SLOW VIRUS DISEASES

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I. INTRODUCTION

The concept of slow virus infections was first introduced by Sigurdsson less than 20 years ago in his classic descriptions of scrapie, visna, maedi, and pulmonary adenomatosis, as transmissible chronic diseases of Icelandic sheep (172). These disorders are characterized by an insidious onset, chronic and unrelenting progressive course, and inexorably fatal outcome. Since then, additional slow virus infections have been described in humans and domestic animals.

Numerous excellent reviews have dealt with many aspects of slow virus infections (8, 38, 47, 55, 58, 60, 96, 108, 164, 185). This presentation attempts to synthesize conclusions which have been drawn from more recent investigations, and will be restricted to infections which involve the central nervous system. Consideration will also be given to the possible viral etiology of chronic degenerative diseases of unknown or obscure causation. Emphasis will be placed on recent developments in the area of specific host and virus factors as determinants of slow disease, and, when possible, virus-host interactions at the cellular level shall be stressed.

It has been observed that while virologists may refer to slow virus diseases, and while slow virologists may certainly exist, it is highly doubtful that there are in fact any slow viruses (51). The term slow refers to the clinical course of the disease in question, rather than to basic properties of the inciting agent.

II. CONVENTIONAL AND UNCONVENTIONAL AGENTS OF SLOW DISEASE

The agents of slow virus diseases can be divided into conventional and unconventional (Table 1). The conventional agents resemble classic viruses, and include measles virus (subacute sclerosing panencephalitis), papovaviruses (progressive multifocal leukoencephalopathy), visna virus (visna), and arenoviruses (lymphocytic choriomeningitis). All of these agents replicate in vitro and induce cytopathic effects (CPE) under appropriate conditions; all are antigenic, and with rare exception, are associated with inflammatory lesions of the central nervous system (CNS). In contrast, the unconventional agents of scrapie, kuru, Creutzfeldt-Jakob disease, and transmissible mink encephalopathy have not yet been demonstrated by electron microscopy, nor do they induce unequivocal in vitro CPE. The scrapie agent replicates in vitro, as determined by dilution experiments. Attempts to demonstrate antigenicity either by artificially stimulating animals inoculated with diseased tissue fractions, or by directly testing infected animals for neutralizing antibody, have been uniformly unsuccessful. Considering scrapie agent as the prototype of unconventional agents, there is little evidence that it has a nucleic acid genome, and this is assumed to be true for the other related unconventional agents. It should be noted, however, that this view is based primarily upon inactivation studies of relatively unpurified scrapie preparations. Since the protective effect of contaminating tissue materials against many harsh treatments is a well-known phenomenon, highly purified scrapie material must be utilized before this conclusion can be accepted without reservation. The agent is considered to be extremely resistant to a wide
range of physicochemical treatments that would quickly inactivate most viruses. Its basic biochemical composition is unknown, and infectivity is uniquely associated with plasma membrane fractions of disrupted, infected cells.

III. VIROLOGICAL AND CLINICOPATHOLOGICAL FINDINGS IN CHRONIC INFECTIONS OF THE CENTRAL NERVOUS SYSTEM (CNS)

A. Chronic Infections of the Human CNS with Conventional Agents

Table 2 is a compilation of the chronic infections of the human CNS with conventional agents.

1. SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE)

SSPE (Dawson's inclusion body encephalitis, Van Bogaert's subacute sclerosing leukoencephalitis, Pette and Dorings panencephalitis) is a rare disease of children and young adults. Its estimated frequency is 1 per 1,000,000 in the United States (168). It occurs more frequently in males than females (4 to 1), and 85% of the cases are children from rural areas (93). Once characteristic clinical features are observed diagnosis is confirmed by the demonstration of measles antibody in the cerebrospinal fluid (CSF) (95).

Evidence that SSPE results from chronic measles virus infection is now extensive, and the isolation of rubeola virus from SSPE brain biopsies and autopsy specimens has been accomplished by several different laboratories (92, 155). Antigenic identity between SSPE virus isolates, wild measles virus strains, and the Edmonston vaccine strain has been demonstrated. However, neutralizing antibody titers of SSPE patients' sera were found to be four to eight times lower against SSPE isolates than against a wild measles virus strain (94). In general, the biological characteristics of SSPE isolates more closely resemble those of laboratory adapted vaccine strains of measles, than those of the wild virus. Recent biochemical studies suggest that differences may exist between the 18S RNA of SSPE virus-infected cells and measles virus-infected cells (202). Whether such findings reflect basic biological differences between the SSPE virus and wild rubeola virus, or simply represent modifications resulting from long-term in vivo residence and propagation of virus within brain cells, remains to be determined.

The pathogenesis of SSPE is not well understood. The availability of an animal model would be a valuable adjunct in the study of the pathogenesis of this disease. Information derived from the study of such a model might provide a rationale for therapeutic trials in this uniformly fatal disorder. It has recently been demonstrated that a latent measles infection of the newborn hamster brain can be established if the animal has a high level of maternally transmitted measles neutralizing antibody before receiving the intracerebral inoculation (195). Animals without antibody developed acute encephalitis. When these chronically infected hamsters were treated with cyclophosphamide, the majority developed persistent myoclonic tremors, and virus was isolated from their brains for at least ninety days postinoculation. In another investigation, weanling hamsters inoculated intracerebrally with SSPE virus
Table 1  The agents of slow virus disease

<table>
<thead>
<tr>
<th>Property</th>
<th>Conventional</th>
<th>Unconventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Morphology</td>
<td>Identifiable virus structure and substructure by electron microscope</td>
<td>Demonstration of virus not accomplished</td>
</tr>
<tr>
<td></td>
<td>(Example: measles virus in subacute sclerosing panencephalitis)</td>
<td></td>
</tr>
<tr>
<td>2. Antigenicity</td>
<td>Stimulate production of specific antibody in naturally infected host, and in artificially immunized animals</td>
<td>Specific antibody not demonstrable in infected host or artificially immunized animals</td>
</tr>
<tr>
<td></td>
<td>(Example: scrapie and other agents of transmissible spongiform encephalopathies)</td>
<td></td>
</tr>
<tr>
<td>3. Biochemical composition</td>
<td>Virus composed of basic nucleocapsid consisting of protein, plus either RNA or DNA, with or without lipid envelope</td>
<td>Biochemical composition unknown</td>
</tr>
<tr>
<td></td>
<td>(Example: scrapie and other agents of transmissible spongiform encephalopathies)</td>
<td></td>
</tr>
<tr>
<td>4. Mode of replication</td>
<td>Generally analogous to the virus family of which it is a member</td>
<td>Unknown, but various hypotheses have been suggested, including that of the replicating membrane; replicating polysaccharide; and nuclear histone, among others</td>
</tr>
<tr>
<td>5. In vitro characteristics</td>
<td>Produced CPE under appropriate conditions, and are inhibited by known metabolic inhibitors of viral replication</td>
<td>CPE not produced, even though replication occurs in appropriate tissue culture systems</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Property</th>
<th>Conventional</th>
<th>Unconventional (Example: scrapie and other agents of transmissible spongiform encephalopathies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Minimal infectivity requirements</td>
<td>Naked virion capable of initiating infection</td>
<td>Infectivity uniquely associated with plasma membrane fraction of disrupted, infected cells</td>
</tr>
<tr>
<td></td>
<td>(Example: measles virus in subacute sclerosing pancephalitis)</td>
<td>(i.e. chemical disruption of plasma membrane may result in total loss of infectivity)</td>
</tr>
<tr>
<td>7. Sensitivity to physicochemical agents</td>
<td>Susceptible to most known virucidal agents</td>
<td>Resistant to a wide range of physicochemical exposure</td>
</tr>
<tr>
<td>8. Natural host range</td>
<td>Restricted</td>
<td>Relatively restricted</td>
</tr>
<tr>
<td>9. Experimental transmission</td>
<td>Limited success—Visna (Icelandic sheep), LCM (Rats &amp; Mice), SSPE (Hamsters), and PML—None</td>
<td>Long incubations. Transmitted to some laboratory animals and/or a few subhuman primates</td>
</tr>
<tr>
<td>10. Salient neuropathological features</td>
<td>Relatively characteristic for each disease. In general, inflammation (except in immunologically-compromised hosts, as in PML), and may demonstrate demyelination and the presence of viral inclusions (SSPE and PML); budding virus (visna, LCM); or viral crystalline aggregates (PML)</td>
<td>Extremely similar, including neuronal vacuolation and astrocytic proliferation in the gray matter. Primarily noninflammatory in nature</td>
</tr>
<tr>
<td>Disease</td>
<td>Virus</td>
<td>Hosts affected</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Subacute sclerosing panencephalitis</td>
<td>Measles virus</td>
<td>Human</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
<td>Papovavirus (SV 40; JC virus)</td>
<td>Human</td>
</tr>
</tbody>
</table>
at 21 to 22 days of age developed a chronic neurological disease characterized by myoclonus in some animals (23). When virus was inoculated into younger, suckling animals, acute encephalitis developed. It was impossible to culture virus from brains of chronically infected animals by conventional virological methods, but using co-cultivation techniques, as successfully utilized in SSPE isolations, virus could be isolated up to 81 days after inoculation. These observations strongly suggest that immune mechanisms may be key determinants in the pathogenesis of chronic measles infection of the brain, as will be discussed later.

2. PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY (PML) PML is a rare central nervous system disorder of man first described in 1958 (9), and characterized primarily by demyelinating lesions. With rare exceptions, patients who contract PML have severe secondary immunodeficiency states resulting from lymphoproliferative disorders, such as Hodgkin's disease, and chronic lymphocytic leukemia, sarcoidosis, or miliary tuberculosis (163). However, there have been reports of primary PML (43). The first clue suggesting a viral component to PML was the electron microscopic demonstration of oligodendrocyte nuclei containing particles resembling papova virions (204, 205). Since then papovavirus has been isolated in cultures of human fetal spongioblasts (148). Two serologically distinct isolates have been recovered: one which is indistinguishable from SV-40 (197, 198), and another which represents a new agent, the JC virus (148). However, most PML brains studied show evidence of JC virus rather than SV-40, and a recent report has summarized the pertinent findings (139). There is some indication that the clinical picture of PML associated with these two agents may differ according to which one is involved, in that SV-40 may be associated with more protracted disease.

Although demyelinating disease has not been transmitted to laboratory animals with PML isolates, there is evidence indicating an etiological relationship between papovavirus and PML (139).

Viral antigen is most concentrated in cells surrounding demyelinated foci, and virions have been observed within oligodendrocytes (205). These cells, which are the myelin-forming elements of the CNS, are depleted within areas of demyelination. Papovaviruses in association with PML brains may exceed $10^7$ to $10^9$ virions per gram of tissue. Furthermore, papovaviruses have not been identified in normal human brain, or in other pathological conditions of humans, except in warts.

Upwards of 70% of 400 normal adults tested had hemagglutinating antibody to JC virus (147). This suggests that PML does not result from infection by a highly lethal or exotic virus. The highest rate of seroconversion appeared to occur before 14 years of age, further supporting the ubiquity of that agent. Antibody to SV-40 virus also occurs with variable frequency in human populations (169, 170). The critical question of whether either of these two agents assume residence in normal brains, inciting the neuropathologic process of PML only under very specific circumstances, as in an immunologically compromised host, is unknown.

The possible association of PML, a degenerative disease of the brain, and malignancy has stimulated a great deal of interest (205). The fact that giant astrocytes usually seen in the lesions of PML cannot be distinguished from malignant as-
trocytes of pleomorphic glioblastomas is consistent with an oncogenic potential of isolated papovaviruses. It has been recently reported that 83% of newborn hamsters inoculated with JC virus developed malignant gliomas within six months. Virus was not recoverable from cultured tumor cells, but could be rescued when tumor cells were fused with permissive cells (193).

B. Chronic Infections of the Human CNS with Unconventional Agents

Table 3 provides a listing of the chronic infections of the human CNS with unconventional agents.

1. KURU  Kuru was the first chronic degenerative disease of the human CNS to be transmitted to an experimental animal (58). This disease was originally discovered to be endemic among the Foré people, a tribe in the eastern highlands of New Guinea (56). The name kuru means to shiver, and refers to the tremors noted in this disease.

The clinical and pathological picture of kuru in man is analogous to the other transmissible spongiform encephalopathies, namely Creutzfeldt-Jakob Disease (CJD), scrapie, and Transmissible Mink Encephalopathy (TME). Clinically, kuru is characterized by progressive ataxia, tremors, emotional lability, and mental deterioration, usually terminating in death within six to nine months (91). All of the spongiform encephalopathies are atypical in that they elicit no inflammatory response, nor leave any of the classic hallmarks of viral infection, such as inclusion bodies or glial nodules (120). There are no febrile phases in the disease and no abnormalities of the CSF, serum, or peripheral blood cells. To date, no antigenic properties of any of the transmissible agents of spongiform encephalopathies have been found (66).

Kuru primarily affects children and adult females, and until the successful transmission of this disease in 1966, it had been considered a heredofamilial disorder of the CNS. The decline in the incidence of kuru now observed reflects the elimination of the practice of cannibalism among the Foré people since 1957. This completely interrupted the natural transmission of the disease (60). Transmission resulted from self-inoculation of the women and their infants during the mourning ritual when highly infective brain was handled before cooking. Infection probably occurred via cutaneous and conjunctival routes of inoculation. In recent years, kuru has occurred in less than one third of the victims than it did during the early years of investigation. The disease is virtually absent among children under 12 years of age (69).

Chimpanzees inoculated with human brain suspensions from kuru victims developed a stereotyped neurological disease within 10 to 59 months (70). They showed neuropathological changes remarkably similar to those observed in human kuru victims (8).

2. CREUTZFELDT-JAKOB DISEASE  CJD, a rare presenile dementia, was originally described by Creutzfeldt in 1920 and Jakob in 1921. Most victims are between the ages of 35 and 65. Males and females are affected equally. Most cases are sporadic, but there are several pedigrees in which multiple family members have
<table>
<thead>
<tr>
<th>Disease</th>
<th>Virus</th>
<th>Hosts affected</th>
<th>Incubation period</th>
<th>Clinical signs</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuru</td>
<td>Infectious agent passes filters with pore size of 220 nm</td>
<td>Human chimpanzee</td>
<td>9-240 months</td>
<td>Incoordination, progressive ataxia, tremors, loss of emotional control, dementia</td>
<td>Progressive vacuolation in the dendritic and axonal processes of the neurons, and to a lesser extent in astrocytes and oligodendrocytes; extensive astroglial hypertrophy and proliferation, ends in status spongiosus of gray matter; no inflammatory reaction or primary demyelination. PAS—positive plaques in cerebellum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>New World monkeys</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Old World monkeys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creutzfeldt-Jakob</td>
<td>Infectious agent passes filters with pore size of 220 nm</td>
<td>Human chimpanzee</td>
<td>4-47 months</td>
<td>Progressive dementia, ataxia, myoclonus</td>
<td>Similar to kuru; cortex, basal ganglia most prominently affected as well as changes in thalamus, cerebellum, brain stem, and spinal cord</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td>New World monkeys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old World monkeys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>domestic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cat</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
been affected over several generations (131). Both sporadic and familial cases have been successfully transmitted to subhuman primates. The disease resembles kuru both clinically and neuropathologically (8) and it has been suggested that they may have identical etiologies.

Since the initial successful transmission of CJD to chimpanzees, a total of 14 of 26 CJD cases have been inoculated into 42 chimpanzees, 18 of which developed the disease (69). Peripheral and intracerebral routes of inoculation of brain tissue produced essentially the same disease, although the incubation period was longer after peripheral inoculation. More recently spider and squirrel monkeys have also developed CJD after inoculation, with incubation periods ranging from 23 to 29 months (71).

Electron microscopic studies of the spongiform encephalopathies have been unrevealing in the search for virus particles. One study, however, described two types of virus-like particles in biopsy tissues from two patients with CJD (192). The larger of the particles resembled myxoviruses of the murine and avian tumor types. However, particles of this general type have also been seen in brains from patients with a variety of unrelated diseases, including cerebral degeneration, Tay-Sachs, and Schilder's disease (20, 74, 75). The smaller particle resembled the papovavirus-like particles found in PML, although there was no evidence of intranuclear particles.

A human brain culture from a patient with CJD transformed spontaneously after 60 days (89). Virus particles morphologically similar to oncogenic RNA viruses were present in the transformed cells. The 110th subculture of these cells still contained virus particles (90). Inoculation of the transformed cell line has not induced CJD in primates after 20 months of observation. It was suggested that this agent is probably not etiologically related to CJD.

C. Chronic Infections of the Animal CNS with Conventional Agents

Table 4 provides a list of the chronic infections of the animal CNS with conventional agents.

1. VISNA Visna, first reported in 1935, is a subacute viral encephalomyelitis of Icelandic sheep. It was completely eradicated by 1951. Visna virus is serologically identical to the agent causing maedi, a slow pulmonary infection of Icelandic sheep (187). Zwoegerziekte, a neurological disease occurring in the Netherlands, is comparable to visna, and may be caused by a similar virus (36). Although it has been suggested that visna is the encephalitic form of the lung disease, further study is necessary to identify the pathogenetic factors which determine the major organ system involved (81).

Visna is readily transmitted by intracerebral inoculation of brain material obtained from diseased sheep; the incubation period may extend from several months to years (173, 174). The virus has been propagated in cultures of sheep choroid plexus or sheep testes cells. Virus replication in cell culture produced CPE characterized by multinucleated syncytia appearing within several days post-inoculation, followed by complete disintegration of the cell culture several days later (175).

Visna virus is a RNA-containing virus with a lipid envelope, replicating by budding from the plasma membrane (32, 183). Morphologically, it resembles agents
<table>
<thead>
<tr>
<th>Disease</th>
<th>Virus</th>
<th>Hosts affected</th>
<th>Incubation period</th>
<th>Clinical signs</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visna</td>
<td>70-100 nm myxovirus-like</td>
<td>Sheep</td>
<td>months to years</td>
<td>Ataxia, paresis of hind legs progressing to total paralysis</td>
<td>Patchy demyelination; meningoleukoencephalomyelitis; high titered serum neutralizing antibody with persistent virus; marked pleocytosis with increased gamma globulin in CSF</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis</td>
<td>50 nm arenavirus</td>
<td>Mouse</td>
<td>days to months</td>
<td>Death preceded by convulsive diathesis</td>
<td>Brain has marked mononuclear cell infiltration in meninges, ependyma, and choroid plexus; virus induced immune-mediated pathology</td>
</tr>
</tbody>
</table>
of the myxovirus group (125, 186). Two types of particles have been observed: one contains a dense core enclosed by a single membrane; the other is a somewhat larger form without a core, enclosed by a double membrane. Visna virus may be inactivated by chloroform, ether, trypsin, formaldehyde, heat, or ultraviolet radiation (184).

The virion has a sedimentation coefficient of 64S, and also contains a RNA-dependent DNA polymerase (reverse transcriptase) (86, 124, 178). It has been stressed that visna virus shares many features with known oncogenic viruses, and indeed it has caused transformation of murine cell cultures (180). In this latter situation, classic viral CPE does not occur, and evidence of complete virus was not detectable in cells or supernatant fractions. However, when such transformed lines were co-cultivated with normal sheep testes cells, virus was subsequently rescued and identified as the visna agent. It retained the capacity to transform mouse cells. The implications of this important observation are unknown, but it demonstrates that viral carcinogenesis and slow virus infections may share important pathogenetic mechanisms, since the agents of both may have the capacity to incorporate viral genome into host cells.

The possible relationship of visna virus to human neurological disease has been considered, and it has been proposed that visna may be a useful model for the study of human subacute and chronic demyelinating disease (85). It has also been suggested that subacute lesions of visna may be indistinguishable from the acute lesions of multiple sclerosis, but no direct evidence in support of an etiological relationship has been described (172).

2. LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCM) LCM infection in mice may occur as a slow virus infection of the CNS (100). This infection has served as a model of the virus carrier state, and of virus-induced immune-mediated pathology. In nature, the virus is indigenous to mice who may harbor it for life, and vertically transmit it to succeeding generations. Intracerebral inoculation of adult mice with LCM virus produces a rapidly fatal generalized infection showing characteristic CNS pathology. However, mice infected transplacentally or inoculated shortly after birth develop a persistent infection with no damage to the host. After 10 months of age, many of the inoculated mice develop a progressive disease involving the CNS. These animals have high levels of LCM virus in the brain, blood, and other tissues. Serological methods failed to detect antibodies, and for years this infection was thought to represent a state of immunological tolerance. Recently, however, LCM carrier mice have been shown to produce antibodies (99). The antibody response results in a large accumulation of antigen-antibody complexes in the kidneys which leads to chronic glomerulonephritis and death (144).

Electron microscopy of infected tissues shows pleomorphic virions containing electron-dense granules budding from cell membranes. They have been given the name arenovirus (165).

Little is known about the relationship between the virions seen by electron microscopy, and infectivity tests or the various antigens demonstrated by serological
techniques. Intact virus and complement-fixing (CF) antigens appear to be immunologically distinct from one another. Animals inoculated with soluble CF antigen are not protected against challenge with infectious virus (98). Whether CF antigen circulates freely in the persistently infected mouse, or is complexed with antibody, is unknown. However, CF antigen can be readily solubilized from infected cells or tissues and remains in the liquid phase after centrifugation. Evidence is available that the lesions in LCM-infected mouse are immune-mediated. However, elucidation of the precise mechanisms involved in viral persistence and in the selected destruction of rodent cerebellum presents an area for further investigation (31). A relationship of an autoimmune phenomenon and disease in infected LCM mice could be comparable to a number of pathological disorders of man, such as glomerulonephritis in systemic lupus erythematosus.

D. Chronic Infections of the Animal CNS with Unconventional Agents

Table 5 is a compilation of the chronic infections of the animal CNS with unconventional agents.

1. SCRAPIE  Scrapie is one of four transmissible subacute spongiform virus encephalopathies, which also include TME, kuru, and CJD (68). All are caused by undefined transmissible agents which can be detected only by animal inoculation after extensive incubation periods. As mentioned, they are all characterized by chronic, progressive, ultimately fatal neurological diseases. Scrapie is the colloquial name given to a disease of sheep, and denotes a persistent tendency of affected animals to scratch or scrape against fixed objects. The disease was first recognized in Scotland nearly two centuries ago, but has spread to Canada, Australia, and the United States in recent years. It does not represent a significant economic problem at present. Since certain breeds of sheep have demonstrated a high incidence of disease, while others are totally resistant, it had been proposed that vertical transmission is controlled by an autosomal recessive gene (37). It has also been suggested that scrapie may occur as an intrauterine infection (54). Spread of the natural disease probably occurs by contact, although the specific routes are unknown (19). Despite the proposed genetic influence, scrapie has been experimentally transmitted to a wide range of diverse and unrelated animal species, and the agent clearly has the capacity to cross wide biological barriers (70, 71).

In spite of the vast amount of work done with scrapie, it has not been completely possible to characterize the transmissible agent. Electron microscopic studies of diseased tissues have failed to provide clues as to the identity of the scrapie agent (27). It is remarkably resistant to formalin, heat, and fluorocarbons (103), and to virucidal levels of ultraviolet radiation (54) and ionizing irradiation (49). These observations have led to suggestions that the agent is not a virus, but instead a self-replicating membrane, polysaccharide (72), or a small nucleic acid core with polysaccharide coat (1). Most recently, it has been suggested that the scrapie agent is a viroid, a term designating a class of naked infectious RNA molecules causing a variety of plant diseases (39, 40).
<table>
<thead>
<tr>
<th>Disease</th>
<th>Virus</th>
<th>Hosts affected</th>
<th>Incubation period</th>
<th>Clinical signs</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapie</td>
<td>Infectious agent passes filters with pore size of 30 nm</td>
<td>Sheep, goat, mouse, rat, gerbil, mink, cynomolgus and squirrel monkey, skunk, hamster</td>
<td>30–60 months</td>
<td>Ataxia, tremors, hyperexcitability, incoordination</td>
<td>Similar to kuru with some PAS-positive doubly refractile birifringent amyloid plaques; subcortical regions particularly medulla affected; vacuolation of nerve cells outstanding</td>
</tr>
<tr>
<td>Transmissible mink encephalopathy</td>
<td>Infectious agent passes filters with pore size of 50 nm</td>
<td>Mink, skunk, raccoon, ferret, hamster, goat, sheep, albino ferret, Old World monkey, New World monkey</td>
<td>4–48 months</td>
<td>Slowly progressive locomotor incoordination; excitability, convulsions</td>
<td>Similar to scrapie; cerebrum most prominently affected especially its more rostral parts; marked astrogliosis and spongy degeneration of the gray matter</td>
</tr>
</tbody>
</table>
Attempts to detect immunological responses by various techniques have been
unsuccessful (54). Antibody formation is not suppressed in infected animals, since
they respond normally when immunized with other antigens (62). Interferon synthe­
sis is not induced by scrapie infection (110). The prominent feature of the histopa­
thological lesions supports the negative immunological findings in that the disease
produces a degenerative process with the absence of an inflammatory reaction.

The agent replicates in vitro without evidence of CPE. Cultures passed up to 41
times, representing a dilution well in excess of the original inoculum, still contained
infectious material (29).

2. TRANSMISSIBLE MINK ENCEPHALOPATHY  TME was first recognized about
25 years ago in U.S. ranch mink, but the disease was not described until 1965 (84).
Perpetuation of the natural disease is not well understood. The disease may be
transmitted under laboratory conditions by subcutaneous or intraperitoneal inocula­
tion, or by feeding infectious material to a variety of animals (22, 70).

Clinicopathological findings combined with the lack of demonstrable humoral
and cellular host responses are reminiscent of scrapie, and it has been proposed that
TME is the mink equivalent of scrapie in sheep and mice (47, 130).

Various animal species have been infected with TME (70). However, clinical
disease did not develop in cats, calves, or chickens, even though the agent persisted
in their lymphoid tissues for up to two years (127). The infective agent was found
in many organs of terminally infected mink, with the highest infectivity in the brain.
Cell cultures of brain retain infectivity after as many as eight passages. Electron
microscopy has failed to reveal the etiological agent (128). The physical and chemi­
cal properties of the agent are similar to those of scrapie. It is partly sensitive to
ether, phenol, and pronase, but relatively resistant to 10% formalin and ultraviolet
radiation (128).

Recently it was found that circulating lymphocytes from infected animals were
noninfectious, although the transmissible agent could be demonstrated within intact
lymphoid organs. The lack of immunological response in these animals, therefore,
could not be the result of infection of circulating lymphocytes (129).

The transmission of TME to primates suggests that a closer relationship to the
human spongiform encephalopathies may exist, and the transmission to skunk and
raccoon could indicate a natural reservoir in wild animals for human disease (71,
185).

IV. THE POSSIBLE ROLE OF VIRUSES IN CHRONIC
DEGENERATIVE DISEASES OF UNKNOWN ETIOLOGY

Since the experimental transmission of the human spongiform encephalopathies to
subhuman primates (59, 70), the recovery of measles virus from SSPE (92), and the
isolation of papovaviruses from PML brains (197, 205), there has been a renewed
effort to determine if other subacute and chronic degenerative diseases of the CNS
<table>
<thead>
<tr>
<th>Disease</th>
<th>Virus suspected</th>
<th>Clinical signs</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>Measles virus?</td>
<td>Incoordination, dysarthria, nystagmus, paraplegia</td>
<td>Acute foci of primary demyelination associated with infiltrates of lymphocytes and macrophages scattered throughout the CNS; chronic sclerotic plaques completely demyelinated with gliosis, shadow plaque with partial demyelination.</td>
</tr>
<tr>
<td>P. paramyxovirus?</td>
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<tr>
<td></td>
<td>Other paramyxovirus?</td>
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<tr>
<td>Guillain-Barre syndrome</td>
<td>Echovirus?</td>
<td>Polyneuritis, ascending paralysis</td>
<td>Foci of primary demyelination associated with infiltrates of lymphocytes and macrophages scattered throughout the peripheral nervous system.</td>
</tr>
<tr>
<td>P. EB virus?</td>
<td></td>
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<tr>
<td></td>
<td>Mumps?</td>
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<tr>
<td></td>
<td>Rubeola? other viruses?</td>
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<tr>
<td></td>
<td>Also <em>Mycoplasma pneumoniae</em></td>
<td></td>
<td></td>
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<tr>
<td>P. Rabies virus?</td>
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<tr>
<td>Parkinsonism</td>
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<tr>
<td>A. idiopathic</td>
<td>?</td>
<td>Akinesia and bradykinesia; muscular rigidity; tremor</td>
<td>Neuronal loss in basal ganglia and brainstem; hyaline inclusions (Lewy body).</td>
</tr>
<tr>
<td>B. postencephalitic</td>
<td>Influenza virus?</td>
<td>Same as above</td>
<td>Same as above.</td>
</tr>
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<tr>
<td>C. Parkinson-dementia complex of Guam</td>
<td>?</td>
<td>Bradykinesia; rigidity; dementia</td>
<td>Neuronal loss widespread throughout brain; neurofibrillary tangles.</td>
</tr>
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<tr>
<td>Presenile dementia</td>
<td></td>
<td>Memory loss; dysarthria; disorientation; dementia</td>
<td>Diffuse neuronal degeneration of cerebral cortex. 1. Alzheimer: senile plaques and neurofibrillary tangles. 2. Pick: ballooned neurones and argentophilic Pick bodies.</td>
</tr>
</tbody>
</table>
have viral etiologies (Table 6). Several such neurological disorders of humans are candidates for possible viral causation.

Arguments favoring a viral etiology for these disorders consist primarily of epidemiological, immunological, or pathological evidence. A review of the most important of these conditions follows:

A. **Multiple Sclerosis (MS)**

The original report of measles antibody occurring in the CSF of MS patients (2) focused attention on the possible etiological role of measles virus in this chronic demyelinating disease of humans. Subsequent serological investigations have supported this original finding (26), and while other viruses have also been implicated to a lesser degree, the measles association is still the strongest relationship. Recently, measles (48) and parainfluenza type-I (181) viruses were reported to have been isolated from passaged MS brain explant cultures, but these reports have not yet been confirmed. Electron microscopic studies of acute MS lesions have demonstrated intracellular virus-like particles (41, 161, 194). It may be that they represent a nonspecific host response to cellular injury, since they are seen in other unrelated disorders (41).

Epidemiological evidence provides the strongest argument for an infectious basis for MS. The disease is generally most common in northern latitudes (16, 119). Migration studies have also determined that the propensity to develop MS is almost certainly acquired before 15 years of age (3, 119). Transmission experiments have been unrewarding, although the induction of a scrapie-like illness in sheep inoculated with MS brain suspensions after many months of incubation represents an interesting, albeit questionable, observation which needs confirmation (150). The occurrence of MS in laboratory personnel working with swayback of sheep is an interesting but unexplained observation which occurred many years ago (24).

Study of the cellular immunity of MS patients may provide important information concerning the pathogenesis of MS. Recent work has suggested that the lymphoreticular cells of the MS patient may have an inability to recognize measles antigen, and other lipid-containing viruses (28, 191). The addition of transfer factor obtained from immune donors to the in vitro system has restored immunocompetence to the deficient cells, and thus it has been suggested that transfer factor therapy may be a rational approach to the treatment of MS (191).

Although the pathogenesis of SSPE and MS are almost certainly different, there are important parallels between these two disorders. Both conditions may have increased IgG in the CSF, and oligoclonal IgG has also been demonstrated in the CSF (25, 121). However, such patterns are occasionally found in other diseases of infectious or immunological etiology (25, 121). Some MS patients have measles antibody in CSF, but SSPE patients always have antibody and in much greater quantity than do MS patients (95, 189). In both conditions immunoglobulins are produced locally in diseased brain (188). At this time the most significant data in support of a viral etiology for MS is the presence of measles antibody in CSF of some MS patients. It is to be hoped that future imaginative studies will provide additional clues to solve the enigma of this intriguing disease.
B. Guillain-Barré Syndrome (GBS)

The GBS, or postinfectious polyradiculitis, has been associated with a multitude of infectious agents, including mumps, influenza, rubeola, enteroviruses, varicella-zoster, Epstein-Barr (EB) virus, and *Mycoplasma pneumoniae* (33, 50, 78, 82, 122, 123, 134, 190). It has also been observed in association with numerous immunization procedures, including tetanus antitoxin, smallpox, rabies, and poliomyelitis vaccines, and more recently with the mumps and rubella vaccines (82, 134, 166). The more consistent associations have been with the enteroviruses, EB virus, and *M. pneumoniae* infections. Echo viruses have been isolated from the CSF of patients with GBS, and increases in serum antibody titers were also noted coincident with neurological dysfunction (190).

For a number of years there have been reported epidemiological associations between acute and subacute CNS disorders, and infectious mononucleosis. Recent work has demonstrated the relationship of the EB virus with infectious mononucleosis (14, 78), and has thus permitted serological correlations between this virus and the GBS. In a recent study, patients with the GBS were found to have high levels of antibody to EB virus, as compared to age-matched controls (78). In another study, peripheral white blood cells from GBS patients were found to have an increased tendency to become continuous self-proliferating lines (14). When examined by electron microscopy, these cells contained herpes-like viral particles resembling EB virus. Inoculation of hamsters with these cells failed to produce neurological disease, however.

Elevated *M. pneumoniae* CSF antibody levels have been associated with the GBS (122, 177), and there is also one reported isolation of *M. pneumoniae* from the CSF of a patient with this disorder (50). It is estimated that up to 7% of patients hospitalized with *M. pneumoniae* infections may develop some type of subacute CNS disorder, including the GBS (177). Although the pathogenesis of CNS disease associated with *M. pneumoniae* is unknown, it has been shown that antibody to this agent cross-reacts with human brain antigen (13). A significant percentage of patients infected with *M. pneumoniae* have complement-fixing antibrain antibody in the serum which may be removed by adsorption with mycoplasma antigen (13). Although *Mycoplasma neurolyticum* and *Mycoplasma gallisepticum* elaborate neurotoxins thought to be pathogenetically important in neurological disease of lower animals, no such toxin has been discovered in the case of *M. pneumoniae* (176).

In view of the variety of infectious agents associated with GBS, this syndrome may represent a nonspecific hyperimmune response, demyelinating in nature. However, much work remains to be done before such an hypothesis can be readily accepted.

C. Amyotrophic Lateral Sclerosis (ALS)

ALS occurs primarily as a sporadic disease, but 5 to 10% of all cases occur in families and are believed to be associated with an autosomal dominant mode of inheritance (142). Certain foci of ALS also occur in other parts of the world in
SLOW VIRUS DISEASES

exceedingly high frequencies, as among the native Guam population (17), and on the Kii peninsula in Japan (171).

ALS is a fatal chronic degenerative disorder affecting the motor neurons of the spinal cord and brain, with a primarily noninflammatory neuropathology. Death and drop-out of motor neurons of affected CNS tissues and demyelination of the motor tracts are hallmarks of this disorder (87, 88). The predilection of poliovirus for anterior horn cells makes it a logical choice as a possible candidate for the etiology of ALS. Proponents of such an association might suggest chronic poliovirus infection of the motor neuron as the underlying pathogenetic mechanism. Although direct evidence for such an association is lacking, it has been suggested that ALS patients gave a history of anteecedent paralytic poliomyelitis at rates up to ten times that observed in controls (159). The association is also supported by the existence of a syndrome following documented paralytic poliomyelitis by fifteen years, or more, where the patient demonstrates a slowly progressive, relatively benign, motor neuron disease, not totally unlike that seen with classic ALS (7, 138). It is possible that this may represent reactivation of an old latent poliovirus infection, or simply an increased rate of cellular decompensation by elements previously injured by the virus many years earlier. These patients should obviously be studied for the presence of poliovirus antibodies in their CSF, and when possible, for poliovirus antigen in affected neural tissues.

Intraneuronal inclusion bodies indistinguishable from those seen in rabies have been described in ALS patients, but the likelihood that they may represent non-specific findings has been suggested (87, 88).

The successful transmission of ALS to rhesus monkeys was first reported by Russian investigators (203), but the study of pathological tissues and affected animals by a United States delegation to Russia failed to document their findings (15, 105). Furthermore, inocula prepared in collaboration with these investigators did not induce CNS disease in animals inoculated in the United States (68). Since then, an additional report has appeared in the Russian literature, again claiming the successful transmission of ALS to rhesus monkeys in subsequent experiments (61).

Recently, C-type virus particles have been found to cause motor neuron disease as well as lymphoproliferative disorders in a wild mouse colony in California (63, 143). This model is currently under investigation.

ALS, as seen in Guam, occurs at rates more than 50 times those seen in the United States (17). Furthermore, neurofibrillary tangles are seen in the brains of virtually 100% of ALS patients in Guam (171). This finding is complicated since a significant proportion of normal individuals in Guam also have these tangles. The significance of these structures is unknown. A percentage of ALS cases also occurs in conjunction with the Parkinson-dementia complex (171). The extraordinarily high incidence of these diseases among the Guam natives is consistent with an infectious etiology, or some toxic environmental exposure. However, a genetic contribution should also be considered.

D. Parkinson’s Disease

Idiopathic Parkinson’s disease, paralysis agitans, represents a significant cause of chronic neurological disease in the adult population, and is the most common form
of Parkinsonism. Its etiology is unknown. The postencephalitic variant of Parkinsonism which occurred as a complication of von Economo's encephalitis could indicate a possible infectious basis (133). Recently, influenza virus antigen was demonstrated in brains of postencephalitic Parkinson patients by the fluorescent antibody technique (64). Tissue culture investigation of these brains was not reported, but would constitute an important step in identifying a virus etiology for this variant of Parkinson's disease. Although similar immunological evidence does not exist to suggest an infectious basis for idiopathic Parkinson's disease, it has been proposed that it may also be caused by persistent influenza virus in the CNS, possibly as a defective virus (52). Viral seroepidemiological studies and tissue culture investigation of Parkinsonism patients would be most worthwhile.

E. Presenile Dementias

The presenile dementias occur as a group of disorders affecting middle-aged adults, having an insidious onset, and no clear etiology (132). They may occur in familial patterns, but most are sporadic cases. CJD, as a presenile dementia, has been shown to be transmissible, although Alzheimer's disease and Pick's disease, as the main constituents of this category, have not been successfully transmitted. Many specimens have been inoculated into subhuman primates, and now must await the lapse of suitable incubation periods (68).

V. SPECIAL FEATURES OF SLOW VIRUS DISEASE

A. General Considerations

In studying the complex and inadequately understood virus-cell relationship, the possibility emerges that virtually all viruses studied in vitro can either convert to, or be experimentally manipulated to exist as agents of chronic persistent infections (141). The clinical spectrum of slow virus infections probably results at least in part from host-related factors, but more likely from a combination of host and virus-related influences. Accordingly, attempts to separate completely host from parasite factors may be futile. But certain host variables may be more consistently associated with some slow virus infections than with others. These shall be briefly reviewed.

B. Host-Related Factors in Slow Virus Infections

1. GENETIC FACTORS  Following the discovery of kuru, a subacute degenerative disorder of the CNS occurring in the highlands of New Guinea, it was initially felt that this represented a genetic affliction (12, 57). Victims of this disease were most often adult females and children, but a precise mode of genetic transmission had not been established. Members of other groups married to, or in close contact with the Foré people, did not contract kuru. In spite of the unequivocally proven infectious nature of kuru, and of the more than 2500 cases studied, no evidence refutes the possibility that a single gene may be responsible for the expression of this disease. Such a gene would be dominant in the adult female and male, but only partially penetrant in the adult male. It would produce disease with an earlier and more acute
onset in children having the homozygous condition (53). More recent investigations of blood groups and serum proteins in which the Foré people were compared with their unrelated neighbors continue to suggest that a genetic determinant may possibly govern expression of disease (118, 201).

CJD, a closely related spongiform encephalopathy, occurs primarily as a sporadic disease, but there are several pedigrees in which multiple family members have been affected over several generations. The distribution of cases is consistent with an autosomal dominant inheritance (131). Perhaps the precise relationship between kuru and CJD and genetic tendencies may be explainable in terms of a recently proposed hypothesis (52). It has been speculated that the original source of the kuru agent may have been a case of CJD occurring among the Foré, and later transmitted as a consequence of the mourning ritual. The disease would then be perpetuated in a pseudogenetic pattern reflecting the familial patterns of the tribal ceremony. It has also been suggested that the kuru agent may represent the modification of a known human virus. The cannibalistic mourning rites of the Foré would have caused it to be passed from human to human serially by ingesting brain, thus selecting for enhanced neurotropism (52).

The expression of scrapie in both sheep and mice has been thought to depend upon a particular genetic allele imparting susceptibility to the host. Natural scrapie in Great Britain has been explained completely in genetic terms, with the expression of clinical scrapie dependent upon homozygosity for an autosomal recessive gene (153). Additional support for possible genetic contributions to slow virus disease expression comes from the range of host susceptibility observed in experimental transmission. In general, marked specificity is seen; although scrapie causes disease in most breeds of sheep tested, breeds do vary in relative susceptibility, and some are completely resistant (153). Although a variety of rodents are susceptible to scrapie, guinea pigs and rabbits are not (70).

Significant genetic influences upon the expression of visna in sheep probably exist. Visna is considered to have been imported into Iceland during the early 1930s in a small flock of apparently healthy Karakul sheep from Germany (73). After the importation of those animals, visna became a recognized disease of Icelandic sheep. Neither visna nor maedi, the pulmonary analog of visna, had ever been observed in Karakul sheep, either in Iceland or in the flocks currently maintained in Germany (179). There is little reason to doubt that Icelandic sheep differ considerably genetically from other European breeds, since Icelandic sheep were brought into that country by settlers more than 1000 years ago, and have lived in relative isolation since (149). Numerous early attempts to transmit visna infection to a variety of laboratory and domestic animals, including non-Icelandic sheep, have been unsuccessful. Most recently, however, evidence of CNS infection in American lambs inoculated with visna virus has been presented (140).

While a possible genetic component of SSPE has been suggested, a recent report describing proven SSPE in only one of two identical twins represents strong evidence against a significant genetic contribution to that disorder (199). The living unaffected twin has no serological evidence of SSPE, nor any other of its laboratory or clinical stigmata.
Aleutian disease of mink is a transmissible slow disease discovered in ranch mink (76, 158). The term Aleutian has reference to the animal's gun metal gray color, similar to that of the Aleutian fox. The gene for this color is inherited as an autosomal recessive trait designated "aa," and mink with the Aleutian color are afflicted with the disorder. Features of this disease include some of those observed in connective tissue disorders of man, and the disease may also be a model for multiple myeloma (158). As with most other slow virus diseases with possible genetic components, the precise mechanisms which predispose such animals to disease expression are unknown. It is of interest, however, that the mortality rates vary among genotypes, according to whether "aa," "Aa," or "AA" mink are involved. Mink with "aa" usually die within 150 days, while over half of "Aa" and "AA" animals live a year or more (158).

2. IMMUNOLOGICAL FACTORS PML, a chronic and fatal neurological disease characterized by focal areas of cerebral demyelination, primarily affects older individuals suffering from a variety of lymphoproliferative disorders of malignant origin, as well as miliary tuberculosis or sarcoid (75, 205). Although the PML-immunodeficiency association may represent one of the strongest relationships existing between host susceptibility and propensity to slow virus disease, the questions it has stimulated far outnumber the solutions it has provided. PML is a very rare disease, in spite of the fact that medically immunosuppressed patients, as well as those with secondary immunodeficiencies, far outnumber those afflicted with this slow disease. If exposure to JC virus and SV-40 virus are as common as seroepidemiologic evidence would indicate, how can it explain the comparative rarity of PML? Does only a small percentage of exposed individuals harbor virus in the brain, available to induce disease later under certain conditions? Must residence of virus in the brain coincide with the onset of the immunodeficient state? The isolation of numerous and diverse viral agents from normal subhuman primate brains (59, 189) raises the question of whether there is a normal viral flora of the human brain.

There are other slow virus diseases which are characterized by unusual immunological features. SSPE is noteworthy in this regard in that afflicted patients usually have exaggerated serum antibody responses to rubeola virus. Hemagglutination inhibition and complement-fixing antibody to this agent are also detectable in the CSF (95). Detection of measles antibody in CSF combined with appropriate clinical and electroencephalographic findings is virtually pathognomonic for this disorder. It is not known whether these abnormal antibody responses to measles virus represent the host reaction to chronic stimulation of antibody synthesizing clones due to persistent viral antigen, or alternatively signify the production of biologically ineffectiv e immunoglobulins.

Based on experimental models of chronic measles infection of the hamster brain, it has been suggested that the SSPE patient may have a deficiency of the cell-mediated immune system, rendering him unable to eliminate viral antigen (23, 195). Alternatively, a blocking factor has been demonstrated in the plasma and CSF of SSPE patients, which has the ability to suppress normal lymphocytes in vitro, rendering them incapable of recognizing measles antigen (167). The blocking factor
may be a complex of immunoglobulin and measles virus antigen. The factor is ten times as concentrated in CSF as in plasma. If this blocking factor is fully characterized, and its pathogenetic role demonstrated, it would represent the first instance in which a blocking factor is centrally involved in the pathogenesis of a human disease.

Approximately 95% of IgG found in CSF of SSPE patients is probably due to local synthesis in the brain (188). Presumably it comes from perivascular mononuclear cells in the brain and meninges. Although it is not known if such antibody damages brain cells of the SSPE patient harboring measles antigen, the immunolysis of HeLa cells infected with measles virus has been demonstrated with measles antibody and complement (135). This mechanism could presumably also occur in vivo. Theoretically, antibody could be directed against released virus containing fragments of the infected cell plasma membrane, or could be directed against the cell itself which has been antigenically altered by virus infection. The possible pathogenetic role of measles antibody in the SSPE patient is complicated by reports of SSPE patients with hypogammaglobulinemia (83, 200), and the occurrence of SSPE-like neuropathology in a child with a combined immunodeficiency syndrome (5).

SSPE may reflect a generally depressed cell-mediated immune system, or a specific defect in cell-mediated immunity against measles virus (126). This has indirect support in that measles infection temporarily suppresses cellular immune mechanisms against a variety of unrelated antigens (45). There are conflicting reports regarding the cellular immune mechanism in SSPE patients (137). One study of four such patients demonstrated impaired delayed hypersensitivity to a variety of skin test antigens including measles virus, and also showed slow rejection of skin allografts (65). In another investigation, eight SSPE patients had positive skin test responses to *Candida*, while those to measles antigen were negative (104). Two of three patients tested became sensitized to dinitrochlorobenzene. These results must be interpreted with caution, since it is known that an adverse nutritional status, as seen in end-stage SSPE patients, may depress delayed hypersensitive reactions (11).

In all naturally occurring cases of visna virus infection, CSF pleocytosis is noted, and an elevation in CSF gamma globulin may also be present (149). The specificity of the increased gamma globulin has not been described, and it is known that visna virus persists in the presence of high titered neutralizing antibody. This is consistent with the claim that the immune response in visna is deficient as compared with that in other viral infections (157). In this disorder, neutralizing antibodies are usually observed well in advance of clinical disease, and it has been proposed that an antigen-antibody reaction occurs on the surface of infected CNS cells, causing cell destruction and secondary demyelination (79, 149). Visna virus has the property of causing cell fusion and subsequent cell disintegration in vitro, even when virus does not replicate (21, 85). In protracted experimental visna disease, if a reisolated virus is compared with the original strain used for inoculation, it is found that sera obtained early in the disease are unable to neutralize the reisolated agent as well as the original one (80). This situation has some analogy to that of SSPE, where the patient has a diminished ability to neutralize SSPE virus isolates when compared
with wild measles (94, 154). These alterations of virus strain, occurring over pro-
longed incubation periods in the host, are also analogous in that the SSPE isolates
usually resemble vaccine strains of measles virus, rather than the wild type (93). As
with SSPE, it has been suggested that a search for serum blocking factors might be
worthwhile. Again, in both SSPE (34, 35) and visna (5), virus may be demonstrated
within CSF cells.

C. Virus-Related Factors

As mentioned, it is difficult to separate host from virus factors in slow disease. Such
a division, though arbitrary, has been made to facilitate an understanding of the
relative contributions of host and parasite to the evolution of slow disease. Some of
these features, and the special problems relating to their study, are the following:

1. LONG INCUBATION PERIOD  Slow virus diseases, by definition, are character-
ized by very long incubation periods, lasting from months up to 20 years or more,
as in human kuru. The events occurring during this interim are poorly understood.
The critical primary interactions between host and virus may occur many years
before the first detectable clinical manifestations are noted. Animal transmission has
enabled investigators to study infected tissues in a sequential fashion, beginning with
inoculation and ending with death. Most retrievable information obtained directly
from human slow virus disease, however, has been limited to pathological descrip-
tions of end-stage tissues. Very little is known concerning the pathologic sequences
in the human brain, although one may infer this from animal investigations. The
study of unconventional agents, in particular, is hampered by an inability to recog-
nize them in infected tissues. Furthermore, the futility of attempting to reconstruct
the complex interactions taking place over a period of months to years prior to
development of clinical disease is self-evident.

2. VIRAL PERSISTENCE  All slow virus diseases are characterized by agents
which persist in host tissues throughout most, if not all, of the clinical disease.
Virtually all of the meaningful laboratory data attributing a viral etiology to slow
disease depends upon this property. Persistence may be demonstrated by morpho-
logically identifiable virions, as in SSPE, PML, visna, and LCM, or by the transmis-
sion of disease, as with scrapie, TME, kuru, and CJD. The demonstration of viral
persistence as a consistent feature of slow virus diseases does not imply an under-
standing of their pathogenesis, and certainly not of their successful prevention and
treatment. A knowledge of the precise mechanisms involved, and the means of
terminating or modifying the relationship will be important requirements if this is
to be achieved.

Persistence of viral antigen within host cells does not necessarily imply a deleteri-
ous effect on the parasitized elements. Consider the classic example of LCM infec-
tion of the rodent CNS, in which fatal neurological disease can be prevented by
immunosuppressive treatment with a variety of chemical and physical agents (31).
Not until the host mounts an immunological attack against the LCM virus does
tissue destruction ensue (31, 144, 145). Unfortunately, most persistent viral infec-
tions, many of which are associated with slow disease, have not been defined to the extent of the LCM system. It is often assumed that if one were to rid the host of persistent viral antigen, the underlying basis for slow disease might likewise be eliminated.

The nature of the unconventional agents of slow disease is so poorly understood that it is difficult to think in terms of their mechanisms of persistence. Immunosuppressive treatments given to mice infected with scrapie had no effect on the incubation period or pathologic development. This suggests that immunological mechanisms neither contribute to the pathogenesis of scrapie, nor play any significant role as defense against the agent (67). If the agent of scrapie resembles a small fragment of nucleic acid in intimate contact with a portion of plasma membrane (52), it would probably have little or no antigenic properties. It would not be recognized by the host's immunological surveillance system, thus explaining its persistence in a wide range of tissues.

Although substantive evidence is lacking to explain viral persistence in slow disease caused by conventional agents, a number of possibilities might account for this phenomenon. As discussed, a defect in the cell-mediated immunological system of the SSPE patient has been postulated (65). The recent demonstration of a serum and CSF blocking factor in these patients (167) represents an alternative mechanism to explain failure of the SSPE patient to eliminate chronic viral infection. Both possibilities must be further explored.

The problem of viral persistence in visna has been studied less extensively than in SSPE, but it has been suggested that viral antigen within mononuclear cells of the CSF may explain this phenomenon (5). In view of antigenic changes in visna virus following extended residence in the sheep CNS, perhaps the infected animal loses the capability it once had to eliminate the original infecting strain. As previously noted, a similar situation may exist in the SSPE patient, in that isolated SSPE viruses more closely resemble vaccine strains than wild virus. Analogous to the situation postulated with visna, perhaps the SSPE patient also loses the ability to eliminate virus because of its prolonged residence in the SSPE brain, and presumed changes in antigenic and biological character. Measles virus may possibly reach the CNS even in uncomplicated infections, as noted by electroencephalographic changes in patients with natural measles infection (151), or given measles vaccine (137, 152).

In the PML patient, it is easier to account for a mechanism of viral persistence, since the majority of these patients have immune deficiencies (205). Most of their underlying deficiencies primarily affect the cellular immune system. Since this is a significant component of the immunological surveillance system in recovery from virus infection (4), perhaps papovavirus persistence is a result of a grossly ineffective antiviral mechanism. Other possibilities certainly exist, however.

3. ANTIGENICITY OF AGENTS All conventional viruses are antigenic; they stimulate the proliferation of immunocompetent lymphoreticular cells and elicit the production of specific antiviral antibody. Specific clones recognize viral antigen upon subsequent exposure, and thus assume a host-protective function. To what extent the host immune system malfunctions, and fails to eliminate virus, is not
known in the cases of SSPE and visna. The PML patient probably manifests diseases because of a deficient immune system.

Both the SSPE (95) and the visna-affected (149) host produce antibody to their respective viruses, often in greatly exaggerated quantities. The PML victim may likewise produce antibody to the papovavirus infecting him, but usually not at increased levels (139). One patient with SV-40 in her brain demonstrated an eightfold rise in antibody titer to that agent during her clinical course, whereas this has not been observed in JC virus PML (139).

Efforts to demonstrate antigenicity of the unconventional agents have been futile. There is no indication that they are able to provoke any trace of immunological response in the infected or artificially immunized host, nor to produce interferon (110). As recently reviewed, many classic immunological methods to detect antibody in affected subjects have been employed, including complement fixation, neutralization, precipitation, gel diffusion, and direct and indirect fluorescent antibody techniques (60, 120). All have been uniformly unsuccessful, even though the affected animals have unimpaired immunological and interferon responses when tested with known antigens. Mouse spleen and kidney tissue suspensions from scrapie-infected mice have also been unsuccessfully used in neutralization tests, and attempts to demonstrate incomplete antibody or antigen-antibody complexes have failed. However, the cell-mediated component of the immune system has not been studied in detail.

Perhaps, as speculated, the actual activating agent in the transmissible spongiform encephalopathies is a small, or helper, molecule rather than a conventional viral particle (52). This would not be expected to elicit the immune response generally observed with complete virions.

VI. VIRUS-HOST INTERACTIONS AND THE PATHOGENESIS OF SLOW DISEASE

The understanding of basic pathogenetic mechanisms involved in slow virus disease is still fragmentary. With most such infections the precise cellular specificity of infection, if any, is unknown. The infectivity of unconventional agents is demonstrable in many pathologically unaffected non-neural tissues, as well as the CNS, in which primary pathological changes occur. Reticuloendothelial tissues, in particular, are a fertile source of these agents. Evidence has been presented that the scrapie agent first replicates in tissues such as spleen, and only secondarily reaches the brain, where it may then be found for an extended period of time before clinical disease develops (42).

It is apparent that the host range of susceptibility to unconventional agents is much more extensive than originally suspected, as described in a recent review (70).

These general areas of study, and the special problems associated with them, form the subject of the following discussion.

A. Cellular and Organ Specificity

The information available concerning the pathogenesis of unconventional slow virus diseases is derived primarily from their transmissibility to a wide variety of labora-
tory and domestic animals, and particularly to the smaller species. Unfortunately little data exist regarding specific host-destructive mechanisms operating at the cellular and molecular levels. It has been suggested that scrapie infection of the CNS causes neuronal degeneration by an indirect route, that is, by disturbances of astrocytic-neuron regulatory mechanisms following infection involving the astrocytes (42). These cells typically hypertrophy and proliferate, and in doing so may adversely affect homeostatic mechanisms of the neurons with which they are in intimate physical contact by foot processes. Whatever the underlying pathogenetic mechanisms, there is no evidence of acute neuronal destruction.

Biochemical study of mouse scrapie brain has demonstrated no abnormalities in RNA and protein metabolism, and total contents of dry matter, protein, and nucleic acid do not differ from normal mouse brains (136). However, radioisotope study of scrapie brain demonstrated an enhanced rate of DNA synthesis not observed in other organs, including the spleen (115, 117). Although there was an increased rate of DNA synthesis or turnover in glial cells, there was no increase in total DNA. DNA synthesis was not considered to represent replication of the scrapie agent, and so the precise interpretation of this observation remains obscure (116).

In both PML and SSPE, intracellular virions have been demonstrated within CNS cells, including oligodendrocytes (196). Interference with the metabolism of this cell presumably leads to disruption and loss of integrity of the cell membrane which, in fact, constitutes myelin. Demyelination is a characteristic feature of both disorders, although it is not the sole neuropathological alteration.

SSPE virus has been isolated from lymph nodes of affected patients; this is consistent with a possible extra-neural site of viral replication in lymphoid organs (97). Similar search for PML viral antigen in extra-neural tissues has been unsuccessful (139), although an exhaustive study has not been conducted.

Cellular pathology with visna occurs in the CNS with the appearance of meningeal and subependymal infiltrates. Myelin degeneration occurs secondary to extensive microglial infiltrations which have a tendency to necrose and cavitate (149).

B. Transmissibility and Range of Host Susceptibility

Knowledge of the complete spectrum of hosts capable of supporting infection by the agents of slow disease would be useful. The conventional agents appear to have a limited range of natural host susceptibility. Thus, SSPE and PML are afflictions restricted entirely to man as natural host. Disease somewhat similar to SSPE has been produced in cynomolgus monkeys (156), calves and lambs (182), ferrets (111), and hamsters (23, 195). Visna, a well-characterized RNA virus sharing many physicochemical and biological properties with onocogenic viruses, produces clinical disease only in Icelandic sheep. Visna virus infection causes subclinical inflammatory lesions in the brains of experimentally inoculated American lambs (140).

The unconventional agents of scrapie, kuru, CJD, and TME produce very similar neuropathological lesions both in naturally infected hosts and experimental animals (8, 120). The comparative neuropathology and overall similarity of clinical disease support the theory that all the transmissible spongiform encephalopathies may be manifestations of infection by a single agent, or very similar agents.
A recent report has summarized the results of transmission studies and indicates that all four spongiform encephalopathies are transmissible to at least some species of Old and New World monkeys (70). Although the incubation period of CJD in man is estimated to be four to five months, CJD tissues inoculated into chimpanzees caused clinical disease in 11 to 26 months. Recently, clinical and pathological criteria for CJD developed in a corneal transplant recipient 18 months after the operation (41a). Post-mortem study of the donor, who had a neurological disorder, revealed CJD after the transplant had been performed. Natural kuru in man, with an estimated incubation period of 60–240 months, caused disease in the chimpanzee in 10–59 months. The domestic cat is also susceptible to experimental inoculation with CJD tissues, manifesting clinical disease within 30 months following inoculation (70). This latter observation raises the possibility of domestic and wild animals serving as natural reservoirs for viruses of slow disease in man (18, 182). It is also of great interest that familial CJD has recently been transmitted to the chimpanzee (46), and suggests the possibility that other familial chronic degenerative disorders of man, having obscure or unknown etiologies, may also be of infectious origin.

Visna is readily transmissible to Icelandic sheep, but not to other animals. PML, with a probable etiological association with papovaviruses, has not been experimentally transmitted. The oncogenicity of the JC isolate for hamsters, while of great interest, does not demonstrate the transmissibility of PML (193). Perhaps future attempts to transmit PML will utilize highly concentrated and purified preparations for inoculation.

The relative ease by which spongiform encephalopathies are transmitted compared to slow diseases caused by conventional agents, may be significant. Perhaps host factors may be more important determinants of disease caused by conventional agents than is the case with the unconventional agents.

C. Viral Replication

While innumerable elegant experiments have demonstrated the intricate biochemical mechanisms involved in the replication of animal and bacterial viruses, no such descriptions exist for the unconventional agents of slow disease. The perplexing features of the scrapie agent have led to the question whether it should be classified as a virus (6, 102), even though it is filtrable, transmissible, and self-replicating. It has been suggested that the agent is a replicating membrane, polysaccharide, or protein (1, 6, 72, 77). It has also been stated that the scrapie agent may not have a nucleic acid genome (6). Such notions do not follow the dicta of classical virology. They have probably resulted from frustration with efforts to characterize an agent which has defied analysis. These earlier hypotheses are in contrast to one recently proposed, which suggests that the scrapie agent is a viroid (40), a term designating naked infectious RNA noted to cause a variety of plant diseases (39). The diversity of these currently held views attests to the paucity of leads in the identification and characterization of the unconventional agents.

Barring some breakthrough, an understanding of the nature of the unconventional agents may be at some distance in the future. However, until these agents have been exhaustively studied, and every reasonable possibility that they contain some
remnant of classic viral material has been eliminated, there is no urgency to seek new biological systems to account for them. It has been stated that even considering the most unusual properties of the scrapie agent, it is not inconceivable that classic viruses with atypical properties may be involved (67). The recent isolation and characterization of the viruses of Aleutian mink disease (112), and hepatitis A (44) and B (160) support this notion.

As mentioned earlier, scrapie-infected mouse brain has demonstrated an increased rate of DNA synthesis, and organ cultures of brain material from scrapie mice have shown marked tendencies for continued cell growth and multiplication not observed in normal tissues. Multiplication of the agent seemed closely linked with division of the infected cells (30). At the macroscopic level, a number of important variables are recognized which affect the preclinical period of experimental scrapie, and have been reviewed (185). These include the size of the inoculum, route of administration, previous passage, history of the agent, species differences, and, in the mouse, even strain differences between donor and recipient. These data, plus genetic predispositions serving as determinants of clinical scrapie, indicate that this infection represents a very complex host-agent interaction.

In SSPE it is believed that defective viral particles may play an important role in persistence in brain cells. A number of questions have been raised regarding the nature of SSPE infection, as well as that occurring in PML (10). The recognized prerequisite for fusion or co-cultivation of SSPE or PML-infected brain explant cultures with indicator cells for induction of virus may be evidence for its defective nature. It is not known whether defective virus particles initiate the chronic infection, or whether they arise secondarily as a result of prolonged residence in the host. A question has been whether the etiologic agents of slow infections may arise from activated viral genes contained within cellular genomes (10). Such a relationship would be analogous to the oncogenes of RNA tumor viruses (101). That some agents of slow disease share common biological mechanisms with oncogenic viruses is demonstrated by the ability of visna virus to transform murine cell tissue cultures (180), and by the presence of a reverse transcriptase enzyme (124, 178).

The exact role that PML-associated papovaviruses occupy in the total biology of infected hosts is not understood. The giant astrocytes associated with papovaviruses in this disorder are indistinguishable from those in malignant glioblastomas. One must wonder if any relationship exists between these viruses and the underlying malignant disorders which frequently precede the onset of PML.

VII. THE FUTURE OF SLOW VIRUS RESEARCH

At the Workshop and Symposium on Slow, Latent, and Temperate Virus Infections held at the National Institutes of Health in 1965, it was observed that the sole criterion to establish the presence of scrapie virus was the induction of scrapie with infected cells (57). Since then, although nearly a decade has passed, less stringent criteria for the demonstration of the unconventional agents have not been developed. This limitation has dictated the slow progress in certain areas of slow virus research. An otherwise straightforward virus titration often measured in days for most con-
ventional viruses may take from months to years for the unconventional agents. These titrations require an in vivo indicator system since they must be conducted with living susceptible hosts. Inoculated animals must be maintained over a prolonged time period. The possibility of nonspecific deaths, as by bacterial infection, constantly looms as a potential threat to the successful completion of long-term studies.

Probably the greatest obstacle impeding the progress of slow virus research is the inability to characterize the unconventional agents of slow disease. Until some biochemical, immunological, or histochemical marker is identified, research in vital areas of slow disease has arrived at a temporary stalemate.

Tissue culture searches for unconventional agents in brain explant cultures are complicated by the isolation of numerous viruses now known to inhabit subhuman primate brains as latent inapparent infections (59, 89). Their relationship, if any, to slow disease is difficult to ascertain.

A better appreciation of host-predisposing factors should be attempted, such as the influence of genetic constitution on disease expression. Further search for aberrant immunological reactions of pathogenetic significance should be conducted. Indeed, there is strong evidence suggesting such a role for the immune system, as discussed earlier.

Perhaps the greatest challenges are represented by the vast spectrum of chronic degenerative disorders of man which occur in either sporadic or heredofamilial patterns. Most are in desperate need of etiological description. It is conceivable that at least some members of this group are caused by agents no longer present in, or recoverable from, diseased tissues. Accordingly, successful recovery of virus and experimental transmission would not be possible (106). These infections occur among lower animals. Examples include mumps-induced obstructive hydrocephalus (107, 109), cerebellar hypoplasia caused by feline panleukopenia virus (113, 114), and bluetongue virus-induced encephalopathies (146, 162). These defects are often noninflammatory in nature, and resemble classic defects of embryogenesis.

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