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Influence of Environmental pH on the Preservation and Inactivation of Herpes Simplex Virus (40656)

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Successful primary isolation of a virus from clinical specimens is enhanced by inoculating cell cultures as soon as possible. During the period between sample collection and culture inoculation, it has been recommended that the clinical specimen be refrigerated or frozen to preclude inactivation and maintain virus viability (1–3). The use of transport media has been proposed for clinical specimens that are delayed in transit to the laboratory, especially if they are stored at ambient temperatures. Although herpes simplex virus (HSV) is reputed for its lability (1), recovery of this virus from clinical specimens is facilitated by utilizing a transport medium (4–6). Thus, these media apparently stabilize the HSV by retarding inactivation and thereby promote the successful recovery of virus.

Inactivation of virus in clinical samples has been attributed to chemical substances in the specimen, e.g., enzymes and/or to the conditions of sample storage and handling (2). Temperature as an effector of the inactivation of HSV, has been the subject of numerous investigations (7). However, the facility and the kinetics of HSV inactivation at a given temperature has been shown to be modulated by the composition and pH of the medium in which the virus is suspended (8–10). This study was performed to characterize further the concerted effects of environmental pH and incubation temperature as a means of specifically promoting the inactivation or preservation of the replicative capability of HSV.

Materials and methods. The virus employed in this study was isolated from lesions associated with a recurrent infection of the volar surface of the hand. *In vitro* cultivation of this HSV isolate was limited to five passages in an attempt to minimize the selection of or elimination of virus subpopulations as previously suggested (11). Antiserum neutralization, plaque diameter, and pock size (12) all indicated the virus was an HSV type 2 (designated Strain SJ).

Stocks of HSV ($>10^8$ plaque-forming units (pfu) per milliliter) were prepared by chemical concentration of virus from infected cell sonicates, using polyethylene glycol (13). The concentrated virus was diluted 10^{-2} into tissue culture medium containing 5% newborn calf serum and antibiotics, and supplemented with 0.025 *M* *N*-2 hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (hepes buffer (10)). The pH of the medium, adjusted to pH 6.3, 7.0, or 7.8 immediately prior to use, was unaltered as a result of the addition of virus. The HSV samples were incubated at 4, 36, or 40°. At select times, an aliquot of each sample was removed, diluted in Hanks' balanced salt solution (14), pH 6.6, and then plated on monolayers of rabbit skin cells. Following a 2-hr adsorption period at room temperature, the cultures received tissue culture medium and were incubated at 36° for 3 days. Plaques were visualized by crystal violet staining of the cell sheet as previously described (12). On occasion an HSV sample was stored at −85° prior to assay for pfu or a sample had to be reassayed for pfu content. In these instances, all three samples for that time point were treated in an identical manner. Preliminary observations indicated there was no difference in the stability of HSV suspended in tissue culture medium adjusted to pH 6.3, 7.0, or 7.8 when stored at −85°.

Results. The relationships between the environmental pH, the incubation temperature and the inactivation of this HSV-2 strain were examined. The data indicate the following: At each temperature (4, 36, and 40°) as the pH of the medium was increased from 6.3 to 7.0 to 7.8, the HSV surviving fraction decreased. In addition, as the incubation temperature was increased the HSV surviving fraction decreased at each pH tested (Figs. 1A–C). Thus, the combination of elevated temperature and an alkaline pH promoted HSV inactivation, while the combination of lower temperature and acidic pH promoted HSV survival.

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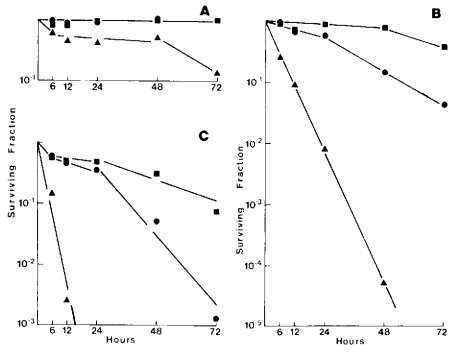

FIG. 1. A stock HSV-2 (Strain SJ) preparation was diluted 10^{-2} into tissue culture medium adjusted to pH 6.3 (■), 7.0 (●), or 7.8 (▲) and incubated at 4 (panel A), 36 (panel B), or 40° (panel C). The pfu content in each sample was determined at the times indicated. The 0-hr virus content for all samples was approximately 6×10^5 pfu/ml.

The reversibility of the pH-associated inactivation effects was also examined. An HSV preparation, diluted into medium adjusted to pH 7.8, was incubated at 36° for 24 hr. At that time, the pH of the medium was adjusted to 6.3 and the sample was reincubated at 36° for an additional 48 hr. Virus survival during the 72-hr incubation period was determined by assaying the HSV pfu. The data indicate that the HSV inactivation at pH 7.8 is irreversible and unresponsive to the subsequent exposure of the virus to an environment which promotes virus stability (pH 6.3). The data further reflect the influence of environmental pH (7.8 vs 6.3) on the rate of HSV inactivation (Fig. 2).

Discussion. The results of previous studies indicate thermal inactivation of HSV is influenced by three factors: the temperature, the composition, and the pH of the medium in which the virus is suspended (8–10). The results presented in this report provide evidence reflecting upon the antagonistic or protagonistic effects of pH on the inactivation of HSV replicative capability suspended in tissue culture medium and incubated at various temperatures. The calculated postlag period half-lives at 36°, pH 6.3, 7.0, and 7.8, of this limited passage recent clinical HSV isolate agree with the values obtained for the plaque-purified laboratory strains of HSV types 1 and 2 (10). In addition, the influence of pH on the preservation or inactivation of HSV has been shown to include 4 and 40°. Thus, these observations permit the selection of an appropriate environment to promote or retard the inactivation of HSV, commensurate with the desire of the investigator.

Preservation of HSV replication potential is requisite for primary virus isolation from clinical specimens. Considering delays associated with transporting samples to the clinical laboratory, a number of investigators have examined the utilization of transport media to enhance HSV recovery when clinical samples are held at ambient temperature for varying periods of time (4–6). Most of the media employed were modified bacteriologic transport media which have a particulate consistency due to the presence of charcoal, agar, agarose, and/or DEAE-dextran. The transport medium pH (7.6) was listed for only one of the media tested, in only one report (4). In addition, one study compared Stuarts medium with a virus transport medium (VTM) which consisted of tissue culture medium containing 10% fetal calf serum (5). In this latter instance the recovery of HSV from clinical specimens held in VTM for 1 to 2 days prior to processing was less than from specimens processed on the day the swabs were taken. This decrease in the efficiency of primary HSV isolation may be a reflection of HSV inactivation at pH >7.0, although the

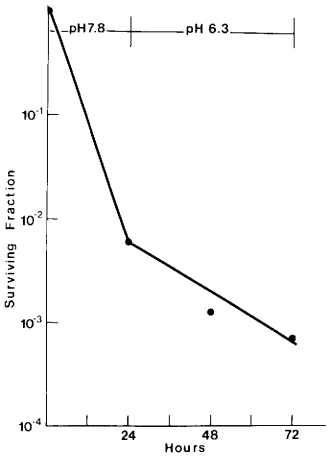

FIG. 2. A stock HSV-2 preparation was diluted 10^{-2} into tissue culture medium adjusted to pH 7.8 and incubated at 36° for 24 hr. The pH of the medium was then adjusted to 6.3. The pfu content was determined at the times indicated. The 0-hr virus content was 3×10^6 pfu/ml.

pH of the VTM was not specifically listed (5). Considering the influence of the composition of the suspending medium on the inactivation of HSV (8–10), a direct comparison of the previous studies with the data in this report is not meaningful. However, the data presented here clearly indicate the lack of HSV inactivation at 4° when virus is suspended in tissue culture medium adjusted to pH 6.3 or 7.0. The utility of this finding as a means of enhancing the primary isolation of HSV from clinical specimens is anticipated.

The ability of a virus to induce the oncogenic transformation of a cell is dependent upon survival of the cell following infection by the virus. In the case of HSV, this has been accomplished by infecting cells with virus previously inactivated by ultraviolet light (15), photodynamic dye (16), or by incubating HSV-infected cells under environmental conditions which preclude virus replication and cytotoxicity (17). A recent report also documented the ability of heat-inactivated (56°) HSV to transform mammalian cells to a thymidine kinase-positive phenotype (18). These observations imply that despite a loss of HSV replicative capability, at least a portion of the thermally inactivated HSV was capable of infecting these cells. Studies in our laboratory on the physical integrity and adsorptive capability of thermally inactivated HSV support this support notion (19). Thus, the observations presented here provide specific means to promote the thermal inactivation of HSV. The utility of this procedure, as a means of producing a virus population which could be employed to examine the transformation and/or oncogenic potential of HSV, remains to be investigated.

Summary. Incubation temperature and environmental pH were evaluated as independent and interdependent effectors of HSV inactivation. Kinetic studies at 4, 36, and 40° showed HSV inactivation was increased as the pH of the suspending medium was increased from 6.3 to 7.0 to 7.8. Conversely, the rate of HSV inactivation was antagonized and significantly reduced at each of these temperatures by adjusting the pH of the suspending culture medium to an acidic level. The utility of these observations as a means of enhancing the successful recovery of HSV from clinical specimens warrants consideration.

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