubation at 37°C.

Fig. 2. Thermal inactivation at 37°C of HSV in HBSS or 1 m Na$_2$SO$_4$. Highly purified and PEG concentrated HSV were diluted $10^{-2}$ into HBSS or 1 m Na$_2$SO$_4$, previously adjusted to either pH 6.3 or 7.8. At the times indicated the surviving fraction (PFU) was determined. ○—○ PEG concentrated HSV, pH 6.3; ●—● PEG concentrated HSV, pH 7.8. △—△ highly purified HSV, pH 6.3; ▲—▲ highly purified HSV, pH 7.8. ○—○ highly purified HSV, pH 6.3 supplemented with a $10^{-2}$ dilution of a PEG precipitate from mock infected cells; ●—● highly purified HSV, pH 7.8 supplemented with a $10^{-2}$ dilution of a PEG precipitate from mock infected cells. ○—○ highly purified HSV, 1 m Na$_2$SO$_4$, pH 6.3; ●—● highly purified HSV, 1 m Na$_2$SO$_4$, pH 7.8.
from mock infected RS cells on the thermal inactivation of highly purified
HSV that was suspended in HBSS was monitored. The addition of this
material readily stabilized the HSV to inactivation at 37 °C at both pH 6.3
and 7.8 as the thermostability of this purified HSV preparation now
approximated that of the HSV-PEG preparation (Fig. 2). This thermosta-
bilizing effect was not due to PEG itself as the thermal inactivation of
highly purified HSV in HBSS was not affected by the presence of 0.001
to 1 percent PEG (data not shown). Thus, the data presented in this report
support the following conclusions: First, the alkaline sensitivity to thermal
inactivation is not an inherent characteristic of HSV but is medium de-
pendent. Second, the rate of HSV thermal inactivation in vitro is in most
instances interdependently affected by composition and the pH of the
suspending medium.

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