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Viruses and Rickettsiae

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In this review I shall try to provide a brief, up-to-date, account of the neuropathology of those viral and rickettsial diseases that are particularly prevalent in tropical regions. These diseases are not, however, exclusive to the tropics. Some, such as AIDS, are common in temperate regions as well, though others are closer to being exclusively tropical, such as some of the arthropod-borne (ARBO) virus encephalides. The latter are dependent for their dissemination on an existence during part of their infectious cycle in insects which are, in turn, climatically and seasonally sensitive. This necessarily limits their geographical distribution. Factors that influence some of the other diseases are less closely dependent on climate and geography and reflect more the social or cultural conditions under which people live. Thus, diseases that depend for their spread on forms of human behavior such as promiscuity or drug abuse (AIDS), or poor hygiene and living conditions (polio, rickettsial diseases) or on contact with domestic and other animals (rabies) may occur in a more widespread distribution, for the tropics are not the only places that afford opportunities for these diseases to flourish.

I shall select for discussion aspects of the pathology of these diseases that are currently undergoing investigation but will aim to present these against the backdrop of more established aspects of their pathology. Recent reviews of the pathology of viral encephalitis can be found in Hamilton and Wiley (33) and Esiri and Kennedy (20) and of HIV-1 infection in Price & Sidtis (78) and Scaravilli (85).

Retrovirus Infections

Retroviruses are enveloped RNA viruses which replicate in cells by means of a reverse transcriptase enzyme that allows synthesis of a complementary DNA which is integrated into the host cell DNA. They remain in the cell for its lifetime and cause lifetime infections in humans.

The recently investigated retroviral infections human immunodeficiency virus (HIV) - 1, HIV-2 and human T lymphotropic virus (HTLV) - 1 are those to be discussed here. HIV-1 is the cause of the world epidemic of AIDS, first reported in the USA in 1981. HIV-2 is a similar but molecularly and serologically distinct virus from HIV-1 which has been described as a pathogen principally in West Africa. HTLV-1 is a virus that causes endemic infection in many parts of the world, but particularly in the tropics, which causes adult T cell leukemia and a form of chronic myelopathy.

HIV-1 Infection and AIDS

HIV-1 infection is the most common central nervous system (CNS) viral infection in tropical countries. There are estimated to be over 18 million adults and 1.5 million children infected with this virus world-wide (103). About 3 million of these people are living in sub-Saharan Africa and 4 million in South and South-East Asia. Over 6 million of those infected with HIV-1 have developed AIDS, the clinical manifestation of HIV-1 infection and its consequences, and AIDS is now the commonest cause of death in those aged 15-49 years in many African localities (80).

HIV-1 infection is associated with a remarkably wide range of neuropathological changes, some of which are due to opportunistic infections resulting from the failure of immune function which HIV-1 infection promotes. Others are neoplastic (lymphoma) or directly attributable to the effects of HIV-1 on the nervous system. It is only with the direct effects of HIV-1 infection that this review is concerned. There are three different manifestations of HIV-1 infection to consider: HIV-1 encephalitis, myelopathy and peripheral neuropathy. For the most part, all are seen relatively late in the course of HIV-1 infection. While the prevalence of opportunistic CNS infections in AIDS sufferers in the tropics is quite high and comparable to that in Western countries the prevalence of HIV-1 encephalitis in tropical countries is lower than in the West, probably because fewer of the systemic infections that occur in AIDS are treated effectively and patients therefore die

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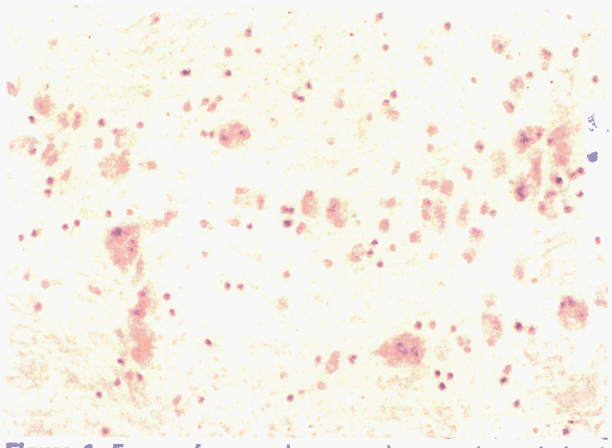

Figure 1. Focus of macrophages and macrophage-derived multinucleated cells in the basal ganglia from a case of HIV-1 encephalitis. H & E stain.

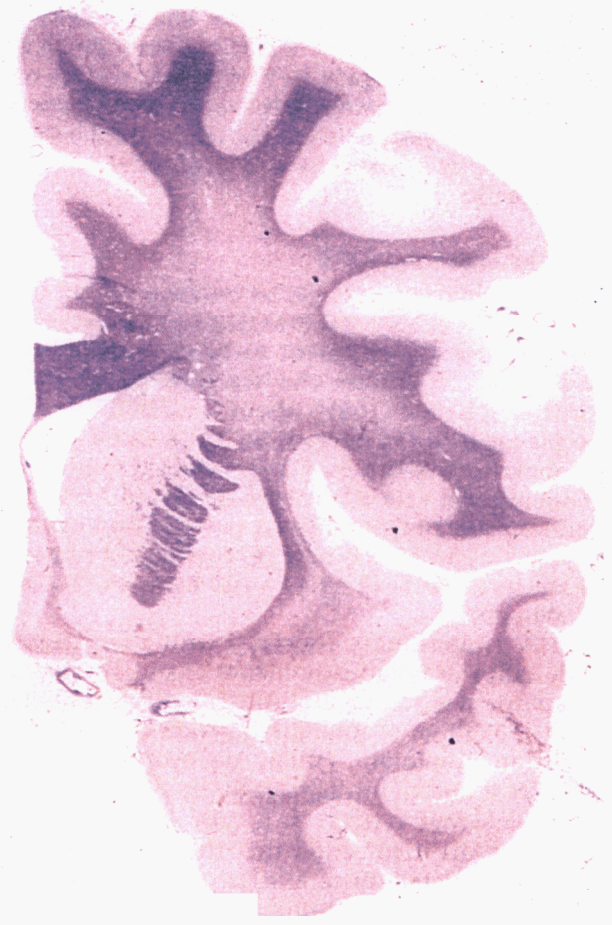

Figure 2. Myelin-stained section through the frontal and anterior temporal lobes of a case of HIV-1 encephalitis. Note diffuse pallor of myelin in the frontal lobe white matter. Luxol fast blue stain. (Reproduced with permission from Esiri MM. Oppenheimer's Diagnostic Neuropathology, 2nd Ed. Blackwell Science 1996).

at an earlier stage of the disease, before HIV-1 encephalitis has had an opportunity to develop. Thus, HIV-1 encephalitis was seen in only 3% of subjects with AIDS undergoing autopsy in Abidjan, West Africa (60) compared with 23-41% in recent autopsy series in Europe (9, 61, 63).

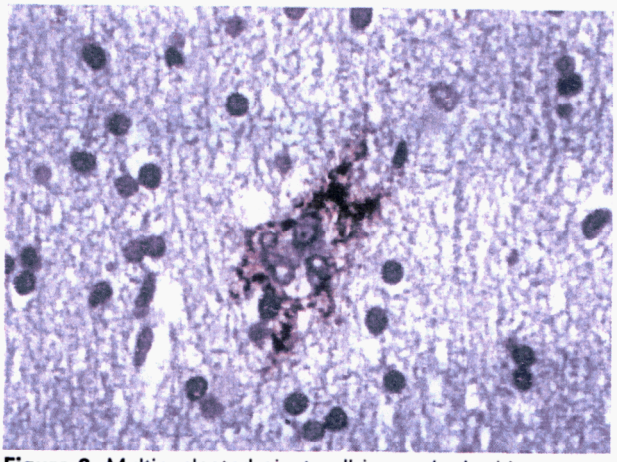

Figure 3. Multinucleated giant cell in cerebral white matter containing HIV-1 p24 core protein antigen (red reaction product). Haematoxylin counterstain. (Immunostained section prepared by Dr CS Morris).

A recent prospective study in the USA showed that the incidence of clinical AIDS dementia depended on the severity of depression of the CD4 lymphocyte count and ranged from 7.3 to 0.5 cases per 100 person years (5). About 20% of cases of advanced AIDS in Western countries can be expected to develop dementia. The prevalence of HIV-1 encephalitis at autopsy varies depending on whether HIV-infection has been treated (9, 32, 61).

No reliable figures for the prevalence of myelopathy and peripheral neuropathy complicating HIV-1 infection in tropical countries are available. In selected series from the USA the prevalence of myelopathy has been estimated as 2-22%, while in an unselected prospective study the incidence of sensory peripheral neuropathy was found to be 1.5/100 person years (5).

HIV-1 Encephalitis

Early in the course of the AIDS epidemic it was noted that HIV-1 can be detected in the brains from some sufferers (89). The presence of the virus in the brain in adults is associated with the clinical development of a diffuse encephalopathy termed the *AIDS dementia complex* (77) or *HIV-1 - associated cognitive/motor complex* (42). This usually occurs towards the terminal stages of infection but it is not seen in all subjects with the pathology of HIV-1 encephalitis. Death usually occurs within 6 months of the development of severe symptoms. Infants and children infected with HIV-1 before or at the time of birth or later are also susceptible to developing HIV-1 encephalitis (88).

HIV-1 encephalitis presents a unique pathological picture. Because the patients are severely immunosuppressed by the effects of HIV-1 on the immune

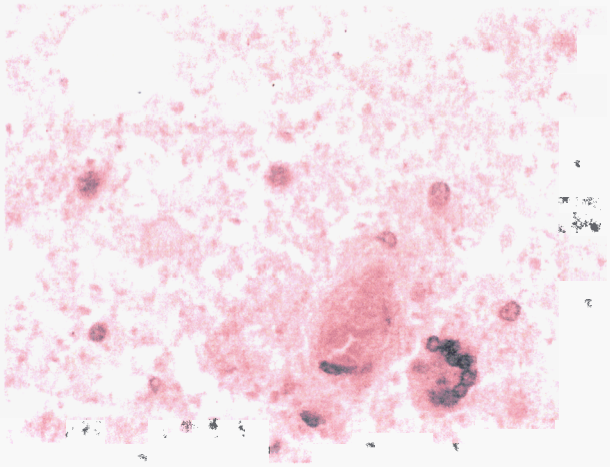

Figure 4. HIV-1 myelopathy. Lateral column white matter showing vacuolar change and a multinucleated macrophage adjacent to a blood vessel. H & E stain.

system there is remarkably little lymphocyte infiltration in the brain and the pathology can be overlooked if it is not specifically sought. There is a diffuse or multifocal accumulation of microglia, macrophages and, in most cases, macrophage-derived multinucleated cells (Fig 1). These are found in a widespread distribution in the brain but with a predilection for the cerebral white matter and deep grey matter. The white matter, in addition, shows diffuse myelin pallor (Fig 2) and reactive astrocytosis. HIV-1 structural proteins can be demonstrated immunocytochemically in multinucleated cells, macrophages and microglia (Fig 3). Because multinucleated cells are not always evident and the other pathology is non-specific, HIV-1 encephalitis cannot be definitely excluded without performing immunocytochemical or *in situ* hybridization reactions for detection of HIV-1. In addition to these alterations in white matter and deep grey matter that are clearly associated with the local presence of virus, more subtle changes not so clearly localized to sites of demonstrable HIV-1 infection, have also been described. Thus, pyramidal neuron loss has been found in parts of the cerebral cortex (21, 22, 101, 105). Subcortical nuclei including the cerebellar dentate nuclei and inferior olives have also been shown to lose neurons (1). These studies of neuron loss have relied on morphometric analysis and simple inspection of sections rarely shows clear evidence of cell loss. In cerebral cortex reduction of dendritic spines on cortical neurons, reduction in synaptic density (64) and apoptosis of neurons (3, 27, 75) have also been found.

The clinical features of the AIDS dementia-complex (73, 79) are predominantly those of a subcortical dementia with slowed responses, apathy, and poor judgement and concentration. This suggests that the subcortical rather than cortical pathology contributes more to the clinical syndrome. Motor impairment can range from mild slowing or clumsiness of rapid or complex movements to severe weakness.

HIV-1 infections of the brain in infants can be associated with severely impaired brain development and delayed or curtailed mental or motor development. The brain weight is normally reduced. The histological features seen in affected infants resemble those seen in adults with the added common feature of mineralization in the walls of blood vessels or as extracellular deposits particularly in the basal ganglia and frontal lobe white matter. Some lesions may be necrotic (30).

HIV Associated Myelopathy

Difficulty walking due to an ataxic-spastic gait and incontinence in AIDS sufferers is usually due to a myelopathy. Like HIV-1 encephalitis this myelopathy tends to occur late in the course of AIDS and there is a weak association with HIV-1 encephalitis (71). The pathology consists of vacuolar change and myelin degeneration in the lateral and posterior column white matter, maximal in the thoracic region (74, 87, 91). The myelin damage is associated with focal macrophage infiltrates. The pathology is reminiscent of that of subacute combined degeneration of the cord due to vitamin B_{12} or folate deficiency. Occasionally multinucleated macrophages are also present (Fig 4), and HIV-1 can be demonstrated in such cells (17).

Peripheral Sensory Neuropathy

The commonest form of peripheral neuropathy in AIDS is a progressive distal sensory neuropathy, sometimes accompanied by pain, seen late in the course of the disease. The pathology consists of a 'dying-back' type of axonal degeneration, but in some cases there may also be evidence of segmental demyelination and inflammation. HIV-1 has occasionally been demonstrated in nerves in such cases (6).

Other Findings

Neurological symptoms can occur during the latent phase of HIV-1 infection, before the onset of AIDS. Peripheral demyelinating neuropathy, either acute or chronic, of the immune type that also occurs in uninfected people as acute or chronic inflammatory polyneuropathy, is more common in HIV-1 infected subjects. Mononeuritis multiplex and cranial neuropathy also occur in this phase and have been attributed to immune complex vasculitis (29). In the terminal stages a sensory ganglionitis and autonomic neuropathy are also seen occasionally. In the CNS in the pre-AIDS phase of HIV-1 infection the brain usually appears normal but it may show mild mononuclear cell infiltrates in meninges and perivascular spaces and increased microglial cell activation (3).

Myopathy or myositis may also occur in subjects with AIDS. In polymyositis HIV-1 has been demon-

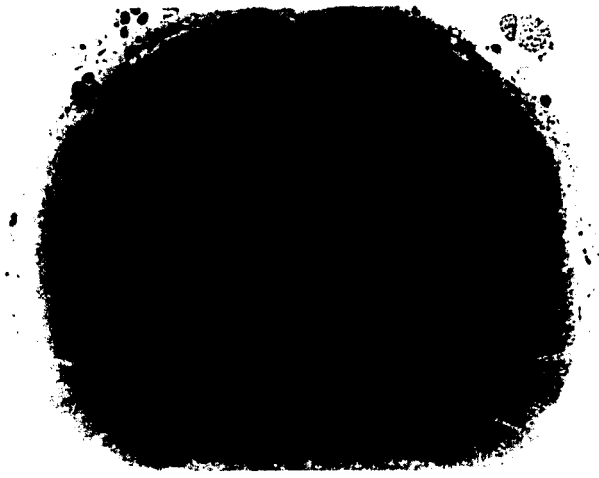

Figure 5. Myelin-stained section from the lumbar spinal cord from a chronic case of tropical spastic paraparesis (TSP). There is pallor of myelin staining, which is also accompanied by axonal loss, in the lateral white matter columns. Inflammation in this case was relatively sparse. Weil stain for myelin.

strated in lymphocytic infiltrates in muscle but not in muscle fibers. Zidovudine therapy for AIDS is associated with a myopathy which is reversible on discontinuing the drug and which appears to damage mitochondria with development of 'ragged red' muscle fibers (90).

Pathogenesis of HIV-1 associated neuropathology

Understanding is still lacking for many aspects of the pathology of HIV-1 encephalitis, myelopathy and neuropathy. It is clear from studies of cerebrospinal fluid (CSF) that HIV-1 reaches the meninges if not the brain in most subjects at an early stage of HIV-1 infection but the relevance of this finding for the development of brain or spinal cord damage is unclear. Symptoms of CNS disease are not correlated with the amount of HIV-1 RNA detectable in CSF but are correlated with the amount of HIV-1 RNA detectable in serum and with the severity of depression of the blood CD4 lymphocyte count (15). This suggests that severe immunosuppression and high HIV-1 levels systemically are necessary conditions for the development of HIV-1 associated CNS damage. However, since not all terminal AIDS sufferers develop CNS disease these conditions cannot on their own be sufficient to cause CNS disease, and it seems likely that an additional requirement is the evolution of the virus. Infectious virus differs in culture requirements and genotype. Genetic variants of the original infecting virus develop in any individual under the stimulus of immune attack during the latent phase of the infection. In particular, the development of variants that are macrophage tropic may be important to the development of CNS disease, since macrophage lineage cells are overwhelmingly the most common site of detection of HIV-1 in the brain. Furthermore, the most likely mechanism for entry of HIV-1 into the brain is as an infection of monocytes traversing the blood brain barrier (37, 94). Since the quantity of HIV DNA in brain was not correlated with prospectively assessed measures of dementia in one recent study (46) it was suggested that the quality rather than the quantity of HIV-1 that is present may be more important for promoting the CNS damage that underlies dementia (46). Furthermore, certain amino-acid changes in the V3 Loop of the envelope protein appear more commonly in brain-derived DNA from neurologically affected than non-neurologically affected individuals (76). Thus, a macrophage tropic variant of the virus with distinctive envelope protein structure and severe immunosuppression late in AIDS are the likely requirements of HIV-1-induced CNS brain damage.

There remains considerable uncertainty over the means by which HIV-1 infected macrophages in the brain bring about the alterations in grey and white matter that have been found in HIV-encephalitis. Recent studies using the highly sensitive methods of polymerase chain reaction (PCR) and PCR - *in situ* hybridization have suggested that other cells in the CNS, particularly astrocytes, are subject to infection by HIV-1, albeit at a much lower level and with more highly restricted expression than macrophages (72, 81, 84, 95). The significance of such low-level infection still needs to be assessed. The much more abundant macrophage infection has been suggested to be capable of causing neuron and myelin damage via a number of different mechanisms. These have been largely investigated *in vitro* and the relative importance of each *in vivo* is not yet clear. Such mechanisms include synthesis and release of cytokines (97), release of soluble neurotoxic viral products such as the envelope protein gp120, and interference with production of growth factors (reviewed by 2, 59, 78).

The likelihood is that similar mechanisms to those operating in HIV-1 encephalitis are also responsible for HIV-associated myelopathy and neuropathy. The similarities between HIV-associated myelopathy and subacute combined degeneration of the cord have prompted speculation that metabolic factors such as local biotin deficiency, possibly exacerbated by its high local consumption by macrophages, may also play a part (92).

HIV-2 Infection

HIV-1 and HIV-2 viruses share approximately 40% of their genome in common. HIV-1 infections are far more widely distributed than HIV-2 which has mainly been described from West Africa. However, cases of HIV-2 infection have also been recently identified in India (8). In many regions of Africa facilities are not available for accurately distinguishing between HIV-1 and HIV-2 infections. The disease produced by HIV-2 closely resembles that produced by HIV-1, the main difference being that the natural

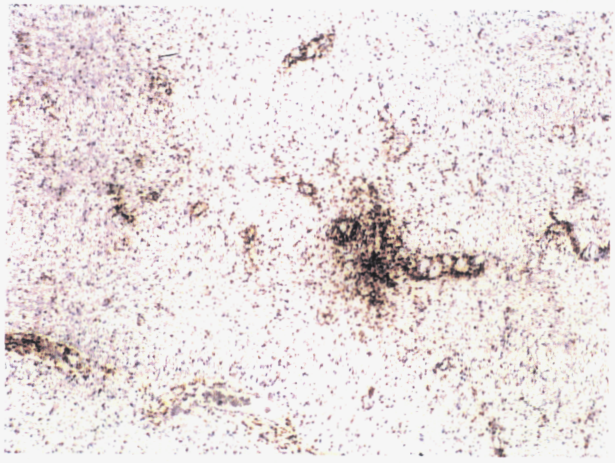

Figure 6. Low power view of the spinal cord inflammatory cell infiltrate in a case of TSP, shown here with a reaction for leucocyte common antigen (LCA). The inflammation is both perivascular and parenchymal in distribution. Counterstained with hematoxylin. (Section prepared by Dr CS Morris).

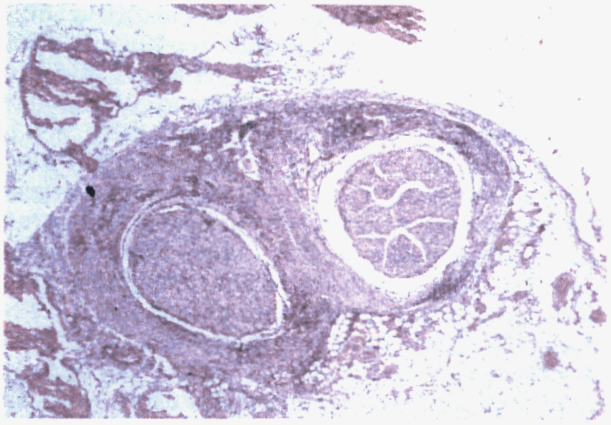

Figure 7. Fibrosis and inflammation in the perineurium surrounding these spinal nerve roots from a case of TSP. H & E stain.

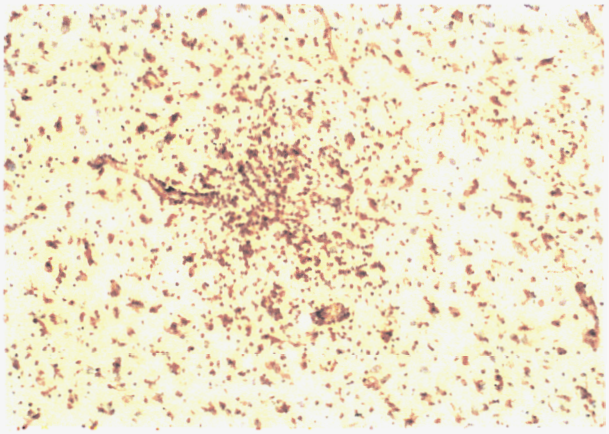

Figure 8. Exceptionally severe inflammatory lesion in the claustrum from the case of TSP illustrated in Fig 6. Reactive microglial cells and macrophages demonstrated by use of the lectin Ricinis Communis agglutinin 1. Counterstained with hematoxylin. (Section prepared by Dr CS Morris).

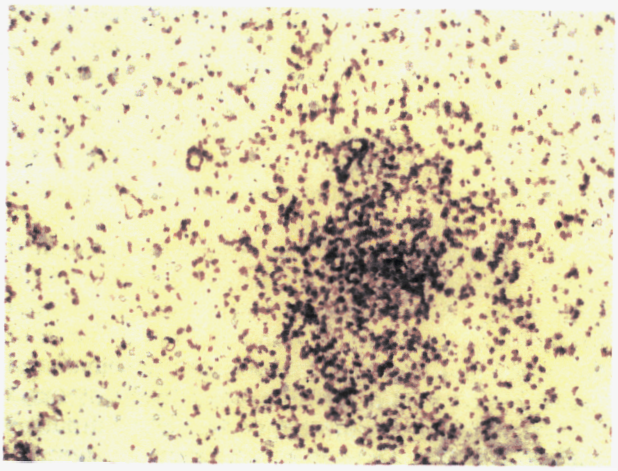

Figure 9. Lymphocytic infiltrate inthe same lesion as that shown in Fig 8, demonstrated with LCA antibody. Counterstained with hematoxylin. (Section prepared by Dr CS Morris).

history of HIV-2 infection seems to be rather more prolonged (102). An autopsy study from Africa suggested that multinucleated giant cell encephalitis is more common there in HIV-2 infection than in HIV-1 infection. A possible explanation for this difference is that the prolonged natural history of HIV-2 infections allows time for the encephalitis to develop (60).

HTLV-1 Infection

HTLV-1 is a human retrovirus found in many parts of the world, particularly in southern Japan, the Caribbean, Africa and South America. In some parts of these areas that harbor endemic virus the seroprevalence rate can be as high as 30% with the majority of those infected being totally asymptomatic (41). However, in about 3% of those infected an aggressive T cell leukemia develops and in about 0.25% of those infected a paralytic disease, tropical spastic paraparesis (TSP), also known as HTLV-1-associated myelopathy (HAM), develops. The incubation period between infection and development of TSP, when known, can vary from months to many years. Patients with TSP have high serum antibody titres to HTLV-1 - a feature that first drew attention to the possible link between HTLV-1 and TSP (28). Clinically TSP is characterized by slowly progressive spastic weakness of the lower limbs, sensory disturbances and difficulties with bladder control.

Pathologically, the most striking feature of TSP is a myelopathy which affects the white and grey matter of the cord, particularly at thoracic and lumbar levels (Fig 5). The pathology varies depending to some extent on the length of history with a well developed inflammatory response being seen in the spinal cord, leptomeninges and spinal roots in cases with shorter histories, and less inflammatory, more gliotic spinal cord lesions and fibrotic meningeal and nerve root lesions in those with a very long history.

The inflammation affects both grey and white matter of the cord and consists of perivascular and more widespread lymphocyte and macrophage infiltration (Fig 6) accompanied by increased numbers of MHC Class II - expressing microglial cells and astrocytes. The majority of lymphocytes are CD8 T cells. The inflammatory lesions extend into the proximal nerve roots and leptomeninges around the cord (Fig 7). HTLV-1 p19 core protein has been demonstrated in spinal cord and proviral DNA has been detected in infiltrating lymphocytes (34, 66) and in occasional astrocytes (53) but not in neurons. White matter of the lateral and posterior columns of the spinal cord shows loss of myelin and to a lesser extent of axons (Fig 5). Similar inflammatory and demyelinating lesions may also be seen in the brain occasionally (Figs 8 and 9) and in the optic nerves. The meningeal inflammatory infiltrate extends to the meninges around the brain stem not uncommonly.

In attempting to explain why a tiny fraction of subjects infected with HTLV-1 should develop this progressive neurological disease studies have been performed on peripheral blood lymphocytes infected with the virus and on the host immune response to the virus. It seems to be generally accepted that TSP sufferers have a higher frequency of CD8 + HTLV-1-specific cytotoxic lymphocyte responses in both blood and CSF than do healthy carriers of the virus (18, 40, 48). There is also a greater amount of HTLV-1 proviral DNA detectable in peripheral blood lymphocytes of TSP subjects than in healthy carriers (52). The evidence suggests that HTLV-1 is present in the spinal cord in TSP and that a brisk immune response to it takes place locally possibly damaging the spinal cord indirectly through release of cytokines and directly by cytotoxic killing of neuroglial cells harboring the virus. At present there is no clear evidence for a specifically neurotropic strain of virus (7).

HTLV-1 infection is associated with the development of polymyositis as well as TSP. In polymyositis, which clinically resembles the same disease in uninfected individuals and is thought to be immunologically mediated, HTLV-1;virus has been detected in the infiltrating lymphocytes in muscle but not in muscle fibers themselves (57).

Polio

Polio viruses are members of the picornavirus family, part of the enterovirus group of human pathogenic viruses, the group of viruses that most frequently infects the central nervous system in the form of aseptic meningitis. Polio virus, a single-stranded RNA virus, which occurs as three different serological subtypes, has long been recognized to be capable of causing paralytic disease in a small minority of subjects whom it infects. Initially described as a sporadic disease in young children this pattern of disease gave way to epidemics of infection particularly in late summer and autumn in Western countries in the early part of this century. Infection occurs by the fecal-oral route and in the 1-2% in which paralytic disease develops this takes the form of an initially non-specific febrile illness with sore throat and headache followed by back pain, stiffness, vomiting and muscle weakness. Limb involvement is often asymmetric and in a minority of subjects bulbar and respiratory muscles are also involved.

This form of disease carries a high mortality rate. Whilst paralytic polio has become exceedingly rare in Western countries as a result of effective immunization policies, it still remains relatively common in less developed countries of the tropics (38).

The usual site of infection with polio virus is the gastrointestinal tract and its associated lymphoid tissue. Confined to this region, the infection is commonly subclinical or trivial. How polio virus reaches the CNS to infect and kill motor neurons remains poorly understood. Early experimental studies in monkeys showed that a phase of viraemia was involved and that introduction of circulating antibody could prevent the infection reaching the CNS (11). A wealth of anecdotal and systematic evidence has recorded an excess susceptibility to paralysis due to polio in muscles that have recently been strenuously exercised, injured or injected (35, 106). Experiments on infection in monkeys some decades ago demonstrated the capacity of the virus to spread along axonal pathways (12). When these observations are coupled with the recent finding that polio virus receptors are present at neuromuscular junctions (56) a likely route for virus to take from blood to CNS would seem to be via muscle (particularly mildly inflamed muscle) and motor axons to spinal cord anterior horn cells. Such a route is supported by recent experiments in transgenic mice expressing human polio virus receptor which could be shown to have the development of paralysis after intramuscular inoculation with polio virus aborted by section of the sciatic nerve (82). The earlier explanation put forward to account for the enhanced susceptibility of injured muscles to paralysis was the unconvincing one that the blood supply to motor neurons innervating these muscles was increased (70). According to this hypothesis the selective vulnerability of motor neurons was assumed to be due to expression of polio virus receptors being confined to motor neurons. However, this has not been found to be the case. The polio virus receptor has now been identified, cloned and sequenced and found to be a member of the immunoglobulin superfamily (65). Its function other than as a polio virus receptor is not known. Northern blot analysis has indicated that its mRNA can be found in many human tissues in addition to brain (49) and the protein has likewise been detected in a wide variety of tissues (26). Immunocytochemical analysis suggests that the receptor is present in the brain not only in motor

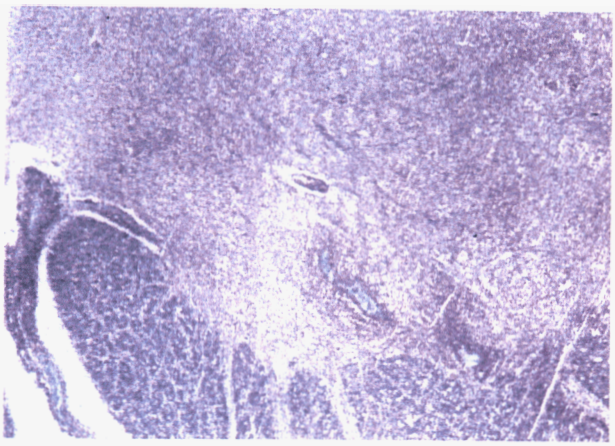

Figure 10. Intense inflammatory cell infiltrates in the anterior horn of the spinal cord in a case of acute polio. Luxol fast blue/cresyl violet-stain.

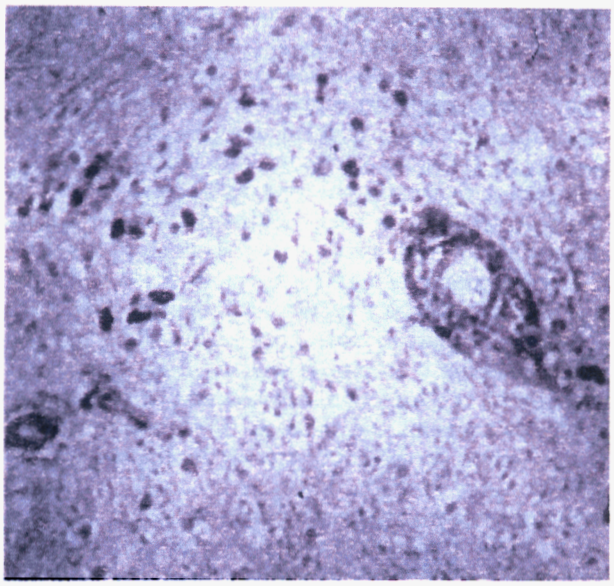

Figure 11. Perivacular and parenchymal IgG-containing plasma cells in the brain stem of a case of bulbar polio. Counterstained with hematoxylin.

cortex and cerebellum (known to be susceptible to infection by polio viruses) but also in olfactory bulb (thought to be refractory to infection) (25). Thus, the distribution of receptors, as recently analysed, does not readily explain the selective susceptibility of CNS cells to infection by polio virus, and this underlines the need to examine alternative explanations such as the route via muscle suggested above.

In the acute phase, polio is characterized pathologically by congestion and a severe inflammatory reaction concentrated particularly on the anterior horns of the spinal cord and, in cases with bulbar symptoms, in motor nuclei of the brain stem. Motor neurons show swelling or necrosis, accompanied by neuronophagia (Fig 10). A little later a severe loss of motor neurons at affected cord levels is detected, with continuing severe inflammation and microglial nodules. By 4-6 weeks following onset of symptoms many plasma cells can also be seen in the same areas (Fig 11) (19). The leptomeninges also participate in the inflammatory process. In the brain inflammatory cell infiltrates are not confined to motor cortex but may also be seen in the pontine and medullary reticular formation, midbrain, deep grey matter of the cerebral hemispheres, and cortex or dentate nuclei of the cerebellum.

Recent studies using *in situ* reverse transcriptase PCR of archival material have shown that polio virus RNA is present exclusively in motor neurons in the spinal cord anterior horns of subjects dying at the time of the acute phase of the disease (39), confirming earlier similar findings using immunocytochemistry and in situ hybridization in experimentally infected monkeys (16, 35).

In subjects dying after an attack of acute polio the inflammation has usually subsided and the sites of damage are marked by anterior horns largely devoid of motor neurons at levels corresponding to sites of origin of the innervation of severely paralyzed muscles and with increased numbers of glial cells in their place. In severe cases the whole anterior half of the cord tends to be severely atrophied and anterior roots wasted. Recent studies undertaken in the context of increasing recognition of a post polio syndromes in which further muscle fatigue and weakness may develop many years after an attack of acute polios have revealed persistent perivascular inflammation in some cases (47). Experiments to examine the possibility that polio virus may persist long after an attack of acute polio have produced suggestive evidence that it may, based on some positive PCR studies of CSF from post-polio syndrome patients (55, 58, 68). The significance of this recent finding for the development of the post polio syndrome remains to be assessed.

Rabies

Rabies is a unique CNS viral infection of human and other mammals due to infection with rabies virus, the most prominent of the lyssa viruses which are members of the rhabdo (rod-shaped) virus family. The virus has a bullet shape, being rounded at one end and flat at the other. It is an enveloped virus with a protruding surface transmembrane G glycoprotein, which is a target for immune responses, and a matrix protein lining the inner surface of the envelope. The matrix protein differs between different rabies virus strains whereas the G protein is shared in common. The envelope encloses the nucleocapsids or infectious components of the virus. They contain a single-stranded RNA genome coding for envelope and additional proteins including the RNA polymerase required for its own transcription.

Rabies virus is capable of infecting a wide variety of hosts, including man, though human infection can be regarded as incidental to infection of other

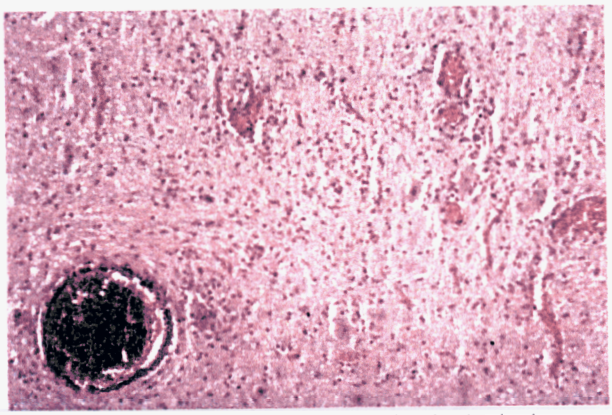

Figure 12. Congestion and inflammation in the brain stem from a case of rabies. H & E stain.

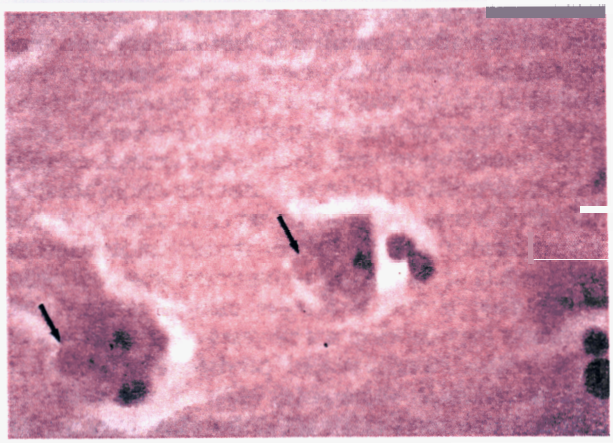

Figure 13. Negri bodies (arrows), eosinophilic rounded bodies in perivascular cytoplasm of two hippocampal pyramidal neurons from a case of rabies. H & E stain.

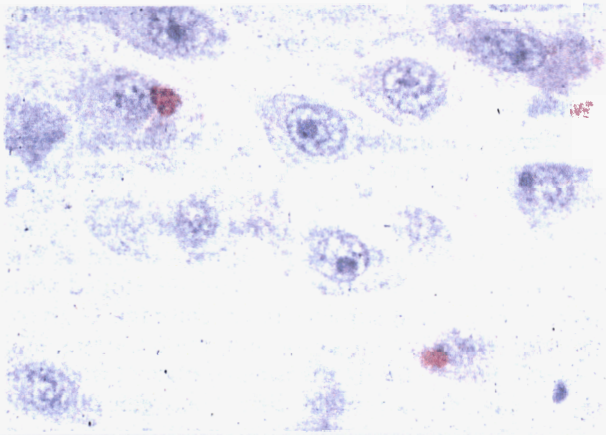

Figure 14. Immmunoreaction for rabies nucleocapid antigen in hippocampal pyramidal cells from a case of rabies. Counterstained with hematoxylin. (Photograph courtesy of Prof G Gosztonyi).

wild and domestic mammals. There are a few island communities that have eliminated the risk of rabies by quarantine procedures that exclude infected animals from their territory. Elsewhere, in different geo-

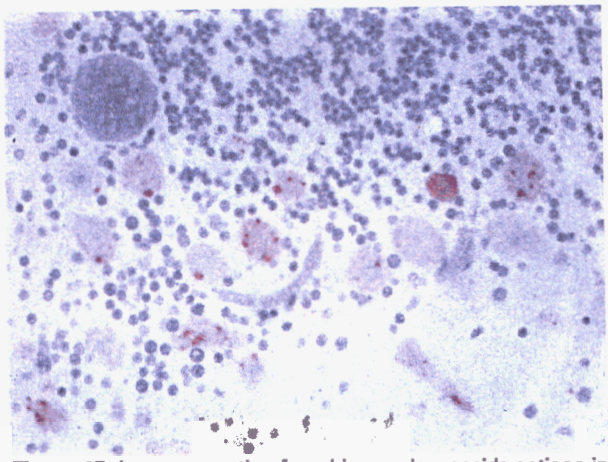

Figure 15. Immunoreaction for rabies nucleocapsids antigen in cerebellar Purkinje cells from a case of rabies. Counterstained with hematoxylin (photograph courtesy of Prof G Gosztonyi).

graphical regions of the world the threat of human infection is associated with infection in different groups of animals which act as reservoirs for the disease. For example, in India and South-East Asia the main threat is from infected dogs, in the USA from skunks, in Europe from foxes, in the central plateau of South Africa from the yellow mongoose, and in South America from bats. Infected animals start to secrete the virus in saliva a few days before developing symptoms and may continue to do so throughout the symptomatic period and beyond. Infection is usually spread by bite from an infected animal, whose tendency to bite is enhanced by aggression induced by the infection, but sometimes licking will suffice to transmit the virus or, if the source is bats, there may be no direct contact at all required for the disease to be spread. Many thousands of human infections occur world-wide each year (67).

Following infection due to a bite an unusually variable, often long, incubation period ensues before symptoms in the human develop. The modal length for such an incubation period is 40 days but it tends to be shorter in children or if the bite was on the head or neck. First symptoms are non-specific, consisting of fever, headache and nausea but there may also be paraesthesiae at the site of the bite. These are followed by meningism, convulsions, unwillingness to swallow due to a tendency to develop pharyngeal spasms, agitation and eventual coma, cardio-respiratory disturbance and death. The mortality rate in untreated infection with no prophylactic intervention is 100%.

Experimental infection following intramuscular injection of virus has clarified the events leading to CNS infection (69) although the reason for the prolonged incubation period is not well understood. The virus proliferates initially locally after being taken into muscle fibers probably by receptors provided by the acetylcholine receptors at motor end plates (54). From this site it spreads via axons to the

Summary of pathological features in various forms of arbovirus encephalitis (reproduced from Ref. 13, with permission)

Disease	Glial nodules	Perivascular infiltrate	Foci of rarefaction necrosis without inflammation	Meningeal infiltrate	Sites of most severe damage	Vasculitis	Foci of tissue necrosis with inflammation	Neuronophagia	White matter lesions	References
EEE	Yes	Neurophils, mononuclear	Yes (may be late calcification)	Neurophils, mononuclear	Basal ganglia, substantia nigra, cerebral cortex, hippocampus	Yes, with thrombi	Many, especially in grey matter	Yes, neurophils, macrophages	Only near affected grey matter	5, 12, 22, 38
WEE	Yes	Mononuclear	Yes	Sparse, mononuclear	Striatum, thalamus, cerebral and cerebellar cortex, base of pons	Not usually	No	Yes, macrophages	No	2, 7, 12, 22, 25, 26, 37, 38
VEE	Yes	Sparse, mononuclear	No	Sparse, mononuclear	Putamen	No	No	Rare	No	12, 22, 28, 35
SLE	Yes	Mononuclear	Rare	Mononuclear	Thalamus, basal ganglia, brain stem, especially midbrain	No	No	Rare	Mild inflammation	4, 12, 18, 19, 22, 29, 31, 34, 36
JBE	Yes	Neurophils only in fulminating cases. Mononuclear	Yes (may be late calcification)	Mononuclear	Thalamus, substantia nigra, cerebral cortex, hippocampus, cerebellar cortex, spinal cord	No	Yes (may be late calcification(	Yes	Only near affected grey matter	12-16, 21, 22, 32, 39, 40
MVE	Yes	Neurophils only in fulminating cases. Mononuclear	Yes (may be late calcification)	Mononuclear	Cerebral and cerebellar cortex, basal ganglia, brain stem	No	Yes	Rare	No	1, 8, 22, 27, 30
TBE	Yes	Mononuclear	Yes	Mononuclear	Upper spinal cord, brain stem, cerebellum, thalamus, basal ganglia	Yes	Yes	Yes	No	3, 6, 9-12, 17, 20, 22, 25, 33
CalE	Yes	Mononuclear	No	Mononuclear	Cerebral cortex, basal ganglia, thalamus, mid-brain, pons	Yes	Few	Yes	No	16

Table 1. EEE: Eastern equine encephalitis; WEE: Western equine encephalitis; VEE: Venezuelan equine encephalitis; SLE: St Louis encephalitis; JBE: Japanese ß encephalitis; MVE: Murray Valley encephalitis; TBE: tick-borne encephalitis; CalE: Californian encephalitis.

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spinal cord. Within the axons the virus is transported as a nucleocapsid, without its envelope (93). Having reached the neuronal perikaryon virus proliferation proceeds. Nucleocapsids are initially formed and later acquire their envelopes at sites on internal cell cisternal membrane or at the cell surface. From the spinal cord, infection spreads rapidly via axonal pathways to all parts of the CNS and, eventually, as enveloped particles at this stage, back into the peripheral nerves. One method of diagnosing the disease is to perform immunofluorescence on sections of skin in which dermal nerves can be seen to harbor the virus. This spread back into the periphery allows infection of salivary glands to occur, hence affording opportunity for spread of virus in saliva. Infection of limbic areas of the brain is said to generate altered behavior with aggression and a tendency to bite. A myocarditis also not uncommonly develops, and this may be fatal. At no stage does a viraemia develop and an immune response is not generated until the infection reaches the CNS.

The macroscopic appearances of the brain in rabies are not diagnostic consisting only of swelling and severe congestion. The distinctive changes are present on the microscopy. There is a variable amount of inflammation concentrated mainly in grey matter with perivascular lymphocyte, macrophage and plasma cell cuffing, neuronophagia and microglial nodules (Fig 12). The most severely affected regions are the brain stem and spinal cord. In some cases the inflammation is not conspicuous. The most useful diagnostic feature is the presence of rabies virus antigen which is distributed widely in the brain and can be suspected from routinely stained sections by the presence of Negri bodies, intracytoplasmic, rounded eosinophilic inclusions which are most prominent in large cells such as Purkinje cells, large cortical and hippocampal pyra-

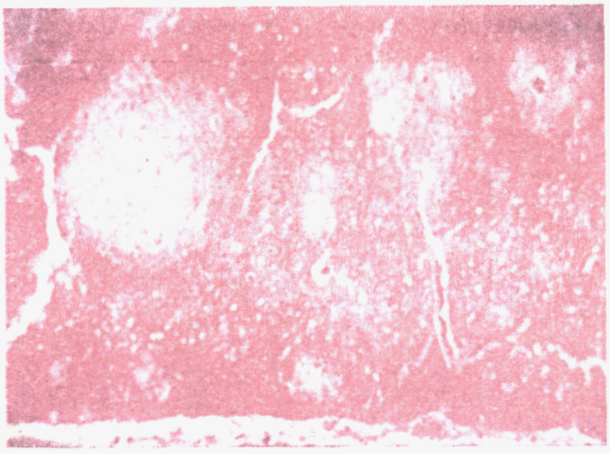

Figure 16. Foci of acellular necrosis in the cerebral cortex from a case of Japanese B encephalitis. H & E stain.

midal cells and spinal cord neurons (Fig 13). Ultrastructural studies have revealed Negri bodies to be ring-shaped aggregates of nucleocapsids (31), and they react immunocytochemically for rabies nucleocapsid antigen (Figs 14 and 15).

There are several puzzling aspects about the nature of rabies infection. One of these concerns the long incubation period. Another is the lack of an explanation for fatal brain stem involvement. This is not readily explicable solely on the basis of the distribution of the virus, for this is much more widespread and does not necessarily give rise to neuronal lysis at other sites. In tissue culture the virus is not in fact highly lytic. The disastrous consequences for the CNS of rabies infection may instead lie in the interference it produces to normal neurotransmitter regulation. Thus, binding capacity of acetylcholine receptors in rat brain was found to be reduced twofold by rabies infection (50), and rabies-infected neuroblastoma cells were shown to have a reduced affinity of opiate receptors and major disruption of aspects of cell metabolism controlled by opiate receptors (51). Another mechanism of neuronal damage that has been proposed is stimulation by rabies virus of production of nitric oxide by inducible nitric oxide synthase (23).

There is a further pattern of rabies pathology rather different from that described above in which the most severe pathology is found in the spinal cord. In this form of the disease, symptoms are predominantly those of an ascending paralysis resembling Guillain-Barré syndrome (14). About 10-20% of cases are of this type. The pathology in this form consists of inflammation, microglial nodules and neuronophagia in the anterior horns of the spinal cord and in the dorsal root ganglia with some extension of this pathology into the brain stem. The spinal leptomeninges are also inflamed. Negri bodies can usually be found in Purkinje cells and hippocampal neurons, as in the more common form of the disease.

Diagnosis during life depends on isolation of virus from saliva, demonstration of viral antigen in corneal cells or in nuchal dermal nerves, around hair follicles, or demonstration of rising serum and/or cerebrospinal fluid titres of anti-rabies antibody. Immunoperoxidase techniques can be used to detect virus in formalin fixed tissue (67). Post-exposure prophylactic immunization and administration of hyperimmune globulin can be provided before development of clinical symptoms and safe pre-exposure immunization is available to those at special risk. It needs to be emphasized that the possibility of rabies infection may be overlooked if a high level of clinical suspicion is not maintained. The diagnosis is not uncommonly made on post mortem examination, when again, the diagnosis may be missed if there is no history of exposure to rabies and a careful examination of the brain with this diagnosis in mind is not made.

Arthropod-borne (Arbo) Viruses

Arbovirus encephalitis (96) is an important cause of mortality and morbidity in many tropical regions of the world where the tendency of these viruses is to cause epidemics (13, 44, 104). The viruses responsible for these diseases are heterogeneous and classified among four main virus groups: the alpha virus subgroup of togaviruses (86) (responsible for Eastern, Western and Venezuelan equine encephalitis (VEE)), the flavivirus subgroup of togaviruses (responsible for St Louis, Japanese B (45) and Murray Valley encephalitides), bunyaviruses (responsible for California and La Crosse encephalitis) and a reovirus (responsible for Colorado tick fever encephalitis (43)). Many of these viruses circulate between mosquitoes and birds or mosquitoes and horses or small mammals and others between ticks and birds or small mammals. The viruses multiply both in the vertebrate and insect stages of their infectious cycles. Humans are incidentally infected, particularly under conditions in which insect breeding is prolific - conditions that were, for example, fulfilled before a recent epidemic of VEE after an absence of such epidemics for 20 years (99). Most cases of infection are subclinical but a minority of people (varying from around 1/100 to 1/1000) experience an acute, febrile illness of varying severity which may or may not be complicated by neurological involvement. Vaccines are available in some countries with populations at risk for arbovirus encephalitis but in other countries many thousands of cases continue to occur each year (104). Abortions and stillbirths are increased during and shortly after epidemics and virus may be recoverable from the brains of aborted fetuses (99).

The diseases are ushered in clinically with nonspecific flu-like symptoms lasting three or four days. This is quickly followed or simultaneously accompanied by features of neurological involvement, particularly seizures, confusion, drowsiness and

Main groups of rickettsial infections (adapted from ref 98)

Disease	Agent	Distribution	Transmission to man
Rocky Mountain spotted fever	R. rickettsii	N Central and S America	Tick bite
Boutonneuse fever	R. conorii	Mediterranean, Africa, Asia	Tick bite
North Asia tick typhus	R. sibirica	Russia, China, Mongolia	Tick bite
Oriental spotted fever	R. Japonica	Japan	Tick bite
Queensland tick typhus	R. australis	E Australia	Tick bite
Rickettsial pox	R. akari	USA, Ukraine, Croatia, possibly worldwide	Mite bite
Typhus fevers			
Sylvate typhus	R. prowazeki	USA	Flea of flying squirrels to man
Epidemic typhus	R. prowazeki	S America, Asia, Central America, Mexico	Louse feces scratched into skin
Recrudescent typhus	R. prowazeki	Worldwide	None (re-activation of previous infection)
Murine typhus	R. typhi	Worldwide predominantly tropical and subtropical	Flea feces scratched into skin, rubbed into conjunctiva or inhaled
Scrub typhus	R. tsutsugamushi	Japan, S and E Asia, N Australia, islands of Western and Southwestern Pacific	Chigger bite

Table 2.

meningism.

The pathological findings in fatal cases of arbovirus encephalitis have many features in common though there are also some important differences (Table 1). The viruses probably proliferate initially at local inoculation sites and are then spread in the blood to the brain and other organs. In the brain, virus infects neurons and, at least for some viruses, endothelial cells. Viral antigen can be detected in neurons early in the course of infection (83). Perivascular infiltrates of predominantly mononuclear inflammatory cells occur throughout the brain but with some predilection for grey matter in most forms of the disease, and for deep cerebral grey matter, cerebral cortex, hippocampus, substantia nigra and cerebellar cortex in particular (Table 1). The spinal cord may also be involved in some cases. Foci of acellular necrosis may be prominent (Fig 16) and a vasculitis with endothelial cell swelling or necrosis of small vessel walls and fibrin deposition in their lumen is seen. Neuronophagia, microglial nodules, astrocytes and patchy neuronal loss are common, but inclusion bodies are not seen. The leptomeninges participate in the inflammatory reaction. In experimental studies the immune response is important in controlling mortality for some viruses, eg, louping ill (107) but less important for others, eg Western and Eastern equine encephalitis (107), Sindbus virus (44) and Semliki Forest virus (100). Other factors such as age of host and strain of virus and its replication rate are also important determinants of disease severity (86).

Diagnosis depends on viral culture from serum or brain, or PCR analysis of such samples and on serological evidence of rising antibody titres during the course of the illness.

Rickettsial diseases

Rickettsial diseases are a heterogeneous group of infections caused by gram-negative bacterial microorganisms of different serological subtypes (Table 2). The organisms are transmitted to humans by insect vectors whose prevalence in particular locations and seasons is a strong determinant of human disease. Most of the different forms of rickettsial disease are found in tropical regions and are generally under-recognized in these countries (98). Apart from the presence of infected insect vectors the other most important factor determining risk of rickettsial disease is the social and living conditions of the local population. Wars, famines and refugee migrations are liable to lead to severe outbreaks of rickettsial disease. Some of these diseases are spread by infected saliva inoculated by a tick or mite bite and others by insect (lice, fleas) feces scratched into skin (Table 2).

These diseases are a cause of substantial illness particularly in developing countries. They present as acute, febrile illnesses, often accompanied by a rash, and, if undiagnosed and untreated, have significant mortality rates. Neurological involvement may be absent or amount only to mild photophobia, headache and drowsiness in mild or moderately severe cases but is more marked in severe cases in which there may be confusion, coma, epilepsy, ataxia or focal neurological signs. A pleocytosis in CSF is sometimes present. The diseases are divided into different groups on the basis of epidemiology, mode of transmission and clinical features (98). However, they all share similar neuropathological features, though these vary in severity from one group to another. The neuropathology was reviewed by Manuelidis and Krigman (62).

After entry of the organisms through the skin they are spread to all parts of the body in the blood. They attach to endothelial cells, mainly in small blood vessels, and are taken into these cells by endocytosis. They proliferate in the cytosol until the cell bursts, allowing spread to other cells, both endothelial and others, including white blood cells. Experimental studies suggest that cells are damaged by lipid peroxidation of their membranes by a combination of free radical production, protease activity and rickettsial phospholipase A_2 production (98). Host cytokines are produced and an immune response generated which leads to focal, mixed inflammatory infiltrates consisting predominantly of T lymphocytes but with some neutrophils and later macrophages admixed with them. These cells accu-

mulate around and within the walls of affected vessels which often include those of the leptomeninges and brain. Other organs are also affected including lungs, heart, skin, liver, pancreas and skeletal muscle. Some rickettsia generate a more severe reaction than others; thus, Rocky Mountain spotted fever is associated with more severe, necrotizing lesions than typhus fever. The organisms themselves can be seen in endothelial cells using an immunofluorescence or immunoperoxidase technique. Cytokine-activated nitric oxide production by host cells in experimentally infected mice appears to be crucial in overcoming infection. Depletion of β-interferon and tumor necrosis factor - α in these animals resulted in fatal overwhelming infection by a normally non-fatal dose of Rickettsia conorii (24). At the inflammatory foci that develop around infected endothelial cells in small blood vessels platelet plugs are liable to form but as these are usually non-occlusive they are not usually associated with microinfarcts. However, in severe cases microinfarcts may be seen at post mortem examination. More commonly there are only congestion and petechial hemorrhage formation to be seen in the leptomeninges and brain on macroscopic examination.

Laboratory diagnosis can be made in the acute phase of the diseases by skin biopsy of any rash and demonstration of rickettsiae by immunofluorescence or immunoperoxidase techniques. Immunostaining of white blood cells may also enable a diagnosis to be made. Retrospectively a diagnosis can be confirmed by demonstrating a four-fold rise in antibody titre to rickettsial antigens between acute and convalescent sera. Effective antibiotic treatment is available and should be given without awaiting laboratory confirmation of diagnosis.

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