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IMMUNOSUPPRESSION AND EXPERIMENTAL VIRUS INFECTION OF THE NERVOUS SYSTEM*

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I. Introduction

The fate of the host has long been an important concern of studies of parasitism by animal viruses. A critical component in this interaction is the complex of changes which constitute the response of the host to the infectious process. The immune response is probably the most extensively studied of these components, and a central theme of this discussion is the role of the immune response in the outcome of primary viral infections of the nervous system.

The altered responsiveness, usually favoring host survival, which occurs on second exposure to viruses, has been discussed in detail in reviews of immunization against viruses (World Health Organization, 1966; Fenner, 1968; Evans, 1969), and will not be considered here.

Descriptive studies of the sequential evolution of virus infection and of the immune response have shown that, with many host-virus combina-

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tions, antibody can be detected early, often before clearance of virus. These observations have suggested that the outcome of infection may be due to a race between virus and the immune response (Mims, 1964; Fenner, 1968). However, such descriptive data are inevitably inconclusive (Bodian, 1961), and the subject has remained dormant for lack of more incisive experimental approaches. The recent burgeoning of immunology, and specifically of methods for immunosuppression, has reopened the question to productive exploration.

It should also be noted that, with a few exceptions, this review draws mainly upon results of experimental studies. Slow infections of the CNS, such as scrapie and visna, are not considered in any detail since the paucity of data on the role of the immune response during their progression does not justify extensive discussion.

II. Pathogenesis of Acute Virus Infections of the Central Nervous System (CNS)

The pathogenesis of CNS virus infection has been thoroughly discussed in an excellent recent discussion (Johnson and Mims, 1968) and additional useful information may be found in several other reviews (Bang and Luttrell, 1961; Mims, 1964; Albrecht, 1968; Fenner, 1968; Baer, 1969).

A. *Peripheral Virus Infection and CNS Invasion*

1. *Routes of CNS Invasion*

Since the CNS is considered to be a relatively sequestered tissue, the means by which viral invasion takes place has aroused recurring interest. Excellent reviews of this topic are presented by Wright (1953), Johnson and Mims (1968), and Baer (1969).

a. Viremia. In most instances, viral invasion of the CNS occurs from the blood. Passage across the blood-brain barrier can take place in several ways. A rather wide variety of viruses have now been reported to grow in endothelium of cerebral capillaries and small vessels (hog cholera virus, Seifried and Cain, 1932; herpes virus, Anderson, 1940; Bang, 1942; distemper virus, Coffin and Liu, 1957; infectious canine hepatitis virus, Cabasso, 1962; influenza virus, Hook *et al.*, 1962; arbovirus, Johnson, 1965b; K. P. Johnson and Johnson, 1968; reovirus, Kundin *et al.*, 1966; vesicular stomatitis virus, Bruno-Lobo *et al.*, 1968; rat virus, Cole *et al.*, 1970; vaccinia virus, Montasir *et al.*, 1966; adenovirus, Rabin and Jenson, 1967).

It seems likely that, in some instances, viruses may move passively across the cerebral capillaries, as suggested by the failure of careful observers to note endothelial infection (Albrecht, 1962, 1968), or across

choroid plexus with subsequent infection of ependymal lining of ventricles prior to involvement of neural parenchyma (Mims, 1960c). Additional evidence of passive crossing of the blood-brain junction is the potentiation of invasion by treatments which increase vascular permeability. Among those shown effective have been carbon dioxide inhalation (Sellers and Lavender, 1962), bacterial endotoxin and hypernatremia (Rahman and Luttrell, 1963), provoking injections (Bodian, 1954), microembolism (Cooke *et al.*, 1942), and preceding parasitic or rickettsial infection of the brain (Mochizuki *et al.*, 1954; MacLeod, 1962).

b. Neural Spread. The other important route of CNS invasion is along peripheral nerves, following introduction of virus by parenteral or olfactory routes. There are probably relatively few instances of naturally occurring infection in which neural spread plays a role. Examples (Johnson and Mims, 1968) of parenteral infection include injection by bite (herpes virus simiae, Sabin and Hurst, 1935; rabies virus, Johnson, 1959) and by syringe inoculation (poliovirus, Nathanson and Langmuir, 1963). Olfactory or trigeminal invasion may be operative for herpes simplex encephalitis (Johnson and Mims, 1968; Kibrick and Gooding, 1965).

Experimentally, neural spread is readily demonstrated by direct immunofluorescent observations (Johnson and Mims, 1968) and, indirectly, by use of nerve section to prevent CNS invasion following either olfactory or parenteral virus introduction (Howe and Ecke, 1937; Nathanson and Bodian, 1961a; Dean *et al.*, 1963; Baer *et al.*, 1965).

Elucidation of the mechanism by which viruses move along nerve trunks has provided a challenge for over 40 years (Goodpasture, 1925; Hurst, 1933; Wright, 1953; Johnson and Mims, 1968; Baer, 1969). Viruses may move at high speed centripetally along nerve trunks to the CNS. Rates have been estimated at 2.4 and 3 mm per hour, respectively, for poliovirus and rabies virus (Bodian and Howe, 1941b; Dean *et al.*, 1963; Baer *et al.*, 1965). Such movement almost certainly implies passive transport of virions. More direct evidence is provided by observations on herpes virus (Sabin, 1937) and on rabies virus (Johnson and Mims, 1968) where neural spread was operative in the apparent absence of replication within the implicated nerve trunks.

There has been a longstanding debate as to the site of passive movement of virions within peripheral nerves (Johnson and Mims, 1968; Baer, 1969). The two sites considered most likely are tissue interspaces (extracellular space) and the axoplasm. Centripetal flow of certain components within the axoplasm has been described and can be of the same order of speed as movement of virus (Lubinska, 1964). The active contractions of myelin recently described (Singer and Bryant, 1969) suggest one possible mechanism for such movement. The clearcut replication of virus within the

perikaryon of sensory ganglion cells without demonstrable involvement of supporting elements (Johnson, 1965a; Yamamoto *et al.,* 1965; ElDadah and Nathanson, 1967) is also consistent with axoplasmic spread. On the other hand, Baer *et al.* (1965), in a series of carefully executed experiments, concluded that the sciatic nerve could conduct rabies virus centripetally, following footpad inoculation, even though the axons had degenerated due to a prior nerve crush at the level of the sciatic notch (however, see Nathanson and Bodian, 1961a). Final resolution awaits further experimental evidence.

Concomitantly with neural spread, certain viruses replicate within Schwann cells and fibroblasts (endoneurium and perineurium) of the implicated peripheral nerves (Hurst, 1933; Sabin, 1937; Johnson, 1964a; Rabin *et al.,* 1968). It seems likely that, in some instances, infection of these supporting cells is secondary to axoplasmic movement of virus. In other virus-host interactions, it is possible that replication along a chain of supporting cells in the peripheral nerve is the primary mechanism of neural spread (Johnson, 1964a).

2. Relationship between Peripheral Phase of Infection and Behavior of Virus in the CNS

There is no regular relationship between the ability of a virus to replicate in nonneural tissues or to spread along peripheral nerves and its ability to gain access to the central nervous system. As a consequence, readily available experimental models may differ qualitatively as well as quantitatively in (*i*) the interval between initiation of infection in the periphery and in the CNS; (*ii*) the relative growth rates of virus in periphery and CNS; and (*iii*) the onset and acceleration of immune induction and other host defenses in relation to growth of virus in the CNS. Albrecht (1968) has reviewed these points in detail with respect to the arboviruses, and has collected examples of host-virus relationships in which (*i*) virus replicates readily in the peripheral tissues but only poorly in the CNS, so that even intracerebral inoculation of large doses produces a sublethal infection with a brisk viremia; (*ii*) virus replicates readily in CNS but relatively poorly in peripheral tissues; such virus strains have a high intracerebral titer, but a very low parenteral LD_{50} titer; (*iii*) arbovirus-host combinations in which virus replicates poorly in both peripheral tissues and in CNS (see ElDadah *et al.*, 1967, and references therein; Cole and Wisseman, 1969a); and (*iv*) finally, combinations in which virus replicates readily in both extraneural tissue and CNS, with regular occurrence of acutely fatal encephalitis.

B. Virus Replication in the CNS and the Pathological Response to Infection

1. Spread of Virus Within the CNS

The initial distribution of virus within the CNS following extraneural infection differs markedly from the distribution of virus which follows intracerebral inoculation. Mims (1960a) has shown that, in mice, an intracerebral inoculum is forcibly dispersed through the ventricular system and the subarachnoid space (as well as into the blood, Cairns, 1950) at the time of inoculation. Virus inoculated in this manner may invade the parenchyma both from the site of inoculation and through the ependymal and meningeal surfaces, particularly if it is capable of replicating in these superficial tissues (Mims, 1960b). In contrast, following extraneural infection, virus usually invades the CNS through (or across) the vascular wall, probably simultaneously at many sites. For viruses which can be identified replicating in endothelium, this widespread vascular distribution within the CNS can be observed directly (Cole *et al.*, 1970).

In those instances where a virus strain of high neurotropism invades the CNS along neural pathways, virus may at first be confined to one CNS area, with subsequent localization of early pathological changes or of early dysfunction to the same area (Bodian, 1959; Nathanson and Bodian, 1961a). Even where CNS invasion follows viremia, the earliest site of invasion may be somewhat circumscribed if there is an area of abnormally high vascular permeability (Bodian, 1954; see discussion in Nathanson and Bodian, 1961a).

Once CNS invasion has occurred, some viruses can spread rapidly through the parenchyma. Immunofluorescent observations (e.g., Johnson and Mercer, 1964; ElDadah *et al.*, 1967) indicate that infection develops simultaneously in parenchymal cells throughout the brain. Additional indirect evidence for ready dissemination is the widespread distribution of infection following intracerebral inoculation, even of viruses of low neurovirulence (Nathanson *et al.*, 1966; ElDadah and Nathanson, 1967).

There are several potential mechanisms of viral dissemination within the parenchyma of the CNS. Viruses which replicate in cytoplasm can be readily observed within dendrites of infected cells (e.g., ElDadah and Nathanson, 1967; Johnson and Mims, 1968). If axoplasmic streaming can passively transport viruses along peripheral nerves, the same mechanism could operate within the CNS. Movement within extracellular spaces, particularly for small viruses, is also possible (Boyse *et al.*, 1956; Brightman, 1965; Johnson and Mims, 1968).

2. Variation in CNS Localization of Infection

Widespread use of immunofluorescent staining has permitted detailed documentation of localization of viral antigen within the infected brain (Albrecht, 1968; Johnson and Mims, 1968). It is now clear that there are a number of sharply different patterns of localization of infection.

(*a*) Among the viruses which replicate within *neurons,* the proportion of infected cells may vary from very high to very low (ElDadah and Nathanson, 1967; Albrecht, 1968; Johnson and Mims, 1968). The proportion of infected cells can be relatively constant throughout the CNS (ElDadah and Nathanson, 1967) or there may be marked differences in the severity of infection in different CNS areas (Hurst, 1936). For instance, among arboviruses, there are some which severely attack cerebellum (louping ill, Hurst, 1931; Brownlee and Wilson, 1932), spinal cord (tick-borne viruses, particularly Russian spring-summer encephalitis, Silber and Soloviev, 1946; Zilber, 1962), or basal ganglia (Eastern equine encephalomyelitis, Nathanson *et al.,* 1969), while others produce more uniformly distributed lesions (Japanese B encephalitis, Nathanson *et al.,* 1966). Rabies virus produces spectacular infection of Purkinje cells (Johnson and Mercer, 1964); rat virus attacks cerebellar granule cells (Margolis and Kilham, 1968; Cole *et al.,* 1970); polioviruses produce their most severe lesions in spinal cord motoneurons (Bodian, 1959); and herpes simplex virus tends to select the olfactory and limbic systems of the forebrain (Hughes, 1969).

(*b*) Infection of *nonneuronal* cells also may vary greatly, without any necessary relation to location or severity of neuronal infection (Johnson and Mims, 1968). For instance, herpes virus encephalitis often involves all glial and neuronal elements in a devastating infection (Johnson, 1964a,b), while rabies virus may produce severe neuronal with little glial infection (Johnson, 1965a; Yamamoto *et al.,* 1965). Nonneuronal infection is considered in greater detail in the section on pathology.

3. Diversity of Pathological Lesions Produced by Virus Infection of the CNS

a. Individual Cells. (*i*) The most familiar cellular response to virus infection is *necrosis of the cell,* which may be produced by most classes of viruses. Tissue culture studies of the mechanisms by which virus infection causes cytopathic effects are reviewed by Fenner (1968) and Godman (1966). These include: (*i*) disruption of transcription or translation of host mRNA or of other essential cellular processes (Bablanian *et al.,* 1965a,b); (*ii*) activation and release of lysosomal enzymes (Allison, 1967); and (*iii*) insertion of virus-specific elements into the cell membrane, which causes "late" polykaryocytosis (Roizman, 1962).

There are innumerable descriptive studies of the evolution of virus-induced cytopathic changes, both in tissue culture (Bang, 1959; Pereira, 1961; Bernhard, 1964; Fenner, 1968) and in the nervous system (Hurst, 1936; Innes and Saunders, 1962; Johnson and Mims, 1968).

It should be recognized that virus-infected cells may undergo functional abnormalities prior to development of architectural changes; also early changes may not be recognized in the light microscope. Thus, in laboratory models in which rapid overwhelming infections occur, the heavily infected CNS may show relatively minor pathological changes (rabies virus, Johnson, 1965a; arboviruses, Albrecht, 1960; ElDadah and Nathanson, 1967).

Also cytolytic viruses may, upon occasion, produce nonnecrotic infection or infection from which the cell recovers. One example is poliovirus infection of motoneurons in the primate spinal cord (Bodian, 1948).

A cell population (even though homogeneous) may respond differently to infection than do the individual cells of which it is composed. Thus, individual cells may undergo lytic infection, and yet the population survives exposure to the virus. Such differences can arise when a relatively small proportion of the susceptible cell population is initially infected; if infection spreads sufficiently slowly to additional cells, host defense mechanisms may lead to eradication of the virus prior to infection of the whole population. Abortive arbovirus infection of the CNS (ElDadah and Nathanson, 1967; Cole and Nathanson, 1968) provides one example of this type of host-virus interaction in the brain.

A similar situation can be seen in certain persistent infections of tissue culture, but here the virus may be carried indefinitely, if the multiplication of uninfected cells occurs at a rate sufficient to replace infected cells (Wheeler and Canby, 1959; Lockart, 1960; Glasgow and Habel, 1962; Henle, 1963; Walker, 1968).

(*ii*) Many viruses are capable of producing *nonlytic* infections. In the CNS, immunofluorescent staining may indicate infection in certain areas in which no cellular destruction is seen (e.g., mumps, Johnson, 1968; certain arboviruses, Albrecht, 1962; rabies virus, Johnson, 1965a; Miyamoto and Matsumoto, 1967). Nonlytic infections have been most thoroughly documented in tissue culture, where they have been produced by a number of enveloped RNA viruses (Walker, 1964, 1968; parainfluenza, Choppin, 1964; Holmes and Choppin, 1966; mumps, Walker and Hinze, 1962a,b; rabies, Fernandes *et al.*, 1964; measles, Rustigian, 1966a,b; rubella, Downie and Oxford, 1969).

(*iii*) Certain viruses can affect cells, in the *absence of replication* of the virion or its components (Fenner, 1968). Among such phenomena, the one most relevant to this review is the cell-fusing action exhibited by a num-

ber of enveloped viruses (Roizman, 1962): measles, canine distemper, rinderpest (Hopper, 1959; Toyoshima *et al.*, 1960; Plowright, 1962; Warren *et al.*, 1962; Cascardo and Karzon, 1965); mumps, Newcastle disease (Henle *et al.*, 1958; Warren *et al.*, 1962; Kohn, 1965); parainfluenza (Okada and Tadokoro, 1962; Holmes and Choppin, 1966); herpes viruses (Roizman, 1962); and visna (Harter and Choppin, 1967). Virus-cell interactions of this type may be relevant to the pathogenesis of certain infections of the CNS in which demyelination is prominent (see discussion below).

b. Central Nervous System. (*i*) *Neuronotropic infection.* The most familiar pathological response of CNS to viral infection is the necrosis and outfall of neurons, together with an associated inflammatory response. Detailed descriptions of this type of response have been reviewed periodically over the last 30 years (Hurst, 1936; Bodian, 1959; Haymaker, 1961; Innes and Saunders, 1962; Johnson and Mims, 1968). A classical example is Bodian's studies of the sequence of changes which poliovirus produces in the anterior horn cell of the spinal cord (Bodian, 1948, 1959, 1964); principal stages are central chromatolysis followed by diffuse chromatolysis, nuclear changes culminating in pyknosis, and cytolysis followed by neuronophagia.

In addition to the necrobiotic sequence in neurons, a variety of other changes may be associated with neuronotropic infections, particularly when the CNS is subject to a devastating attack. These include: focal areas of demyelination or softening, sometimes perivascular in location; breakdown in the vascular wall with thrombi or small hemorrhages; and late atrophy, glial scars, or cysts (Haymaker, 1961; Innes and Saunders, 1962; Hughes, 1969). Variation in severity of neuronal destruction, duration of illness, physiological status of host, and probably other factors, account for the considerable individual differences in the pathologic picture.

(*ii*) *Primary attack upon meninges, ependyma, or vessels.* Attack upon meninges, ependyma, or vascular endothelium occurs in many CNS virus infections, but in some instances it stands out as the salient aspect of the disease process.

Leptomeningitis may be the site of most severe pathological change in certain experimental infections with virus strains which do not readily spread to the CNS parenchyma. Of particular note are unadapted strains of myxoviruses (Fraser *et al.*, 1959; Mims, 1960b; Johnson and Johnson, 1969) and of vaccinia virus (Rosenau and Andervont, 1931; Mims, 1960a; Kristensson and Sourander, 1969). The pathogenesis of influenza infection in the mouse is an interesting example which has been elucidated by the work of Mims (1960b). Unadapted strains of influenza, following intra-

cerebral inoculation, infect the meninges, where they undergo a single cycle of replication, apparently producing progeny which are unable to infect additional cells, either meningeal or neuronal. A single large inoculum can infect enough meningeal cells to produce an acute leptomeningitis. The relative importance of leptomeningitis and of a "toxic" effect of the virus in producing the clinical syndrome is still unclear (Johnson, personal communication, 1969). Lymphocytic choriomeningitis and perhaps other related viruses (such as Machupo, Junin, Lassa) may also produce a selective meningeal infection under certain circumstances (Weissenbacher *et al.,* 1969). Certain distinctive clinical features often characterize leptomeningeal infections, particularly a marked convulsive diathesis such that animals often die in "status epilepticus."

Ependymitis may or may not be associated with simultaneous infection of leptomeninges. Severe ependymal infection has been described for myxoviruses (Johnson and Johnson, 1969) and for reoviruses (Margolis and Kilham, 1969; Kilham and Margolis, 1969). Of particular interest are the studies by R. T. Johnson and Johnson (1968) dealing with intracerebral inoculation of suckling hamsters with a benign nonneuroadapted strain of mumps virus. Immunofluorescent staining indicated that this strain of mumps produced an infection which involved ependyma, with essentially no infection of parenchymal cells. As a result of ependymal destruction the aqueduct was frequently obliterated, with subsequent development of internal hydrocephalus.

Endothelial infection can be associated with severe hemorrhagic lesions. If CNS parenchymal infection is minimal, a hemorrhagic encephalopathy results (rat virus in suckling rats, Cole *et al.,* 1970; Margolis and Kilham, 1970; NWS strain of influenza virus in chicken embryos, Hook *et al.,* 1962). If concomitant parenchymal infection occurs, hemorrhage is superimposed upon an encephalomyelitis (herpes, Bang, 1942; hog cholera, Seifried and Cain, 1932; infectious canine hepatitis, Cabasso, 1962; blue tongue, Young and Cordy, 1964; arboviruses in chicken embryo, Bang, 1943). It should be noted that it is not clear whether hemorrhages are simply due to cytocidal endothelial infection; it has been postulated that a consumptive coagulopathy may play a role in evolution of hemorrhage (Margolis and Kilham, 1970; Cole *et al.,* 1970). Furthermore, there are several instances where endothelial infection is not associated with hemorrhage (Coffin and Liu, 1957; Johnson, 1965b; Kundin *et al.,* 1966; Bruno-Lobo *et al.,* 1968).

(*iii*) *Demyelination prominent.* There are a number of CNS infections in which destruction of myelin is a prominent and regular aspect of the pathological process. Examples vary as to the severity of concomitant attack upon neurons or the intensity of inflammation. *Visna* is a slow infec-

tion; the etiologic agent is an enveloped RNA virus, with sufficient characteristics in common with conventional viruses to justify its inclusion. Following inoculation of sheep (the natural host) neurological symptoms develop in months to years; the CNS lesion is a severe infiltration of round cells which begins in perivascular, periependymal, and leptomeningeal sites; destruction of underlying tissue, including demyelination, usually appears later in the progress of the disease (Sigurdsson *et al.*, 1957, 1962). Harter has suggested that the membrane-fusing action of visna virus might play a role in production of the demyelinating lesion (Thormar, 1961; Harter and Choppin, 1967; Bunge and Harter, 1969).

Mouse hepatitis virus (a coronavirus) occurs in nature in neurotropic variants; these may also be produced by CNS passage. The most detailed description is presented in the original studies of Cheever and colleagues (Cheever *et al.*, 1949; Bailey *et al.*, 1949) of the JHM strain of MHV. This strain produced an acute necrosis of neurons of hippocampus and olfactory cortex, and simultaneous acute patchy demyelination in the brainstem and cord. White matter lesions appeared to commence with destruction of myelin, leaving axons relatively intact; inflammation followed breakdown of myelin; and giant cells were occasionally seen in a variety of tissues. Many of these features are similar to the demyelination produced by RNA viruses with cell-fusing properties (see above), and bring to mind the hypothesis of Harter and Choppin (1967) regarding the pathogenesis of visna.

Border disease is an infection of fetal sheep due to an agent, possibly a virus, transmitted across the placenta after parenteral inoculation of the pregnant ewe (Barlow and Gardiner, 1969). The pathological picture (Barlow and Dickinson, 1965; Cancilla and Barlow, 1968, 1970) is primarily one of severe demyelination; axons appear normal, but there is a decrease in myelin lamellae and degeneration of some myelin sheaths, as well as astrogliosis. There is little inflammation or involvement of neuronal perikarya.

(*iv*) *Panencephalitis.* An attenuated strain of *blue tongue* virus produced an asymptomatic immunizing infection in adult sheep, but if administered to pregnant ewes between 4 and 8 weeks of gestation caused a severe encephalopathy in the fetus (Cox, 1954; Schultz and Delay, 1955). The disease is characterized by an acute loss of neurons and other cells, producing severe atrophy with ventricular dilatation and large subcortical cysts; the architecture of the residual grey and white matter is loose and spongy, and diffuse round cell infiltrates and endothelial changes are present (Young and Cordy, 1964). The reports of Richards and Cordy (1967) and Svehag (1962) suggest that the overwhelming panencephalitis produced by blue tongue virus in fetal lamb or suckling mouse is similar to

the lethal panencephalitis produced in suckling mice by many other viruses (for example, arboviruses, ElDadah and Nathanson, 1967; vesicular stomatitis virus, Bruno-Lobo *et al.*, 1968; herpes virus, Johnson, 1964a). The fetal lamb differs in that it may remain viable *in utero* for some time after the devastating panencephalitis, permitting pathological study of the late residua of widespread necrobiosis.

Subacute sclerosing panencephalitis (*SSPE*) is a rare disease which generally follows a progressive course leading to death (Sever and Zeman, 1968). The disease occurs in children who usually have experienced uneventful measles months to years before onset. During active disease, measles serum and CSF antibody titers are elevated; in the brain measles antigen, which stains specifically with measles immunofluorescent conjugates, is seen in the nucleus or cytoplasm of parenchymal cells, and may be visualized in the electron microscope as myxoviruslike nucleocapsids.

Although speculative, present knowledge suggests that SSPE is due to infection with a defective measles variant which is able to replicate once inside a permissive cell, but which cannot spread through extracellular fluids to infect potentially susceptible cells (Rustigian, 1966a,b; Baublis and Payne, 1968).

Histologically, the lesions of SSPE can be divided into two groups. In the grey matter there is cytolytic infection of neurons and glia with an associated inflammatory reaction. In the white matter there are large areas of partial demyelination with diffuse and perivascular infiltrations of cells. It appears that the demyelination may be due to destruction of oligodendroglia and that it cannot be explained as merely secondary to neuronal outfall (Herndon and Rubinstein, 1968).

The possibility of an immunopathological component in the pathogenesis of the disease, is supported by the studies of ter Meulen *et al.* (1969) and Saunders *et al.* (1969). Katz and associates (1968, 1970) have produced a subacute encephalitis in ferrets by intracerebral inoculation of brain homogenates from patients with SSPE. Since the affected ferrets fail to show evidence of measles antigen or myxovirions in the CNS, although they do develop measles antibody, the relationship of these findings to SSPE is not clear (for discussion see Johnson, 1970b).

Distemper occurs in nature as a respiratory infection of dogs; the causal agent is closely related to measles virus. A certain proportion of canine distemper infections involve the CNS, where several different pathological entities may be produced (Gorham, 1960; Gillespie, 1962; Innes and Saunders, 1962). The recent study by Appel (1969) helps to clarify this confusing situation. Susceptible dogs were infected by aerosol; after an incubation period of 3 to 4 weeks, about 50% of animals developed an acutely fatal disease with severe respiratory and intestinal symptoms.

These animals had a very widespread infection with a minimal antibody response; an acute encephalitis was present, with a pantropic (nondemyelinating) infection of meninges, ependyma, neurons, and glia. Most of the remaining animals underwent a mild or subclinical immunizing infection, with a detectable antibody response by 3 weeks; in these animals there was little or no infection of the CNS. A small number of dogs (2 of 55) also experienced an apparently silent immunizing infection; however, 40 to 60 days after infection they developed convulsions, with a panencephalitis, including demyelination as well as neuronal destruction and inflammation; at this time viral antigen was present in Purkinje cells and other neurons (but absent from most extraneural tissues); antibody titers were high in CSF as well as serum. The pathogenesis of distemper demyelination poses a number of challenging questions, including the hypothesized role of an immunopathological process (Choppin, 1968); the persistence of virus in spite of an active immune response; and the possible direct attack of virus on either oligodendroglia (Moulton, 1956) or on the myelin sheath itself (Gorham, 1960).

Postinfectious encephalitis. The rare occurrence of severe neurological symptoms shortly after acute infection with vaccinia, measles, and possibly other nonneurotropic viruses has been repeatedly reviewed (Miller *et al.,* 1956; Scott, 1967). This term has been used for a heterogeneous group of pathological entities (van Bogaert *et al.,* 1961), but is probably best limited to cases in which the cardinal pathological features are perivenular demyelination and cuffing, usually with minimal neuronal involvement (Turnbull and McIntosh, 1926, Perdrau, 1928; van Bogaert *et al.,* 1961; Blackwood *et al.,* 1967). Speculation about the pathogenesis of demyelination has included cytocidal infection of oligodendroglia, direct interaction of virus and myelin membranes, or an immunopathological process attacking myelin (Koprowski, 1962; Isacson, 1967; Choppin, 1968; Paterson, 1969). The similarities between postinfectious encephalitis, distemper, and SSPE suggest that there may be common factors in their pathogenesis.

C. The Inflammatory Response

1. Acute Neuronotropic Infections

An inflammatory response is regularly seen in the CNS as part of the pathological picture characteristic of infections with viruses which produce cytocidal infection of neurons and (often) also of glia. Detailed descriptions have repeatedly documented the salient features of inflammation. In experimental poliomyelitis (Bodian, 1948, 1959), histological changes in neurons occur first; these are followed by the appearance in the CNS of polymorphonuclear leukocytes and mononuclear cells (both lymphocytes

and macrophages). Polymorphonuclear cells are usually most common in the first few days of the inflammatory response, and even then may be infrequent, while mononuclear cells may persist for months, particularly around blood vessels. Inflammatory cells typically assume several characteristic configurations: as perivascular cuffs, particularly around small vessels; as focal accumulations often at sites of neuronophagia; and as scattered cells distributed diffusely throughout the affected tissue. Inflammation is seen in both white and grey matter, but the most severe infiltrates are usually in grey matter.

Although excellent descriptions of the CNS inflammatory response have been available for over 50 years (Harbitz and Scheel, 1907), the mechanisms which underlie this response are not yet well understood.

(*a*) *Cellular destruction* may be an important *stimulus* to the response, since there is a general tendency toward correlation of distribution and severity of inflammation with severity of neuronal loss. The neuronophagia which is so prominently associated with neuronal destruction in some CNS viral infections (Bodian, 1948) indicates that cellular destruction is one of the stimuli responsible for inflammation. The inflammatory response to neuronal destruction due to toxins or anoxia is consistent with this view (Innes and Saunders, 1962). However, in local areas of the infected CNS there may be a marked discrepancy between cellular infection or cellular destruction and inflammation (Bodian, 1959; Johnson, 1968); likewise, inflammation may be seen in white matter distant from any apparent neuronal outfall (Nathanson *et al.*, 1965).

At present, there is no clear evidence whether the *immune response* plays any role in initiation of the viral inflammatory response; there are a few observations (Bodian and Howe, 1941a; Cole and Nathanson, 1968; Johnson, 1970a) which fail to indicate that the immune response is important, but these do not provide definitive information. It has been suggested by Webb (Webb and Smith, 1966; Webb *et al.*, 1968a,b; Webb, 1969) that the inflammatory response in neuronotropic viral encephalitides is associated with the formation of virus-antibody complexes in and around the small vessels of the CNS, but direct evidence for this association is lacking. Berge and associates (1961a) in an earlier series of studies also advanced a similar view.

(*b*) Recent observations by Johnson (1970a) shed considerable light on the *source and nature of cells* participating in the inflammatory response. Using tritiated thymidine and India ink to label, respectively, proliferating cells and phagocytic cells, it appears that the majority of inflammatory cells are recruited from outside the CNS; they are probably mainly monocytes, although some may be lymphocytes. Most inflammatory cells are derived from rapidly proliferating populations, and proliferation occurs

both before and after they enter the CNS; cells with phagocytic activity are found, not only at sites of neuronophagia, but also in perivascular cuffs where they may be histologically indistinguishable from lymphocytes.

Zlotnik (1968) has recently described marked and regular astrogliosis accompanying acute rabies and arbovirus encephalitis in laboratory rodents, which was often seen as early as 24 hours after infection. Since earlier workers did not use stains appropriate for visualizing astrocytes, there is little information about the astroglial component of the inflammatory response. Further studies are needed to assess the significance of these findings.

(*c*) The relationship of *inflammation to outcome* of neuronotropic infection is currently controversial. There are at least two cell types in the inflammatory lesion which could play a role in recovery from infection; these are plasma cells (Bodian, 1948) and macrophages. Heremans (1968) has reviewed evidence that during infections of the CNS, immunoglobulins are produced locally which are specific for the infecting agent (Morgan, 1949a; Bell *et al.*, 1966). However, there does not seem to be any regular relationship between the outcome of infection (or titer of virus in the brain) and severity of inflammation.

Taking a quite different viewpoint, Webb and Smith (1966; Webb *et al.*, 1968a,b; Webb, 1969) have suggested that an immunopathological process is responsible for the inflammatory response (or at least certain components thereof), and that immunopathology or inflammation (Nahmias *et al.*, 1969; Hirsch and Murphy, 1968) may play a role in viral encephalitic death.

2. Other CNS Virus Infections

The diverse pathological syndromes which can be seen with nonneuronotropic virus infections of the CNS are associated with a variety of degrees and types of inflammation.

(*a*) *Inflammation is minimal* in certain infections of mesodermal elements in the CNS, such as the hemorrhagic encephalopathy produced by certain strains of influenza and rat viruses (Hook *et al.*, 1962; Cole *et al.*, 1970). Infections primarily of leptomeninges or ependyma are usually accompanied by inflammation; this is seen in the areas of cellular infection, but may also be present in the uninfected parenchyma of the CNS (R. T. Johnson and Johnson, 1968).

(*b*) *Demyelinating processes* may be associated with hypercellularity in affected areas of white matter. In some instances this is mainly due to increase in size or number of indigenous glial elements (Border disease, Barlow and Gardiner, 1969). In MHV-initiated demyelination,

which is a more acute process, polymorphonuclear and mononuclear leukocytes are prominent initially, with later appearance of gliosis (Bailey *et al.*, 1949). In the slow infection, visna, hypercellularity is regularly seen in areas of demyelination, and may be the initial histologically apparent change (Sigurdsson *et al.*, 1962).

(*c*) *Panencephalitides* are regularly characterized by severe inflammation, both in grey and white matter. A heterogeneous population of cells is involved, which may represent several quite different pathological processes occurring simultaneously (Perdrau, 1928; Miller *et al.*, 1956; Innes and Saunders, 1962; Sever and Zeman, 1968; Appel, 1969).

D. *Variables Which Influence the Outcome of CNS Infections*

The course and outcome of CNS infection are influenced by the host, the virus, and the mode of infection. The effects of these variables are described below, but discussion of the underlying mechanisms is deferred to a later section.

1. *Virus Variation*

In recent years virus variation has become the province of workers interested in the genetics of animal viruses. Studies of this type, reviewed by Fenner and Sambrook (1964), Takemoto (1966), and Cooper (1967), have often been concerned with the biochemical mechanisms of genetic markers. In some instances, studies of mutants have produced instructive examples of the ways in which variations in the virus proliferative cycle can influence pathogenicity for host cells (Wagner *et al.*, 1963; Rapp, 1963; Finter, 1964a; Cooper *et al.*, 1966; Lwoff, 1969). Such studies suggest the type of information which should eventually be sought to explain the molecular basis of virus pathogenicity.

a. Origin of Virus Variants. More relevant to the theme of this review are virus variants which manifest differences in pathogenicity for experimental animals. Fenner and Cairns (1959) assembled a useful review of the older literature on this subject. Most variants presumably arise by mutation; the literature on their origin pertains mainly to the selection of variant subpopulations from heterogeneous parental populations.

It is convenient to consider variants as derived either by (*i*) segregation of individual infectious particles (plaque-forming centers) or small populations (terminal dilution), or by (*ii*) sequential passage of a heterogeneous population in a host which will support virus replication. No attempt will be made to catalog here the numerous histories of modified virus strains which have been published. Rather, a few selected examples will be noted.

(*i*) *Plaque mutants.* The search for attenuated virus variants optimal

for human immunization against poliomyelitis involved one of the most extensive explorations of plaque mutants. Much of this work is summarized in Sabin (1957), Plotkin *et al.* (1962), and in two symposia (Live Poliovirus Vaccines, 1st and 2nd Int. Conf., 1959, 1960). Although no marker was found which invariably correlated with monkey neurovirulence, there was a rough correlation between the ability to replicate at high or low temperatures and virulence or avirulence, respectively (Lwoff, 1959, 1961, 1969; Dubes and Wenner, 1957; Sabin, 1961; Vonka *et al.*, 1967).

(*ii*) *Strains derived by passage.* Many of the "classical" strains used prior to the era of animal virus plaquing (Dulbecco, 1952) were derived by sequential passages in animals or cell cultures. Passage in the CNS, when sufficiently prolonged, yielded strains which were designated "fixed" or "obligatory neurotropes," referring to their tendency to replicate or spread only in the peripheral or central nervous system. However, it is not entirely clear whether such strains can infect glia, Schwann cells, or endoneural fibroblasts, in addition to neurons. Some examples are: the MV strain of poliovirus (Flexner, 1931; Bodian, 1959; Nathanson and Bodian, 1961a,b), the French neurotropic strain of yellow fever virus (Strode, 1951), and fixed strains of rabies virus (Johnson, 1959). Certain other viruses, even without many serial CNS passages, show a tendency toward obligatory neurotropism in certain hosts (herpes simplex virus, Sabin, 1937; Dean *et al.*, 1963; Wildy, 1967; vesicular stomatitis virus, Sabin and Olitsky, 1937a,b).

In contrast, strains of reduced animal virulence have also been derived by serial passage. Among these are the 17D strain of yellow fever virus (Strode, 1951), the LSc strain of poliovirus (Sabin, 1957), the E5 strain of Langat virus (Thind and Price, 1966), the MD-1 strain of dengue-1 virus (Wisseman *et al.*, 1963), and an attenuated strain of Venezuelan equine encephalitis virus (Berge *et al.*, 1961b). Experience has shown that attenuation for a given animal host is best achieved by passage in a different animal host or in tissue culture. Furthermore, passage leading to reduced virulence is often an irregular phenomenon, occurring in only a few of several parallel passage lines originating from a single virus stock (Thind and Price, 1966).

b. Comparative Pathogenesis of Viral Variants. The majority of studies reporting virus strain differences in animal virulence are limited to titrations of lethality and of infectivity. Detailed descriptions of the evolution of infection, which might suggest points of difference in comparative pathogenesis, are not often undertaken.

Attenuated poliovirus strains have been extensively studied in humans, chimpanzees, and monkeys (Live Poliovirus Vaccines, 1st and 2nd Int.

Conf., 1959, 1960); they are capable of regularly infecting oropharynx and intestine, but produce only minimal and infrequent viremia, in comparison with the most virulent wild strains (Melnick *et al.*, 1966). Growth of attenuated strains in the monkey spinal cord is greatly diminished in comparison with neurovirulent strains, and this in turn is reflected in mildness of histological lesions (Live Poliovirus Vaccines, 1st Int. Conf., 1959; Bodian, 1961).

A number of studies of arbovirus variants have been published. Thind and Price (1966) found that the attenuated E5 strain of Langat virus, when injected intraperitoneally, produced only trace viremia, and, occasionally, invaded the CNS where low levels of virus appeared for a short time; mice survived without symptoms. In contrast, the parent M3 strain produced a marked viremia, invaded the CNS early, and regularly replicated to high titer, with consequent fatal encephalitis. Cole and Wisseman (1969a) compared several passage levels of dengue-1 virus, following intracerebral inoculation of adult mice. The MP-3 strain (3 mouse passages) replicated slowly and then disappeared, often without producing clinical symptoms. The MP-125 strain replicated rapidly, to high titer, and regularly killed adult mice. Thus virulence may be associated both with greater neuro-invasiveness and with enhanced replication in the CNS. At present, little is known of the mechanisms which underlie such variations.

2. Host Variables

A number of host variables have been shown to markedly influence the pathogenesis of experimental virus infection. At a descriptive level, considerable information is available with regard to comparative pathogenesis (Bang and Luttrell, 1961).

a. *Age.* Because the dramatic effect of age on susceptibility to CNS viral infection is seen in so many laboratory models, this variable is probably the most thoroughly studied (Sigel, 1952). Age-related decrease in susceptibility has been measured in several ways: (*i*) intracerebral LD_{50} titer (Lennette and Koprowski, 1944; MacDonald, 1952; Schlesinger and Frankel, 1952; Nir and Goldwasser, 1961); (*ii*) intracerebral incubation period, and maximum titer attained by virus in infected brain (Meicklejohn *et al.*, 1952; Sabin, 1952; Overman and Kilham, 1953; Overman, 1954a,b; ElDadah *et al.*, 1967; Cole and Wisseman, 1969a); (*iii*) intracerebral susceptibility to viruses of relatively low virulence or freshly isolated strains prior to passage (Schlesinger and Frankel, 1952; Cole and Wisseman, 1969a); (*iv*) intracerebral susceptibility of relatively resistant rodent species (Duffy, 1951; ElDadah *et al.*, 1967); and (*v*) intraperitoneal LD_{50} titer (Lennette and Koprow-

ski, 1944; Johnson, 1964b; ElDadah *et al.*, 1967), or severity of disease following other extraneural routes of infection (Sabin and Olitsky, 1937a,b).

b. Species. Animal viruses vary markedly in the breadth of their host range. Thus, human polioviruses are well known for their relative limitation to primate hosts (Holland, 1961; Plotkin *et al.*, 1962; Kunin, 1962). However, spider monkeys (genus *Ateles*) show a nonimmune resistance to types 2 and 3 (but not type 1) human polioviruses, even following intracerebral inoculation of highly virulent strains (Jungeblut and Bautista, 1956; Nathanson *et al.*, unpublished, 1969). In contrast, rabies virus, if inoculated intracerebrally, has high virulence for most mammalian species regardless of age (Johnson, 1959). Human enteroviruses show marked differences in their ability to infect mice: polioviruses usually fail to infect, group A coxsackie viruses produce a fatal poliomyelitis and severe myositis, and group B coxsackie viruses attack brain and brown fat. Group B arboviruses are much more virulent for certain species of laboratory rodents (mice and hamsters) than for others (rats and guinea pigs) (ElDadah *et al.*, 1967).

c. Genetic Differences Within Species. There may be dramatic differences in the viral susceptibility of different animals of the same species. Studies of the genetics of susceptibility have dealt mainly with mice, with particular references to mouse hepatitis virus (Bang and Warwick, 1960; Kantoch *et al.*, 1963), and group B arboviruses (reviewed in ElDadah *et al.*, 1967; Fenner, 1968). Arbovirus susceptibility has been studied by Webster and associates (Webster and Clow, 1936), Sabin and associates (Sabin, 1954), and Koprowski and associates (Goodman and Koprowski, 1962; Vainio, 1963; Groschel and Koprowski, 1965). Adult mice from resistant strains are not killed by intracerebral inoculation of certain group B arboviruses, although suckling animals are susceptible. Resistance is carried by a single dominant autosomal gene.

d. Physiological Status of the Host. Susceptibility can be markedly influenced by the physiological condition of the animal host. *Body temperature* can be raised or lowered by residence in a warm or cold environment. Elevated temperature usually favors the host, which may survive a potentially lethal infection (poliovirus, Lwoff *et al.*, 1960; coxsackie virus B1, Walker and Boring, 1958; Sindbis virus, Kirn *et al.*, 1967; dengue virus, Cole and Wisseman, 1969b; herpesvirus, Carmichael *et al.*, 1969; Carmichael and Barnes, 1969). Conversely, low temperatures may enhance susceptibility (Boring *et al.*, 1956).

Corticosteroids or stress can enhance susceptibility of experimental animals to infection (see discussions of poliovirus, coxsackie virus, and arbo-

virus in subsequent sections). *Sex* differences in susceptibility have been reported (encephalomyocarditis virus, Glasgow, unpublished, 1969) and *pregnancy* may be associated with increased risk (Siegel and Greenberg, 1955; Farber and Glasgow, 1968). Finally, *nutritional status* can affect the outcome of infection (Scrimshaw *et al.*, 1968; Woodruff, 1970).

Trauma of various types has been associated with enhanced risk of symptomatic CNS infection. The influence of several types of trauma upon poliomyelitis has received detailed epidemiological and experimental study (Habel, 1955). Parenteral injections, particularly of irritating materials, clearly enhance the risk of clinical poliomyelitis following extraneural virus infection (Hill and Knowelden, 1950; Bodian, 1954). Likewise, tonsillectomy enhances the risk of bulbar poliomyelitis, even years after the operation (Adams *et al.*, 1953; Paffenbarger and Wilson, 1955).

III. Effect of Immunosuppression on Selected Experimental Models of CNS Viral Infection

A. *Lymphocytic Choriomeningitis*

Lymphocytic choriomeningitis (LCM) of mice is of particular importance as the prototype of CNS viral infections in which the disease process is mediated by an immunopathological mechanism (Hotchin, 1962, 1965; Volkert and Larsen, 1965a). LCM is an unclassified, enveloped virus (Dalton *et al.*, 1968) which occurs in nature as an enzootic infection of mice (Traub, 1936b, 1939). Experimentally, the virus will produce symptomatic infections in a number of animal species (Findlay and Stern, 1936; Armstrong, 1942), but the following discussion is focussed on studies in mice.

1. Course of Infection in Mice

Depending on the age of mice, and upon numerous other variables, LCM infection can follow several markedly different courses:

(*a*) *Persistent infection with antigen excess* occurs following exposure to virus *in utero* or shortly after birth. Animals so infected carry high virus titers in blood, brain, and other tissues throughout their lives, and yet appear to develop and behave almost normally (Traub, 1936a,b, 1939; Hotchin, 1962, 1965; Pollard *et al.*, 1968a,b). Such carrier animals can transmit infection vertically, initiating persistent infections in their offspring (Pollard *et al.*, 1968a). In addition, they continually excrete virus in urine, resulting in occasional infection of other animals, including man (Farmer and Janeway, 1942). By conventional techinques it is difficult to demonstrate antibody in the serum; this type of infection was

therefore termed persistent tolerant infection or PTI (Hotchin, 1962; Volkert and Larsen, 1965a). More recently, it has been shown that antibody is formed in this condition (Oldstone and Dixon, 1969; Benson and Hotchin, 1969). Such antibody may be bound to circulating infectious virus, and can be detected by use of antiserum directed against mouse immunoglobulins (Oldstone and Dixon, 1969).

(*b*) *Persistent infection with antibody excess* occurs in juvenile or adult mice inoculated by a parenteral route. Such animals usually undergo silent immunizing infection, with the disappearance of infectious virus and appearance of CF antibody within several weeks inoculation. Persistence of small amounts of virus may be demonstrated directly (Haas, 1954; Rowe, 1954), but special techniques can dramatically unmask virus. Thus, when such immune animals are treated with antilymphocyte serum (ALS) viremia reappears and persists until treatment is terminated (Volkert and Lundstedt, 1968). Likewise, animals in which neonatal "tolerant" infections have been "cured" by grafting immune isologous lymphoid cells, continue to show traces of virus in spite of high levels of circulating N and CF antibody (Volkert and Larsen, 1964, 1965a).

Finally, if an appropriate balance is struck between virus and antibody, persistent infections may be created in which both infectious virus and CF antibody can be found in the serum over long periods of time (Hirsch *et al.*, 1968).

(*c*) *Acutely lethal choriomeningitis* occurs in mice, 1 week or older, following intracerebral inoculation of virus. Typically, following an incubation period of about 1 week, nonspecific symptoms develop, accompanied by a characteristic convulsive diathesis. Paralysis or other localizing neurological signs are uncommon and animals often die during a seizure. Histologically, there may be lesions in liver and other viscera, but the salient lesions are found in the central nervous system, where a severe choriomeningitis occurs, consisting primarily of lymphocytes and other mononuclear cells (Findlay and Stern, 1936; Lillie and Armstrong, 1945).

A number of variables have been shown to play an important role in the outcome of LCM infection. Different *strains* of virus vary in their virulence; virulence is influenced by passage history (intracerebral-brain or intraperitoneal-visceral). An "aggressive" brain-passaged virus is more apt to kill acutely, while a "docile" visceral-passaged virus is more likely to produce persistent infection (Hotchin *et al.*, 1962; Hotchin, 1965). Host *species* is of importance, since persistent infections with antigen excess have not been reported in animals other than mice (Volkert and Larsen, 1965b), although choriomeningitis is readily produced in a variety of species (Findlay and Stern, 1936; Armstrong, 1942). More recently,

it has been shown that there are marked differences between strains of mice, with respect to the titers which the virus reaches following neonatal infection. This, in turn, may play a critical role in the outcome of infection (Oldstone and Dixon, 1968, 1969). As noted above, *age* of host and *route* of virus injection are also critical determinants. Following intraperitoneal inoculation of adult mice, there is a variable mortality, depending upon strain of virus. Virus replicates in the brains of both fatally affected and surviving animals; however in survivors the titers are relatively lower and choriomeningitis, if present, is mild (Rowe, 1954; Lehmann-Grube, 1964).

2. Mechanism of Persistent Infection

Although persistent infection with LCM virus is not completely understood, several essential aspects have been delineated. LCM virus is enveloped; its interaction with host cells probably has features in common with other enveloped viruses, such as the myxoviruses and rhabdoviruses (Marcus, 1962; Lehmann-Grube *et al.,* 1969). Infected cells in culture can remain viable while supporting virus replication over an extended period (Benson and Hotchin, 1960; Benda and Cinatl, 1962; Seamer, 1965; Lehmann-Grube, 1967). The plasma membrane of such infected cells contains virus-specific antigenic determinants (Dalton *et al.,* 1968) which can bind antiviral antibody, thereby activating complement with subsequent cellular injury (Oldstone and Dixon, 1970). Cytopathology has also been observed in infected cultures to which have been added LCM immune lymphoid cells (Volkert and Lundstedt, 1968; Oldstone and Dixon, 1970) but, at present, evidence for the viral immunospecificity of this event is lacking.

In vivo a virus-cell interaction of this type could conceivably result in either persistent infection without pathological changes or in disease, depending on whether sensitized lymphoid cells and/or antibody and complement can gain access to infected cells. The fact that skin grafts from LCM carrier mice are rejected by uninfected syngenic recipients (Holtermann and Majde, 1969) is persuasive evidence for role of virus-specific surface antigen in the pathogenesis of disease.

Another factor of potential importance in persistence of LCM infection is the ability of the virus to infect susceptible cells even after it has been bound by virus-specific immunoglobulin. This is suggested by the observation that viral infectivity and CF activity may coexist in the blood over a long period (Volkert and Lundstedt, 1968; Hirsch *et al.,* 1968; Larsen, 1969a,b). More recently, it has been shown (Oldstone and Dixon, 1969) that in persistent infections with antigen excess, the bulk of infectious virus is inactivated by antiserum directed against mouse

immunoglobulins. Analysis of the neutralization kinetics of other viruses (Notkins *et al.*, 1966, 1968; Ashe *et al.*, 1968; Krummel and Uhr, 1969) suggests that such infectious virus-antibody complexes may be a relatively common phenomenon.

3. *Pathogenesis of Acute Choriomeningitis*

a. Immunosuppression. Probably the most dramatic evidence that acute lymphocytic choriomeningitis is mediated by an immunopathological process comes from use of immunosuppression to manipulate the outcome of infection in adult mice. Originally Rowe (1954, 1956) and subsequently Hotchin and Weigand (1961) showed that X-irradiation converted a potentially fatal intracerebral virus inoculation into a benign infection. Similar results have since been reported for immunosuppressive drugs (Haas and Stewart, 1956; Hotchin, 1962; Gilden *et al.*, 1971); antilymphocyte serum (ALS, Lundstedt and Volkert, 1967; Gledhill, 1967; Hirsch *et al.*, 1967, 1968); and thymectomy (Rowe *et al.*, 1963; Levey *et al.*, 1963; East *et al.*, 1964).

Following immunosuppression with X-irradiation, intracerebally inoculated adult mice may develop persistent infections with antigen excess (Rowe, 1954; Hotchin, 1962). If short-term immunosuppression is applied, with ALS (Hirsch *et al.*, 1967; Gledhill, 1967) or cyclophosphamide (Gilden *et al.*, 1971), there can be either a transient sparing effect followed by fatal choriomeningitis upon the recovery of immunoresponsiveness, or permanent protection. Since the growth curve of virus in the CNS is similar in mice destined to die and in those protected by suppression (Rowe, 1954; Hotchin, 1962) protection cannot be attributed to differences in the number of infected cells in the brain.

Further evidence of the dynamic relationship between virus replication and immune induction is provided by the studies of Gilden *et al.* (1971), which demonstrate that, following intracerebral virus injection, the timing of immunosuppression is critical to the outcome. Thus, a single immunosuppressive dose of cyclophosphamide given to 6-week-old BALB/c mice between 3 to 5 days after LCM virus results in permanent survival of up to 20% of animals; drug given 1 or 2 days after virus increases mean survival time from 1 week (in controls) to 2 weeks, but all mice die of acute choriomeningitis.

b. Adoptive Immunization. To completely establish the immunopathological nature of choriomeningitis, it would be necessary to convert an asymptomatic persistent infection into overt choriomeningitis by immune serum or cells. Recently Oldstone and Dixon (1970) have shown that the intrathecal inoculation of immune serum into persistently infected mice can produce histologically apparent choriomeningitis, with-

out death of animals. Additional evidence that choriomeningitis may in part be antibody-mediated is the observation that a proportion of adult mice, when fully depeleted of the third component of complement with cobra venom, are protected against intracerebral inoculation of LCM virus (Oldstone, 1970). Similar decomplementation experiments by others (Gilden *et al.*, 1969) have failed to show significant protection from acute LCM, indicating that precise experimental conditions are required to demonstrate this effect.

Warranting further explanation are the results of studies of the effect of adoptively immunizing persistently infected mice of the C3H strain (Volkert and Larsen, 1965a; Larsen, 1969a,b). Such animals, following inoculation of lymphoid cells obtained from immunized syngenic donors, develop persistent high levels of both CF and neutralizing antibodies but CNS disease does not occur. Infectious virus disappears from the blood as well as from the spleen and lymph nodes, where the bulk of the grafted cell inoculum apparently localizes. Infectivity persists in the kidneys and presumably in the brain.

Current observations by Oldstone and Dixon (1970) provide a possible explanation. They found that passive intravenous immunization of persistently infected mice, with either immune serum or syngenic cells from an immunized donor, did produce leptomeningitis and perivascular cuffing. These effects could be demonstrated in the SWR/J strain but not in the C3H strain of mice. It had been previously shown (Oldstone and Dixon, 1968) that persistently infected SWR/J mice carried much higher levels of virus than did persistently infected C3H mice. However, Lehmann-Grube (1969) has failed to find any effect of mouse strain on the titers of virus in the brain.

At present it is difficult to assess the relative contributions of the humoral and cellular components of the immune response to the pathogenesis of acute LCM. Although the available evidence suggests that both components can play a role in the immunopathological process, the precise cause of death needs further study.

c. Intraperitoneal Virus Inoculation. The inability of some strains of LCM virus to produce fatal CNS disease following peripheral inoculation of adult mice, particularly when involvement of the brain is regularly demonstrable, is still not entirely explained (Rowe, 1954; Lehmann-Grube, 1964). Here, infection of the CNS is secondary to visceral infection. Under these conditions the initiation of extraneural immune induction, before infection of the CNS reaches a critical threshold, may serve to abort the infectious process; clinical choriomeningitis does not occur, but relatively mild inflammatory lesions can be observed histologically. Apparently, the absence of acute disease is due to in-

sufficient amounts of virus or viral antigen on "target" cell surfaces which can subsequently interact with virus-specific humoral and/or cellular components of the immune response of the host.

4. *Late Disease and Glomerulonephritis*

Although not central to the subject of this review, it should be noted that persistent LCM infection with antigen excess may, under certain circumstances, result in an immune complex disease. Originally called "late" disease by Hotchin (1962), since it often develops after many months after infection, it may be accelerated under circumstances which favor the formation of high levels of circulating antigen-antibody complexes (Hirsch *et al.,* 1968). Oldstone and Dixon (1967, 1969) have shown that the glomerulonephritis which is a cardinal feature of this disease is associated with deposits of LCM virus, LCM antibody, and complement components in the mesangial zone just outside the basement membrane of the glomerular capillary.

B. Arbovirus Encephalitis

1. *Pathogenesis*

Because of their marked ability to produce CNS infections in several rodent and primate species, arboviruses (Chamberlain, 1968), particularly those in groups A and B, have been extensively employed in studies of the pathogenesis of experimental viral infections of the CNS. Selection of appropriate host-virus combinations permits the study of different variables, with respect to their influence upon the course and eventual outcome of experimental infection. Age and species of host, route of infection, dose, strain, and passage history of virus, can be manipulated to provide laboratory models of CNS infections which range from inapparent and abortive to progressive and lethal. Abortive infections are particularly interesting since they probably represent the analog of many naturally occurring viral infections of man, in which virus gains access to the CNS, undergoes a limited period of replication, and is then eliminated.

2. *Effect of Immunosuppression on Factors Related to Resistance*

a. Rationale. A number of studies of abortive CNS infections produced by arboviruses have documented an association between the appearance of detectable humoral antibody and the disappearance of virus from the CNS, thereby suggesting that the immune response may be an important determinant in the outcome of infection (Kundin, 1966; Webb *et al.,* 1968a,b; Thind and Price, 1969a; Cole and Wisseman, 1969a; Weiner *et al.,* 1970). Studies utilizing procedures which modify or sup-

TABLE I

IMMUNOSUPPRESSIVE TREATMENT OF EXPERIMENTAL ANIMALS INFECTED WITH ARBOVIRUSES

Virus group	Virus	Experimental host	Method of immunosuppression	Reference
A	Venezuelan equine encephalitis	Mouse	X-Irradiation	Kundin (1966)
	Venezuelan equine encephalitis	Monkey	Cortisone	Gleiser *et al.* (1961)
	Western equine encephalomyelitis	Mouse	Cyclophosphamide	Weiner *et al.* (1971)
	Western equine encephalomyelitis	Mouse	Cyclophosphamide	Thind and Price (1969b)
	Semliki forest	Mouse	Cyclophosphamide	Cole, Bradish, and Allner (unpublished, 1969)
B	St. Louis encephalitis	Mouse	X-Irradiation	Goldberg *et al.* (1935)
	St. Louis encephalitis	Mouse	Cyclophosphamide	Thind and Price (1969b)
	St. Louis encephalitis	Hamster	Cortisone	Imam and Hammon (1957a, b)
	Japanese encephalitis	Hamster	X-Irradiation	Imam and Hammon (1957a)
	Japanese encephalitis	Hamster	Cortisone	Imam and Hammon (1957a, b)
	Japanese encephalitis	Mouse	Cyclophosphamide	Thind and Price (1969b)
	Japanese encephalitis	Mouse	Cortisone	Vollmer and Hurlburt (1951)
	Japanese encephalitis	Monkey	Cyclophosphamide	Nathanson and Cole (1970)
	Dengue-1	Mouse	Cyclophosphamide	Cole and Nathanson (1968)
	Dengue-2	Mouse	Cyclophosphamide	Thind and Price (1969b)
	West Nile	Mouse	X-Irradiation	Goodman and Koprowski (1962)
	West Nile	Mouse	Cortisone	Goodman and Koprowski (1962)
	West Nile	Mouse	6-Thioguanine	Goodman and Koprowski (1962)
	West Nile	Mouse	Cyclophosphamide	Cole, Weiner and Nathanson, (unpublished, 1968)
	West Nile	Mouse	Cyclophosphamide	Weiner *et al.* (1971)
	West Nile	Mouse	Cyclophosphamide	Thind and Price (1969b)
	West Nile	Rat	Cyclophosphamide	Cole and Nathanson (1968)
	Yellow fever	Mouse	Antilymphoid serum	Hirsch and Murphy (1967, 1968)
	Yellow fever	Mouse	Antimacrophage serum	Panijel and Cayeux (1968)
	Ilheus	Mouse	Cyclophosphamide	Thind and Price (1969b)
	Langat	Mouse	X-Irradiation	Webb *et al.* (1968b)
	Langat	Mouse	Antibody	Webb *et al.* (1968a)
	Langat	Mouse	Cyclophosphamide	Thind and Price (1969a, b, c)
	Langat	Mouse	Thymectomy	Thind and Price (1969c)
	Langat	Mouse	Antilymphoid serum	Thind and Price (1969c)
	Tick-borne encephalitis	Mouse	X-Irradiation	Malkova (1962)
Ungrouped	Vesicular stomatitis	Mouse	Antimacrophage serum	Hirsch *et al.* (1969)

press the host immunological apparatus were designed to clarify this association.

As shown in Table I, numerous reports have appeared which describe the use of various immunosuppressive procedures to alter the outcome

of experimental arbovirus encephalitis. Most studies have shown that significant depression of immune reactivity is associated with enhanced virus-specific morbidity and mortality. Suppressed animals often show a prolonged viremia, elevated virus levels in target tissues, and a reduced or undetectable antibody response.

To illustrate these effects, the remainder of this section will review a series of studies in which arbovirus infection was compared in the normal and immunosuppressed host. Emphasis is placed on laboratory models of self-limiting infections of the CNS in which a single host- or virus-associated variable appears to account for the nonfatal outcome. Immunosuppression was accomplished using cyclophosphamide, a particularly potent agent (Schwartz and Borel, 1968), and one whose effect is consistently reproducible.

b. Methods. Acute immunosuppression was accomplished by initiating drug treatment 24 hours after live virus injection. Since the duration of cyclophosphamide-mediated immunosuppression is limited, one or two additional drug doses were usually given at 5- to 8-day intervals. Drug was administered by the intraperitoneal or subcutaneous route, and dosage (milligrams per kilogram body weight), was adjusted on the basis of host age and species.

c. Host Species. Certain strains of Japanese encephalitis (JE) virus have a high intracerebral neurovirulence for young adult rhesus monkeys; about 75% die, with an average survival time of 8 to 10 days (Nathanson *et al.*, 1966). In contrast, young adult spider monkeys fail to develop clinical disease following intracerebral inoculation of JE virus, although histological evidence of encephalitis is readily demonstrable. Minimal amounts of virus can infrequently be isolated from CNS tissue and blood during the first week of infection, but virus does not appear in oropharyngeal secretions.

Cyclophosphamide was given to spider monkeys on day 1 (100 mg/kg) and day 9 (50 mg) after an intracerebral inoculation of JE virus. All animals receiving drug developed acute paralytic disease within 12 to 14 days, preceded by several days of viremia and oropharyngeal shedding of virus. Histological examination of spinal cords from immunosuppressed animals revealed very severe neuronal destruction, in comparison with untreated monkeys in which cord lesions were relatively mild (Nathanson and Cole, 1970).

Of particular interest was the almost complete absence of neuronophagia even though many neurons showed severe diffuse chromatolysis. This damping of inflammatory elements was associated with drug-induced leucopenia. All immunosuppressed monkeys had virus in their spinal cords, and levels were as much as 1000 times above those seen

in infected control animals. Only infected nonsuppressed monkeys developed neutralizing and hemagglutination-inhibiting antibodies, which were first detected 14 days after infection. Drug control animals remained well and were free of CNS lesions at sacrifice.

d. Host Age. The association of decreasing host susceptibility with increasing host age is described in a foregoing section. One well-studied host-virus combination, in which age-related resistance is absolute, is West Nile virus infection of rats (Sabin, 1952, 1954; ElDadah *et al.,* 1967; ElDadah and Nathanson, 1967). Suckling animals display an equal susceptibility to West Nile virus given by any route, and uniformly die from a fulminating infection of the CNS. At 16 days of age death no longer occurs, although a small percentage of rats develop transient neurological symptoms. Adult rats remain asymptomatic following intracerebral inoculation of West Nile virus, but undergo an abortive CNS infection. A limited period of viral replication can be detected in the brain by direct assay or by immunofluorescent staining, and virus disappears from the brain shortly after serum-neutralizing antibodies appear. Histologically, only minimal perivascular cuffs and focal infiltrates are present with little or no evidence of neuronal outfall.

Adult rats were given cyclophosphamide, 100 mg/kg, on day 1 and 50 mg/kg on days 8 and 14 after intracerebral inoculation of West Nile virus (Cole and Nathanson, 1968). Approximately 75% of these animals developed fatal CNS infection; virus eventually reached a level in the brain which was 100-fold or greater than that found in normal animals. Of particular significance was the fact that in both suppressed and normal rats virus growth curves and number of infected neurons in the brain were similar through day 7, indicating that immunosuppression had no direct effect on the numbers of susceptible cells or on the rate of spread of the infection. Following the appearance of serum-neutralizing antibody the amount of virus in the brain of normal animals subsequently fell to undetectable levels by the 11th day. Virus titers continued to increase in the brains of drug-treated animals, and by the 12th day reached a maximum level which remained essentially unchanged until death. An increase in the number of fluorescent cells was seen, which paralleled the increase in virus. Histologically, brains revealed severe destruction of the cerebral cortex and hippocampus, with almost total loss of neurons. Rats given drug alone showed no histological abnormalities of the CNS, but 20% died of drug toxicity.

e. Virus Strain and Passage History. As mentioned earlier, prolonged serial brain-to-brain propagation of some arboviruses can result in the emergence of a virus population with markedly enhanced neurovirulence.

Dengue-1 virus, isolated from acute phase serum from human patients, usually produces no overt symptoms when first inoculated intracerebrally in suckling mice. After 2 or 3 additional blind intracerebral passages, the virus is lethal for sucklings but innocuous for adult mice. Additional passage eventually leads to a virus strain (MP-125) capable of producing uniform mortality in mice of all ages (Cole and Wisseman, 1969a).

Infections with low-passaged dengue-1 virus (MP-3) were studied in normal and immunosuppressed adult mice, the latter receiving 150 mg/kg of cyclophosphamide on days 1, 7, and 14 after intracerebral inoculation of virus. In both groups of mice minimal levels of virus were first detectable in the CNS several days after infection. Virus persisted at a low level in the brains of normal animals, disappearing shortly after the appearance of serum antibody on the 12th day; no animals showed signs of clinical illness. In contrast, all immunosuppressed mice developed CNS disease and died 19 to 25 days after infection, without a detectable antibody response. Virus in the brain reached high levels and persisted until death. Lethal MP-3 infection in suppressed mice is characterized by slow virus replication and long incubation period, which differentiates it from lethal MP-125 infection in normal mice (Cole and Nathanson, 1968).

f. Route of Inoculation. The effect of this variable is best illustrated by employing arboviruses which exhibit a low or negligible peripheral neurovirulence, but which are highly virulent when inoculated directly into the CNS. Both dengue-1 (MP-125 strain) and West Nile virus fail to kill adult mice following intraperitoneal inoculation with very large doses, while relatively small doses given intracerebrally are lethal. Unlike dengue virus, West Nile virus gains access to the CNS following peripheral inoculation and produces a transient self-limiting infection preceded by a minimal viremia (Weiner *et al.,* 1970).

A single dose of cyclophosphamide (250 mg/kg), given 1 day after peripheral West Nile virus inoculation, completely overcomes this route-dependent resistance. Animals develop fatal CNS disease, with high levels of virus in blood and brain prior to death (Cole, Weiner, and Nathanson, unpublished, 1968). In comparison, repetitive drug doses following peripheral inoculation with dengue virus produce no effect. Dengue virus, with its minimal potential for replication in extraneural tissue (Cole and Wisseman, 1969a), lacks the ability to invade the neural compartment; resistance in this case is independent of immune responsiveness.

g. Virus Dose. The role of immune responsiveness in the relationship between virus dose and outcome of CNS infection can be readily demonstrated by titrating selected arboviruses in normal and immunosuppressed

mice. Keeping host age and route of inoculation constant, the 50% lethal end points for West Nile, Langat, Semliki Forest, and Western equine encephalomyelitis viruses were all found to increase in immunosuppressed animals (Thind and Price, 1969b; Cole and Nathanson, unpublished, 1969).

C. *Miscellaneous Viruses*

This section reviews data on certain other experimental models of virus infection of the CNS which have been used for studies of immunosuppression. Reference is also made to problems which might be fruitfully probed with immunosuppressive techniques in future studies.

1. *Picornaviruses*

Encephalomyocarditis (EMC) virus. Glasgow and associates (Murphy and Glasgow, 1967, 1968; Farber and Glasgow, 1968) have reported the effect of several immunosuppressive measures upon the outcome of EMC infections in adult mice. Intraperitoneal inoculation of EMC virus produces an infection which kills a small proportion of mice (large virus dose) or no mice (small dose). Combined treatment with cyclophosphamide and thioguanine enhanced viremia and replication in target organs and over 80% of the mice died. In treated animals the onset of serum antibody was delayed by 2 days; serum interferon titers were slightly higher in treated than control mice.

Preparation of mice with 350 R whole body X-irradiation (Murphy and Glasgow, 1968) had a similar effect. Depression of neutralizing antibody induction was marked; in controls, antibody appeared on day 4 and reached titers over 1000 by day 6; at this time antibody had not appeared in treated mice. Passive administration of anti-EMC serum (recipients had a neutralizing antibody titer of 2000), to X-irradiated, EMC-inoculated mice, produced complete protection if given on the day of virus and partial protection when given up to 3 days after virus. Glasgow concluded that the immune response played a more important role than interferon in the recovery of mice from EMC infections.

Poliovirus and coxsackie virus. The sensitivity of monkeys to the minimal amounts of live poliovirus which remained in the incriminated lots of Cutter vaccine (Nathanson and Langmuir, 1963) was increased by cortisone and/or X-irradiation (Syverton *et al.* 1956; Eklund *et al.*, 1956; Bodian, 1956). It appears, from the fragmentary data in those reports, that viremia was enhanced by the treatments, but it is not clear whether there was a significant suppression of serum antibody response.

Other studies, showing that cortisone, X-irradiation, or stress enhanced

susceptibility of a variety of experimental animals to poliovirus or coxsackie virus, reported higher virus titers in blood and brain, but also failed to include data on antibody (Kilbourne and Horsfall, 1951; Syverton *et al.*, 1952; Shwartzman and Fisher, 1952; Shwartzman, 1953; Melnick, 1953; Shwartzman *et al.*, 1955; Cajal *et al.*, 1959; Johnsson and Rasmussen, 1965). Smith and Cheever (1959) administered 400 R whole-body X-irradiation to weanling mice and, 24 hours later, inoculated them intraperitoneally with coxsackie virus B4; a more widespread infection was seen (apparently without death) together with a reduced neutralizing antibody response in the X-irradiated animals, in comparison to infected but untreated control mice.

2. Rabies Virus

Although, as the first animal virus isolated, rabies has received extensive study, its pathogenesis still presents some provocative problems. Wiktor, Koprowski, and associates (Fernandes *et al.*, 1964; Wiktor *et al.*, 1968; Campbell *et al.*, 1968) have demonstrated that rabies virus can produce persistent noncytocidal infections in certain tissue culture systems, and that rabies virus antiserum plus complement will cause immune lysis of such infected cells. Johnson (1965a) studied the pathogenesis of the fixed CVS strain of rabies virus in mice. After subcutaneous inoculation, high titers of virus were present in the CNS on the third day and immunofluorescent antigen was seen on the fourth day; however, little or no evidence of neuronal cytopathology or inflammation was seen up to death of mice 8 to 12 days after infection. The lack of necrotic changes in neurons infected for 4 days or more suggested that, in this experimental model, the virus may not be cytopathic.

Nonneuroadapted strains of rabies virus ("street virus") can produce subclinical infections when inoculated by extraneural routes (Johnson, 1966; Bell *et al.*, 1966). Following subclinical infection the virus may persist as a latent infection, which can be activated by stress or by administration of ACTH (Soave 1962, 1964).

Recovery from acute symptomatic rabies infection of the CNS has been studied by Bell (1964; Bell *et al.*, 1966). Disappearance from the brain of detectable infectious rabies virus is associated with appearance of rabies antibody in brain homogenates, reminiscent of Morgan's (1949a) findings for poliomyelitis and Schlesinger's (1949a,b) for arboviruses.

This brief synopsis suggests several questions regarding rabies virus-host interactions which might be explored with immunosuppression. Among these are: (*i*) What is the role of the immune response in recovery from active rabies virus infection of the CNS, or in maintaining persistent rabies infections in a latent form? (*ii*) Can rabies infection initiate an immunopathological process?

3. *Herpes Viruses*

The comparative virology of the herpes viruses has been reviewed by Plummer (1967), while Nahmias and Dowdle (1968) have compared types 1 and 2 (oral and genital) strains of herpes simplex virus (herpesvirus hominis) in detail. A number of these viruses (including herpes simplex virus, varicella virus, B virus, and pseudorabies virus) are capable of producing severe encephalitis and have an unusual affinity for first-order sensory neurons. Infection may be localized to one or a few sensory ganglia together with the corresponding innervation area of skin or mucous membrane. Herpes viruses often can produce persistent latent infections and it is postulated that the cell bodies of first-order sensory neurons may serve as the site of persistence of herpes simplex virus (Paine, 1964; Kibrick and Gooding, 1965; Roizman, 1965; Fenner, 1968) and of varicella virus (Weller, 1965; Hope-Simpson, 1965). The limited data available suggest that a continued low level of infectious virus may be found during latency (Schmidt and Rasmussen, 1960; Kaufman *et al.,* 1968). However, virus has not been isolated from trigeminal ganglia of patients with facial herpes (Richter, 1944).

The mechanism of activation of latent herpes simplex or herpes zoster is obscure. Activation may occur following radiation, fever, physiological and psychological disturbances, and section of the sensory root of the trigeminal nerve (Ellison *et al.,* 1959). Likewise, zoster may be associated with a variety of prior precipitating conditions. Hope-Simpson (1965) has postulated that a naturally occurring decline in antibody level may precede zoster, and it is possible that a diminished immune responsiveness due to disease (e.g., Hodgkin's disease, Sokal and Firat, 1965) or immunosuppressive therapy could be one of the precipitating causes. The activation of cytomegalovirus (Schneck, 1965; Craighead, 1969) and of herpes simplex virus (Montgomerie *et al.,* 1969) which has been reported in immunosuppressed patients is consistent with this possibility. However, it seems likely that activation of latent herpes infections is often unrelated to immune mechanisms.

At present there is only limited information on the effect of immunosuppression upon experimental herpes simplex infection. Nahmias *et al.* (1969) treated weanling mice with antithymocyte serum, before and after infection with varying doses of a type 1 herpes virus strain. Two different effects were seen. Viral inocula adequate to produce approximately 50% mortality from encephalitis were administered by intraperitoneal or intragenital routes; under these circumstances virus probably spreads through the circulation to invade the CNS, and antithymocyte serum increased mortality to 75–100%. Following intracerebral inoculation, antithymocyte serum either had no influence or decreased mortality, depending upon virus dose. The authors suggested that the latter effect

might be due to inhibition of the CNS inflammatory response. In our view this hypothesis (Hirsch and Murphy, 1967), which resembles that advanced by Webb *et al.* (1968a,b), requires further testing.

4. Parvoviruses

Rat virus is an indigenous virus of laboratory and wild rats (Kilham, 1966; Toolan, 1968); some strains cause symptomatic (often fatal) infections in suckling rats, but only inapparent infections in adult animals. There is evidence to suggest that this virus can also produce persistent latent infections accompanied by serum antibody (Kilham and Olivier, 1959; Kilham, 1966; Robey *et al.*, 1968).

The HER strain of rat virus causes silent infections when inoculated into adult rats; however, paralytic disease develops in a significant number following drug-induced immunosuppression (Nathanson *et al.*, 1970). The HER strain was originally isolated from "normal" animals given immunosuppressive drugs only (Nathanson *et al.*, 1970; Paterson and Nathanson, unpublished, 1970); whether this represented activation of latent infection, or potentiation of a coincidental natural acute infection, has yet to be determined.

IV. Discussion and Conclusions

A. Multifactorial Determination of the Outcome of Viral Infection

1. Concept of a Race between Virus and Host Defenses

Apart from the use of immunosuppressive techniques, there are a number of observations in the literature which are consistent with the concept that the outcome of a viral infection of the CNS is determined by a race between the replicating agent and host defenses, including the immune response. If infection is visualized as a race between virus and host defenses, then the outcome is determined by the balance between factors favoring the virus and those favoring the host. Furthermore, different factors, involving quite different mechanisms, can act in concert or in opposition. Several examples illustrate this viewpoint.

a. *Intracerebral Inoculation.* Schlesinger's (Schlesinger *et al.*, 1944; Schlesinger, 1949a,b) series of investigations of Western equine encephalomyelitis (WEE) virus are particularly instructive. Guinea pigs, vaccinated with killed WEE virus, resisted homologous intracerebral challenge, although virus replication in the brain appeared to parallel that in controls for about 24 hours. After this time, virus disappeared from brains of vaccinated animals but rapidly multiplied in controls, which died 2 or 3 days after inoculation.

In a similar experiment using vaccinated mice (Schlesinger, 1949a) the outcome was dependent upon the strain of WEE virus; growth of a rapidly

multiplying strain was retarded in brains of vaccinated animals, but death still occurred. In contrast, a strain which replicated relatively slowly, but which killed unimmunized animals, produced an abortive nonfatal cycle of infection in vaccinated mice, with clearance of virus by about 5 days after infection.

Studies of the protective effect of elevated body temperature offer another example. Cole and Wisseman (1969b) found that mice, incubated at 35°C (body temperature 39°C) survived an intracerebral inoculation of a strain of dengue-1 virus which killed all animals held at 22°C (body temperature 37°C). At elevated temperatures intracerebral virus replication was retarded, brain interferon levels were lower, and appearance of antibody was delayed. Approximately 20 days after infection, virus titers dropped and antibody appeared. In this instance it appears that hyperthermia had a greater inhibitory effect on virus replication (Lwoff, 1959, 1969) than on host defenses, and reversed the outcome of infection. When a more virulent dengue virus strain was used, the effect of hyperthermia was negligible and all animals died.

b. Extraneural Inoculation. For many years it has been well documented that a variety of viruses, which regularly produce lethal infections when inoculated intracerebrally, even in minimal doses, produce only sublethal (often subclinical) immunizing infections when inoculated parenterally (Lennette and Koprowski, 1944). More recently, it has become clear that, in some instances, the potentially lethal virus actually invades the CNS, where it undergoes a transient cycle of replication, reaches only low titer, and then disappears (Gleiser *et al.*, 1962; Huang and Wong, 1963; Webb *et al.*, 1968b; Thind and Price, 1969d; Doherty, 1969; Weiner *et al.*, 1970). From these circumstances it is evident that infection aborts without spreading to a large residual population of highly susceptible cells. It appears that peripheral inoculation (and extraneural infection) triggers host defenses several days prior to CNS invasion, permitting these defenses to anticipate and outrace the infectious process. The role of the immune response in interactions of this type has been described in the foregoing section on arbovirus encephalitis.

The effect of specific factors is dependent upon the mode of virus spread to the CNS. Thus, Nathanson and Bodian (1962) found that a small dose of immune globulin protected monkeys against intramuscular challenge with the highly virulent Mahoney strain of poliovirus, which invaded the CNS from the blood. However, the same treatment failed to protect against the "fixed" MV strain, which spread to the CNS by the neural route. Conversely, sciatic nerve freeze protected against gastrocnemius inoculation of the MV strain but not against the Mahoney strain (Nathanson and Bodian, 1961a).

c. Comment. These examples suggest certain generalizations which, al-

though obvious deserve brief statement. (*i*) Virus virulence and host susceptibility are attributes which can only be characterized for specific virus-host interactions. (*ii*) The relative importance of specific mechanisms in determining the outcome of infection differs markedly in different experimental models, as well as in different instances of naturally occurring infection. (*iii*) It is simplistic to consider a single mechanism as the sole determinant of the outcome of infection, although, for purposes of analysis, it is often possible to experimentally "isolate" individual mechanisms. (*iv*) Experimental intervention designed to demonstrate the importance of a particular mechanism must be interpreted with caution. Exogenous factors (such as antibody, interferon, or immunologically competent cells), introduced into experimental animals, may produce unambiguous effects but do not necessarily prove that the factor under examination is singularly important in the outcome of unmanipulated infection. Attempts to inhibit a specific host defense may suffer from lack of specificity, since it is difficult to markedly impair a single defense mechanism without producing widespread physiological derangements.

2. Role of the Immune Response in Primary Viral Infection: Present Status and Future Directions

The evidence regarding the possible role of the immune response in recovery from primary viral infections with potentially neurovirulent viruses, can be conveniently considered under several heads, based upon differences in experimental approach.

a. Descriptive Sequential Observations. Classical descriptive studies of viral pathogenesis fall into this category. For instance, Bodian (1959) summarized the sequential evolution of poliovirus infection of primates, based upon data accumulated by many laboratories. Antibody appears between 5 and 10 days after infection, often coincident with the clearing of viremia, and about the time that spinal cord virus titers are increasing, that is, a few days prior to paralysis. Such observations may be misleading for several reasons. First, more sensitive methods of detection indicate that serum antibody can appear considerably earlier after exposure to poliovirus antigen (Svehag and Mandel, 1964) and the earliest antibody may be complexed by excess virus in the blood (Nathanson and Bodian, 1962; Melnick *et al.*, 1966). Furthermore, antibody in CNS or CSF may be more relevant than serum antibody (Morgan, 1949a,b). In any event, data of this type permit only weak inferences as to causal relationships.

Descriptive studies provide more important data when a comparison is made of models in which the outcome of infection varies. There are several studies in which naturally occurring differences in the time of appearance of serum antibody were related to the outcome of infection.

Overman and Kilham (1953) studied mumps meningoencephalitis produced by intracerebral inoculation of hamsters with the M-1 (less virulent) and M-2 (more virulent) virus strains. The M-1 strain killed newborn hamsters, while 8-day-old animals survived. Survival was associated with an earlier HI antibody response. The M-2 strain, which had a much shorter incubation period, killed 8-day-old hamsters, and Overman and Kilham suggested that the M-2 strain was able to outrace the immune response. In additional studies with mice, Overman (1954a,b) showed that an inactivated mumps vaccine elicited a more rapid HI antibody response in older mice; furthermore the greatest change in responsiveness occurred between 7 and 10 days of age, which correlated with the age of development of host resistance.

Morgan (1941) found that the response of mice to inactivated Eastern equine encephalomyelitis (EEE) virus vaccine, as measured by neutralizing antibody, increased considerably during the first 10 days of life; this was correlated with a marked reduction, during the first month of life, in the intraperitoneal LD_{50} of the virus. A similar explanation for age-specific resistance of mice to cowpox virus was advanced by Subrahmanyan (1968).

Schell (1960) studied the marked differences in susceptibility of mice of different strains to mousepox virus, and concluded that the relative resistance ofC57BL mice was correlated with their more effective immune response to this agent.

b. Passive Immunization. It is well known that passive administration of immune serum prior to infection can dramatically protect against extraneural viral infection, if viremia plays an important role in CNS invasion (Morgan, 1949b; Nathanson and Bodian, 1962). Furthermore, doses of antibody, so low that no neutralizing activity can be measured in the serum of recipients, can protect (Bodian, 1952). However, if administration is delayed until after infection, the effect is rapidly lost, depending upon the host-virus combination, and upon route of administration and dose of antibody (Murphy and Glasgow, 1968). Thus, experiments with passive antibody are suggestive, but they leave unanswered the question whether the active primary immune response of the unmanipulated host is sufficiently rapid to play a role in the outcome of infection.

c. Active Immunization. Another approach to evidence implicating the immune response is manipulation of the virus inoculum to vary the amount of antigen relative to the number of infectious particles. A classic example is the work of Schlesinger (1949b), who showed that when "lightly immunized" mice were challenged intracerebrally with varying doses of Western equine encephalomyelitis virus, death occurred following small but not large virus inocula. The paradoxical or "zone" effect which has

been described for other host-virus interactions (Schlesinger, 1959), was interpreted as reflecting the greater antigenic stimulus afforded by the larger inoculum. A similar approach was exploited by Bodian (1956), who showed that incorporation of a small amount of virulent virus in a large volume of inactivated vaccine markedly reduced the subsequent frequency of paralysis following intramuscular injection.

d. Immunosuppression. The effects of immunosuppression have been detailed in an earlier section and only the essentials need be recapitulated. A variety of procedures can be used to render experimental animals unresponsive or hyporesponsive to exogenous antigens, including viruses. Application of these techniques to many virus-host models produces several effects: (*i*) The levels of virus in blood, brain, or other tissues are elevated, and virus may appear earlier and persist for longer periods. (*ii*) More cells are eventually infected (immunofluorescent observations) and, if the virus is cytocidal, a greater number are destroyed. Concomitantly, subclinical infections become symptomatic and often fatal. (*iii*) The appearance of antibody in serum or tissues is retarded, and death may intervene before antibody is detected. (*iv*) Interferon levels are often directly related to virus titer, and may be higher in tissues of suppressed animals than in infected but unsuppressed controls. (*v*) The physiological derangements produced by many immunosuppressive techniques may reduce the interferon response: cyclophosphamide, Robinson and Heath (1968); antilymphoid serum, Barth *et al.* (1969) and Sheagren *et al.* (1969); X-irradiation, DeMaeyer *et al.* (1969); cortisone, Rytel and Kilbourne (1966) and Mendelson and Glasgow (1966). However, large doses of immunosuppressive drugs do not always reduce the interferon response (Ho *et al.*, 1967).

e. Comment. The dramatic potentiation of many experimental virus infections by a variety of immunosuppressive techniques strongly suggests that the immune response, in some virus-host interactions, plays a key role in the outcome. In our view, concomitant reduced interferon responsiveness, when it occurs, is not sufficient to account for this potentiation, in light of the elevated interferon levels which regularly accompany enhanced virus titers. At present it is impossible to determine whether the effects of immunosuppressants are due, in part, to damping of host defenses other than the immune response and interferon.

f. Future Directions. To further define the role of immune mechanisms during viral infections, better methods are needed both for immunosuppression and to monitor immune status.

Greater specificity is required of immunosuppressive methodology; the goal is an animal with normal responsiveness to all but one or more selected antigens. Due in great part to the stimulus of tissue transplantation, this goal may soon be achieved.

(*i*) *Immunization-suppression.* Schwartz and Borel (1968) have reviewed evidence indicating that administration of an immunosuppressive drug, during the period of immune induction (for instance, 24 hours after antigen) may selectively destroy immunocytes responding to that antigen (Santos, 1967). Upon recovery from the acute drug effects, the animal regains normal responsiveness to antigens other than the one administered prior to drug. Although this approach has successfully been used with some inert particulate antigens, its success depends on a number of critical variables; repeated administration of the test antigen and drug, or thymectomy, may be required to maintain unresponsiveness.

Preliminary experiments in our laboratory (Weiner *et al.*, 1971) demonstrated that this approach has potential for studies of virus infection. Adult mice were given 3 intraperitoneal inoculations of formalin-inactivated West Nile (WN) or Western equine encephalomyelitis (WEE) virus vaccines, at weekly intervals. One day after each vaccination the mice received cyclophosphamide, 150 mg/kg. Ten days after the last cyclophosphamide injection, animals suppressed in this manner were again inoculated with WN or WEE vaccine; they failed to produce HI antibody to the virus to which they had been suppressed, but responded normally to the heterologous viral antigen. Since WN and WEE viruses exhibit low neurovirulence in adult mice after intraperitoneal inoculation, the specificity of immunosuppression could be further tested. Ten days after immunization-suppression, mice were challenged intraperitoneally with a large dose of WN or WEE virus; animals were killed by the virus to which they had been suppressed, but survived challenge with the heterologous virus.

(*ii*) *Passive administration of antibody* has been successfully used to suppress responsiveness to the corresponding antigen (Uhr and Moller, 1968), presumably by virtue of its ability to bind antigen. The potentiation of Langat virus infection by specific viral antibodies (Webb *et al.*, 1968a) may represent an example of this phenomenon.

Large doses of *antigen* have been shown to induce hyporesponsiveness (Dresser and Mitchison, 1968). Thus, Flick and Pincus (1963) injected inactivated concentrated vaccinia virus intramuscularly into newborn rabbits, and 4 days later challenged intradermally with live vaccinia virus. In contrast to control rabbits which developed a normal primary local vaccinia lesion, animals pretreated with viral antigen developed a generalized vaccinia infection which killed more than 50%.

(*iii*) Tests designed to provide *in vitro* assessment of *delayed hypersensitivity* to viral antigens are a major need. The variety of products released by sensitized lymphocytes *in vitro,* when exposed to the immunizing antigen (David, 1968), and the biological responses evoked by these prod-

ucts, suggest numerous potential *in vitro* assays. When such methods are applied to viral pathogenesis new interpretations of established phenomena may emerge.

3. *Interferon as a Host Defense Mechanism*

The active current interest in interferon has generated a large body of literature. Several symposium volumes provide useful general reviews (Finter, 1967a, 1970; Wolstenholme and O'Connor, 1967). *In vitro* studies of interferon are outside the scope of this discussion, but it is relevant to note the mass of data which documents marked differences between viruses, both in their activity as interferon inducers, and in their sensitivity to interferon. This, in turn, suggests that the importance of interferon will vary in different host-virus interactions.

Particularly relevant to consideration of host defenses is the review by Baron (1970).

a. Descriptive Sequential Observations. Most cells are potentially capable of interferon synthesis, and interferon production usually occurs in those tissues which are supporting virus replication. In addition, there is a tendency for local fixation of interferon (Finter, 1966). Thus, following intracerebral inoculation of adult mice with West Nile virus, virus replication and interferon production are essentially confined to the CNS (Subrahmanyan and Mims, 1966). Conversely, intravenous inoculation of interferon inducers stimulates particularly the spleen, and results in high titers of circulating interferon (Fruitstone *et al.*, 1966; Baron *et al.*, 1966a,b). Intravenously injected interferon rapidly disappears (10–60 minutes), and probably equilibrates with the extracellular fluid compartment (Baron *et al.*, 1966a; Finter, 1966; Gresser *et al.*, 1967; Ho *et al.*, 1967). However, it appears to be taken up more readily by certain tissues and organs (particularly liver) than by others (Subrahmanyan and Mims, 1966; Ho *et al.*, 1967).

Sequential descriptions of the relationship between virus, interferon, and antibody are numerous (e.g., Murphy and Glasgow, 1967, 1968; Cole and Wisseman, 1969a). In general, these show that the rise and fall of interferon follows that of virus quite closely, while antibody appears later, often about the time that virus titers begin to drop. Originally, such observations were provisionally interpreted (Baron, 1963; Isaacs, 1963) as evidence for the role of interferon in recovery from infection. The pitfalls in such an interpretation are illustrated by the comparative study of Cole and Wisseman (1969a), who observed the usual sequence of events, but found the highest interferon levels in lethal host-virus combinations, where virus titers were also highest.

b. Comparative Studies of Variable Host Susceptibility. The marked

influence of *age* on susceptibility of rodents to infection with a wide variety of viruses capable of producing encephalitis has been described above. These observations provide an opportunity for assessment of the possible role of interferon in these age-specific changes in susceptibility. An early report by Heineberg *et al.* (1964), utilizing coxsackie virus B1, suggested that interferon production might be reduced in susceptible infant mice compared to resistant adults. However, subsequent studies with other models (Sindbis virus, Vilvek, 1964; West Nile virus, Subrahmanyan, 1968; dengue virus, Cole and Wisseman, 1969a) fail to suggest that interferon plays an important role in age-specific variation in host susceptibility.

In a preceding section the *genetically determined* difference in susceptibility of different strains to mice to intracerebral inoculation of group B arboviruses was described (Goodman and Koprowski, 1962). Virus replicates in brains of resistant animals, but growth is slower from the outset, indicating some quantitative difference in virus-cell interaction. *In vitro* studies (Vainio, 1963) suggest that cell cultures reflect the susceptibility of the animals from which they are derived, since susceptible cultures yielded at least 100-fold as much virus as did resistant cultures. Cultures from resistant and susceptible animals show equal ability to produce interferon (Vainio *et al.*, 1961). More recently Hanson and associates (1969) have suggested that resistant cells are more sensitive to interferon than are susceptible cells. Further exploration of this provocative finding is needed.

c. Virus Virulence. There are a number of *in vitro* studies comparing virus variants with a greater or lesser virulence, in which the more virulent strain produces less interferon or is less sensitive to interferon (Glasgow and Habel, 1962; Wagner *et al.*, 1963; Finter, 1964a; Aurelian and Roizman, 1965). However, in certain *in vivo* systems, virulent strains are as sensitive to interferon as avirulent strains (Cole and Wisseman, 1969a).

d. Protection by Passive Administration of Exogenous Interferon. Administration of pre-formed interferon to a passive recipient has repeatedly been shown to protect against a subsequent virus challenge. Finter (1966) explored the effect of interferon against intraperitoneal challenge with a strain of Semliki Forest virus which produced a lethal infection in adult mice after intraperitoneal inoculation. When a large dose of interferon was inoculated intramuscularly 3.5 hours prior to challenge with 110 mouse intraperitoneal LD_{50}, about 90% of animals survived. Interferon protection dropped rapidly with increasing virus dose; pretreatment with 2 large doses gave 80, 40, and 0% protection, respectively, against 80, 320, and 1280 LD_{50} of virus. When given after virus, interferon was much less effective. Since interferon is rapidly removed from the circulation, the doses used by Finter produced negligible serum titers in recipients.

Baron and co-workers (1966b) gave juvenile mice passive interferon intravenously, and then challenged with minimal doses (about 1–10 LD_{50}) of encephalomyocarditis (EMC) virus or vesicular stomatitis virus (VSV), by the intracerebral route. Pretreatment with large doses over the 24 hours prior to challenge protected mice completely against an EMC challenge which killed 40% of controls. A similar interferon regime reduced VSV mortality from 95 to 65%.

In evaluating these results, several comparisons should be borne in mind. The amounts of interferon used in passive experiments are undoubtedly less than those produced actively in response to viruses which are optimal interferon inducers (Baron *et al.*, 1966a). Passive protection with interferon given prior to virus challenge, when compared to that afforded by passively administered antibody, is impressive for intracerebral injection (Morgan, 1949b), but not for extraneural routes of infection. In any event, studies of this type only indicate the potential role which interferon might play in the outcome of certain virus infections.

e. Effect of Active Interferon Induction. Finter (1966) tested the effect of virus-induced interferon in protection against heterologous virus challenge of mice. Active induction of interferon with Newcastle disease virus (NDV) injected intravenously at various times from 24 to 4 hours prior to intraperitoneal challenge with 110 intraperitoneal LD_{50} of Semliki Forest virus, protected 30 to 100% of mice. Baron and associates (1966b) found that the protection afforded against intracerebral challenge with about 10 LD_{50} (95% lethal dose) of EMC virus depended on the time of induction, survival being greatest (60%) when NDV was administered 24 hours before virus challenge.

On the other hand, interferon induction has had a variable effect on the outcome of certain experimental rabies infections. Thus, while high levels of circulating interferon did not protect against intramuscular challenge of adult mice with approximately 1 LD_{50} of rabies virus (Soave, 1968; Finter, 1967b), complete protection of rabbits was achieved by a single intravenous injection of the synthetic inducer polyinosinic-polycytidylic acid (Fenje and Postic, 1970).

f. Comment. The experimental data indicate that some viruses are potent interferon inducers, and that the levels of interferon found in blood and tissues during certain virus infections are sufficient to markedly retard virus replication. Combined with the older data on viral interference (Schlesinger *et al.*, 1944), it appears likely that interferon modulates a number of infections. In some instances, interferon may be a critical determinant of the outcome. However, the lack of a method for specific blockade of the interferon aspect of host defenses, makes definitive proof difficult to attain.

4. Other Mechanisms

a. Age Effects. The age-specific decrease in susceptibility of mice to intraperitoneal or other extraneural routes of injection, in the face of relatively high susceptibility to intracerebral inoculation, has already been discussed at several points. This effect is associated with variations in viremia, which in turn reflect differences in the replication of virus in tissues that release virus into the circulation. Little attempt has been made to elucidate the underlying cellular mechanisms, with the exception of a study by Johnson (1964b) of herpes simplex virus. *In vitro,* peritoneal macrophages from suckling and adult mice were equally susceptible to herpes infection, but infected macrophages from young animals were much more efficient as a source of infection for other cells in the culture. Age may also have a decisive effect in experimental models where neural spread, rather than viremia, is operative (e.g., Sabin and Olitsky, 1937a,b). There is a need for further *in vitro* studies of age-determined susceptibility.

b. Virus Receptor Sites on Cell Surfaces. Since the pioneering work of Holland (1961) and his associates on the mechanism of the resistance of nonprimate cells or animals to human polioviruses, a great deal of information on cellular receptor sites has accumulated. In a series of studies Holland showed that while mouse cells cannot be infected with intact poliovirus, viral RNA alone, or viral RNA enclosed within the capsid of coxsackie virus B1, can enter and infect mouse cells (Holland, 1961; Cords and Holland, 1964).

Kunin (1962) found that loss of susceptibility of older mice to group B coxsackie viruses was correlated with age-specific reduction of receptor activity of brain homogenates. Originally it was thought that quantitative differences in attachment might account for more subtle effects, such as the virulence of different poliovirus strains; more recent work (Harter and Choppin, 1965) has failed to confirm earlier impressions.

c. Serum Protective Factor. Thind and Price (1968, 1969c) have described cross-protection between antigenically related group B arboviruses in mice. Protection could be passively transferred by serum from immunized mice which lacked detectable neutralizing antibody, and the activity was designated serum protective factor (SPF). SPF resembles antibody in its antigenic specificity and persistence in the serum of immunized mice; however, it differs in certain physical properties from the best characterized immunoglobulins, and its precise nature awaits further study.

d. Temperature Effects on Virus Replication. There is little information on variation in neurovirulence of virus strains, which goes beyond essentially descriptive data. The effect of temperature on virus replication is an exception. Temperature can be studied both as a virus variable, by

comparing mutants with different growth temperature optima (Fenner, 1968), and as a host variable, by comparing replication of a single strain at optimal and nonoptimal temperatures (Lwoff, 1969). Furthermore, temperature characteristics tend to exert a strong influence on the outcome of infection; that is, thermoresistant mutants tend to be virulent; and elevation of body temperature tends to slow virus growth and favor host survival. Thus, Carmichael and associates (1969; Carmichael and Barnes, 1969) have correlated the high susceptibility of young puppies to canine herpesvirus with their body temperature (about 36°C) which is lower than that of adult dogs (37°–38°C); in tissue culture the virus grows optimally at 35°–36°C.

Lwoff (1959, 1961, 1969) has made an extensive study of the mechanism of temperature sensitivity, based on *in vitro* studies of the replication of poliovirus variants. At supraoptimal temperatures several events were defined (Lwoff, 1969) which contribute to the reduced accumulation of viral RNA: a ribonuclease is activated (perhaps released from lysosomes) which degrades viral RNA; and the activity of viral RNA replicase is markedly decreased.

It has been suggested (Baron, 1970) that the effects of temperature might occur (at least in some laboratory models) because hyperthermia causes a greater reduction in virus replication than in interferon production, with an inverse effect of hypothermia (Stancek, 1965). Thus, Ruiz-Gomez and Sosa-Martinez (1965) found that holding mice at 4°C enhanced their susceptibility to coxsackie virus B1, with concomitant increase in virus titers and decrease in interferon levels. On the other hand, the protection of hyperthermic mice from Sindbis or from dengue-1 virus infection (Kirn *et al.*, 1967; Cole and Wisseman, 1969b) was associated with decreased levels of both virus and interferon in the brain.

V. Summary

This review has summarized current views of the pathogenesis of virus infections of the nervous system, with particular attention to certain aspects of virus-host interactions. Following invasion of the central nervous system, infection can follow a variety of patterns, as to number and distribution of neuronal and nonneuronal cells involved. There is a corresponding diversity in the pathological lesions of the CNS produced by acute virus infection.

Infection can be pictured as a race between virus and host defenses, where many factors, acting through different mechanisms, can influence the outcome. Outcome is always determined by multiple virus and host variables, although single variables can be independently studied under experimentally controlled conditions in the laboratory. A body of evidence has evolved to indicate that, in many virus-host combinations, the

immune response plays an important role in recovery from primary infections. Likewise, it is clear that an immunopathological process mediates the disease which follows certain CNS virus infections. Further refinement to produce virus-specific immunosuppression is required to strengthen the experimental evidence. Finally, *in vitro* correlates of delayed hypersensitivity are needed to delineate the relative roles of humoral and cellular aspects of the immune response in the outcome of virus infections of the central nervous system.

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