The Latent Herpes Simplex Virus

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INTRODUCTION

One of the most interesting concepts in the study of host-pathogen relationships is the subject of viral latency. The elucidation of the mechanisms regulating the establishment and maintenance of animal virus latency has been a tedious process, with definitive explanations still forthcoming. Yet, the importance of this topic is becoming increasingly apparent, since a latent virus may provide the inoculum needed to establish a variety of acute recurring infections and perhaps initiate and maintain an oncogenic process.

Two types of mammalian viruses, herpes simplex virus type 1 (human herpesvirus 1, HSV-1) and herpes simplex virus type 2 (human herpesvirus 2, HSV-2), have received significant consideration regarding viral latency (196, 216, 217, 245). Pathologically, HSV-1 is commonly involved in lesions above the waist and HSV-2, which appears to be venereally transmitted, is primarily associated with genital lesions (121, 132, 177, 211, 267). So common is infection by these viruses that exposure to them occurs in most of the population at an early age, resulting in a variety of primary infections. In the majority of these cases, the virus will assume a position of latency and infections recur in three-quarters of those so afflicted (216). Most of these recurring infections bring only minor discomfort, although both HSV-1 and HSV-2 have the capability of inflicting serious damage, including encephalitis (239), blindness (31), and possibly cancer (136, 137). Indeed, the virus needed to bring about these maladies may be provided endogenously by latent HSV.

In this review, we shall attempt to organize recent reports on the latency of HSV based on previously proposed hypotheses. Beginning with factors that tend to suppress herpetic infections, we shall then follow the virus through the various aspects of the latent state and conclude with a discussion of current methods of treatment and the potential involvement of HSV in the oncogenic process. Hence, by clarifying the various facets of the topic of HSV latency, we hope to bring into focus the current advances and problems in this area, and in so doing assist investigators of this enigma.

ESTABLISHMENT OF A LATENT STATE

Latency may be defined as that state in which a virus remains present within an individual without initiating any overt infection. It seems difficult to reconcile the existence of a latent state with a virus known for the rapidity with which it replicates and destroys its host cell. Thus, it would appear that the most likely explanation for HSV to enter this period of quiescence, after having established a successful focus of infection, is that conditions have arisen or intensified in the milieu such that the surrounding environment is no longer conducive to the production of new virions. Indeed, the host immune response is a prime agency for effecting such a change, for, when it is impaired with immunosuppressive therapy...

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Among the first components of the immune system to arrive at the site of a recurrence are the humoral antibodies whose presence may serve to limit HSV activity (84). These antibodies, coupled with complement, are able to neutralize or destroy virus or infected cells containing virus-specific antigens (29, 60, 97, 113, 175, 214, 258, 266). Furthermore, some studies have suggested that IgA, the secretory immunoglobulin associated with mucous surfaces (22), may also play a role in HSV containment in mucosal tissue (60, 158, 175, 252). Moreover, the complement-antigen-antibody union mediates the release of anaphylatoxin or similar products, and produces a chemotactic factor, which may result in leukocytic infiltration of the affected area (28, 166, 169, 240). By adhering to the endothelium in large numbers, leukocytes may wall off the viral lesion and thus help to localize the infection (60). Macrophages then migrate into the infected tissue where some appear to be abortively infected by HSV (244), and in this unusual manner they may help to prevent the spread of infection.

Also in cases of recurrent infection, thymus-derived sensitized lymphocytes (T-cells) possess the capability of recognizing and responding to stimulation by either HSV-1 or HSV-2 antigens with a high degree of specificity (219, 220). These activated lymphocytes may also release a nonspecific cytotoxin and a macrophage migration-inhibiting factor (MIF; 90), both of which may assist in HSV containment. Support for this concept was recently provided by Wilton et al. (263), who suggested that the absence of MIF and lymphocyte cytotoxin may predispose the cells to the establishment of recurrent infections. Furthermore, lymphocytes, independently or in concert with macrophages, may produce interferon, resulting in reduced or limited viral replication in neighboring uninfected cells (60, 71, 93, 210). In this regard, studies of herpetic encephalitis and conjunctivitis have established both the prophylactic and therapeutic results achieved by interferon in HSV-initiated disease processes (23, 26, 40, 130, 172, 198, 204). Yet, conflicting reports by others suggest that HSV may be somewhat resistant to the effects of interferon (95, 183, 189, 230, 231, 255).

According to the studies of Lodmell et al. (152), both the humoral and the cellular immune systems must operate together in a synergistic fashion to suppress HSV infections. The possibility of such enhanced dual action has gained support from the in vitro work of Ennis, who observed a similar phenomenon (69). These results suggest that neither system alone possesses the capability of terminating the infection. However, when both systems are operating efficiently in association with the inflammatory response, the virus is unable to replicate. Unless it is transmitted to a new host by the unwitting assistance of the infected individual, the virus faces eventual destruction if it continues on its normal course. Therefore, the maintenance of maximum replication potential requires that an apparent latent state temporarily take effect. Then, when the cellular environment is more amenable, the virus can reappear and begin the replication sequence anew.

POSSIBLE MECHANISMS OF LATENCY

Having entered the latent state, in all likelihood as a result of the pressure exerted upon it by the body’s defense system, the virus may be visualized as existing in one of two possible conditions, as set forth by Roizman (216). The static state hypothesis maintains that certain cells (known as virogenic cells), in which the lytic cycle is reversibly interrupted, are capable of sheltering a nonreplicating HSV or its genome between recurrent infections. The alternate proposal, known as the dynamic state hypothesis, suggests that the latent virus appears to be multiplying very slowly, resulting in a chronic subclinical infection, perhaps in the vicinity of the site of the recrudescences.

Dynamic State Hypothesis

Support for the dynamic state hypothesis evolves from observations of both in vivo and in vitro persistent infections. In 1953, Buddingh et
al. (32) reported the isolation of HSV from the saliva and feces of apparently healthy children who presented no clinical evidence of herpetic infections. Other reports have described the isolation of HSV from oral, nasal, and ocular secretions in clinically quiescent individuals suffering from minimal recurrent herpes infections, as well as from individuals with an extensive history of herpetic recurrences (65, 134, 151, 229). Furthermore, Kaufman et al. (128, 129) were able to isolate HSV from the lacrimal glands of rabbits and the tears of human subjects during periods of clinical quiescence.

Although most of the above HSV isolates are undoubtedly type 1, similar reports describe the recovery of HSV-2 from the genitourinary tract during overt as well as latent infections (41, 42, 117, 161, 163, 212). Such findings are significant to the dynamic state hypothesis and demonstrate the body's capability to harbor replicating virus with no apparent ill effects.

A natural extension of chronic in vivo studies is the correlative work performed in vitro. Persistent infections have been established in vitro by Hampir and Burroughs (95) and Nii (183, 184) in different cell types. Although the experimental systems were dissimilar, both underwent cycles of cell destruction followed by periods of cellular repopulation, during which both virus and cells coexisted in what may be described as a fluctuating state of equilibrium. These studies also underlined the importance of genetically determined factors (95, 96) as well as antibody (184) in the maintenance of this dynamic state of cell-virus coexistence. Although the mechanism responsible for maintaining a cell-virus equilibrium is undefined, these systems apparently provide the prerequisites required to establish a chronic cell-virus interaction (indeed, establishment of chronic herpes infection has been observed even when simian virus 5-infected cultures were superinfected with HSV [16]).

An outgrowth of these and other studies was the recent discovery that defective herpes particles generated during a productive infection may be capable of partial interference with the viral replicative cycle (21, 30). Such interference could conceivably result in the termination of acute infections and in the establishment of chronic infections (100, 183), for these particles may induce interferon production or directly interfere with superinfection by HSV, resulting in a controlled but persistent viral infection characteristic of the dynamic state (100, 183). Therefore, it follows that those factors which would tend to increase the ratio of interfering to infectious particles, possibly resulting in the release of large numbers of defective HSV, could be responsible for the onset or maintenance of the latent state.

It should be pointed out that work on defective herpesviruses is in what might be termed an embryonic state. The importance of defective particles in the infectious process has only been appreciated within the past few years (110). Studies on their interaction with the host tissue are currently becoming the focus of widespread investigation.

In summary, in vitro studies suggest that a dynamic relationship exists between the virus and cell population which could be the result of various host cell factors. Consequently, if this hypothesis is valid, the in vivo and in vitro studies taken collectively may support the existence of persistent infections which could account for the reappearance of infection.

**Static State Hypothesis**

One may visualize the inception of a nonreplicating or static state as being initiated by either a specific or a nonspecific host cell-virus interaction. A nonspecific inhibition of viral replication would be equated with the presence of adverse environmental conditions within the cell which preclude the initiation or maintenance of viral multiplication. These conditions, such as a lack of required nutrients or proper growth conditions, are not a direct rebuttal to the presence of the virus but, rather, reflect the fortuitous state of affairs within the cell at the time of viral entry. On the other hand, the cell may specifically respond to the infecting virus by producing a substance which bars virus replication (216). This substance may be interferon or another inhibitory product. Interestingly, extracts which can inhibit the replication of HSV have been obtained from malignant lymphoma cells (206) as well as hamster cells transformed by HSV-2 (64).

It is implicit within the definition of the static state that infectious virus is not observed in the vicinity of infection during quiescence. However, the static state does not necessarily imply a completely inactive viral genome, since latent viruses at times reveal their presence through specific markers. It is the detection of virus-specific products in the absence of infectious virions which often serves to reveal the presence of either part or all of the viral genome and, in this manner, the existence of a static state virus. For example, both simian virus 40 (SV40) and adenovirus induce virus-specific nonvion-
associated proteins (e.g., T-antigen) in transformed cells even though infectious virus is usually absent (24, 87, 111). Such an antigen has yet to be described for HSV, but the day may not be far removed when the existence of a herpesvirus T-antigen is established. At present there are reports suggesting that herpesvirus nonvirion antigens do indeed exist (9, 108, 227). In fact, it has been demonstrated that thymidine kinase, an early enzyme which may be specific for HSV (187), was present in mouse cells (173) and in human cells (55) abortively infected with this virus. These studies may represent a stable association of at least some of the viral genes with the host cell in the absence of infectious virus. In addition, in vitro studies indicate that HSV nucleic acids (47) and structural antigens (66) may reside in HSV-transformed cells, even though here again infectious virions could not be found.

It should be emphasized that studies indicating the presence of viral antigens within a cell merely suggest that at least some portion of the viral genome is present and is being expressed. This does not necessarily indicate that the complete infectious genome is present. Nevertheless, other methods have been used to determine whether an infectious but latent viral genome exists within a cell. Basically, these methods involve altering the latent system such that infectious viral progeny are recovered. The presence of the SV40 genome in certain transformed cells can be demonstrated by utilizing cell fusion techniques. When this occurs, late viral functions can be expressed, resulting in new virus production, whereas prior to this event no infectious virus can be detected (140). Furthermore, in cells of AKR mice in which neither virus nor viral products can be demonstrated, it is possible to induce the production of murine leukemia virus by exposing the cells to X irradiation or ultraviolet irradiation (223). Results such as these set a precedent for the existence of a static state in HSV latency because they established that animal viruses can remain silent within host cells with minimal or no detectable expression of the viral genetic material.

Similarly, several studies of HSV-cell systems have been conducted in which viral synthesis was activated in cells which had previously been devoid of any detectable signs of a viral genome. Fortuitously, Aurelian et al. (8, 11) induced HSV from cervical carcinoma cells by elevating the pH of the culture medium. Prior to this pH adjustment, there was no indication of the presence of virus within the tissue. Also, Stevens and Cook (243, 245, 246) could demonstrate infectious HSV in ganglia of latently infected mice and rabbits during quiescence, but only after these ganglia had been excised and maintained as organ cultures for several days. Moreover, Nii (184) has encountered the static state in an HSV-infected cell system, where no infectious virus was demonstrable for a period of several months before reactivation. The culmination of this line of experimentation was reported by O'Neill et al. (191), who were able to suppress HSV-2 infection in human embryonic lung cells with cytosine arabinoside (ara-c), an inhibitor of deoxyribonucleic acid (DNA) synthesis (149). During this period of quiescence, the virus was still present, but in a noninfectious form. However, several days after the removal of ara-c, fully infectious HSV-2 reappeared. Hence, it appears as if these investigators had artificially produced, maintained, and terminated a static state system in vitro.

The form HSV assumes while in a nonreplicating state is unknown. Because of the lack of evidence supporting the concept that the complete virion is present within a cell, many investigators have assumed that during the static state the virus exists solely as nucleic acid. Thus, the problem becomes one of determining whether viral nucleic acid exists in an integrated state (i.e., covalently attached to the host's chromosomal DNA) or in a nonintegrated state (i.e., not attached to the chromosomal DNA and possibly existing as an episomal factor). Most work in this area has been done on viruses which have proven to be more amenable to characterization than has HSV. As might be expected, bacteria provided the earliest and most abundant data on integrated phage genomes (80, 147, 264) and nonintegrated episomal factors (114); however, recent work performed on cells transformed with adenovirus or SV40 demonstrate that the DNAs of these viruses are closely associated by covalent linkage with the host genome (92, 98, 228). To attest to this intimate association, Marin and Littlefield (159), utilizing hybrid cultures of normal and transformed cells, demonstrated that with the loss of specific chromosomes the cultures also lost their transformed morphology. Similarly, Croce et al. (49) recently reported that in hybrid cultures of mouse and SV40-transformed human cells the SV40 T-antigen was expressed only when the human C-7 chromosome was present in such cultures.

More directly related to HSV latency has been the demonstration that Epstein-Barr virus
(EBV), a member of the herpes group, can exist as nucleic acid contained within the nucleus. Using nucleic acid hybridization techniques, zur Hausen and Schulte-Holthausen (268), as well as Nonoyama and Pagano (185), have provided strong evidence for the existence of the EBV genome within the nucleus of malignant lymphoma cells.

As can be seen from the above data, most of the work on the location of the viral DNA during latency has involved the host cell’s nucleus. At present there is no evidence supporting the idea that a herpesvirus exists in a latent state as an extranuclear episomal factor. However, the above-mentioned studies on the relationship between EBV DNA and the nucleus do not clearly indicate whether a herpes genome is capable of existing in an integrated state. Yet, with respect to this latter point, it may be significant that characteristic breaks at certain regions of chromosomes in HSV-infected leukocytes have been reported (12). These breaks may only signify weak points in the chromosomal structure (248), but then again they may represent points from which integrated pieces of viral DNA have been activated. In this regard, Frenkel et al. (81) and Roizman and Frenkel (218), using nucleic acid hybridization, examined the virus-free tissue of a cervical carcinoma for the presence of HSV-2 genetic material. Their studies revealed that approximately 40% of the HSV-2 genome was present and is covalently linked to the host chromosomal DNA. Although this indicates that some HSV DNA can exist in an integrated state, it has yet to be shown that an entire nondefective HSV genome can remain in this condition. Also, the problem of whether a latent HSV genome exists as the intact duplex or as a single strand of DNA remains to be clarified, since both possess infectivity (237).

Finally, it should be mentioned that the existence of either a dynamic state or a static state does not rule out the presence of the other in a given individual or population. As described above, experimental data exist to support both states, and the work of Nii (184) may indicate that HSV is capable of reverting from one state to the other.

**LOCATION OF THE LATENT VIRUS**

There are three regions of the body which appear to be particularly susceptible to recurrent HSV infections. These regions are the genital area, the eye, and the oral cavity. Each of these locations has been studied to determine whether HSV is present in the absence of lesions and to locate, if possible, the specific tissue(s) that harbors the latent virus.

**Genital Area**

Unfortunately, little work has been done to investigate latent or recurrent HSV-2 genital infections; however, several current reports describe the isolation of infectious virus from genitourinary specimens. Rawls et al. (212) isolated HSV-2 from smegma of four individuals, although all had penile lesions at the time. Moreover, Centifanto et al. (41) and McIndoe and Churchouse (163) were able to demonstrate the presence of virus even in the absence of specific lesions, but others have had only minimal or variable success in locating HSV-2 in genitourinary tract specimens (91, 117, 135, 182). Consequently, HSV-2 may be able to enter a latent state, but at present no specific sheltering site for the genital type of HSV has been identified.

**Eye**

In work performed on rabbits subject to recurring herpes keratitis, a herpesvirus was isolated or identified during periods of quiescence in ocular secretions, corneal stromal cells, the lacrimal gland, and the Harderian gland (57, 128, 145, 202, 250). Yet, in a similar study Nesburn et al. (180) were unable to recover the virus in any specific eye tissue or associated gland except in organ cultures of the trigeminal ganglion. As previously mentioned, Kaufman et al. (129), working with human subjects, noted that HSV was present in the tears of individuals who did not exhibit herpetic lesions; this fact, in conjunction with his experiments on rabbits, suggested that the lacrimal gland may be the site of chronic HSV multiplication in humans (129). In a more recent report, Dawson et al. (58) were able to locate viral particles in human corneal stromal cells, and they raised the possibility that this mesodermally derived tissue may harbor the latent virus. This latter study does not, of course, preclude the possibility that HSV may simultaneously or alternatively reside in lacrimal glands.

**Oral Cavity—Site of Recurrent Infections**

The majority of work relating to the identification of the location of a latent virus involves the oral cavity (221). Efforts to isolate HSV from sites of recurrent infection on the mucous membranes of the oral cavity in the absence of apparent infection have failed to yield positive
results (226). However, skin excision experiments from other anatomical locations demonstrate that HSV usually does not remain at the exact site of recurrent infection during periods of quiescence (4, 242). This may be due to pressure exerted at the recrudescent site by the immune system during the acute infection, which might destroy all virus at the infection site and leave only that virus located in nearby tissues.

Non-Neural Tissue in the Vicinity of Recurrent Infection

The isolation of HSV from oral secretions of healthy individuals (32, 65, 128) provides the initiation point for attempts to localize the site where this virus is produced, since this location is believed to be synonymous with the focus of latency (i.e., the cell[s] harboring the latent virus). It was obvious from these studies that the virus released from the sheltering tissue must have relatively facile access to the oral cavity if it were indeed the inoculum used to establish the recurrent infection. Although it appears that some virus-harboring tissue may release virions for long periods of time (6), thus facilitating the detection of the sheltering region, many tissues have been implicated as harboring sites, and at present no conclusive data pinpointing the specific location of the latent virus in humans can be presented.

With regard to suspected latency foci, Douglas and Couch (65) have suggested that HSV may be intimately associated with the salivary glands (even though attempts to isolate HSV from parotid gland secretions proved unsuccessful) or with cells of the oropharynx (151). In support of the latter possibility, HSV has been secured from pharyngeal washings of adults with upper respiratory illness (167, 170) as well as from the nasopharyngeal region of healthy infants and young children (229).

In addition, HSV has been found deeper in the respiratory tract. Herout et al. (101), after studying autopsy samples, identified HSV in tracheal, bronchial, and alveolar specimens. Other studies of individuals with respiratory illness yielded similar results (94, 194, 195). However, Lindgren et al. (151), who isolated HSV in 5% of a random distribution of individuals with respiratory infections, suggested that HSV is rarely the causative agent of respiratory illness, but that HSV-bearing respiratory secretions may provide the inocula needed to initiate recurrent infections. Consequently, HSV has been isolated from a number of areas in the oral cavity and the respiratory tract. Unfortunately, none of these regions can be clearly designated as the major shelter for the latent virus. The possibility exists that the several locations mentioned above can serve with equal efficiency as foci of latency.

Yet, these experiments indicating the presence of a virus in vicinal areas have one basic shortcoming; namely, they fail to account for the fact that recurrences often take place at the same site (125, 235). If the virus were in a gland or respiratory passageway during latency, one would expect eruptions to take place anywhere in the vicinity of the virus-harboring tissue. Hence, one would not expect to find a preponderance of apparent recurrences taking place in one particular location. One possible explanation is that, during latency, the virus exists at a site which has direct and/or specific access to the location of the recurrences. Such a tissue might be the stratum germinativum proximal to the site of recurrent infection or in the neighboring nerve network (196).

Neural Tissue and HSV

Herpes simplex virus may be considered a neurotrophic virus (141, 142), and it has been implicated in several neural disorders (27, 99, 120, 156, 162, 215, 241, 257). Although HSV is regarded as a rather neurovirulent virus (13, 155, 157, 190, 222), it has been shown that some herpesviruses, including HSV, may replicate within the nervous system while causing little or no visible damage (62, 201, 202). Since nerve cells are unique in that they do not reproduce in the adult and have a long life span, a temporarily inactive virus would be able to survive within a nerve, with little risk of being lost through cell death or division. Hence, nearby nerve cells could provide an excellent site in which to establish a focus of latency.

Because primary and recurrent HSV infections of the facial area occur in the superficial layers of the skin, the virus is closer to peripheral nerves than it is to main nerve branches. Thus, one would expect the virus to enter the nervous system by way of a terminal nerve pathway. Studies have demonstrated that the virus can migrate centripetally along terminal nerve endings after initial infection (119, 174, 236, 262). Yet, conflicting reports exist with regard to whether the virus travels mainly through the axon (62, 105, 143) or through the nerve-supporting cellular network (13, 20, 119, 205, 236), for available evidence supports both of these migratory pathways. Since the peripheral nerves of the face eventually lead to the trigeminal nerve (the fifth cranial nerve) which in turns forms the trigeminal ganglion (also known as the Gasserian or semilunar ganglion),
it follows that upsetting the physiological balance anywhere within this nerve tract might disturb a cell-virus association (assuming the virus is present within this tissue), such that a herpetic infection would recur. Evidence favoring this concept originates from cases in which a trigeminal sensory root rhizotomy was performed on individuals with intractable neuralgia; it was found that a large percentage of these patients displayed herpetic vesicles in the area innervated by the branches of this nerve (particularly by the second and third branches; 36, 68). Furthermore, herpetic vesicles are not found and cannot be induced in denervated skin (35, 68). Consequently, these results suggest that the trigeminal nerve network or one of its components may in fact shelter a herpesvirus.

The ganglion of the trigeminal nerve has been considered the primary site for a latency focus, since from experimentation on animals it is well established that HSV can remain latent in the sensory ganglia (15, 180, 243, 246, 247). Moreover, studies on human trigeminal tissue resulted in the recovery of HSV from this ganglion, but not from other areas of the trigeminal nerve network (14, 17). These results have led Paine (196) and Stevens and Cook (246) to propose the trigeminal ganglion as the sheltering site for the latent virus. Furthermore, Schwartz and Elizan (233) have suggested that glial cells, which are also associated with the ganglia, may be the specific harboring site of the latent virus. According to these investigators, when glial cells fuse with neurons, the viral replicative cycle is activated and the released virus may then initiate a reinfection. This concept might derive support from Stevens and Cook’s (246) ability to recover virus only from intact ganglia but not from specific cells of the ganglia. Unfortunately, one now encounters a difficulty that was mentioned previously; that is, if the virus resides in the Gasserian ganglion, one would not expect to find the majority of recurrences taking place at the same location; rather, they should occur with equal frequency in all areas within the sphere of influence of this ganglion (i.e., the face; 36). Yet, if one postulates that HSV remains dormant in the peripheral nervous system (162), a dilemma appears. Although a peripheral residence would explain the specificity of the infection site, it does not account for the fact that virus is recoverable only from the ganglia (17, 246). On the other hand, it has been shown by in vitro studies that all cells in the nervous system are capable of supporting HSV replication (74, 148). Thus, the nervous system can indeed harbor a latent virus, but the definition of the exact cell type involved, and the resolution of whether or not most recurrent facial HSV infections are due to a nerve-harbored virus, require continued study.

Much of the research reviewed above involves the isolation of infectious virus from various regions of the body (especially from ocular, genital, and non-neural tissues). Hence, most investigators tacitly assume that the latent virus exists in a dynamic state (i.e., as a chronic low-grade infection). Interestingly, the three proposals suggesting a viral residence in neural tissue seem to favor a static state type of existence for the latent virus. Unfortunately, with present techniques it is not possible to scan a large number of cells in search of a “static state” or inactive virus, especially if the virus is incorporated into the host genome. Until this gap between theory and technology is bridged, data supporting either concept (i.e., the static state or the dynamic state) cannot be placed in proper perspective, and so the form as well as the principal sheltering site of the latent virus continues unresolved.

**REAPPEARANCE OF INFECTION**

Having considered the commencement of latency, the possible forms of the latent state, and the suspected locations of the latent virus, one may now inquire about the nature of the agents responsible for the onset of recrudescence. A great deal of research in this area has dealt with recurrent herpetic infections of the lip and oral cavity (i.e., cold sores or fever blisters). One inducer of fever blisters is of course fever, whether artificially induced or occurring “naturally” (43, 259). However, this in itself is somewhat perplexing since several recent publications have demonstrated that HSV replicates poorly in vitro at elevated temperatures (50, 51, 85, 107). The explanation for this discrepancy may lie in the fact that the virulence of HSV is correlated with its ability to grow at a higher than normal temperature (37). In other words, the better a particular virus can replicate at an elevated temperature (e.g., during a fever), the greater potential it possesses for initiating an infection. Thus, only the more heat-resistant strains of any given HSV type would replicate well during a fever. Such interstrain variations with respect to temperature sensitivity may explain the method by which HSV, noted for its intolerance of elevated temperature, can continue to replicate during a fever, but this does not, of course, indicate the viral structures or functions responsible for conferring heat resistance. An interesting hypothesis, put forth by
Lwoff (154), suggests that virulence may be associated with the ability of a virus to replicate rapidly at elevated temperatures, perhaps as a result of the presence of an especially active viral replicase. Thus, a virulent or heat-resistant virus may be capable of completing its lytic cycle before ribonucleases released from altered cellular lysosomes can incapacitate the viral ribonucleic acid. Thus, the hypothesis associates virulence or ability to replicate at elevated temperatures with both the genetic makeup of the virus and the stability of the cellular lysosomes.

With respect to this latter point, it is interesting to note that Lwoff has taken into account the physiological condition of the host cell. Whereas attention is generally directed toward the virus during the onset of infection, the host's response to the latent virus cannot be ignored, since it could conceivably be altered by adverse conditions including fever. Hence, any specific control which the body's defense system or an individual cell has over the virus during latency could be affected in such a manner that the formation and release of large numbers of infectious viral particles would result. Noteworthy in this regard is Herriott's proposal which suggests that an infected cell may release viral nucleic acid into the extracellular environment, where it is normally incapacitated by humoral nucleases (102). However, during a fever the nuclease activity may be hampered by a deoxyribonuclease inhibitor released from leukocytes (103). When this occurs, free viral nucleic acid is then able to spread and infect surrounding tissues, which results in the reappearance of herpetic vesicles. Although this is an interesting concept, it has yet to receive direct experimental support and requires further development to account for the fact that not all febrile illnesses induce acute herpetic infection with equal frequency (75).

Of course, in addition to viral nucleic acid, it is probable that antigenic viral particles are also released from some sites of latency, since an increased rate of recurrence takes place soon after impairment of the host immune system, as previously noted. Thus, reduced levels of immunoglobulin (252), immunosuppressive hematological disorders (7, 39, 106), the administration of immunosuppressive drugs (112, 146, 153), and possibly steroid therapy (134, 188), all of which have been shown to result in immunological deficiencies, would allow a latent virus to establish a focus of acute infection. The modus operandi of an immunological disorder in causing a recrudescence can be understood by visualizing a host-virus equilibrium (i.e., a type of dynamic state) regulated by the immune system. An impairment of this regulatory mechanism could create an imbalance in the equilibrium favoring the virus moiety and result in an apparent infection.

The relationship between a static state virus and the immune system is not as amenable to interpretation. If the static state exists, then the effect of an agent which induces viral replication may be more indirect, although at present the sparsity of data relating to this point precludes speculation.

Other factors which may bring about the reappearance of a herpetic infection include trigeminal nerve rhizotomy (36, 68), pesticides (249), trauma (160, 178), anaphylactic shock (89), the Arthus reaction (3), adrenalin (232), pituitary hormones (76), menstruation (234), streptococcal infection (33), excessive exposure to cold, wind, or sunlight (76), and ultraviolet light (216). Also implicated as possible stimulators of a latent herpesvirus are multiple sclerosis (38, 52), nasopharyngeal carcinoma (77, 197), and even Cannabis spp. (122). Interestingly, several reports have suggested that emotional stress (115) as well as depression (213, 225) may precipitate recurrences (however, in a recent report by Pokorny et al. [203] such claims were not substantiated). The exact mechanisms of operation of these factors in inducing recurrent herpetic eruptions are for the most part unknown. It is possible that these agents may assist a dynamic state virus by weakening the resistance of the cells subject to HSV infection, thus making these cells more "permissive" to reinfecion (216). However, if the latent virus is in a static state, then these agents may act either directly or indirectly on the virus, in order to initiate its replicative cycle.

One may ponder whether in fact these recurrent eruptions are nothing more than new infections caused by recontact with the virus from the external environment, rather than the reactivation of a latent virus. Current evidence seems to weigh heavily against this idea, for, if these later infections were caused by virus from an external source, it is unlikely that they would occur mainly in the same specific regions where past herpetic eruptions have taken place (125, 234, 235). If the inocula were provided from the environment, it would be especially difficult to rationalize the fact that emotional stress can precipitate an outbreak (115), or to explain the manner in which psychotherapy can effect a cessation of recurrences (25). Thus, the most suitable explanation for these observations is that HSV exists in a state of latency which provides the virus needed to establish a recurrent infection.
TREATMENT OF RECURRENT HSV INFECTIONS

As is the case with most viral infections, therapeutic procedures for herpetic lesions are still in the early stages of development. The treatment of HSV infections has run almost the entire gamut of recognized pharmacological techniques. A wide range of agents and procedures which are able to inhibit or suppress HSV replication in vivo or in vitro with varying degrees of success have emerged (with others being added to the list continually). Noteworthy among these are: several antimitotic chemicals (253), cancer cell extracts (2), human gamma globulin (1), trifluorothymidine (261), trifluorodeoxyuridine (124), lysine (123), iododeoxycytidine (144), iododeoxyuridine (IudR or IDU; 56, 138, 261), polyinosinic-cytidylic acid (179, 231, 260), interferon (126), ara-c (109), ara-c with L-asparaginase (34), arabinofuranosyladenine (138, 200), phosphonoacetic acid (238), adenine arabinoside (45, 199), rifamycin (181), ethyl ether (186), various photodynamic dyes (109), X irradiation (7, 139), a vaccinia virus vaccine (72), a herpesvirus vaccine (127, 251), cryo-therapy (19, 83, 104), and psychotherapy (25). However, HSV has proven to be resistant to glutathione (124), beta-methazone (124), pyran copolymers (231), lysozyme (124), 6-azauridine (138), and kethoxal (256).

Of the above listed efficacious drugs, two have gained fairly wide appeal in clinical practice. IudR, which has been shown to inhibit HSV in vitro (116, 124, 200, 207, 231), has yielded promising results in the treatment of herpes encephalitis (18, 44, 160, 165, 254) and herpetic keratitis (5, 53, 61, 261). Similar results have been obtained with ara-c both in vitro (78, 193, 207) and in the treatment of a variety of herpetic eruptions (109) including oral lesions (158) and herpes encephalitis (73, 86, 257). Of the two drugs, ara-c is slightly favored, because of the ease of administration and its reduced toxicity to the host (73, 109), even though it may synergistically act with HSV to effectuate increased chromosomal damage (192, 193). Also, recent studies indicate that the therapeutic effects of IudR therapy may not be as beneficial as earlier thought (46, 59, 63, 70, 79, 118, 133, 257). Thus, chemical therapy alone has to provide a clinically proven, potent but safe agent for treating herpetic recurrences, and so other approaches have been investigated.

One of the more successful innovations used to combat HSV infections has been the combined administration of both chemical and physical agents in the suppression of herpetic lesions. The chemicals are often photodynamic dyes such as proflavine (164, 168) and neutral red (82, 164), although IudR has also been tested (53), which are used in conjunction with fluorescent, incandescent, or ultraviolet light. Although the results of clinical trials have been encouraging (131), this method of treatment has resulted in an inadvertent yet serious oversight. With the gradual delineation of the agents responsible for activating the oncogenic potential of the herpesviruses, it is becoming apparent that these methods of treatment resemble the procedures used to demonstrate the malignant transforming ability of HSV in vitro. For example, the oncogenicity of HSV was first demonstrated by the transformation of hamster cells in vitro with ultraviolet light-irradiated virus (66, 208), and yet ultraviolet light is currently being utilized, in combination with IudR, to treat recurrent herpetic lesions (53). Furthermore, a procedure similar to neutral red-fluorescent light therapy has recently been used to transform hamster cells oncogenically in vitro (150, 209).

However, it must be emphasized that, although in vitro studies can provide valuable information pertaining to the mechanics of various cell systems, the results obtained do not necessarily correlate to a significant degree with actual in vivo events. Obviously, more work is needed to substantiate any relation between the results of these transformation experiments and therapeutic regimes. Follow-up studies of the incidence of cancer in individuals who have received dye-light therapy for herpetic infections would be the next logical step to determine whether a danger does indeed exist, yet these procedures may be of too recent origin to obtain statistically significant epidemiological data.

LATENT HSV AND CANCER

Although there is no direct proof that HSV is an etiological agent of human cancers, its in vitro transforming ability and oncogenic potential cannot be denied (54, 66, 67). This latter fact, in conjunction with considerable circumstantial evidence implicating the virus with several types of neoplastic processes, has stimulated research on the possible relationship between HSV and human malignancies (9, 10, 224). Because of the failure to isolate HSV from a given type of neoplasm, and the observation that viral replication connotes the cessation of cellular macromolecular synthesis and eventual cell death (217), it follows that neither an acutely nor a chronically (i.e., dynamic state) infected cell while supporting HSV replication can initiate a malignancy. If this assumption is
correct, then only defective viral particles released from a chronic infection, chemically altered viruses, static state HSV, or cells resistant to the destructive lytic effects of HSV would be involved in the HSV oncogenic process. If defective or chemically altered particles induce cancer, it is likely that they operate from within the cell, occupying the cell as nonreplicating units. Thus, defective particles may in effect be equated with a type of static state virus.

Support linking a static state virus with oncogenesis is supplied by recent studies of HSV-2-transformed hamster cells (47) and human uterine cervical tumor cells (81, 218). In neither case could infectious virus be detected, yet in both experiments the presence of HSV genome segments was established by use of nucleic acid hybridization techniques. Moreover, Aurelian et al. cultured cervical tumor cells in vitro for 6 months prior to their spontaneous production of HSV-2 (11). These data, strengthened by epidemiological studies, suggest that a static state HSV-2 appears to offer a promising subject for those investigating human cancer using a virological approach.

Although considerable evidence is accumu-
lating to associate HSV-2 with uterine cervical carcinoma (176), little attention has been di-
rected to the possible in vivo oncogenicity of latent HSV-1, even though its carcinogenic capability has been demonstrated in vitro (67). However, Wyburn-Mason (265) has presented incriminating evidence from cases in which carcinomas had developed at sites of recurrent HSV lip infection.

Yet, if the latent virus is harbored within the components of the nervous system, as previ-
ously discussed, then one might expect to find HSV markers associated with nerve-related tu-
ors. Accordingly, Munk et al. (171) studied seven types of central nervous system tumors for viral markers and were able to find HSV antigens in cells from a malignant glioma (gli-
omas are neoplasms originating from ectoder-
mal supporting tissues that include glial cells which, it may be recalled, have been proposed by Schwartz and Elizan [233] as a possible focus of HSV latency). It would be interesting and possibly profitable to conduct studies of nervous system tumors utilizing a more sensitive procedure such as nucleic acid hybridization in order to substantiate and expand upon the above results.

Although these experiments do not prove a viral etiology of human cancer, they do suggest that the relationship between HSV and cancer may be more than just coincidental. This in itself justifies a closer examination of the life-
long and intimate association between HSV and its host cell during the state of latency.

CONCLUDING REMARKS

Throughout this review, we have attempted to organize recent reports on the latency of HSV into a coherent body of information. In retrospect, this review may have raised more questions than it has answered, but rather than reiterate the queries and ambiguities that have been presented, it may be more profitable to sketch several promising lines of investiga-
tion which the literature cited appears to fore-
shadow.

The efficacy of interferon in inhibiting HSV infections should be clarified. If it does have therapeutic or prophylactic value, then the use of interferon inducers during herpetic recurrences may be warranted. However, the conflicting data on this point, cited earlier, may suggest an inconsistent response so that interferon may be of value in only some herpetic infections. This varied sensitivity could be due to several factors, including subtle differences in herpes types or subtypes.

The cell(s) in which the virus is sequestered continues to remain an enigma whether one is considering the dynamic or static state. Al-
though it is easy to imagine that research in this area would be best served by more sensitive procedures for detecting infectious virus, it appears that such techniques have resulted in increased controversy. As these procedures have evolved, the virus seems to be present in several areas without specific identification of any one area as the main sheltering site. Perhaps it would be worthwhile to utilize more precise methods to detect virus markers, including nucleic acid hybridization, or to attempt to induce the appearance of viral products in specific cells lacking infectious virus.

Although many agents have been held re-
sponsible for the precipitation of recurrent HSV infections, the mechanism of induction may be similar for all. The agents responsible for such recurrences are many and varied, but all have the ability to stress the cells and/or the body, resulting in a physiological imbalance. If the cell is controlling the virus by a suppressive mechanism during periods of quiescence, then an alteration in this control process may result in the reappearance of virus. Whether a cell factor exists and functions in such a manner is unknown, and the stressing of cells in attempts to activate virus have met with minimal suc-
cess.

Although treatment of herpetic infections is warranted, a serious consideration must now be
directed toward the effects of therapy on the virus. Although the virus may be inactivated so that it is no longer able to induce visible cytopathic alterations, it is not necessarily destroyed beyond the point of some genomic expressions which may result in the transformation of a cell. If the virus enters a state of latency after exposure to various physical or chemical agents, it may carry an increased ability to transform cells. The continuing presence of the virus, altered or otherwise, in close association with a given cell type, may allow sufficient interaction for the development of a carcinoma; however, chemically or physically altered HSV may increase such potential, and extensive studies of these possibilities are certainly warranted.

Some reflection upon these and other comments presented in the body of this review are indicative of the state of affairs regarding HSV latency at this time. The groundwork has been completed, and the basic hypotheses along with relevant data have been set forth. However, continuing research is required before the major factors involved in the establishment of latency as manifested by HSV are finally elucidated, and before effective prophylactic agents can be developed to prevent this bothersome and at times serious threat to human health.

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LATENT HERPES SIMPLEX VIRUS


