Medical
PROGRESS

Refer to: Adams JM: Persistent or slow viral infections and related diseases (Medical Progress). West J Med 122:380-393, May 1975

Persistent or Slow Viral Infections and Related Diseases

JOHN M. ADAMS, MD, PhD, *Los Angeles*

The discovery of persistent transmissible agents by veterinarians has led to striking advances in the infectious cause of neuropathies of human beings. There is evidence for persisting infection in congenital rubella and the herpes group of viruses including cytomegalovirus infections. Hepatitis types A and B are candidates for inclusion in the category of persisting viral infections.

The rubeola or measles virus is established as a persistent virus which causes elevated antibodies in the serum and cerebrospinal fluid of many patients with severe demyelinating disease such as subacute sclerosing panencephalitis and multiple sclerosis. Elevated antibodies against vaccinia virus have been found in the cerebrospinal fluid of some patients with multiple sclerosis and neuromyelitis optica, a rare form of multiple sclerosis.

BOTH THE CONCEPT and the reality of slow virus infections was first brought to the attention of the scientific community by Sigurdsson,[1] who successfully isolated viral agents from Icelandic sheep afflicted with a progressive degenerative disease of the nervous system. He defined a slow virus infection as, "an infection of the host in which the agent continued to multiply, slowly producing progressive abnormality over the course of months and years, usually with a very long incubation period, and often with a localization of the infectious process to a single organ." The concept elaborated by Sigurdsson was a relatively strange one to biologists concerned with human diseases. However, it was recognized that rabies may have a long incubation period followed by an overwhelming and fatal illness; lymphocytic choriomeningitis and viral hepatitis also seem to persist causing subacute disease.

Studies by Hotchin[2] on lymphocytic choriomeningitis (LCM) virus infection in mice showed that animals which had been inoculated at birth could suffer from a long continued infection despite high titers of virus. This persistent tolerant infection (PTI) leads, after approximately ten months, to a debilitating fatal disease; in the infected mice the situation is unique in that the LCM virus infection is continuously active at a high level during the life span of the infected animal. In ordinary latency, the level of virus in the in-

From the Department of Pediatrics, University of California, Los Angeles, Center for the Health Sciences, Los Angeles; and Rancho Los Amigos Hospital, Downey.

Some of the investigations reported here were supported in part by Grant #R01-NS09024-04 and various donors, particularly Dorothy and Milton Hart, Joan and Forrest Adams and John M. Adams, Jr.

Reprint requests to: J. M. Adams, MD, PhD, Department of Pediatrics, UCLA Center for the Health Sciences, Los Angeles, CA 90024.

ABBREVIATIONS USED IN TEXT

ALS = amyotrophic lateral sclerosis
CNS = central nervous system
CSF = cerebral spinal fluid
EBV = Epstein-Barr virus
HAA = hepatitis associated antigen
HB = hepatitis B antigen
HI = hemagglutination-inhibition (test)
HSV = herpes simplex virus
Ig = immunoglobulin
IH = infectious hepatitis
IM = infectious mononucleosis
LCM = lymphocytic choriomeningitis
ME = measles encephalitis
MS = multiple sclerosis
NO = neuromyelitis optica
PTI = persistent tolerant infection
SH = serum hepatitis
SSPE = subacute sclerosing panencephalitis
V-Ab = virus antigen-antiviral antibody

fected animal is low or minimal and, indeed, evidence of active virus is often undetectable. In persistent tolerant state, in contrast, virus titer is high, comparable to titers found at the peak of acute viral illness.

PTI also differs in that in PTI infection there is very little antibody. Mice, in which PTI has been induced with certain strains of LCM virus, exhibit a slow virus infection after an incubation period of approximately ten months. After ten months, the mortality rises sharply, reaching approximately 90 percent by 20 months after inoculation, much higher than in control groups. LCM virus is, therefore, an example of a persistent virus disease which results only if the agent is introduced neonatally with consequent production of immunologic tolerance.

Rubella viremia in man might qualify as a persistent tolerant infection since the virus can persist in human infants for 12 to 18 months before the immune system of the infected child is finally able to eliminate it.

The following tentative conclusions have been reached by Hotchin[2] concerning the general properties of slow viruses: (1) they actively multiply to high titers in the host for long periods of time without apparent disease; (2) these viruses are not readily recognized as foreign by the host; (3) an immune reaction to these viral agents is either very late or it does not occur at all; (4) the disease produced may be an autoimmune response caused by termination of tolerance, although the final disease may be a gradual process of erosion of the affected cells by the virus.

Because the host finds great difficulty in recognizing these viruses as foreign, the PTI state is a fundamental prerequisite for slow virus disease. Hotchin's[2] hypothesis of slow virus pathogenesis can be summarized as follows: Slow viruses multiply for a long period of time in the host with access to virtually all types of cells. Infection of certain cell types is followed after a long period by nonmalignant transformation (or other change) of these cells which possibly aided by immune attack, results in loss of function. When the functional reserves are exhausted, clinical disease ensues.

In a recent report Hotchin and associates[3] recorded infection of hospital personnel by lymphocytic choriomeningitis virus, the source of which was shown to be a colony of Syrian hamsters. In persons working with the research colony, acute febrile illnesses developed, some severe. They conclude that the outbreak emphasizes the dangers of working with experimental hamsters. Commercially distributed hamsters have been found to carry LCM virus and have caused human infections. As pointed out previously by Hotchin[2] the LCM virus represents a model for the study of persistent or slow virus infection.

In several chronic viral infections of animals, infectious virus travels in the circulation complexes to host immunoglobulin (Ig), presumably antiviral antibody. According to Oldstone and Dixon[4] virus antigen-antiviral antibody (V-Ab) complexes are competent pathogenic agents and V-Ab immune complex disease probably occurs frequently in chronic viral infections.

The above authors state that, clearly, generalized viral infection with viremia may provide viral antigens which form circulating complexes with host antiviral antibody and complement. They conclude that this form of disease is neither truly a viral nor autoimmune but rather an immune complex disease.

Notkins[5] has proposed the theory that viruses induce immunopathologic lesions by the following mechanism: (1) the virus is a foreign antigen, (2) it is a self-replicating agent and thus can produce a continuous supply of antigen over a long period of time and (3) certain viruses can induce new antigens on the surface of infected cells. These properties may initiate immunopathologic changes through several mechanisms, one of which is to infect the cells of the immune system and thereby produce immunologic derangements.

Studies reported by Notkins[5] showed that herpes simplex virus, vaccinia, influenza and Newcastle disease virus all induced new antigens on the surface of infected cells; the interaction of specific antiviral antibody and complement with these antigens resulted in cell injury. He further suggests that immunological injury may be associated with a variety of acute, recurrent, and persistent viral infections.

Thus, one of the most perplexing aspects of the pathogenesis of persistent virus infections has been the long interval between the initial exposure and the eventual expression of the disease.

Scrapie

Scrapie in sheep (the name refers to compulsive rubbing and scraping against fixed objects) was recognized by farmers in Lincolnshire who petitioned Parliament in 1755 to make it illegal to mix distempered sheep with healthy animals. Affected animals suffer from progressive ataxia, especially in the hind legs, and collapse. Typically, the disease affects sheep at about four years of age and is fatal in a few weeks or months. Sheep which are infected naturally with scrapie virus have an asymptomatic incubation period of three to five years. In the animals infected, there is no febrile phase and there are no changes in cerebrospinal fluid. Lesions are limited to the central nervous system and are concentrated in the cerebellum, where widespread neuronal degeneration and prominent hypertrophy and proliferation of astrocytes are typical (Figure 1). These neuropathological changes differ from those customarily seen in viral infections of the central nervous system.

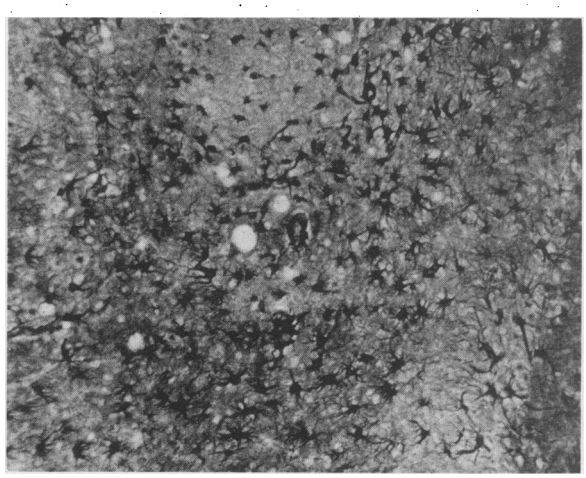

Figure 1.—A photomicrograph of the hippocampus of a scrapie affected rat showing pronounced hypertrophy and proliferation of astrocytes (Cajal reduced from × 200 approx.). (Picture was provided by I. H. Pattison and published by the Journal of the Royal College of Physicians of London, Vol. 1, 93-98, 1966.)

Experimental studies with scrapie have failed to show evidence of an agent-producing disease in primates including chimpanzees. Transmission studies in sheep indicate a very lengthy incubation period extending from four months to four years. However, the passage of scrapie agent to sheep can be continued indefinitely. The agent has been detected in most tissues including brain, spinal cord, pituitary gland, adrenal gland, salivary glands, spleen, liver and lymphatic glands.

The disease is always fatal. Although the agent is filterable and apparently self-replicating, no one has as yet been able to demonstrate it by tissue culture, serology or electron microscopy. Experimental studies with scrapie agent indicate that it has properties that are generally associated with viruses; however, it is remarkably resistant to a number of physical and chemical treatments that destroy conventional viruses.[6] The possibility that it may not contain nucleic acid suggests that a hitherto unrecognized agent may be involved. Its striking similarity to Kuru in man has stimulated great interest in its applicability to other slowly progressive degenerative diseases of unknown cause in both man and animals.

Visna

Visna—a chronic, paralytic and fatal disease of sheep—was the first disease in which Sigurdsson[1] was successful in isolating a persistent virus. The disease is insidious on onset; the earliest symptom is a slight abnormality of gait. The hind limbs become involved and total paralysis gradually occurs. In spite of being unable to rise, the animals live for a considerable length of time after total paralysis, retaining full consciousness. Blindness has been observed in a few cases. The temperature is normal or only slightly increased; the period from onset of first signs of visna to complete paralysis may vary from several months to several years. Remissions are rare and the disease is inevitably fatal.

Lesions in the brain and spinal cord are detectable only by microscopic examination (perivascular infiltration of lymphocytes, profound and widespread demyelination of the white matter around the ventricles, cerebrum and cerebellum, and in the white fibers of the spinal cord).

Sigurdsson[1] transmitted the disease from brains of infected animals and observed that several

months or even years elapsed before any outward signs of illness appeared in the inoculated animals. Signs then developed that were identical with those observed in the natural disease. About one to two months after inoculation but before signs of visna appeared, increased cells were present in the spinal fluid. Cells in the spinal fluid are common in the natural disease and failed to occur in sheep inoculated with normal sheep brain or with other central nervous system diseases. Antibodies developed which were comparable to those found in the natural disease.

In 1957, the virus was isolated in tissue cultures from visna infected brain.[6] In an experiment in which four sheep were inoculated by the pulmonary route they showed no signs of spinal fluid changes or histologic lesions in the brain and spinal cord. In three of the four, the virus apparently multiplied somewhere outside the nervous system since neutralizing antibodies appeared in the serum about 3 to 12 months after inoculation. After inoculation, visna virus may be isolated during the preclinical period from both blood and spinal fluid. Visna virus has been isolated from the blood in spite of high concentration of neutralizing antibodies in the serum.

The viruses of visna and of maedi (a chronic pulmonary disease of sheep) appear to be quite similar and have a close antigenic relationship. In tissue culture they produce a characteristic cytopathic effect which leads to complete cell destruction. Visna virus in tissue culture forms syncytia or multinucleated giant cells (Figure 2). The demyelination which occurs in visna-infected sheep is similar to certain postinfectious encephalitides in man. Other viruses associated with demyelination such as measles, mumps and canine distemper may also cause giant cell formation in tissue culture. Many of the pathological features —such as lymphocytic and microglial proliferation, perivascular infiltration and demyelination of white matter in addition to the occurrence of multinucleated giant cells—suggest similarities which have been observed in measles encephalitis and in some patients with multiple sclerosis.[7]

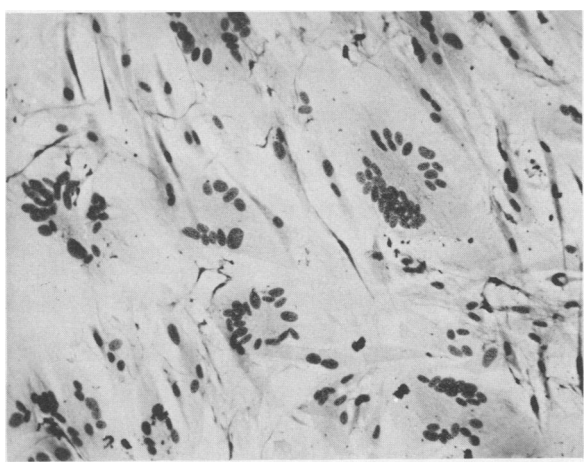

Figure 2.—A photomicrograph of visna virus showing cytopathic effect produced in sheep choroid plexus cells, stained with giemsa. (Picture was provided by Halldor Thormar, and published in Virology 14:463, 1961. Copyright is held by Academic Press.)

Visna antisera with a neutralizing titer of 1:256 failed to neutralize the measles virus, using the sensitive hemagglutination-inhibition (HI) test. Sera from 12 different sheep, considered to be normal and free of disease, likewise failed to reveal measles antibody when tested by the HI method. Indirect fluorescent antibody studies with measles and visna antisera likewise failed to show any relationship. (Unpublished data, J. M. Adams and H. Thormar)

Recently Narayan and associates[8] reported that in American lambs inoculated intracerebrally with visna virus, a persistent infection developed in which the pathologic changes were similar to the early lesions of visna in Icelandic sheep. They concluded that the disease in the experimental lambs might provide a possible model for the study of virus-induced demyelinating disease.

Mink Encephalopathy

On farms in Wisconsin, Washington and Idaho a slowly progressive locomotor incoordination of mink has been known for nearly 20 years. The encephalopathy is characterized by excitability and latent somnolence, rarely with convulsions. The illness is fatal in a few weeks to two months, and the pathologic findings are limited to the central nervous system with evidence of widespread neuronal degeneration, astrogliosis and spongy degeneration of the gray matter.[9]

Following an incubation period of five to ten months, the disease may be transmitted from mink to mink by subcutaneous and intraperitoneal inoculation of either brain or spleen suspensions. The agent is small, unusually resistant to ether and formaldehyde and apparently as resistant to heat as the agent of scrapie. Its similarity to scrapie has suggested that the disease may have spread from infected sheep to mink.

Slow virus infections in animals have not been limited to the central nervous system. For ex-

ample, Aleutian disease of minks is characterized by histiocytosis and bile-duct proliferation. Maedi, which involves the lungs, causes a chronic progressive pneumonia, having characteristics of a slow virus infection similar to visna, both occurring in sheep.

Kuru and Creutzfeldt-Jakob Disease

Studies of Sigurdsson[1] stimulated Gajdusek[10] to pursue the concept of slow virus infection particularly as it is related to a disease known as kuru, which was under investigation. This disease represented an exclusive involvement of the central nervous system in cannibalistic stone-age man in the high interior of New Guinea. Gajdusek[10] estimated that over half of the deaths among the 7,000 South Fore people of New Guinea could be accounted for by kuru.

The clinical features are predominantly cerebellar, including ataxia, disturbed balance and tremor; death occurs in most cases in a few months or less than one year. The patients themselves recognized difficulty in walking and coordination before detection of the disease by physical examination. Shivering was a characteristic symptom. Slurring of speech occurred early and finally resulted in inability to talk. Strabismus and jerky eye movements (but no true nystagmus) were present in children. Mentality appeared to remain normal, but the disease progressed in all patients to death, usually from thirst and starvation, infected ulcers and pneumonia. The pathologic changes in kuru are confined to the nervous system where they are widespread, characterized by a pronounced proliferation and hypertrophy of the astrocytes throughout the brain. Neuronal degeneration is most severe in the cerebellum and demyelination is minimal. Typical astroglosis is shown in Figure 1.

Since the recognition and beginning of investigations of kuru, the high mortality rate has declined significantly. The death rate in children has declined dramatically. Indeed, no child under 12 years of age has died of the disease in the most recent survey, no children are ill with kuru under 14 years of age. This is in striking contrast to the situation when kuru was first recognized; it was then primarily a disease of women and of children of both sexes. The sharply reduced mortality from kuru undoubtedly is related to the great reduction in cannibalism, and suggests virus contaminated brain—this organ considered a particular delicacy by the Fore people.

The clinical features and findings in kuru failed to suggest that the disease might be infectious; however, the successful experimental research with scrapie and visna stimulated Gajdusek and associates to pursue the possibility of a slow virus infection. Hadlow, in 1959, called attention to similarities between scrapie in sheep and the reported symptoms of kuru in man. It was apparent, however, that although transmission studies had been attempted with multiple sclerosis, amyotropic lateral sclerosis, parkinsonism, Schilder's disease and subacute sclerosing panencephalitis, no one had followed their experiments for an extended period of time. In 1966, Gajdusek and associates[11] at the National Institute of Health initiated long-term studies on primates inoculated with suspensions of frozen kuru brain from fatal cases. After incubation periods of 18 to 30 months, seven of eight chimpanzees developed a kuru-like syndrome. Incubation periods in second passage animals ranged from 10 to 18 months.

The duration of the disease did not vary at different passage levels, and remained remarkably similar to the human cases. Gajdusek, Gibbs and Alpers[12] concluded that kuru is a progressive degenerative disease of the central nervous system in man, experimentally transmissible only to the chimpanzee, in which the disease is remarkably similar to that observed in human victims. Kuru, undoubtedly, represents the first clear example of a chronic degenerative disorder of the central nervous system in man that is caused by a slow virus infection.

Gibbs and Gajdusek[13] reported that fatal spongiform encephalopathy occurred in four chimpanzees, 12 to 14 months after inoculation with suspensions of brain from four patients with Creutzfeldt-Jakob disease. Their successful transmission studies strongly indicate that the disease of these patients should be included with the virus infections which may be slow in nature, such as kuru, scrapie and mink encephalopathy. They were successful in passing the disease from chimpanzee to chimpanzee with the same long incubation period. Success was also obtained with an inoculum which had been stored at $-70°C$ ($-94°F$) for over two years.

All four original patients with Creutzfeldt-Jakob disease died with a similar fatal spongiform encephalopathy, characterized by unremitting and rapidly progressive brain disease with associated severe dementia, disturbances of vision, myoclonic jerks and ataxia. Transmission experiments have

now been successful with brain suspensions from each of six of the eight patients in the original report. The transmissible agent has not been isolated in tissue culture nor has it been clearly identified.

Vernon and associates[14] reported virus-like particles in brain tissue obtained from two patients with Creutzfeldt-Jakob disease. They reported aggregates of nucleoprotein-type filaments which were observed in scattered unidentifiable cytoplasmic fragments or in intracellular spaces.

Further evidence implicating Creutzfeldt-Jakob disease as a slow virus infection was reported recently. A corneal transplant from a 55-year-old man who died with this disease resulted in the death of the recipient from Creutzfeldt-Jakob disease two years later.*

Herpes Group of Viruses

Herpes simplex virus (HSV) type 1 infects most of the population of the United States, usually between six months and five years of age. It is estimated that about 1 percent of those infected suffer mild or severe illnesses lasting one to three weeks. The initial infection is inapparent in the remainder of the population. However, Roizman[15] states that as many as 75 percent of those who have contracted primary infection as evidenced by antiviral antibody are affected at sometime during their lives with recurrent herpetic eruptions. In patients with recurrent herpes infections, circulating antibody almost invariably is present. Lesions tend to occur at the same portion of lips or other areas and persons subject to recurrent herpetic episodes can often predict the recrudescences accurately. It appears clear that following primary infection, the HSV is harbored in an inapparent form at some particular site. Specific stimuli associated with physical or emotional provocations of the host may be manifested in the form of typical herpetic lesions. Perhaps a selective defect in cell mediated immunity is present in these patients.

Of particular interest in these diseases is the mechanism whereby the virus persists in the host in the interval between recurrences. The problem of termination of virus spread is important in view of the apparent ineffectiveness of antiviral antibody. The question arises as to how the infection becomes arrested. It is possible that the virus spreads by direct extension from cell to cell and thus avoids contact with antibody. Interferon and possibly other nonspecific responses of the host may be responsible for termination of the viral infection. It has been been observed by Roizman[15] that cells resistant to HSV produce interferon following infection. Type 2 herpes virus is usually responsible for genital herpes, skin lesions below the waist, neonatal herpes and meningitis.

Cytomegaloviruses (herpes-like) are a major cause of mental retardation according to a recent report by Weller.[16] Indeed he suspects that "we have yet to define the late sequelae of infection with the cytomegaloviruses." Infection by these agents occurs in about 1 percent of newborn infants, but rapidly rises to 10 to 20 percent during the third or fourth month of life; this suggests that infection acquired at birth may become evident slowly. Whether the increasing prevalence with advancing age is a consequence of acquired infection or is related to reactivation of a latent agent is undetermined. Obviously we have much to learn about these common but elusive viruses.

Leider and associates[17] suggested that herpes simplex virus encephalitis is possible, due to a reactivated latent infection. "Since most of the primary herpetic infections which occur in childhood are commonly unrecognized and are not followed by recurrent herpes labialis, it is conceivable that patients without a history of herpes labialis could contract encephalitis from reactivation of a latent infection." Of 52 patients with signs of central nervous system disease in association with herpes simplex virus, they studied 18 subsequently. Of the 18, three had a history of recurrent herpes labialis before the onset of encephalitis, suggesting that their disease may have been a manifestation of reactivation of herpes simplex infection (Figure 3).

A herpes-like virus is associated with Burkitt's lymphoma and may also be linked to infectious mononucleosis (IM), raising the possibility that an extremely common virus may produce malignancy as well as a benign infection, presumably due to differences in host resistance. Recent studies have shown that the Epstein-Barr virus (herpes-like) has growth stimulating capability. Burkitt's lymphoma, a childhood disease occurring in certain areas of Africa, is considered to be the first instance in which an infective agent could be linked to malignancy in man. Peripheral blood lymphocytes from healthy donors, or those with diseases other than infectious mononucleosis, can

San Francisco Chronicle, March 13, and *New York Times*, March 16, 1975.

be cultured only with difficulty, whereas those of IM patients developed readily into permanent lines of lymphoblastoid cells thus suggesting a growth-stimulating effect of the causative agent.

The Epstein-Barr virus (EBV) undoubtedly has worldwide distribution.[18] The Henles found that in about 80 percent of the low-income population in Philadelphia, antibodies to EBV are present. The antibodies appear in some children early in life unassociated with specific illness (presumably the virus producing a silent infection). Further studies soon disclosed a significant association between EBV and infectious mononucleosis (IM). Indirect fluorescent antibody tests also confirmed the association of EBV and IM. Among a group of 150 Yale students, 97 lacked both EBV antibody and a history of IM as freshmen. During the following four to eight years, 28 had frank IM associated with elevated heterophil and EBV antibody responses, and 15 others acquired antibody without evidence of associated illness. Thus the incidence of EBV infection was 44 percent in this population; in contrast none of 53 persons with EBV antibody when they entered Yale subsequently developed clinical IM. Since IM appears to be associated with relatively high socioeconomic standing (being high in college and medical students), the causative agent of IM may be one that spreads early in life, especially under low socioeconomic conditions when the infection might not lead to a characteristic disease but might leave permanent immunity.[19]

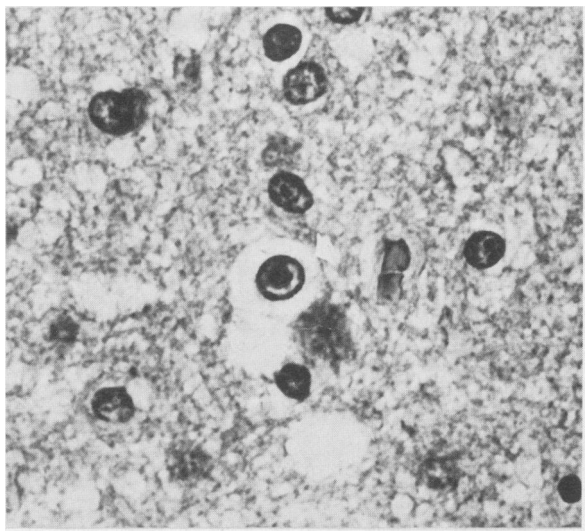

Figure 3.—A photomicrograph showing Cowdry, type A intranuclear inclusion bodies from the right temporal lobe of a patient from whom a strain of type I **herpesvirus hominis** was isolated (reduced from × 855). (Provided by A. Martin Lerner, MD, and taken from an article published by Bailey EJ, Nolan DC, Lerner AM: J of Immunol 104:607-615, 1970. Williams and Wilkins Co., Baltimore, Md. U.S.A.)

Applebaum and associates[20] cite the occurrence of a varicelliform eruption on four of their patients with herpes zoster encephalitis. Von Bokay[21] suggested a close relationship between zoster and chicken pox. The observed occurrence of zoster and chicken pox is considered to represent an unusual clinical response in the same patient to the same virus. By electron micrography, the viral bodies of varicella and zoster are identical in appearance and size.[22] Agents which are indistinguishable from the varicella virus[23] have been isolated from zoster lesions.

Congenital Rubella

Congenital rubella is a chronic, generalized infection, causing frequent defects of the eye (including chorioretinitis, cataracts and glaucoma) and heart (patent ductus arteriosus often with peripheral pulmonary stenosis). Dental abnormalities, microcephaly and mental retardation are also common. The frequency of defects is greatest when infection occurs in the first month of pregnancy. Signs of congenital rubella include primary thrombocytopenia, enlarged liver and evidence of bleeding with petechiae commonly present. The bilirubin may be elevated with fatal termination in some patients.

In utero acquired rubella infection, in contrast to that acquired postnatally, is characterized by a *persistence* of the virus.

In children born with congenital rubella the virus persists despite serum antibodies.[24] Virus may be isolated readily from urine, feces, throat swabs and cerebrospinal fluid as well as from peripheral blood, bone marrow, lens tissue and middle ear fluid. Viral persistence tends to terminate spontaneously when the child reaches about 12 to 18 months of age. Virus has been isolated postmortem from essentially every organ and gland examined including the brain, and tissues obtained postmortem and grown in tissue culture were found to be infected with rubella virus. The virus was not cytolytic; both the cells and virus survived for many months establishing virus carrier cultures.

Although the virus-infected cells were morphologically indistinguishable from noninfected cells, examination of the infected cells indicated that they had a slower growth rate. Doubling time of infected cells was 48 to 72 hours as compared

with 24 hours in noninfected cells. The noninfected cells could be maintained for about 40 passages while infected cells could only be maintained for 20 passages. Thus, not only was growth rate of the infected cells affected by the virus but the ultimate doubling potential of the cells was also reduced. Careful analysis of the virus-carrier cultures showed that every cell in the culture was infected. Rubella antiserum added to the culture fluid did not rid the culture of virus. These findings suggest that the virus was passed from parent cell to daughter cell during cell division.

Organs of infants with congenital rubella contained fewer cells than organs from infants without congenital rubella. The cells appear normal in size and shape but were present in subnormal numbers. Involvement in early embryonic life would result in a slower growth rate and thus smaller organs and a smaller baby. A significant correlation was found between failure to thrive and virus persistence.

Cells derived from embryonic tissues are neither sensitive to nor capable of producing interferon. The possibility exists that in early embryonic life the cells of the human fetus are not capable of an interferon response upon exposure to the rubella virus. The chronically infected cells appear to be refractory to interferon systems since they could not be stimulated to produce interferon when superinfected with another virus which readily produced interferon in noninfected cells. A parallel exists between the persistent infection in rubella and that which has been established with lymphocytic choriomeningitis infection in mice. It appears that chronicity may occur only by acquiring the virus *in utero* or neonatally.

The neurological aspects of congenital rubella were studied in 100 infants by Desmond and associates,[25] who found evidence of central nervous system involvement in 81 of 100 infants studied. The infants were lethargic and irritable with tone disturbances, seizures and delays in motor development and socialization. A full fontanel, increase in concentration of spinal fluid protein and persistence of the rubella virus in spinal fluid were the outstanding manifestations of encephalitis. Twenty of the patients died, but 64 were followed regularly for 18 months, at which time 69 percent had some degree of neurological impairment. At postmortem, the most prominent lesions were leptomeningitis, vasculitis and multiple focal areas of necrosis and perivascular calcification (Figure 4).

Possible Slow Virus Infections of Man

Amyotrophic lateral sclerosis (ALS) has been described as a syndrome with symptoms and signs of lower and upper motor neuron degeneration rather than a single disease entity. Kurland[26] outlined three groups or types of ALS: (1) classic or sporadic, (2) familial and presumably hereditary and (3) Mariana Islands form. He observed that in the hereditary form there is predilection for males not present in the other types. Also in this form there is a greater likelihood that the initial weakness and wasting will occur in the lower extremities. Demyelination of the posterior columns is signifiant without sensory changes; the histochemical changes in the skin described in some forms have not been observed in the familial or hereditary type, in which a family history may show a pattern compatible with dominant inheritance. In the Mariana Islands the disease tends to occur more often in younger persons than in the classical form, and spasticity is more likely to be present initially.

The first concept of ALS on the island of Guam was that the disease was genetic in origin, but the recognition of ALS in other Western Pacific Islands suggested a possible exogenous factor. Further epidemiologic studies in the Pacific Islands as well as elsewhere are clearly needed.

Viral Hepatitis

The viral cause of infectious hepatitis remains to be proved, but the possibility that serum hepa-

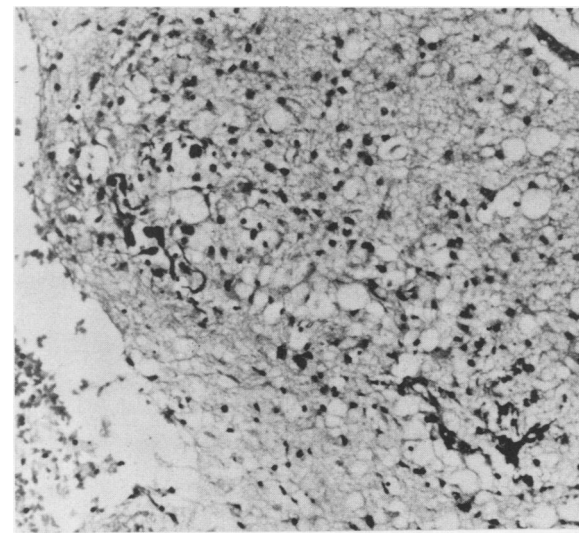

Figure 4.—A photomicrograph from a patient with congenital rubella showing a focal area of necrosis and perivascular calcification. (Stain: hematoxylin-eosin, reduced from × 580.)

titis (SH) is a slow or latent virus infection seems to be widely accepted. Serum hepatitis differs from infectious hepatitis (IH) in many clinical epidemiologic and immunologic respects. The incubation period of SH ranges from about 50 to 180 days; it may occur in any season, infecting all age groups.[27]

The recent discovery of Australian antigen, also referred to as hepatitis associated antigen (HAA) or hepatitis B antigen (HB) is a major advance in this still very confused group of diseases. The immunodiffusion and complement fixation tests for HAA are useful in some cases in differentiating SH from IH and in identifying the carrier state following SH infection. The hepatitis agent is highly infectious; the tests available for HB have practical value in detecting the antigen in blood banks and in screening potential donors.

" . . . though the discovery of Australia antigen and the establishment of its link with SH represents a major breakthrough in hepatitis, we remain several breakthroughs removed from a more complete understanding of the etiology of either IH or SH."[27] The hepatitis viruses are now designated type A for infectious hepatitis and type B for serum hepatitis. The type A virus has been transmitted to marmosets and type B to chimpanzees. A specific hepatitis B immune serum globulin is now under trial for passive immunization and standard gammaglobulin is used to prevent type A hepatitis. It is interesting to note that at a recent symposium on viral hepatitis held in Milan, Doctor Maurice R. Hilleman, of the Merck Institute for Therapeutic Research, showed for the first time electron microscopic pictures of the hepatitis A virus which is smaller than the B type.

Vaccinia Virus in Central Nervous System Disease

One recent study[28] showed vaccinia virus neutralizing antibodies in the cerebral spinal fluid (CSF) of 30 percent of 187 patients with multiple sclerosis; in only 3 percent of 87 patients with other neurologic diseases, and in none of 30 normal control subjects.

A case of neuromyelitis optica (Devic's disease) was reported by Adams and associates[29] in which the vaccinia virus was directly implicated 11 years after primary vaccination of the patient against smallpox. Antibody titers against vaccinia virus were extremely high in the patient's serum (1:5,000) on several occasions before death; likewise, very elevated titers were found in CSF, ranging from 1:16 to 1:64. Brain tissue, obtained promptly following death, was inoculated along with Freund's complete adjuvant subcutaneously into three rabbits. In all three, vaccinia antibodies developed, whereas in ten control animals there was no antibody response. Subsequently, three rabbits were inoculated in a similar fashion with normal brain tissue plus adjuvant and viral antibodies failed to develop. To our knowledge, this patient represents the first known case in which the vaccinia virus may play an etiologic role in severe demyelinating disease.

At postmortem, the brain showed a severe form of subacute disseminated sclerosis and the presence of Guarnieri's bodies, typical of infection caused by vaccinia virus. Much further study of patients with demyelinating disease will be required before the role of vaccinia virus and other possible persistent agents such as the measles virus can be implicated as etiologic agents in degenerative brain diseases.

The pathological findings in variola and vaccinia encephalitis appear to be identical.[30] The commonest neurological syndrome in these conditions is that of disseminated encephalomyelitis. In 1968, it was pointed out by Osuntokun[31] that "neurological complications of smallpox vaccination are more likely to occur in infants (under 1), after primary vaccination and in adults revaccinated after a long interval following primary vaccination in childhood."

Persistence of Measles Virus and Demyelinating Disease

The primary demyelinating disorders have posed a singularly intractable problem for the physician; their classification is unclear and their cause, until quite recently, was unknown. It now appears that many and perhaps all of these disorders—that is, multiple sclerosis (MS), subacute sclerosing panencephalitis (SSPE) and neuromyelitis optica (NO)—may not be primary at all, but represent long-delayed sequelae of viral infections.

Viral infections in general, and measles rubeola in particular, may severely affect the central nervous system (CNS). Meigs and Pepper in 1877[32] described a number of atypical measles cases with such encephalitic symptoms as violent nausea and vomiting, frontal headache, delirium and convulsions. Measles encephalitis appears within a few days of the infection and ends, in one way or an-

other, within days or weeks. The condition can persist and progress for months or even years. That some protracted cases of encephalitis are caused by persistent infection with measles virus, rather than by some other concomitant agent or process, has been shown by portmortem studies in which CNS tissues show characteristic traces of the virus: multinucleated giant cells and inclusion bodies in both the cytoplasm and nucleus (Figure 5). The same cytological changes have been observed in other viral diseases—for example, canine distemper and rinderpest (a disease of cattle), both have symptomatologic and immunologic similarities to measles. A disease of mature dogs known as old dog encephalitis (ODE) has been shown to be similar immunologically and pathologically to distemper and measles and consequently is of importance as a possible animal model for the study of severe demyelinating diseases of human beings such as MS, SSPE and NO.[33]

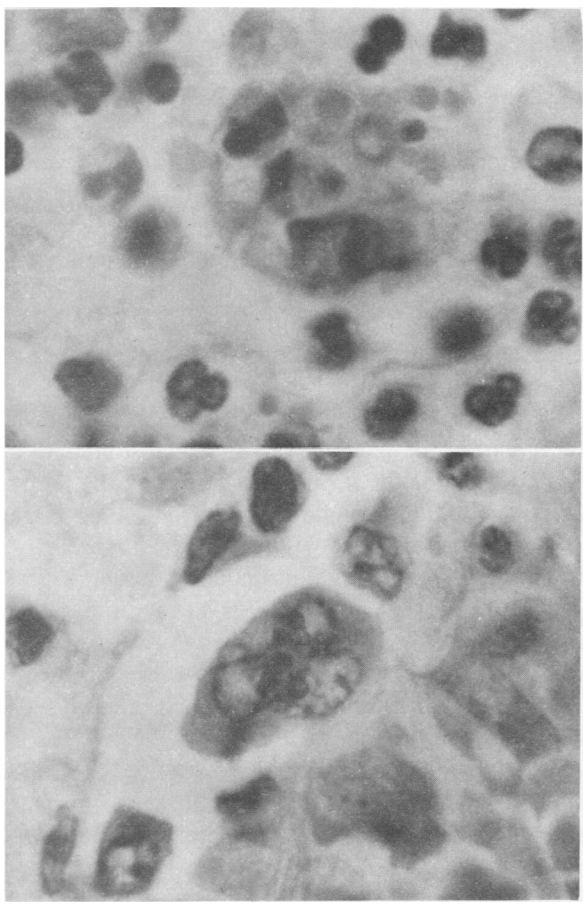

Figure 5.—Upper, Photomicrograph from the meninges of a child, age 13 months, who died following measles encephalitis, showing a multinucleated giant cell with cytoplasmic and intranuclear inclusion bodies. (Stain: hematoxylin-eosin, reduced from × 2100.) **Lower,** Photomicrograph from a 5½-year-old child who died following measles encephalitis, showing a small multinucleated giant cell in the perivascular space. (Stain: picro-Mallory reduced from × 2100.)

Two other features of measles encephalitis merit mention. First, in all but the most fulminating cases, where death intervenes very rapidly, a consistent pathologic feature is demyelination, often accompanied by scarring; this feature is probably responsible for the high incidence (up to 50 percent) of permanent neurologic impairment in patients who survive the initial encephalitis. Second, it appears that the affinity of measles virus for the CNS may be far greater than the infrequency of frank encephalitis (0.5 percent or less of all measles cases) would suggest. Electroencephalographic studies of patients with measles indicate that in from 30 to 50 percent there are temporary brain-wave changes characteristic of those seen in unambiguous encephalitis cases.[34]

Measles encephalitis can in many cases be classed as a demyelinating disease and, in at least some cases, as subacute or chronic progressive disease—as can vaccinia encephalitis and the other encephalitides associated with childhood diseases. It is possible that some or all of the other chronic demyelinating diseases might result from the same or related processes. This concept is strengthened by the pronounced similarity between some forms of primary demyelinating disease and certain conditions clearly linked to viral infection. For example, Ferraro[35] found no significant difference, clinical or pathologic, between acute multiple sclerosis and subacute varieties of postexanthematous and postvaccinal encephalomyelitis. Similar observations have been made by other investigators.

The fundamental observations of J. M. Charcot established neurology on the basis of pathological changes.[36] His students (Pierre Marie probably being the most eminent) continued to expound his teachings and correlated typical symptom complexes with neuropathological findings. In 1895, Pierre Marie,[37] in lectures to the New Sydenham Society, London, stated: "Eruptive fevers must also be mentioned, namely measles, scarlatina, and above all—smallpox. Cases are numerous in which insular sclerosis has been known to occur during convalescence after the affection: tremor in the limbs with more or less paresis, disorder of speech which becomes slow and scanning, nystagmus and in short, all the characteristic symptoms

of insular sclerosis may exist. At times these symptoms cease and entirely disappear, but they may also continue and confirmed insular sclerosis occurs."

In 1912 Schilder[38] reported a very slowly progressive degeneration of the white matter characterized by demyelination in the main parts of the brain. Similar cerebral sclerosis has since been reported by Scholz,[39] Balo[40] and Greenfield.[41] It is apparent that neuromyelitis optica and disseminated sclerosis exhibit certain similarities to these conditions. The reason for grouping them together is that the histologic changes are, in their incipiency, of the same nature in all.

A common feature of many of these diseases of the nervous system is the presence of foci of destruction of the myelin sheaths of the nerve fibers. The areas of destruction vary in size, shape and distribution, and also in the acuteness of the pathologcal process. Lord Brain[42] points out that "the axis cylinders often suffer as well as their myelin sheaths . . . the most acute forms often follow acute infections, especially the exanthemata caused by viruses such as measles, smallpox, and vaccinia."

Measles encephalitis (ME) was studied in its acute, subacute and chronic forms and evidence of invasion by the measles virus was indicated by the hallmark of the virus, namely, intranuclear and cytoplasmic inclusion bodies and multinucleated giant cells (Figure 5). The studies provided evidence for persistence of the measles virus months and years following the initial measles illness. These findings were reported by the writer early in 1966.[43] Following this report, several studies on Dawson's "inclusion encephalitis" appeared in 1967 pointing directly to an etiologic association with measles virus. Connolly and coworkers[44] reported high levels of measles antibody with rising levels as the disease progressed. They were also successful in demonstrating specific fluorescence of inclusion bodies in nerve cells by measles antibody, indicating clearly the presence of measles virus in some form.

Dawson[45] reported two cases of "inclusion encephalitis" characterized by involuntary jerking movements of the limbs and mental regression and by the presence in many brain cells of intranuclear inclusion bodies. Examination showed a diffuse inflammatory reaction in the white matter in certain areas of the brain. The characteristics of the inflammation are those of viral encephalitis. The areas of damage to the white matter may resemble to some extent the early stages of Schilder's disease. Perivascular infiltration with lymphocytes and plasma cells occur both in the gray and the white matter. Both nuclear and cytoplastic inclusion bodies may be found in the same cell, or one type may occur without the other.

These patients have very high levels of measles antibodies in their serum and spinal fluid, indicating the overwhelmng nature of their infection. In recent brain biopsy studies, inclusion bodies similar to those found in measles encephalitis are present and are found to be positive for the presence of measles virus when examined by the fluorescent antibody technique. Electron microscopic studies of brain sections have shown filaments or tubules similar to the structure known to be present in measles infected cells.[46,47]

An early clue to the origin of subacute sclerosing panencephalitis came from the inclusion bodies that gave it its original name. These are seen in both the nucleus and the cytoplasm—a phenomenon now known to be uniquely associated in man with measles virus. In the late 1960's, several investigators began following up the possible relationship of measles and SSPE. They demonstrated high levels of measles antibodies in both the serum and especially the spinal fluid of patients with SSPE. More important, they turned up conclusive evidence of measles virus in the brain tissue of some terminal cases.[48-50]

The presence of measles virus has been shown by a variety of techniques. The inclusion bodies are positive for the virus when tested by the fluorescent antibody technique. Electron microscope studies of brain sections have shown filaments or tubules similar to the structures known to be present in measles infected cells[46] (Figure 6). The measles virus has been identified by propagation of the patients' brain cells and co-cultivation with other cell lines such as HeLa, human embryo kidney and green-monkey kidney cells. Many details of the pathologic processes in SSPE are still obscure, but that measles virus *in some form* is deeply implicated seems beyond question. Presumably, this disease may represent a genetically determined defect in cellular immunity to the measles virus.

Multiple sclerosis strikes most commonly in the years between 20 and 40, although it is known to occur occasionally in children. It appears to be influenced by latitude in both hemispheres and is more common in the temperate zones than the tropics. It also seems to be associated with par-

ticular localities rather than with any special group of persons living there. The epidemiology of measles encephalitis is unknown, but the meager information which is available would correspond to what is known about MS. Graphs constructed by epidemiologists have suggested a relationship of MS to a common childhood illness probably caused by viruses. Their studies show that the latent period of the disease is prolonged and may extend over decades.

Dean states that "an environmental factor would appear to be mainly responsible for multiple sclerosis, and this environmental influence may be associated to some extent with latitude."[51]

Other epidemiologic studies have focused on the familial occurrence of MS and have determined that the incidence of the disease in families where a parent or a sibling already has the disease is something like eight times normal (general population) incidence.[52]

However, these data are consistent with what one would expect of some environmental agent exerting its influence in childhood—specifically, a virus that could be transmitted from child to child, less often from parent to child, and never, or almost never, from parent to parent. Operating on this assumption, Poskanzer[52] has calculated from a summary of known cases in siblings that the disease, if acquired from a common exposure in childhood, would have an incubation period averaging about 21 years (with the range of incubation from 12 to 32 years).

Can measles virus remain latent in the human body for as long as 21 years before causing clinical manifestations? There is evidence that it can. In some cases of SSPE, the disease developed many years after childhood measles. Enders-Ruckle[52] reported that persistent virus has actually been isolated from lymphoid tissues (lymph nodes and spleen) in a monkey and two adult humans—all three possessing naturally acquired measles antibodies. A genetic defect resulting in a selective defect in cellular immunity to the measles and related viruses is postulated. The defect may represent immunodeficiency of the T cells making it difficult for the patient to eradicate the infectious agent. The defect may be associated with the presence of the LD-7a determinant. In collaboration

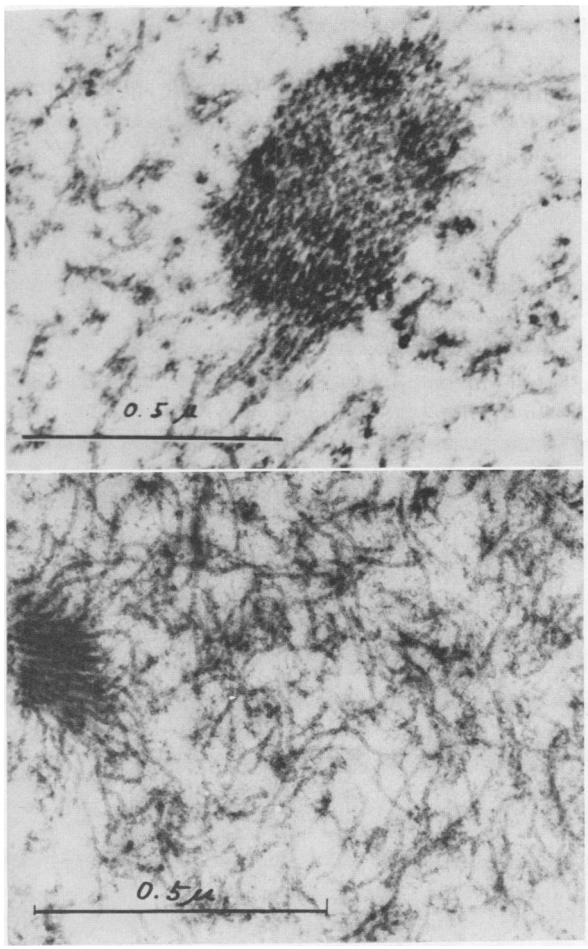

Figure 6.—Electronmicrographs of the fine structure of the measles virus (made from specimens of infected tissue cells) show filaments of high density which are frequently seen in a crystalline arrangement (reduced from × 126,000). (Republished by permission from Adams JM: Persistence of measles virus and demyelinating disease. Hosp Pract, May 1970)

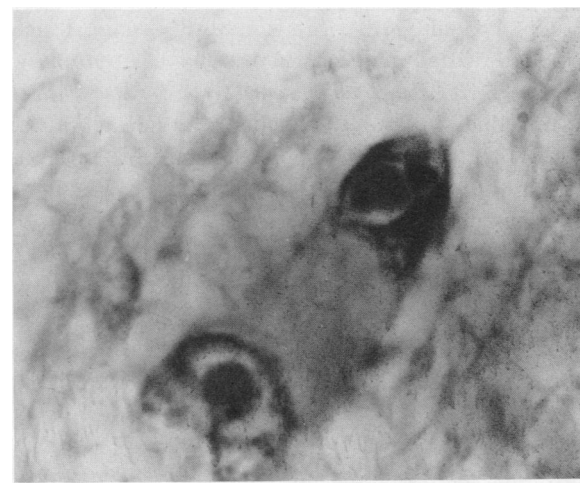

Figure 7.—A photomicrograph from a 14-year-old child who died from sequelae of measles encephalitis which occurred at the age of 6 years, showing an astrocyte with two intranuclear inclusion bodies. (Stain: hematoxylin-eosin, reduced from × 2100.)

with Finkelstein and Walford we found that the histocompatibility antigen type HLA-7 was overrepresented in 64 patients with MS. This finding is compatible with the results reported by others.[54]

Previously we described two children with fatal measles encephalitis in whom were seen the hallmarks of measles: characteristic inclusion bodies and multinucleated giant cells 8 and 11 years after their initial encephalitis[55] (Figure 7).

In 1966, Rustigian[56] reported that measles virus persisted in HeLa cell cultures for up to 500 generations, over a period of six years. Continued subculturing led to a virtual disappearance of the virus's destructive effect, as well as to a decrease in the amount of virus recovered from the persistently infected cells. The virus could persist for long periods when in the presence of high levels of measles antibody. In this case, the infected cell developed a "non-yielder" state, in which there was no detectable virus that could infect normal cultures. Yet virus continued to be synthesized intracellularly and to form characteristic inclusion bodies. Rustigian[56] described this anomalous virus as incomplete, lacking all or part of its normal protein envelope; virion apparently was passed from cell to cell in the course of normal cell division.

It is apparent that the epidemiology of MS shows certain suspicious features—some of them similar to those of measles encephalitis. It is likewise clear that some viruses, in particular measles virus, can persist in cells over long periods, both *in vitro* and *in vivo*. Finally there is evidence that in some persons the measles virus cannot only persist but can cause demyelinating disease (such as SSPE) after a relatively lengthy asymptomatic period.

The hallmark of the measles virus has shown up on postmortem brain sections from some patients with MS.[57] As mentioned earlier, inclusion bodies and multinucleated cells have also been found in measles encephalitis as well as in SSPE and in animals infected with the related distemper and rinderpest viruses, measles virus and the like. Measles virus may be one cause but not the only cause of MS. The disease could be produced by similar persistent infections with other agents or combination of agents. This disease may occur only in persons with a genetically determined defect in cellular immunity to measles or related viruses. However, we must know much more about the possible relationship between the virus and the CNS pathology in MS, before reaching definite conclusions. Demyelination might result directly from viral damage to the cells, but it might instead be due to an antigen-antiviral antibody complex postulated in some other demyelinating diseases.

The importance of optic neuritis (ON) has been emphasized recently by McAlpine,[58] Link and associates[59] and Arnason.[60] This event is not uncommon in childhood, particularly in association with childhood diseases such as measles, and acute respiratory diseases of viral origin. When patients are followed for many years, the early signs of multiple sclerosis (MS)—often vague and confusing—should be seriously considered. The long-range follow-up of ON has eventually led to a diagnosis of MS. The occurrence of optic neuritis at some time during the course of disease in every patient with MS is not unusual and when it occurs early in life, recovery may be complete.

Summary

Outstanding contributions in the field of veterinary medicine led to the discovery of transmissible agents as the cause of a new group of human diseases. Persistent virus infections of sheep such as scrapie and visna, and mink encephalopathy, stimulated a search for the infectious cause of human neuropathies. Kuru, a disease of cannibalistic tribes in the highlands of New Guinea, and Creutzfeldt-Jakob diseases are now clearly shown to be infectious in nature with long incubation periods. The pathologic changes in each are apparently limited to the nervous system.

Other slow, latent and temperate infectious diseases are possible candidates for inclusion in the category of persisting viral infections. Evidence for persisting infection in congenital rubella and cytomegalovirus infections in the newborn is established in spite of high antibody titers. The herpes group of viruses (which includes cytomegalovirus) is recognized as causing possible recurrent, latent and temperate infections. Many of the same characteristics are evident in infectious hepatitis type A, and serum hepatitis type B.

The possibility that measles may become a slow or persistent infection is supported by immunological and pathological findings in experimental studies as well as in man. Several clinics have reported increased levels of measles antibodies in the sera and spinal fluid of many patients with subacute sclerosing panencephalitis and multiple sclerosis. Measles virus has been isolated from patients with subacute sclerosing

panencephalitis, occurring years following childhood measles. Clinical concepts in the early literature support the relationship of measles and primary demyelinating diseases.

REFERENCES

1. Sigurdsson B: Observations on three slow infections of sheep. Br Vet J 110:3, 7, 9, 255, 307, 341, 1954

2. Hotchin J: Immune and autoimmune reactions in the pathogenesis of slow virus disease. Curr Top Microbiol Immunol 40:33, 1967

3. Hotchin J, Sikora E, Kinch W, et al: Lymphocytic choriomeningitis in a hamster colony causes infection of hospital personnel. Science 185:1173, 1974

4. Oldstone MBA, Dixon FJ: Virus-antiviral antibody complexes, *In* Progress in Immunology—First International Congress of Immunology. New York and London, Academic Press, 1971, pp 763-777

5. Notkins AL: Immunological injury of virus-infected cells by antiviral antibody and complement, *In* Progress in Immunology—First International Congress of Immunology. New York and London, Academic Press, 1971, pp 780-786

6. Pattison IJ: Scrapie—An experimentally transmissible degenerative disease of the central nervous system in sheep. J R Coll Physicians Lond 1:93, 1966

7. Adams JM: Persistence of measles virus and demyelinating disease. Hosp Pract p 87, May 1970

8. Narayan O, Silverstein AM, Price D, et al: Visna virus infection of American lambs. Science 183:1202-1203, 1974

9. Hartsough GR, Burger D: Encephalopathy of mink—I. Epizoologic and clinical observations. J Infect Dis 115:387-392, 1965

10. Gajdusek DC: Kuru in New Guinea and the origin of the NINDB study of slow, latent and temperate virus infections of the nervous system of man, *In* NINDB Monograph No 2: Slow, Latent and Temperate Virus Infections, Public Health Service Publ. #1378. Washington, DC, Government Printing Office, 1965, p 3

11. Gajdusek DC, Gibbs CJ Jr, Alpers M: Experimental transmission of kuru-like syndrome to chimpanzees. Nature (London) 209:794-796, 1966

12. Gajdusek DC, Gibbs CJ Jr, Alpers M: Transmission and passage of experimental "kuru" to chimpanzees. Science 155:212-214, 1967

13. Gibbs CJ, Gajdusek DC: Infection as the etiology of spongiform encephalopathy (Creutzfeldt-Jakob disease). Science 165: 1023-1025, 1969

14. Vernon ML, Horta-Barbosa L, Fuccillo DA, et al: Virus-like particles and nucleo-protein-type filaments in brain tissue from two patients with Creutzfeldt-Jakob disease. Lancet 1:964, 1970

15. Roizman B: An inquiry into the mechanisms of recurrent herpes infections of man, *In* Perspectives in Virology IV. New York, Harper and Row, 1965, pp 283-304

16. Weller TH: Cytomegaloviruses—The difficult years. J Infect Dis 122:532, 1970

17. Leider W, Magoffin RL, Lennette EH, et al: Herpes-simplex-virus encephalitis—Its possible association with reactivated latent infection. N Engl J Med 273:341-347, 1965

18. Henle G, Henle W: EB virus in the etiology of infectious mononucleosis. Hosp Pract, 33, Jul 1970

19. Evans AS, Niederman JC, McCollum RW: Seropidemiologic studies of infectious mononucleosis with EB virus. N Engl J Med 279:1123-1127, 1968

20. Applebaum E, Kreps SI, Sunshine A: Herpes zoster encephalitis. Am J Med 32:25-31, 1962

21. Von Bokay J: Ueber den atiologischen Zussammenhang der Varizellen mit gewissen Fallen von Herpes Zoster. Wien Klin Wochenschr 22:1323, 1909

22. Rake G, Blank J, Coriel LL, et al: The relationship of varicella and herpes zoster—Electron microscope studies. J Bacteriol 56:293, 1948

23. Weller TH: Serial propagation *in vitro* of agents producing inclusion bodies derived from varicella and herpes zoster. Proc Soc Exp Biol Med 83:340, 1953

24. Rawls WE: Congenital rubella—A chronic viral disease. Pediatr Dig, 41-50, Sep 1967

25. Desmond MM, Wilson GS, Melnick JL, et al: Congenital rubella encephalitis. J Pediatr 71:311, 1967

26. Kurland LT: Amyotrophic lateral sclerosis—A reappraisal, *In* NINDB Monograph No. 2: Slow, Latent and Temperate Virus Infections, Public Health Service Publ. #1378, Washington, DC, Government Printing Office, 1965, pp 13-22

27. Krugman S: Etiology of viral hepatitis. Hosp Pract, 45, Mar 1970

28. Kempe CH, Takabayashi K, Miyamoto H, et al: Elevated cerebrospinal fluid vaccinia antibodies in multiple sclerosis. Arch Neurol 28:278, 1973

29. Adams JM, Brown WJ, Eberle ED, et al: Vaccinia virus implicated in diffuse demyelinating disease. Proc Soc Exp Biol Med 143:799, 1973

30. Marsden JP, Hurst EW: Acute perivascular myelinoclasis ("acute disseminated encephalomyelitis") in smallpox. Brain 55: 181, 1932

31. Osuntokun BO: The neurological complications of vaccination against smallpox and measles. W Afr Med J 17:115, 1968

32. Meigs JF, Pepper W: A Practical Treatise on the Diseases of Children, 6th Ed. Philadelphia, The Blakiston Company, 1877

33. Adams JM, Snow HD: Viral demyelinating encephalitis and old dog encephalitis—Possible relationship to distemper, measles, and demyelinating disease of man. Cal Vet 27:8-10, 1973

34. Gibbs FA, Gibbs EL, Carpenter PR, et al: Electroencephalographic abnormality in "uncomplicated" childhood diseases. JAMA 171:1050, 1959

35. Ferraro A: Primary demyelinating processes of the central nervous system. Arch Neurol Psychiat 37:1, 100, 1937

36. Charcot JM: Histologie de la sclerose en plaques. Gaz Hop (Paris) 41:554, 557, 566, 1868

37. Marie P: Lectures on Diseases of the Spinal Cord. London, The New Sydenham Society, 1895, p 134

38. Schilder P: Zur kenntnis der sogennanten diffusen Sklerose. Z Ges Neurol Psychiat 10:1, 1912

39. Scholz W: Klinische, pathologische-anatomische und erbbiologische Untersuchungen bei familiarer, diffuser Hirnsklerose im Kindesalter. A Ges Neurol Psychiat 99:651, 1925

40. Balo J: Encephalitis periaxialis concentrica. Arch Neurol Psychiat (Chicago) 19:242, 1928

41. Greenfield JG: The pathology of measles encephalomyelitis. Brain 52:171, 1929

42. Brain L: Diseases of the Nervous System. London, Oxford University Press, 1962

43. Adams JM, Baird C, Filloy L: Inclusion bodies in measles encephalitis. JAMA 195:290, 1966

44. Connolly JH, Allen IV, Hurvitz LJ, et al: Measles virus antibody and antigen in subacute sclerosing panencephalitis. Lancet 1:542, 1967

45. Dawson JR Jr: Cellular inclusions in cerebral lesions of lethargic encephalitis. Am J Path 9:7, 1933

46. Kallman F, Adams JM, Williams RC, et al: The fine structure of cellular inclusions in measles virus infections. J Biophys Biochem Cytol 6:379-382, 1959

47. Tawara J: Fine structure of filaments in dog kidney cell cultures infected with measles virus. Virology 25:322, 1965

48. Horta-Barbosa L, Fuccillo DA, Sever JL, et al: Subacute sclerosing panencephalitis—Isolation of measles virus from a brain biopsy. Nature 221:974, 1969

49. Baublis JV, Payne FE: Measles antigen and syncytium formation in brain cell cultures from subacute sclerosing parencephalitis (SSPE). Proc Soc Exp Biol Med 129:593, 1968

50. Prineas J: Paramyxovirus-like particles associated with acute demyelination in chronic relapsing multiple sclerosis. Science 178: 760-763, 1972

51. Dean G: Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African born and in white immigrants to South Africa. Br Med J 2:724, 1967

52. Poskanzer DC: Epidemiological evidence for a viral etiology for multiple sclerosis, *In* NINDB Monograph No. 2: Slow, Latent and Temperate Virus Infections, Public Health Service Publ. #1378, Washington, DC, Government Printing Office, 1965, p 55

53. Enders JF, Ruckle G: Methods of determining immunity resulting from measles. Arch ges Virus-forsch 16:182, 1965

54. Jersild C, Fog T, Hansen GS, et al: Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. Lancet 7840:1221-1224, 1973

55. Adams JM: Clinical pathology of measles encephalitis and sequelae. Neurology (Minneap) 18:52, 1968

56. Rustigian R: Persistent infection of cells in culture by measles virus—II. Effect of measles antibody on persistently infected HeLa sublines and recovery of HeLa clonal line persistently infected with incomplete virus. J Bacteriol 92:1805, 1966

57. Adams JM, Brown WJ: Studies on inclusion bodies in early and late demyelinating diseases. Int Arch Allergy Appl Immunol 36:83, 1969. Pathogenesis and Etiology of Demyelinating Diseases, Basel, S. Karger

58. McAlpine D, Lumsden CE, Acheson ED: Multiple Sclerosis —A Reappraisal. Edinburgh and London, Churchill, Livingston, 1972

59. Link H, Norrby E, Olsson J: Immunoglobulins and measles antibodies in optic neuritis. N Engl J Med 289:1103-1107, 1973

60. Arnason BGW: Optic neuritis and multiple sclerosis. N Engl J Med 289:1140-1141, 1973