PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **185**, 484–492 (1987)

Herpes Simplex Virus: Recurrent and Nonrecurrent Strains (42574)

YSOLINA CENTIFANTO-FITZGERALD, DELMAR R. CALDWELL, AND FRED YATES

Department of Ophthalmology, Tulane University School of Medicine, New Orleans, Louisiana 70112

Abstract. A study of three Herpes Simplex strains with different frequencies of recurrent disease was done using the New Zealand white rabbit eye model. Each of the three strains, the McKrae strain (high frequency), the E-43 strain (low frequency), and the CGA-3 (no recurrence) grew well in the rabbit corneal epithelium and produced overt recognizable disease for up to 5 days post-infection, thus minimizing differences in virus reactivation due to a lack of or insufficient ganglionic colonization. Asymptomatic shedding and spontaneous recurrences, as well as iontophoretically induced recurrences, were seen in the E-43 and McKrae strains, but not in the CGA-3-infected animals. The virus strain's optimum temperature was an important aspect of its reactivation process, as shown by the failure of the nonrecurrent CGA-3 to replicate at the host's core temperature (39°C). The fact that these explants yielded infectious virus at 33°C and not at 39°C confirmed that the CGA-3 had colonized the ganglia, and its lack of recurrences or shedding suggests a temperature-dependency relationship. Our observations were further supported by the preferential growth at 39°C of fresh clinical isolates obtained from HSV encephalitis and herpes labialis. Isolates from animals infected with the heterogeneous McKrae were classified as shedders (isolated in the absence of disease) and recurrent (isolated from a recurrent lesion). Both shedders and recurrent isolates were of a homologous nature and retained their phenotype when tested. From this study, we theorize that reactivation and disease may have different regulatory mechanisms. The type of recurrent disease (lesions, asymptomatic shedding, or none) is virus-dependent and frequency of disease may be regulated by host functions.

Primary infection of the corneal epithelium with herpes simplex virus (HSV) results in ocular dendritic disease of varying severity and the concomitant establishment of latency in the corresponding trigeminal ganglia (1). During latency (2, 3), the virus resides in the neuronal cells and is reactivated from time to time through an as yet unknown mechanism. Evidence suggests that upon reactivation the virus replicates in the ganglia which innervates the peripheral site of initial infection and results in either epithelial lesions or asymptomatic shedding. For the sake of clarity, we define recurrence as the reappearance of epithelial lesions and shedding as the recovery of infectious HSV virus from eyes without apparent herpetic disease. Differences in frequencies of lesion reappearance (high, low, or none) and type of lesion (recurrence vs shedding) can be considered as part of the properties which characterize a particular viral strain or phenotype.

In humans with a history of herpetic infection, the HSV can be recovered from tears (4–6), saliva (7), and genital secretions (8), even in the absence of active disease. The virus has also been recovered in individuals without any history of herpetic infection (9). Following primary infection of ocular herpetic disease some patients experience recurrent episodes of disease while others do not (10–11). In the first group, the frequency of recurrence varies greatly, from one to multiple episodes each year. Several studies have attempted to relate recurrence to a variation in the immune system of the host (12), and a cause and effect relationship due to an immune deficiency has been found in some cases (13). However, there is not yet a verifiable explanation of why, in otherwise healthy individuals, some experience episodes of disease while others do not. There is ample evidence to suggest that the inherent characteristics of the virus are determinants in the disease process. Previous work from my laboratory has shown that the type of disease, as well as its severity and duration, is attributable to and characteristic of the infecting virus strain (14–17). Since variations

0037-9727/87 $1.50

seen in humans with herpetic disease can also be seen in New Zealand white (NZW) rabbits (infected with various HSV-1 strains), we chose this experimental animal model for our study of recurrent herpetic disease.

Our study goal was to determine the phenotypic differences between recurrent and nonrecurrent HSV strains, and to identify possible determinants in the recurrence pattern. Specifically, we attempted to find whether failure to reactivate is due to an insufficient colonization of the ganglia or to an inherent incapability of the strain to be reactivated.

Materials and Methods. *Cells and viruses.* Vero and RK-13 cells obtained from Whittaker M.A. Bioproducts (Rockville, MD) were grown in Eagle's basal minimal medium supplemented with 10% fetal calf serum, 1% glutamine, sodium bicarbonate, and antibiotics. From our library of wild-type HSV-1 strains, we chose three strains with differing frequencies of recurrent disease. Those chosen included the McKrae strain (high frequency of recurrence), the E-43 strain (low frequency of recurrence), and the CGA-3 strain (zero recurrence).

For the thymidine kinase studies we included the HSV-1 SC16 strain as a positive control for the thymidine kinase (TK) enzyme. The SC16-SI is a TK mutant strain obtained from the SC16 parent strain and was included as a negative control (18, 19). Both strains were kindly donated by H. J. Field University of Cambridge, United Kingdom. The clinical isolates from herpetic ocular disease used in this study were obtained from our own clinic population. Virus isolated from confirmed cases of encephalitis and herpes labiales were obtained from the Charity Hospital, New Orleans, Louisiana. None of the clinical isolates were repeatedly passed on tissue culture. All the studies were made from a P-1 stock grown at 36.5°C.

Animal model. The characteristics of the New Zealand white (NZW) rabbit as the animal model for ocular herpetic disease have been previously described (20). Three basic types of experiments were designed to assess (1) severity and pattern of primary ocular disease, (2) virus shedding and recurrent disease, and (3) establishment of latency. For the first experiment, each rabbit was infected in both eyes with 10^5 PFU of the virus strain. The animals were examined daily by slit lamp, and the severity and duration of the disease were recorded. The aim of the second experiment was to document ganglionic colonization. The infected animals were sacrificed, and the trigeminal ganglia removed and processed for virus recovery according to described procedures (21). In the third experiment a group of 50 rabbits was infected. At 30 days of postinfection (pi) when the disease had cleared, the survivors were examined for frequency of recurrent episodes of disease as well as for virus shedding. Examinations and culturing were performed three times a week for 4 to 5 weeks.

In vivo titer. To determine the amount of infectious virus present in the cornea during the acute phase of infection, three groups of 10 rabbits were infected (in both eyes) with each of the three chosen HSV strains. At Days 1, 3, 5, 7, and 9 pi, two animals from each group were sacrificed. The eyes were then removed, the epithelium scraped and placed in tissue culture media, and its titer determined in RK-13 cell cultures.

Trigeminal ganglia virus recovery. The trigeminal ganglia was surgically removed from the sacrificed animals and washed with phosphate-buffered saline (PBS) containing antibiotics. The tissue was minced and processed for virus recovery as described above. All cultures were kept for 3 weeks, with weekly sampling of supernatant fluid to determine the presence of infectious virus.

Temperature optimum. Growth of the three virus strains at 39 and 33°C was determined by plaque assay.

Plaque assay. Vero cell cultures in 25-cm^2 flasks were infected with the appropriate amount of virus (100–150 PFU) and allowed to adsorb for 45 min at 37°C. After the adsorption period, maintenance media containing 1% methylcellulose was added. One set of cultures was incubated at 39°C and the other at 33°C. After 48 hr both sets of cultures were stained with crystal violet and the number and size of plaques were noted.

Iontophoresis. Reactivation of latent virus by iontophoresis with 6-hydroxydopamine (6-HD) was accomplished using the method of Shimomura *et al.* (22). The presence of shedding and/or recurrences was recorded daily. Animals were sacrificed after 6 days and the percentages of virus colonization of both tri-

geminal and superior cervical ganglia were determined by cocultivation in RK-13 monolayer culture.

Thymidine kinase studies. For the three HSV prototypes used (i.e., CGA-3, E43, and McKrae), the specific activity of the thymidine kinase enzyme was measured according to described procedures (23). For the various HSV isolates, the TK phenotype was determined by using a rapid assay in tissue culture described by Tenser *et al.* (24) that permits the screening of a large number of strains. In this assay, 100 PFU of the particular virus strain was inoculated onto a rabbit kidney monolayer cell culture and allowed to adsorb for 45 min. The cell cultures were then overlaid with growth medium containing 0.5% methylcellulose. After 2–3 days of incubation and the appearance of discrete, well-defined plaques, the overlay was removed and the maintenance medium containing [^{14}C]thymidine (NE Nuclear) was added. The cultures were incubated again for 5 to 6 hr then the medium was removed and the monolayers washed, stained, and fixed with crystal violet. The stained and dried cultures were then exposed to X-ray film for various periods of time.

Results. *Ocular disease produced by parent strains.* All three HSV-1 strains produced a well-defined herpetic keratitis in the rabbit cornea. Some of the biological characteristics of these strains are illustrated in Table 1. Characteristically, the McKrae strain produced severe epithelial disease that sometimes persisted for more than 10 days and inflicted a 40–60% mortality in the study rabbits. The frequency of disease recurrence was high. In one experiment, 10 of 13 animals had recurrent disease (76%) and in another, 28 out of 30 (93%) had recurrence. The E-43 strain produced mild dendritic keratitis that usually resolved around Day 7 pi, leaving corneas clear and unscarred. Recurrences were less frequent, usually affecting between 30 and 40% of the animals. Mortality was less than 10%. In comparison, the CGA-3 strain produced mild but significant disease but no mortality, virus shedding, or recurrences. All 3 HSV strains had an active thymidine kinase. The presence of thymidine kinase activity of the virus strains was further documented using the rapid method as described above. In this experiment, we included a known positive and negative TK strain as a control for our technique. We found that the McKrae, E43, and CGA-3 strains were positive for TK activity (pictures not shown).

Temperature requirements varied among the strains. At 33°C, the McKrae strain cultures developed twice as many plaques as the cultures at 39°C. The E-43 cultures had a similar number of plaques at both temperatures. In contrast, the CGA-3 cultures had many plaques at 33°C but only about 1% of the plaques developed at 39°C.

Our findings from the recurrent episodes of disease documents and confirms that the McKrae and E-43 strains establish latency, al-

TABLE I. HSV PROTOTYPES OF PARENT STRAINS

Virus strain	No. of plaques/dish		Percentage of mortality	Ocular disease 1 week pi[a]	Recurrences/ No. of rabbits	TK units/ mg protein
	33°C	39°C				
McKrae	330	160	40–60	3.4	10/13 28/30	1.50
E-43	78 75	76 158	>10	2.1	12/30	1.62
CGA-3	150	1	0	1.3	0	1.83

Note. Groups of NZW rabbits were infected in both eyes with 10^{-5} PFU of the HSV strains as described under Methods. All animals were examined daily with a slit lamp biomicroscope. Starting at 30 days postinfection, the surviving animals were examined and cultured three times a week for 45 days to record the appearance of recurrent lesions and shedding episodes. In the temperature experiments, the number of plaques was done in triplicate cultures. The thymidine kinase activity was of the prototypes measured by the method of Lee (23). The TK phenotype of the strains (24), including a positive and negative control, was also included.

[a] Percent of corneal surface involved (average of 10 eyes).

though each causes a different frequency of recurrent disease. The CGA-3 strain, in contrast, does not produce recurrence. It may be that the failure of this strain to produce recurrence is due to low levels of ganglionic colonization which, in turn, may be related to how well the virus grows in the ocular tissue during the primary infection.

HSV in rabbit cornea. To determine the titers of infectious virus present in ocular tissue during primary infection, rabbits in three groups of 10 animals were infected with the McKrae, E-43, and CGA-3 strains. At Days one, three, five, seven, and nine pi, the amount of infectious virus in the cornea was determined as described under Methods.

The results, illustrated in Table II, indicate that all three viruses grew well in the rabbit cornea although more virus was found in the McKrae-infected animals than in the others. The titers of infectious virus in the rabbit cornea correlated well with the observed severity of ocular disease. Although large differences were found between McKrae- and CGA-3-infected corneas, all eyes became infected and exhibited overt disease for at least 5 days. We expect no significant differences in ganglionic colonization for the following reasons: (a) it is known and documented that colonization of the ganglia takes place within the first 24 hr of infection (25) regardless of the subsequent amount of viral growth, and (b) avirulent strains that produced no disease in the eye colonize the ganglia (26). In acute primary infections, only a small number of neurons become latently infected, usually 1% of the total number of neurons in the ganglia (27, 28). From these premises, it seems that the primary ocular infection with CGA-3 strain could confer ganglionic colonization; and the failure to reactivate and shed was due to a factor other than the amount of virus growth at the peripheral site.

TABLE II. *IN VIVO* TITER OF HSV STRAINS IN CORNEAS OF NEW ZEALAND WHITE RABBITS

Days pi	HSV-1 strains CGA-3	E-43	McKrae
3	2×20^4	8×10^4	19×10^5
5	9.8×10^5	8.5×10^6	28×10^6
7	16×10^3	29×10^3	38×10^3
9	5×10^2	6×10^2	3×10^3

Note. NZW rabbits were infected with the HSV strains as described under Methods. At the designated time, two animals were sacrificed, the eyes removed, and the corneal epithelium scraped and placed in tissue culture maintenance media. The amount of infectious virus in each cornea was determined by plaque assays in RK-13 cell cultures.

TABLE III. SHEDDING OF HSV IN INFECTED NEW ZEALAND WHITE RABBITS

Strain	Spontaneous shedding (% eyes)	Induction after iontophoresis (% eyes)	% Positive for HSV after induction (Trigeminal ganglia)
McKrae	92	100	100
E-43	16.7	57.1	100
CGA-3	0	0	25

Note. After iontophoresis induction, 100% of the McKrae-infected animals displayed shedding, including eyes in which spontaneous shedding was not seen. Virus was also isolated from 100% of the trigeminal ganglia. In the E-43-infected animals, an increased shedding of virus was seen, with 100% colonization of the trigeminal ganglia. In contrast, the CGA-3-infected animals failed to shed infectious virus before and after iontophoresis. Only 25% of the trigeminal ganglia released virus.

Ganglionic colonization. Percentages of ganglionic colonization, spontaneous shedding, as well as induced shedding after 6-hydroxydopamine iontophoresis were quantitated and documented for all three prototype strains. To substantiate the establishment of latency NZW rabbits were infected with the HSV strains as described under Methods. During the latent stage following primary infection, the animals were cultured daily for 30 days to record the levels of spontaneous shedding. At the end of that period, all animals were subjected to 6-HD iontophoresis followed by topical epinephrine. The animals were cultured for 5 days immediately following the iontophoresis procedure. They were then sacrificed, and the trigeminal ganglia was surgically removed and processed for virus recovery. The results are shown in Table III. After iontophoresis induction, shedding of virus was observed in 100% of the eyes of McKrae-strain-infected animals, including those in which spontaneous shedding was not previously detected. A similar situation was seen

with the E-43 strain in which shedding of virus after the iontophoresis procedure increased from 16.7 to 57.1% of all eyes. The CGA-3-infected animals did not exhibit virus shedding either before or after iontophoresis.

In this study, 100% of the trigeminal ganglia of McKrae- and E-43-infected animals tested positive for HSV following iontophoresis. In contrast, in rabbits infected with the CGA-3 strain, only 25% of the trigeminal ganglia were positive for the HSV after iontophoresis. The fact that some of the ganglia of the CGA-3-infected animals yielded the infectious virus by cocultivation after iontophoresis indicated that at least in some cases the virus had reached the ganglia and had established latency. Since none of the animals shed or exhibited a recurrent lesion during latency, it appeared that this latent virus could not easily be reactivated at the ganglionic site. It was unclear whether the absence of shedding before and after iontophoresis was due to a lack of ganglionic colonization. Since growth of the CGA-3 strain at the peripheral site was sufficient to expect colonization, recovery of latent virus was attempted at 33°C incubation temperature. This is based on the fact that the core temperature of rabbits is 39°C and the external temperature of the cornea is usually 33°C. Six trigeminal ganglia of latently infected animals were surgically removed, explanted, incubated at 33°C for 3 days, and then seeded onto RK-13 monolayer cultures after which virus was recovered in 100% of the explants. This indicates that colonization of the ganglia took place, but reactivation and replication of the latent virus at the internal temperature of the ganglia did not occur. The fact that the explants yielded infectious virus at 33°C confirmed that CGA-3 had in fact successfully colonized the ganglia and that its apparent lack of recurrence or shedding suggests a temperature-dependency relationship or some other *in vivo* vs *in vitro* difference.

This finding was further tested in another experiment. Surgically removed trigeminal ganglia from CGA-3-latently infected animals were used for explants and cocultivation assays as described under Methods. All cultures were kept at 39°C. The explants failed to release infectious virus in the supernatant fluid. In the cocultivation cultures, a change in morphology of the feeder layer was seen. But again, no infectious virus was released. All the inoculated samplings from these cultures were kept at 36.5°C.

Isolation of shedder and recurrent strains. In these experiments, we have partially characterized three different phenotypes for recurrent disease within wild-type HSV-1 strains. It was also observed that in McKrae-infected rabbits, some shed asymptomatically, some had recurrent disease, and some neither shed nor had disease. It remained unclear why a lesion formed in some cases, while asymptomatic shedding occurred in others. The isolates, obtained from McKrae-infected animals (shedders, recurrent, and ganglionic), were examined for optimum-growth temperature and pattern of ocular disease. Essentially it was found that viruses isolated from shedding episodes produced more plaques at 39°C than at 33°C, while those from recurrent eye lesions grew well at both temperatures or slightly better at 33°C. In contrast, ganglionic isolates displayed the same variability in temperature requirements noted in different stocks of the parent McKrae strain. That is to say, they grew equally at both temperatures or only slightly better at one of the two temperatures.

The McKrae strain, used extensively as a model for recurrent disease, has a high frequency of recurrences and shedding episodes and is also a very heterogenous stock. Thus, a random colonization of the ganglia by any of these virions can be expected. When reactivation at the neuronal cell takes place, different phenotypes are expressed and sent to the peripheral site (hence shedders and recurrent phenotypes can be recovered). This hypothesis was then tested using groups of six rabbits. The eyes in each group were infected with one of these isolates. Six weeks after infection, the animals were subjected to iontophoresis with 6-hydroxydopamine as described under Methods. All the eyes were examined with the slit lamp daily for 7 days after induction. Rabbits infected with the recurrent phenotype produced corneal lesions in five out of six animals with 8 out of 10 eyes exhibiting dendritic ulcers. Rabbits infected with the shedder phenotype showed only small punctate lesions in 3 out of 10 eyes.

Our findings indicate that the phenotype of the infecting strain was conserved, and that "recurrent strains" produced dendritic ulcers

while the "shedders" produced minimal disease in the form of punctate staining.

The optimum temperature of these isolates was of interest to us; the shedders favored a high temperature while the recurrent favored a lower temperature, suggesting perhaps a relationship between type of disease and temperature.

Clinical isolates. The concept that optimum temperature requirements of HSV-1 strains are important factors in the development of clinical disease was reaffirmed by findings in temperature studies with fresh clinical isolates. For these studies, four representative types of isolates were selected and obtained from patients with (1) multiple recurrences per year, (2) sporadic recurrences, (3) herpes labialis, or (4) herpes encephalitis. We found that (a) isolates that produced multiple recurrences grew preferentially at 33°C but also grew at 37 and 39°C, (b) isolates that produced sporadic recurrences were more variable but consistently favored the highest temperatures, and (c) isolates from encephalitis and herpes labialis cases had an optimum temperature range of 37–39°C. Results are presented in Table IV. A true "shedder" isolate was not included in the testing, because study patients already had lesions at the time of their first visit.

TABLE IV. TEMPERATURE OPTIMUM OF CLINICAL ISOLATES AND TYPE OF HSV RECURRENT DISEASE

Patient	No. plaques/dish 33°C	No. plaques/dish 39°C	Clinical history
FA	700	25	Multiple recurrences
GR	880	400	Multiple recurrences
CH	520	110	Multiple recurrences
HO	50	13	Multiple recurrences
1	500	523	Multiple recurrences
2	800	400	Multiple recurrences
3	270	361	Sporadic recurrences
4	76	153	Herpes labialis
5	258	313	Herpes labialis
6	152	210	Encephalitis
7	386	450	Encephalitis
53	152	201	Encephalitis
55	386	448	Encephalitis

Note. Herpes simplex virus strains isolated from our clinical population were tested for their optimum temperature as described under Methods. Isolates from patients with multiple recurrences favor a lower temperature (33°C) while the isolates from encephalitis and herpes labialis grew well at both temperatures and better at the higher temperature.

Discussion. In this communication, we have demonstrated the existence of different phenotypes of ocular herpetic diseases and a possible role for temperature in the recurrence phenomena. The frequency of reactivation at the ganglionic site may be dependent on the strain's characteristics, but the development and frequency of disease may be controlled by host factors.

Central to this study was the question of whether differences in the number of recurrent episodes of disease are due to different levels of colonization of the trigeminal ganglia during primary infection. Several aspects of this work warrant discussion. All three viruses were found to produce overt disease in the NZW rabbit eye. The severity of disease (Table I) and the amount of infectious virus in the rabbit corneas (Table II) was sufficient to colonize the trigeminal ganglia. It is known that colonization of the ganglia occurs within the first 24 hr of infection (25) and that the virus successfully reaches the ganglia even in asymptomatic infections by avirulent strains (29). Therefore, the rate of reactivation and/or recurrences in the three strains studied cannot be ascribed to poor growth of the infecting strains during primary infection or to insufficient colonization of the ganglia.

In the NZW rabbit model, spontaneous shedding and recurrences can be frequent. Our experiments utilized a large number of animals, all of which were subjected to the same conditions or stimuli. Our results suggest that reactivation at the ganglionic site and asymptomatic shedding in the tear film must be determined by the characteristics of the strains in residence in the ganglia (30).

The role of temperature on virus shedding was illustrated by the CGA-3 strain. This particular strain does not shed even after artificial induction by 6-hydroxydopamine iontophoresis. However, when the excised ganglia of

CGA-3-infected animals were incubated at 33°C, rather than at 39°C (which is the core temperature of the host), the virus was recovered in all animals tested. The failure of this strain to shed in the tear film seems to depend on, or be indicated by, the strain's temperature requirement. This phenomenon is similar to that of temperature-sensitive mutants of which some are classified as latency minus for their apparent inability to colonize the ganglia (31). The inability of the CGA-3 strain to achieve strong virus replication at 39°C may account for its failure to shed virus during latency.

The effects of temperature on the pathogenesis of herpes simplex virus infections have been reported by several investigators. Initially, the effect of ambient temperature in the development of the disease was examined (32–33). In these studies, mice were infected intracerebrally with HSV and kept at either 34 or 22°C. It was found that the mice in the group that was kept at 34°C were more resistant to the infection than those kept at the lower temperature. These studies, although significant, were hard to evaluate since any changes in the host core temperature would affect the entire metabolism of the animal.

The studies in which the optimum temperature of the virus is the determinant factor in disease development has been reported for bovine herpes virus (BHV). In this system, it was shown that infection with BHV produced lesions in cooler areas such as the skin and around the udder and teats, while infections in the internal organs did not progress and virus replication was very low. This suggests that temperature effect on viral pathogenesis may operate at a local level rather than by systemic modification of immune responses (34). These observations are in agreement with our findings that the temperature optimum of the infecting strain is a determinant in viral disease.

Recently, Huang and co-workers (35) compared the disease produced by one HSV-2 strain and its cold-adapted variant in intracerebrally infected mice. It was found that although both viruses infected the neurons, the infection produced by the wild type spread through several tissues in contrast with the infection produced by the cold variant, which did not spread. They suggest that the cold variant strain was impaired in its ability to lyse the neuronal cells and spread to other tissues. Recently, Stevens and co-workers (36) have described in their studies that failure to reactivate during latency is due to a neuron temperature-sensitive lesion.

It appears then that the ability of a latent virus to reactivate at the ganglionic site is controlled by the latent virus characteristics and that lesion formations at the peripheral site are more or less determined by host functions.

This peculiar relationship of strain optimum temperature and disease was further illustrated by data derived from fresh cultures obtained from hospital patients. The viral isolates from encephalitis patients and herpes labialis (fever blisters) favored the higher temperature, while those isolated from patients with high frequency of recurrences had a lower optimum temperature.

We theorize that virus shedding and recurrent disease may have different regulatory mechanisms. Our findings indicate that a virus which reaches the peripheral site must find the "proper" environment to further multiply and cause a lesion. For example, viruses that prefer to require a higher temperature can cause a lesion upon onset of a high fever, thermal injury such as sunburn, or because of a preferential localization in the warmer areas of the body, such as the mucocutaneous junctions. The rapid onset of these lesions (from overnight to 24 hr) suggests that asymptomatic shedding may have already occurred, but that upon encountering the "proper" environmental conditions, the virus causes a recurrent episode of disease.

The McKrae strain has a high frequency of both recurrent disease and shedding episodes and is a heterogenous stock. From McKrae-infected animals, isolates were carefully obtained: "shedders" during the absence of disease, "recurrent" from a recurrent corneal lesion, and "ganglionic" from animals with neither shedding nor a recurrence. Most importantly, when these isolates were tested they retained their phenotypes. The "recurrent" isolates produced dendritic ulcers, while the "shedders" produced only punctate staining at best. The homologous nature of these isolates is most likely the result of a small foci of active replication within the ganglia. These data are in agreement with our present understanding of latency in which reactivation

causes a limited foci of viral replication within the ganglia.

It is then apparent that in the pathogenesis of herpetic disease, the HSV strain optimum temperature is important not only on the progress of the primary disease, but in its reactivation from the latent state.

In summary, different aspects of herpetic disease, as well as its clinical course, in otherwise healthy individuals are consistent with and may be determined by the phenotype of the infecting strain.

Supported by Grant 045-2652 from the Eye, Ear, Nose, and Throat Foundation in New Orleans, Louisiana, and in part by the National Institute of Health Grant EY06037. The authors thank Dr. J. M. Hill for technical assistance in some of the iontophoresis experiments.

1. Stevens JG, Nesburn AB, Cook ML. Latent herpes simplex virus from trigeminal ganglia of rabbits with recurrent eye infection. Nature (London) New Biol **235**:216–217, 1972.
2. Cook ML, Bastone VB, Stevens JG. Evidence that neurons harbor latent herpes simplex virus. Infect Immun **9**:946–951, 1974.
3. Galloway DA, Fenoglio M, Shevchuk M, McDougall JK. Detention of herpes simplex RNA in sensory ganglia. Virology **95**:265–268, 1979.
4. Kaufman HE, Brown DC, Ellison EM. Recurrent herpes in rabbit and man. Science **156**:1628–1629, 1967.
5. Nesburn AB, Elliott JM, Leibowitz HM. Spontaneous reactivation of experimental herpes simplex keratitis in rabbits. Arch Ophthalmol **78**:523–529, 1967.
6. Laibson PR, Kibrick S. Recurrence of herpes simplex virus in rabbit eyes: Results of a three-year study. Invest Ophthalmol **8**:346–350, 1969.
7. Douglas RG, Couch RB. A prospective study of chronic herpes virus infection and recurrent herpes labialis in humans. J Immunol **104**:289–295, 1970.
8. Deardourf SL, DeTure FA, Drylie DM, Centifanto Y, Kaufman H. Association between herpes hominis type 2 and male genitourinary tract. J Urol **112**:126–127, 1974.
9. Baringer JR, Swoveland P. Recovery of herpes-simplex virus from human trigeminal ganglions. N Engl J Med **288**:648–650, 1973.
10. Wilhelmus KR, Coster DJ, Donavan HC. Prognostic indicators of herpetic keratitis: Analysis of a 5-year observation period after corneal ulceration. Arch Ophthalmol **99**:1578–1582, 1981.
11. Shuster J, Kaufman HE, Nesburn AB. Statistical analysis of the rate of recurrence of herpes virus ocular epithelial disease. Amer J Ophthalmol **91**:328–331, 1981.
12. Price R, Schmitz J. Route of infection systemic host resistance and integrity of ganglionic axons influence acute and latent herpes simplex virus infections of the superior cervical ganglion. Infec Immun **23**:373–383, 1979.
13. Openshaw H, Sekizawa T, Wohlenberg C, Notkins AL. The role of immunity in latency and reactivation of herpes simplex virus. In: Nahmias AJ, Dowdle W, Schinazi R, eds. The Human Herpes Viruses. Amsterdam/New York, Elsevier, pp287–296, 1981.
14. Wander AH, Centifanto YM, Kaufman HE. Strain specifically of clinical isolates of herpes simplex virus. Arch Ophthalmol **98**:1458–1461, 1980.
15. Centifanto-Fitzgerald YM, Fenger T, Kaufman HE. Virus proteins in herpetic keratitis. Exp Eye Res **35**: 425–441, 1982.
16. Smeraglia R, Hochadel J, Varnell ED, Kaufman HE, Centifanto-Fitzgerald YM. The role of herpes simplex virus secreted glycoproteins in herpetic keratitis. Exp Eye Res **35**:443–459, 1982.
17. Centifanto-Fitzgerald YM, Yamaguchi T, Kaufman HE, Tognon M, Roizman B. Ocular disease pattern induced by herpes simplex virus is genetically determined by a specific region of viral DNA. J Exp Med **155**:475–489, 1982.
18. Field HJ, DeClercq E. Effects of oral treatment with acyclovir and bromovinyldeoxyuridine on the establishment and maintenance of latent herpes simplex virus infection in mice. J Gen Virol **56**:259–265, 1981.
19. Field HJ, Darby G. Pathogenicity in mice of strains of herpes simplex virus which are resistant to acyclovir in vitro and in vivo. Antimicrob Agents Chemother **17** No. 2: 209–216, 1980.
20. Centifanto-Fitzgerald YM. Initial herpes simplex virus type 1 infection prevents ganglionic superinfection by other strains. Infect Immun **35**:1125–1132, 1982.
21. Nesburn AB, Dunkel EC, Trousdale MD. Enhanced HSV recovery from neuronal tissues of latently infected rabbits. Pro Soc Exp Biol Med **162**:398–401, 1980.
22. Shimomura Y, Gangarosa LP, Kataoka M, Hill JM. HSV-1 shedding by iontophoresis of 6-hydroxydopamine followed by topical epinephrine. Invest Ophthalmol Visual Sci **24**:1588–1594, 1983.
23. Lee LS, Cheng YC. Human deoxythymidine kinase. J Biol Chem **251**:2600–2604, 1976.
24. Tenser RB, Jones JC, Rissel SJ, Fralish FA. Thymidine plaque autoradiography of thymidine kinases-positive and thymidine kinase-negative herpesviruses. J Clin Microbiol **17**:122–127, 1983.
25. Klein RJ, Friedman-Kien AE, Kaley L, Brady E. Effects of topical applications of phosphonoacetate on colonization of mouse trigeminal ganglia with herpes simplex virus type 1. Antimicrob Agents Chemother **26**:65–68, 1984.
26. Centifanto-Fitzgerald YM, Kaufman HE. Herpes simplex virus strain specificity and ocular disease. In: Nahmias AJ, Dowdle WR, Schinazi RF, eds. The

Human Herpes Viruses. New York, Amer. Elsevier, p595, 1981.
27. Walz MA, Yamamoto H, Notkins AL. Immunological response restricts the number of cells sensory ganglia infected with herpes simplex virus. Nature (London) **264**:554–556, 1976.
28. Watson K, Stevens JG, Cook ML, Subak-Sharpe JH. Latency competence of thirteen HSV-1 temperature sensitive mutants. J Gen Virol **49**:149, 1980.
29. Centifanto YM, Rayfield M, Tian P, Kaufman HE. Herpes simplex virus latency in the rabbit trigeminal ganglia. Ganglionic superinfection. Proc Soc Exp Med **179**:55–67, 1985.
30. Gerdes JC, Smith DS. Recurrence phenotypes and establishment of latency following rabbit keratitis produced by multiple herpes simplex virus strains. J Gen Virol **64**:2441–2454, 1983.
31. Brown SM, Subak-Sharpe JH, Warren KG, Wroblewska Z, Koprowski H. Detection by complementation of defective or uninducible (herpes simplex type 1) virus genomes latent in human ganglia. Proc Natl Acad Sci USA **76**:2364.
32. Lycke E, Hermodsson S, Kristensson K, Roos BE. The herpes simplex virus encephalitis in mice at different environmental temperatures. Acta Pathol Microbiol Scand Sect B **79**:502–510, 1971.
33. Schmidt JR, Rasmussen AF Jr. The influence of environmental temperature on the course of experimental herpes simplex infection. J Infect Dis **107**:356–360, 1960.
34. Letchworth GJ, Carmichael LE. Local tissue temperature: A critical factor in the pathogenesis of bovine herpesvirus 2. Infect Immun **43**:1072–1079, 1984.
35. Huang DD, Abrams GD, Maassab HF. Neurological involvement in mice after infection with a cold-adapted herpes simplex type 2 virus. Infect Immun **35**:1070–1078, 1982.
36. Cook ML, Thompson RL, Stevens JG. A herpes simplex virus mutant is temperature sensitive for reactivation from the latent state: Evidence for selective restriction in neuronal cells. Virology **155**:293–296, 1986.

Received December 29, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted May 8, 1987.