Archives of Virology 72, 143—168 (1982)

Archives of Virology

The Pathogenesis of Acute, Latent and Recurrent Herpes Simplex Virus Infections

Brief Review

By

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With 4 Figures

Accepted February 17, 1982

The cornerstone of the pathogenetic mechanisms of herpes simplex virus (HSV) infections is the establishment of virus latency in sensory ganglia. The ganglionic location of latent HSV infection was predicted by GOODPASTURE (30) in 1929, and confirmed experimentally in mice 42 years later by STEVENS and COOK (106). It appears that there is a general agreement on the basic features of HSV infection: mucocutaneous primary infection, colonization and acute infection of ganglia, followed by establishment of latency in neurons; the latent virus may be subjected to periodic reactivation associated in some cases with the development of recurrent lesions. However, the details of this mechanism are not fully elucidated and some of its aspects are still the subject of opposing views. The purpose of this review is to highlight some of the unknowns, to analyze conflicting experimental data, and to offer a few ideas which may contribute to a better understanding of the pathogenetic mechanisms of HSV infections.

The discussion of the mechanisms involved in the initiation of the acute ganglionic infection, in the establishment and the maintenance of latency, the reactivation process, as well as the prevention and treatment of the infection as related to pathogenetic mechanisms is based mainly on articles published over the past 5—6 years. Earlier data have been extensively discussed in previous reviews (4, 19, 43, 72, 80, 94, 105, 117), and the immunological aspects of latency have been analyzed recently (3, 95).

The Initiation of the Acute Ganglionic Infection

After peripheral inoculation of experimental animals with HSV, the amount of virus which can be detected in corresponding sensory ganglia shows a continuous increase for the first 4 to 7 days post-infection (p. i.). This increase is

0304-8608/82/0072/0143/$ 05.20

followed by a decrease of free virus titers, so that by the 14th day p. i. virus can no longer be isolated from cell-free ganglia homogenates. At this point the acute phase terminates and the latent phase of the ganglionic infection begins.

The orthodox view is that HSV travels centripetally along the axons from peripheral inoculation sites towards the ganglia, where a productive infection in neurons is initiated (13). The evidence supporting these assumptions is based on electronoptic images showing the presence of isolated virions in neurons and nerves several days after infection (13, 36, 53, 55, 57). However, the electronoptic images of neurons never show the the presence of virus factories similar to those seen in neurons in which virus is reactivated after *in vitro* cultivation of latently infected ganglia (5).

The presence of viral mRNA in ganglia during the acute phase of infection is another indication of a productive infection (88). However, the ratio of viral RNA to viral DNA is only 0.05 to 0.17, indicating either that virus multiplication is restricted to only a few virions, or that virus synthesis is arrested at an early stage. In addition, results obtained with *ts* (62, 64) and Acyclovir-resistant (ACV^r) mutants (47) have clearly shown that a productive infection is not required for the establishment of acute infections in neurons and ganglia. When ACV^r mutants penetrate the ganglia after peripheral inoculation, no increase in virus titer can be observed after the virus becomes detectable by the 2nd day p. i.; instead a continuous decrease in virus titer takes place until the mutant disappears altogether from the ganglia (51).

Another set of observations which might be at variance with the concept of a productive infection in ganglia during the acute phase of infection stems from studies on the effect of antiviral drugs and immune serum on the establishment of acute and latent HSV infections in trigeminal ganglia of mice. Several drugs and immune serum which cannot prevent the invasion of ganglia by HSV have nevertheless the ability to induce a significant reduction in both the titer of free virus during the acute phase and the amount of reactivtable virus during the latent phase, when compared to similar values observed in untreated mice (44, 48, 49, 52).

The above results suggest that the increase of virus titers in ganglia during the acute phase of infection may result from a continuous virus supply originating at the peripheral inoculation site, rather than by a productive infection inside the neurons.

Substantiating this view are experiments which showed that when virus multiplication is arrested at an early stage at the peripheral inoculation site with phosphonoacetic acid (PAA), virus titers in ganglia lag well behind those observed in untreated mice (R. J. KLEIN and E. DESTEFANO, Abstracts. International Workshop on Herpesviruses, July 27—31, 1981, Bologna, Italy, pp. 140 to 141). PAA treatment was started 3 to 18 hours after infection, thus permitting the initiation of local multiplication and a limited invasion of the nervous system. If the virus titer increases in ganglia were caused by a productive infection then the local antiviral treatment could not prevent the intraganglionic process of virus multiplication. Local antiviral treatments can, however, reduce virus titers in ganglia during the acute phase of infection by halting the continuous virus supply from the site of the primary infection.

Sequential Isolation of HSV from Ganglia and Nerves

Studies by WILDY (121), KRISTENSSON *et al.* (56) and COOK and STEVENS (13) have shown that after footpad inoculation of HSV in mice the virus is first detected in ganglia or spinal cord and only afterwards in the sciatic nerve. Our own studies (46) have shown, in addition, the following noteworthy features: i) Free virus was not detectable in the distal and proximal sections of the sciatic nerve during the first 2 days p. i. This failure was not due to very small amounts of virus in the verve, since even when the nerves were kept after removal from the organism for 2 days in explant culture, no virus could be detected. ii) Virus titers were always higher in the proximal than in the distal section of the sciatic nerve. iii) Free virus was detectable in the lumbosacral ganglia by the 2nd day p. i. iv) Virus titers were always higher in ganglia than in the sciatic nerve.

These observations are hard to reconcile with the concept of retrograde axonal migration of HSV from the peripheral inoculation site to the ganglia. In order to do so one has to assume either i) that the migration proceeds at very high speed so that the chance to isolate virus from nerves at any time is very low, or ii) that during migration the virus assumes a non-infectious form. The first assumption would be hard to prove, but the identification of viral DNA in nerves during the first 2 days p. i. would lend strong support to the second suggestion.

The observation that HSV is first detected in ganglia and only afterwards in the nerves is, however, compatible with the hypothesis that colonization of ganglia proceeds by systemic routes. If this were true, then i) HSV should be found in the blood or lymph during the acute phase of infection, and ii) acute ganglionic infections should be initiated in mice after ipsilateral neurectomy.

Viremia During Primary HSV Infection

HSV has been detected in the blood of newborn mice after intranasal inoculation (41) and in adult and newborn mice after footpad inoculation (61, 121). However, there are other studies reporting the absence of virus in the blood after corneal, footpad, intravaginal or intraperitoneal inoculation (53, 56, 57, 92). On the other hand, HSV type 2 inoculated in the footpad of guinea pigs was regularly found by the second day after infection in regional lymph nodes (101). This observation suggests that the lymphatic system can be used in virus dissemination through the organism. Our own attempts to isolate virus from blood were only partially successful: we detected virus in cocultivated lymphocytes isolated from the blood only in about 10 percent of mice inoculated in the footpad (46). In this connection it is worthwhile to mention that HSV was isolated from the buffy coat of patients with meningitis (16, 73). It appears, therefore, that HSV can be present in the blood, but that its isolation is connected with some as yet unidentified difficulties. The importance of the circulatory system in the colonization of ganglia was highlighted by the demonstration that latent ganglionic infections were initiated after virus inoculation in the tail vein followed by tail amputation precluding secondary neural spread (14).

Effect of Neurectomy on the Penetration of HSV in the Nervous System

Surgical section of the sciatic nerve 3 days before virus inoculation prevented the isolation of HSV from the spinal cord in 4 out of 5 mice on the 10th day

after inoculation (121). Detection of virus in the ganglia was not attempted. Ligature, freezing and colchicine treatment of the sciatic nerve 4 to 0 days before virus inoculation into the footpad of mice reduced the mortality to 10 percent, compared to an almost 100 percent mortality when these procedures were applied after virus inoculation. However, the isolation of the virus in ganglia or the spinal cord was not attempted (56). Section of the sciatic and femoral nerves was able to prevent completely the establishment of latent HSV infections in dorsal root ganglia of guinea pigs after footpad inoculation (100). Following the section of the sciatic and femoral nerves of mice inoculated in the footpad with pseudorabies virus, the mortality was reduced to about 70 percent (100 percent mortality in control mice). In addition in all mice examined, the virus was detected in the dorsal root ganglia despite the sectioning of the sciatic and femoral nerves (23).

In view of these rather conflicting results, we have recently reinvestigated the initiation of the acute infection in sensory ganglia of mice after sciatic and/or femoral neurectomy (46). We were regularly able to demonstrate the presence of free virus on the 4th and 7th day after infection in spinal ganglia of mice in which either the sciatic or the femoral nerve was cut. However, by sectioning both nerves the establishment of acute infections in spinal ganglia was prevented in about 80 percent of mice.

It appears, therefore, that axonal migration is the preferred route for colonization of sensory ganglia by HSV. However, the results of COOK and STEVENS (14) regarding the establishment of latency after intravenous virus inoculation, those of FIELD and HILL (23) obtained with pseudorabies virus, as well as the incomplete protection observed in our experiments, indicate that under certain circumstances the virus may use alternate routes to reach the ganglion.

The Orthodox and the Heretic Views and a Pragmatic Attitude

The orthodox view that HSV invades the ganglia through axonal migration and initiates a productive infection in the neurons could be challenged by the heretic view that the virus migrates to the ganglia by systemic routes and accumulates in neurons by a continuous supply from the site of the primary infection. Neither view is based on unshatterable proof. Evidence for either view is circumstantial, incomplete and sometimes contradictory. It does, however, make little difference for the final outcome—the establishment of latency — whether the virus invades the ganglion through axonal migration or systemic spread, or whether the accumulation of virus in ganglia results from a productive infection or through a continuous supply from the site of the primary infection.

The difference between these views becomes, however, important when the treatment of primary HSV infections is considered. A topical local treatment may not suffice if acute ganglionic infections are initiated also by extraneural routes, whereas systemic antiviral treatments would make little sense if the virus reaches the ganglion only through axonal migration. Since the virus may reach the ganglion by multiple or alternate routes, a safe attitude would be the use of both local and systemic antiviral treatments. The local treatment would prevent the penetration of the virus in nerve endings and would run dry the source for continuous virus supply. Systemic treatment may prevent virus

multiplication in extraneural tissues and even in the nervous system if the local treatment is only partially effective.

The Latent Phase of Infection

At the end of the acute phase of infection free HSV can no longer be detected in sensory ganglia (77). However, a very low rate of virus synthesis in ganglionic cells cannot be excluded, since it might escape conventional techniques of virus detection. Discontinuous or continuous virus synthesis during the latent phase of the infection are the two opposite propositions of the static or dynamic state hypotheses of latency respectively (94).

The Transition from the Acute to the Latent Phase of Infection

Mechanisms which determine the transition from the acute phase of the infection, when free virus can be isolated from ganglia, to the latent phase, when no infectious virus can be detected in cell-free ganglia homogenates, have not yet been elucidated. This transition is paralleled by the development of immune responses and since T-cell deficient nude mice invariably develop an acute fatal HSV infection of the nervous system unless protected by passive transfer of immune serum or spleen cells (70), it has been proposed that immune factors play a major role in the switch from the acute to the latent phase of the infection (77, 78, 102).

Three models by which immune factors may act have been suggested (78). i) The immune modulation model assumes that ganglion cells are permissive for HSV replication and that the host's virus-specific antibodies modulate the productive infection so that a nonlytic, latent infection is established in ganglion cells. ii) The immune elimination model assumes that there are two populations of ganglion cells: one permissive in which a productive infection develops, and one nonpermissive in which a latent infection is instated. Antibodies will eliminate the productively infected cells, but will not affect the latently infected cells. iii) The third model assumes that ganglion cells, nonpermissive for virus replication become permissive after receiving signals from the inflammatory reaction in the skin elicited by local virus multiplication. The host's immune response then turns off this signal by decreasing virus replication in the skin. This returns ganglion cells to the nonpermissive state and latency is established.

The basic idea of these models is that all or some ganglia cells are or can be made permissive for virus replication. This, however, has not been demonstrated and it appears that the increase of virus titers in ganglia results from continuous supply from the site of the primary infection (see section Initiation of the Acute Ganglionic Infection). Secondly, by assigning to antibodies the role of switching an acute to a latent infection merely postpones a solution, since no explanation is available on how antibodies mediate this transition.

An alternate model can be proposed by assuming that ganglion cells are nonpermissive for virus replication and that colonization of ganglia is achieved by continuous virus supply from the site of the primary infection (Fig. 1). The development of immune responses restricts virus replication in mucocutaneous tissues, thus halting additional virus penetration in the ganglia. It is likely that

the majority of virions in neurons are inactivated by cellular enzymes, and only a minority will be integrated in a latent form in cellular structures. This quantitative assessment is based on data which show that during the latent phase there are 10 to 20 times less HSV DNA equivalents per cell than during the acute stage of the infection (88).

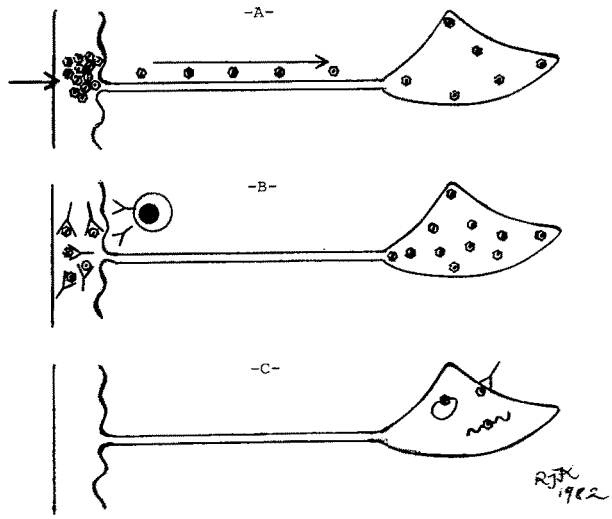

Fig. 1. Hypothetical model for the transition from the acute to the latent phase of HSV injections. *A* Penetration and replication of virus in skin is followed by migration of virus to ganglia and colonization of neurons. *B* Development of immune responses, followeed by neutralization of virus in skin, ends the process of migration and colonization. *C* Virus is eliminated from skin. Immune and/or other mechanisms eliminate the majority of virus from neurons. A few virions become integrated in a latent form in some cellular components

The transition of the latent stage might then be mediated by virus and host factors which have evolved during the long evolutionary relationship between herpesviruses and man (71). Latency confers obvious survival advantages for herpesviruses and may also provide man with some survival benefits, such as protection against infection with other neurotropic viruses.

This model does not offer a precise explanation for the transition from the acute to the latent stage of the HSV infection. It has, however, a heuristic value at least as great as those models which rely solely on immune mechanisms to account for the transition.

Viral Functions Necessary for the Establishment of Latency

It has been shown that temperature sensitive *(ts)* mutants of HSV differ in the capacity to establish latent ganglionic infections in mice. Both DNA-positive and DNA-negative *ts* mutants are capable of establishing latent infections and, conversely some DNA-negative as well as DNA-positive *ts* mutants are latency-negative (62, 116). The data suggest that DNA synthesis is not essential for the establishment of latency.

FIELD and WILDY (24) found that thymidine-kinase negative (TK^-) HSV mutants have a low pathogenicity and a decreased ability to induce latent infections in mice. This finding was confirmed with TK^-, arabinosylthimine resistant HSV mutants in guinea pigs (110) and mice (86, 109), and acyclovir-resistant mutants in mice (21, 47, 51). Based on these facts TENSER and DUNSTAN (109) hypothesized that HSV TK expression is necessary for the establishment of latent sensory ganglion infections. On the other hand, PRICE and KHAN (86) speculate that TK^- mutants establish latency without initial productive infection and without detectable antigen expression, and that the defect in enzyme synthesis results in the inability of the latent virus to become reactivated. This hypothesis predicts that when refined methods will be applied the presence of latent genome will be detected (86).

Our data which showed that mice whose trigeminal ganglia were colonized by an acyclovir-resistant and TK^- (F. RAPP, personal communication) HSV type 1 mutant are resistant to subsequent colonization with the latency-positive parental strain, can be explained by either mechanism: Resistance to reinfection may be mediated by the presence in neurons of the non-reactivtable TK^- mutant, or by some permanent change induced in neurons during the temporary presence in ganglia of the TK^- mutant which never became latently established. Only further experiments will decide between these alternatives; for the present time facts indicate that TK^- mutants colonize the ganglion, but do not multiply and can no longer be detected after their short temporary presence in the ganglia (47, 51).

Finally, WATSON *et al.* (116) analyzing 13 *ts* HSV type 1 mutants for their capacity to establish latent infections in mice concluded that the immediate early viral protein VP175, and at least one additional later functioning virus gene product, are needed for establishment of the latent state. In one of the *ts* latency-negative mutants the production of a 122,000 M. W. protein is affected, but no function has yet been assigned to this gene product.

The Search for Traces of Virus Activity During Latency

Since all attempts, with a single possible exception (98), to isolate infectious virus from cell-free ganglia homogenates have failed, more subtle approaches have been used to detect traces of virus synthesis in latently infected ganglia. One of the methods used was to determine if HSV mRNA is transcribed from the DNA of the latent virus. When RNA extracted from latently infected mouse trigeminal ganglia was hybridized against radiolabeled HSV DNA, viral mRNA could not be detected (88). Since the method cannot exclude transcription of a small portion of the viral genome, the result suggests that there is at least a partial, if not a complete, block of transcription during the latent stage. On the other hand, when human paravertebral ganglia were examined for the presence of viral mRNA by *in situ* cytological hybridization using a nick-translated HSV DNA probe, viral mRNA was detected in 2 out of the 7 sets of human ganglia examined (28).

The discrepancy in the outcome of the above two studies may reside either in a difference between the nature of latency in mice and in men, or may result from a difference in procedures. Since humans are subjected to episodes of

spontaneous reactivation, whereas mice are not, it is quite possible that in human ganglia a low level of virus synthesis, as reflected in the presence of HSV mRNA, is maintained. On the other hand mouse trigeminal ganglia can be processed to a cell-free homogenate within minutes after the killing of animals, whereas human ganglia are removed, at best, within a few hours after death. It is not impossible that during these few hours the process of reactivation is initated in the cadaver, leading thus to the synthesis of viral mRNA.

A second approach was the attempt to detect virus-specific thymidine kinase (TK) in latently infected ganglia. Using latently infected mouse ganglia, YAMAMOTO *et al.* (122) were able to detect the virus-specific enzyme up to 60 days after primary infection, although free virus was no longer detectable after 14 days. The presence of the enzyme during these 60 days could, therefore, result either from a low level virus synthesis, or may reflect the persistence of the enzyme from the acute phase of infection. Working with latently infected guinea pig ganglia, FONG and SCRIBA (26) could not detect virus-specific enzyme either during the acute or during the latent phase of infection. Since in guinea pigs HSV can reside in a latent form also in the footpads (99), and independently from the ganglionic infection (100), the presence of viral TK was examined at this particular site of the primary infection. Viral TK was detected in footpad cells only during the acute phase, thus reflecting active virus multiplication only at the site of the primary infection (26).

The slightly different outcome of experiments regarding the presence of viral TK in latently infected tissue of mice and guinea pigs may again hint at differences in which latency is established and maintained in various species.

Complicating further the problem was the detection of viral polypeptide VP175 in latently infected trigeminal ganglia of rabbits by indirect immunofluorescence (31). VP175 is an immediate early protein which can be detected by 2 hours and reaches its peak rate of synthesis by 4 hours after infection (38). VP175 may be required to block the synthesis of immediate early proteins and to maintain the synthesis of early proteins (18). A *ts* mutant overproducing VP175 was found to be defective in the synthesis of late HSV proteins (15). Given the general regulatory functions of VP175 one is tempted to hypothesize on its role in the maintenance of latency. If continuous synthesis of VP175 is required for the maintenance of latency in sensory ganglia of rabbits, then virus-specific mRNA should also be present in ganglia. However, two other possibilities may be considered: i) since persistent HSV infection in rabbits is associated with frequent recurrences, it might be possible that HSV is maintained in ganglia in a dynamic rather than in a static state. ii) The unavoidable lapse of time between the killing of the animal and the processing of ganglia could be long enough for the initiation of VP175 synthesis and its subsequent detection by indirect immunofluorescence.

Both the experiments with viral mRNA and viral TK suggest that during the latent phase there is a complete or an almost complete block of the expression of the viral genetic information and, that at least in mice, the static state hypothesis is more likely than the dynamic state hypothesis. This still leaves unanswered the question in what form the virus is maintained in this static state. Three possible forms may be considered: i) complete virions, inhibited by unknown

factors in expressing their genetic information; ii) free nucleic acid located in the nucleus or cytoplasm, as a result of early interruption in the growth cycle; and iii) viral nucleic acid integrated in chromosomes of latently infected neurons. RITCHIE and TIMBURY (93) commenting on the similarity between the structure of HSV DNA and that of transposons, suggest that the viral genome could insert into the host DNA by legitimate or illegitimate recombination depending on whether the cellular DNA does or does not contain herpes-like repetitive DNA sequences.

Permanence of the Latent Infection

Latently infected ganglia are the source for reactivtable HSV, and it appears that this source is not depleted for long periods of time. At least four factors, two quantitative (the number of latently infected neurons, and the number of neurons involved during each reactivating event), and two qualitative (the fate of the neuron after reactivation, and the possibility to infect new sets of neurons after each recurrent episode) seem to be involved in maintaining the latent infection (Fig. 2).

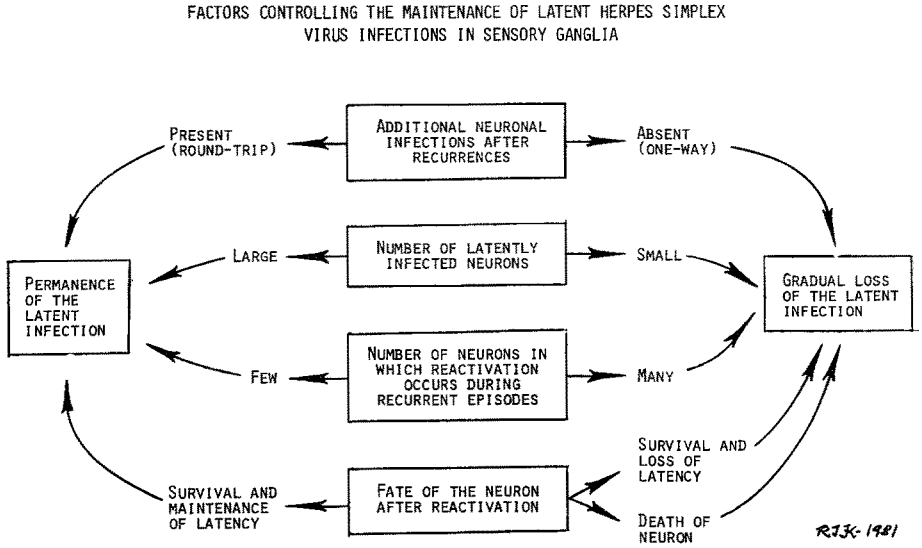

Fig. 2. Quantitative and qualitative factors involved in the prolonged maintenance of latent HSV infections in sensory ganglia

How Many Neurons Become Latently Infected Once the Acute Phase of Infection Is Terminated?

Human trigeminal ganglia contain about 150,000 neurons, those of cats and rats about 50,000 (88). There are no estimates regarding the number of neurons in the trigeminal ganglion of the mouse. Based on their relative number in men, cats, and rats, one might estimate that the number of neurons in the trigeminal ganglion of the mouse is about 10^4 to 2×10^4.

It has been shown (115) that the percentage of latently infected cells in mouse trigeminal ganglia is 0.1 percent (10 to 20 neurons), which is probably an under-

estimate. It is safe to assume that mild primary infection will lead to the establishment of latency in fewer neurons than a severe primary infections. We have indeed shown that several antiviral compounds and immune serum which cannot prevent the invasion of ganglia by HSV, have nevertheless the ability to mitigate the severity of the infection, and protect mice against death. In these mice the relative amount of reactivatable virus in ganglia is significantly reduced when compared to similar values observed in untreated mice (44, 48, 49, 52). Since human primary HSV infections can range from asymptomatic to very severe, one may assume that the proportion of latently infected neurons in humans can be anywhere between 0.1 and 10 percent (150 to 15,000 latently infected neurons in each trigeminal ganglion).

In How Many Neurons Does Reactivation Take Place During Each Recurrent Episode?

No direct data regarding the number of neurons in which reactivation takes place during each recurrent episode are available. It has been shown that after neurectomy the proportion of detectable latent infections in cervical dorsal root ganglia of mice is significantly reduced (64). This would indicate that virus is reactivated in most or all neurons harboring a latent infection, and that neurons are either destroyed or loose the latent virus after the reactivating event induced by neurectomy. However, since latent infections can still be detected in about 25 percent of ganglia after neurectomy (32 to 62 percent in our unpublished experiments), one may argue that latency disappeared precisely from those ganglia in which only a few neurons became latently infected after the primary infection. Indeed, preliminary data suggest that there is a relation between the frequency of persisting latent infections after neurectomy, and the virus dose used in the primary infection. Ganglia in which large numbers of neurons are latently infected, will continue to present evidence of reactivatable virus even after neurectomy. As a working hypothesis I would suggest that the number of neurons in which latent virus becomes activated during each reactivating event is limited, and not dependent on the total number of latently infected neurons. However, it is quite possible that the nature of the stimulus which induces reactivation may influence the number of neurons in which the latent virus becomes activated. On the other hand, the severity of each recurrent episode might be determined by the number of neurons in which reactivation takes place.

What Is the Fate of the Neuron After the Latent Virus Has Been Reactivated?

The fate of the neuron after the latent virus has been reactivated is not yet known. Three possible outcomes can be visualized: i) The neuron is destroyed after virus reactivation. ii) The neuron is not destroyed after reactivation. iii) The neuron is not destroyed, but the latent virus disappears from the cell after the reactivating event.

Several facts hint that the neuron may be destroyed after reactivation: Electronoptic images show active virus synthesis in neurons of latently infected ganglia maintained in explant culture (5). Ganglia in which reactivation was

induced by neurectomy show a strong immunofluorescence of neurons (64). However, neuronal death is not a necessary outcome of a productive virus infection, as demonstrated by the recovery of cells damaged by poliovirus infection of the spinal cord (8). On the other hand, the absence of sensory losses in persons suffering from recurrent herpes is not necessarily an argument against the absence of neuronal death after virus reactivation: Since during each recurrent episode only a limited number of neurons are probably destroyed, this slight and gradual loss over a long period of time could be compensated for by the large number of remaining functionally active neurons.

Is the Number of Latently Infected Neurons Fixed After the Primary Episode, or Can Latency Be Established in New Sets of Neurons After Recurrent Episodes?

Some time ago I proposed two mechanisms by which the maintenance of latency might be explained (43). Both explanations assumed that the neuron is not destroyed after reactivation and that reactivation takes place in all latently infected neurons during each recurrent episode: i) During recurrences the latent virus is activated and the virus travels centrifugally to the skin. The information for renewed recurrences is never lost from the latently infected neuron; each episode is a "one-way" transmission from neuron to skin site. ii) During recurrences the latent virus is activated and eliminated from the neuron. After travelling centrifugally to the skin and inducing lesions, the virus will have completed the first leg of its "round-trip". Virus synthesized in the skin will then migrate centrifugally toward the neuron and reestablish a latent infection.

Neither mechanism has been proved or rejected. However, reconsidering now the maintenance of latency it is clear that "one-way" or "round-trip" mechanisms must be included in the larger context of the quantitative aspects which may control the permanence of latent HSV infections in neurons of sensory ganglia.

Facing the Unknowns

It appears, therefore, that any hypothesis regarding the permanence of latent infections must face the following facts: i) The number of neurons which become latently infected after the primary episode may range from tens to thousands. ii) The number of neurons in which reactivation takes place during each recurrent episode may represent a variable proportion of their total number. iii) After reactivation the neuron may be destroyed or can survive; in the latter case the latent virus can either be eliminated, or can be maintained in the surviving neuron. iv) The number of latently infected neurons can either be fixed and defined after the primary episode or additional neurons can become latently infected after each recurrent episode.

A large number of combinations of these variables can be taken as starting points for a hypothesis explaining the prolonged maintenance of latency. However, we shall describe only three possible scenarios, based mainly on the fate of the neuron after the reactivation of the latent virus and on the absence or presence of neuronal reinfection after each recurrent episode. We will assume that after the primary episode in humans, generally not more than 1 percent of

the neurons become latently infected and that the number of neurons in which reactivation occurs during each episode is low (1 to 10 percent).

Hypothetical Scenarios Which Could Explain the Permanence of Latent HSV Infections in Neurons of Sensory Ganglia

Repeated One-way Trips From Few Out of Many Potential Points of Origin

After the primary infection of the ganglion a relatively large number of neurons become latently infected. Reactivation takes place only in a few out of many latently infected neurons during each recurrent episode. The neurons in which reactivation has taken place may either be destroyed or lose permanently the latent virus. However, the large number of latently infected neurons provides a long-lasting source of reactivatable virus for repeated recurrent episodes. If after the primary infection only 1500 neurons become latently infected and reactivation takes place 5 times a year in 20 neurons at a time, the number of latently infected neurons could still maintain a recurrent disease spanning over 15 years.

Repeated One-way Trips From a Stable Point of Origin

After the primary infection of the ganglion only a small number of neurons become latently infected. Reactivation takes place in all latently infected neurons. The neurons in which reactivation has taken place are not destroyed and the information for renewed reactivation is not lost thus maintaining the recurrent disease for prolonged periods. If this mechanism is operational, a single infected neuron could in theory maintain the recurrent disease.

Repeated Round-trips From Renewed Points of Origin

After the primary infection of the ganglion only a small number of neurons become latently infected. Reactivation takes place in all or most of the latently infected neurons. Neurons in which reactivation has taken place are either destroyed or lose the latent virus. However, the reactivated virus, after inducing recurrent lesions at the peripheral site, will reinfect by centripetal migration a new set of neurons, thus maintaining a source of reactivatable virus for repeated recurrent episodes.

The above mechanisms try to explain the permanence of latency under the least favorable conditions and do not exclude other possible combinations of the variables of the equation. In the first scenario neurons are destroyed, reinfection does not occur, but latency is maintained for prolonged periods through sequential reactivation of the virus in a few out of a relative large pool of latently infected neurons. In the second scenario only a very small number of neurons are latently infected, but latency is maintained by the persistence of the latent virus in neurons not destroyed by the reactivating event. In the third scenario only a very small number of neurons are latently infected, the neurons are destroyed after the reactivating event, but latency is maintained through the reinfection of new sets of neurons after each recurrent episode.

The Spontaneous Healing of Latent HSV Infections

The same combinations of variables which can explain the endurance of latent HSV infections in sensory ganglia, may also account for a possible spontaneous cure from latency (Fig. 2). Three conditions must be fulfilled in order to eliminate a latent infection: i) The number latently infected neurons should represent only a small proportion of the total number of neurons in ganglia. ii) The neurons harboring a latent infection must be destroyed or shall lose the latent virus after the reactivating event. iii) Reinfection of neurons shall not occur after recurrent episodes, or the number of reinfected neurons shall be significantly lower than the number of neurons destroyed after each reactivating event.

Scientific evidence for spontaneous healing of recurrent herpes is not available. Only long-term prospective studies of patients suffering from recurrent disease would provide this type of information. Fortunately, numerous representative groups of patients have been involved over the last years in short-term studies regarding the effectiveness of antiherpetic drugs. These groups could be monitored for the next 10 to 25 years and valuable information might be obtained regarding the persistence or the spontaneous healing of recurrent HSV infections.

The Reactivation Process

The absence of infectious virus in cell-free ganglia homogenates, obtained from humans or experimental animals which have suffered in the past a primary HSV infection, defines operationally the latent phase of the viral infection; the detection of infectious virus in these homogenates indicates that the process of reactivation has been initiated. It is conceivable, although difficult to prove, that the process of reactivation will remain limited to the presence of infectious virus in ganglia. In most cases, however, it appears that this phase is followed either by asymptomatic virus shedding or by development of virus-induced mucocutaneous lesions (Fig. 3).

Experiments with latently infected mice show that infectious virus can indeed be detected in cell-free ganglia homogenates of latently infected mice, within 24 hours after the induction of reactivation. It appears also that the amount of infectious virus is low and difficult to detect: 17 percent (32 out of 187) of mice had virus in the ear tissue, but only 6 percent (19 out of 333) in the superior cervical ganglion after induction of reactivation with cellophane tape stripping or xylene application (W. A. Blyth, Abstracts, International Workshop on Herpesviruses, July 27—31, 1981, Bologna, Italy, pp. 138—139). Virus was also detected in a small proportion of mouse ganglia in which virus reactivation was induced by dry ice application to the lips (77). A similar low frequency of infectious virus isolation from spinal ganglia was observed by Walz, Price and Notkins (114), Price and Schmitz (87) and McLennan and Darby (64) and in our laboratory (unpublished data) after induction of reactivation by sciatic nerve sectioning. Likewise, viral antigen can be detected only in few neurons after induction of reactivation by cyclophosphamide treatment (58) or neurectomy (64). The nature of these experiments precludes the establishment of a direct relationship between the presence of virus in ganglia and the subsequent presence of the virus in the skin. Nevertheless, they strongly suggest that unrestricted

multiplication of reactivated virus does not occur and that infectious virus does not persist for prolonged periods in the ganglia after induction of reactivation.

Asymptomatic virus shedding occurs spontaneously (107), or after surgical manipulations (32, 75) of the trigeminal ganglion of rabbits with latent HSV infections. Asymptomatic shedding of HSV can also be induced by iontophoresis of epinephrine into the cornea of rabbits with latent HSV infections (60). HSV can be isolated from the skin of the footpad of guinea pigs (99) and the skin of the ear of mice (35) with latent infections, but without any clinical symptoms of recurrent lesions.

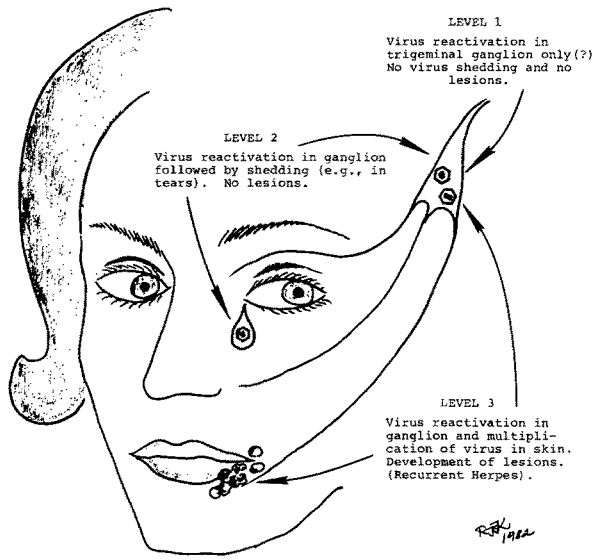

Fig. 3. The different levels at which reactivation of latent HSV can be detected

In humans asymptomatic virus shedding (but also development of recurrent lesions) is observed after microneurosurgical decompression of the trigeminal ganglion nerve root (84). Reactivation of HSV occurs commonly in immunosuppressed patients after organ transplantation (2, 54, 67, 74, 81, 85, 90). Although most of these patients develop herpetic lesions, in some of them reactivation is restricted to asymptomatic virus shedding. Asymptomatic virus shedding in women with latent HSV infection appears to be a frequent occurence; WHITLEY *et al.* (119) have recently found that 70 percent of mothers delivering HSV-infected newborns present asymptomatic viral shedding. However, only 10 percent of non-pregnant women with a history of recurrent herpes presented evidence of asymptomatic HSV shedding (1). Spontaneous asymptomatic HSV shedding in throat, saliva, urine, cervical secretion, and tears of persons with a history of recurrent herpes is mentioned in numerous articles and the frequency varies between 0.5 and 10 percent indifferent populations studied (see for review 19).

There is evidence that the process of reactivation is not necessarily followed by the development of the characteristic mucocutaneous lesions, that reactivation

is frequently associated only with asymptomatic virus shedding and that reactivation might be limited to a transitory presence of infectious virus in sensory ganglia harboring the latent infection.

Reactivation of the latent virus can in some cases be related to specific inducing agents, described and discussed in previous reviews on recurrent herpes (19, 43, 80, 105, 117). When no specific agent can account for the event, the process of reactivation is called spontaneous. "Spontaneous" is on one hand a convenient label to hide our present ignorance regarding not yet identified inducing agents. But "spontaneous" may be closer to a reality, i. e. describing a stochastic process within the neurons in the ganglion which leads to the expression of the latent virus and the synthesis of infectious virus.

When the inducing agents are known, we must ask how they act. Specifically we may question whether they act directly on the latent virus, whether they bring about reactivation through a mediator, or whether they just promote the development of recurrent lesions by an already reactivated virus. The first attempt to explain these complexities was the "skin-trigger" hypothesis forwarded by HILL and BLYTH (34) and based on two assumptions: i) asymptomatic virus reactivation occurs frequently and microfoci of infection are present in the skin at the time of reactivation; and ii) inducing agents do not act directly upon the latent virus in the ganglion. Instead some of the stimuli, such as UV light and trauma induce a local inflammation which is largely mediated by local hormones. These modifications in the skin will then facilitate the development of recurrent lesions. Among local hormones, prostaglandin E2 (PGE2) plays a major role: PGE2 when injected subcutaneously in mice with latent infection is able to induce recurrent lesions (7). In addition PGE2 enhances the spread of HSV in cell cultures, increasing the size of plaques, whereas inhibitors of PG synthesis decrease their size (33). Recently (65) it was shown that antisera to PGE significantly supressed experimental allergic encephalomyelitis in rats and prevented graft-versus-host and host-versus-graft reactivation in mice, suggesting that PGEs are important mediators of the early CMI responses *in vivo*.

The "skin-trigger" hypothesis does not provide an explanation for the mechanism of action of inducing agents which do not act upon the skin: emotional factors (stress, anxiety, depression) or physiological states (menstruation, fatigue). Nevertheless, the "skin-trigger" hypothesis provides for the time being a valid working hypothesis for the mechanism of action of some of the inducing agents.

The frequency of reactivating events, as estimated by the number of clinical recurrent episodes, seems to be related to the antigenic type of the virus and/or the location of the latent virus. Prospective follow-up studies showed that among patients with a first episode of HSV-1 genital infection only 14 percent experienced a subsequent recurrent episode, as compared to 60 percent of those with a HSV-2 infection (91). This observation suggests that latent HSV-1 is less prone to be reactivated in the sacral sensory ganglia then HSV-2, or that these ganglia restrict the reactivation of the latent HSV-1. If the converse would be true for orofacial and eye HSV infections, then the discrepancy between the high frequency of persons with HSV serum antibodies and the lower estimated number of persons suffering from the recurrent disease could be partly explained. Another factor which may contribute to this discrepancy consists from latent infections with

non-reactivatable virus: it was shown that more than 50 percent of ganglia from cadavers, which had been consistently negative for spontaneous release of virus in explant culture, contained latent virus that could be rescued following superinfection of the explant culture with *ts* mutants (9). It is quite conceivable, therefore, that virus which was non-reactivatable in ganglia explant cultures, was likewise not amenable to reactivation in the living organism.

Regarding the discrepancy between the estimated frequency of persons with HSV specific serum antibodies and the estimated number of patients suffering from recurrent herpes, one may ask whether the presence of antibodies reflects the presence of a latent infection.

Examining retrospectively 307 mice inoculated in the orofacial area with HSV-1 which were used over the last 2 years as controls in various experiments done in our laboratory, we found the following: 8 percent of the mice had no antibodies and were not latently infected, suggesting that they were never successfully infected; 2 percent had antibodies, but no latent infections were detected in trigeminal ganglia; 1 percent presented a latent infection, but had undetectable antibody titers; finally, 89 percent had antibodies and were also latently infected. These data suggest that, at least in mice, the presence of HSV-specific serum antibodies is a reliable indicator for the presence of a latent infection, and that the absence of antibodies is in most cases a reflection of the absence of latent infections. If this situation were to hold true in humans, then it would indicate that in the majority of the population latent HSV is not amenable to reactivation. Whether this is due to a special state of the latent virus, or to control mechanisms of the human organisms cannot be determined at the present time.

Prevention and Treatment of HSV Infections

The discussion of the problems related to the prevention and treatment is not intended to provide a review of drugs and their mechanism of action and of therapeutic approaches used or tested in recurrent HSV infections. Several recent reviews cover these aspects extensively (6, 27, 40, 68, 79, 103, 118). Suffice it to say that attempts to treat recurrent herpes have generally failed or yielded meager results in double-blind controlled studies (29, 39, 63, 69, 96, 104). The temporary relief was minimal, and ganglionic latency—the source for future recurrent episodes—was not affected by the antiviral drugs. Studies presently conducted with acyclovir, phosphonoformate, and other antiviral compounds will indicate how much benefit one may derive from their use in the treatment of recurrent herpes labialis and genitalis.

There are, however, situations where the use of antiviral compounds has and will provide definite help. One case is HSV encephalitis successfully treated with arabinosyladenine (119), and the second is the preventive treatment of immunosuppressed patients in order to preclude the recurrence of the latent HSV infection. In these cases acyclovir has already been proven to be highly effective (12, 17, 66, 97), and interferon might also be helpful (11, 83).

Recent studies in experimental animal models have shown, however, that acyclovir (21, 48, 82, 108, 113) does not affect an established latent infection,

and Field and Wildy have stated that "attempts to eradicate the latent infection during the quiescent phase are likely to be fruitless and these drugs should not be used for this purpose".

This being so, the only way to handle the problem of latent infections is to prevent their establishment. Since the discovery of the cell-transforming ability of inactivated whole HSV (20) precludes the use of killed virus as a vaccine, two alternatives have been considered: subunit virus vaccines and attenuated live virus vaccines. Several studies with subunit vaccines in experimental animal systems have shown that these vaccines can prevent the fatal outcome of the infection, but yielded variable results regarding their ability to prevent the establishment of latent infections (10, 37, 42, 45, 59, 68, 76, 89). It appears that subunit vaccines will require multiple administrations of large amounts of viral protein for protection against latency and that protection will wane markedly with time (112). Even if an effective subunit vaccine will eventually be developed, its application will face difficult logistical problems and will be associated with high production costs.

During our investigations on HSV latency we observed that mice with an established latent infection are resistant to subsequent challenge inoculation done at the site of the primary infection, and only rarely develop latent infections when challenged at a different site (50). These observations suggest that latently infected ganglia cannot be reinfected.

Mice do not develop spontaneous recurrent infections, the hallmark of the human disease, and are thus protected for lifetime once they survive a primary infection. In order to confer the same degree of protection in man a non-reactivatable virus should be used. Field and Wildy (24) showed that TK$^-$ mutants have a reduced propensity to induce latent infections in mice. This observation was confirmed in mice and guinea pigs with TK$^-$ mutants or drug-resistant mutants selected by their virtue of being TK$^-$ (22, 86, 109, 110, 111). We have selected an acyclovir-resistant mutant, which proved to be completely latency-negative, and which was able to protect mice against latency when they were reinfected with a high dose of the latency-positive parental HSV type 1 (47, 51) strain. This mutant is latency-negative, non-pathogenic, does not induce cell-transformation *in vitro* (F. Rapp, personal communication) and has the capacity to protect against an otherwise fatal infection with wild-type virus. These characteristics are required properties of a live attenuated HSV vaccine.

A live vaccine against HSV infections raises technical and ethical problems. The technical problems include the following: i) to determine the duration of the protection, ii) to study the remote possibility that the latency-negative virus may become reactivated, iii) to determine the number of inoculations required to confer an overall protection of the body, iv) and to investigate the degree of cross-protection against the heterologous antigenic type. All these and other aspects can be solved in reliable and reproducible animal systems.

The ethical problem consists in the willfull inoculation of a live virus vaccine whose ultimate behaviour in any individual human being can never be predicted. Although we know that natural infection with the wild virus will lead in most cases to harmful consequences, we cannot control and determine in advance whether it will or will not have harmful consequences. However, the ethical

question can also be asked from the opposite point of view: do we have the right not to inoculate an attenuated virus with definite beneficial effect but unknown ultimate fate when we know that the natural infection will lead in most cases to long-lasting detrimental effects?

The ethical problem cannot be settled by discussions alone. Only by solving the technical aspects can we reach a valid ethical decision. Therefore, investigations regarding a live attenuated vaccine are justified, and even if today the opposition to the idea is still strong, it is quite likely that the pressure from the millions suffering from recurrent herpes will require in the near future the use of a vaccine able to prevent the establishment of latent HSV infection in sensory ganglia. The basic idea behind this approach is that only ganglia which were colonized by an attenuated virus can be protected against invasion by wild virus strains; and that immune responses induced by various subunit HSV vaccines will provide only temporary and incomplete protection.

Conclusions (Fig. 4)

Herpes simplex virus enters the host organism in most cases through mucocutaneous surfaces, where virus replication takes place. From the site of the primary infection the virus starts to colonize corresponding sensory ganglia migrating along the nerves and under certain circumstances probably also through the blood and lymphatic vessels. Infectious virus, detectable in cell-free homogenates, persists in the ganglia for a limited time only. This sequence of events characterizes the acute phase of infection. If the infection does not spread further to the spinal cord and central nervous system, the host organism survives and becomes a carrier of a latent viral infection.

It appears that immune responses may mediate the transition from the acute to the latent stage of the infection, but other mechanisms evolved during the long evolutionary relationship between herpesviruses and their vertebrate host could also be involved. The expression of some viral functions, such as the synthesis of an immediate early protein and of viral thymidine kinase, may be necessary for the establishment of latency, but synthesis of viral DNA is not essential. During the latent stage infectious virus cannot be isolated from cell-free ganglia homogenates. However, viral mRNA, enzymes, and antigens can be detected in the sensory ganglia of some host organisms during the latent stage of the infection. Whether these products reflect viral activity during the latent stage, represent footprints of the acute ganglionic infection, or are produced only after the removal of the ganglion from the organism cannot yet be decided. The site of the latent infection are the neurons of sensory ganglia although in some organisms (guinea pigs) latent virus was detected in extraneural tissue.

The latent virus is subjected in some host organisms to periodic bouts of spontaneous reactivation. During reactivation infectious virus can be detected in cell-free ganglia homogenates, and the process can be associated with asymptomatic virus shedding at mucocutaneous surfaces. The hallmark of reactivation is the development of recurrent lesions at the site of the primary infection. Reactivation can be elicited by a series of inducing factors which may act directly or indirectly on the latent virus. Inducing factors acting on the skin

Primary Acute Infection
- Virus multiplication at the site of inoculation (eye, skin, etc.)
- Colonization of ganglia by HSV
- Infectious virus detectable in cell-free ganglia homogenates

Establishment of Latency
- Viral functions probably required:
  - immediate early proteins,
  - viral thymidine kinase,
  - viral DNA synthesis not essential
- Transition from acute to latent phase
  - immunological and/or non-immunological mechanisms might mediate the establishment of latency

Latent Infection
- Infectious virus not detectable in cell-free ganglia homogenates
- Viral functions expressed during latency
  - viral mRNA (present in human but not in mouse ganglia)
  - viral thymidine kinase (temporary presence in mouse, but absent in guinea pig ganglia)
  - VP 175 (in rabbit ganglia)

Reactivation of Latent Virus
- Detection
  - infectious virus in cell-free ganglia homogenates
  - asymptomatic virus shedding
  - development of recurrent lesions
- *In vitro* reactivation of latently infected ganglia
  - mechanism: surgical severance of the nervous connections of the ganglion prior to explantation (?)
- *In vivo* reactivation
  - spontaneous: no obvious inducing factors are incriminated
  - caused by indusing factors
    - acting on skin: mediation by protaglandins (?)
    - acting on the ganglion: mechanism unknown

Maintenance of Latent Infection
- Factors controlling the endurance of latency
  - number of neurons harboring latent virus
  - number of neurons in which reactivation occurs
  - fate of the neuron after reactivation
  - infection of neurons after reactivation

Fig. 4. Summary of pathogenetic mechanism of acute, latent and recurrent herpes simplex virus infections

may mediate the reactivation process through an increased synthesis of prostaglandins. The frequency of reactivating events may depend on the state of the host organism, the nature and intensity of inducing factors, and on the antigenic type of the latent virus harbored in particular ganglia.

Latency, once initiated, persists for prolonged periods or even for the lifetime of the host organism. The permanence of latency is controlled by a series of factors, such as the number of neurons which become latently infected after the primary infection and the number of neurons in which reactivation occurs during each recurrent episode. The duration of the latent infection is also dependent on the fate of the neuron after reactivation (survival or death) and on the possibility of establishing new sets of latently infected neurons after each recurrent episode.

The same factors, when working in the opposite direction, may also lead to a spontaneous cure of the neuron from the latent infection. The cure of latency by antiviral compounds has not been achieved, since the latent virus is not affected by these drugs. However, drugs can prevent the establishment of latency when treatment is applied early after the initiation of the primary infection or can at least reduce virus colonization of the ganglia and, as a consequence, also reduce the number of neurons which will eventually become latently infected. Likewise, antiviral drugs can prevent virus reactivation or reduce virus replication and spread after reactivation has been induced. It is in this area where antiviral drugs have given their best performance. Specific vaccination against HSV infections in human is not yet available. Experimental subunit vaccines might provide temporary protection against primary infection, but a long-lasting protection of ganglia against latency can probably be achieved only by colonization of ganglia with latency-negative virus mutants.

Note added in proof: In continuation of previous studies regarding the presence of HSV mRNA in latently infected human paravertebral ganglia, Galloway, Fenoglio and McDougall [J. Virol. **41**, 686—691 (1982)] have shown that transcripts from the left-hand 30 per cent of the viral genome are present in all of the HSV-positive cases (14 out of 40 patients). RNA homologous to other sequences from the L component of HSV type 2 genome were present less frequently, and no RNA from the S component was detected. Many genes are encoded in the 30 per cent left-hand segment, including the gene for the viral thymidine-kinase. The results suggest that an incomplete, but specific transcription of the HSV genome takes place in latently infected human ganglia.

CENTIFANTO-FITZGERALD *et al.* [J. Exp. Med. **155**, 475—489 (1982)], using stromal and epithelial disease-producing HSV-1 strains and several of their recombinants have shown that the characteristics of ocular herpetic disease in rabbits are genetically determined by the virus strain. The viral functions controlling the oculat disease pattern are situated between 0.70 and 0.83 map units of the HSV-1 genome. This observation provides an important clue in explaining the pleomorphism of HSV-induced symptomatology.

Acknowledgments

I would like to thank Dr. J. Vilček for critically reading the manuscript and Miss M. Cassano for excellent secretarial assistance.

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Received February, 16, 1982