The possible viral aetiology of disseminated sclerosis¹

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The two most attractive current hypotheses concerning the aetiology of disseminated (multiple) sclerosis (MS) are those of (1) an 'autoimmune' process, analogous to experimental allergic encephalitis, or (2) an infection, for which the 'slow virus diseases' of the central nervous system represent a possible model. Experimental data have been advanced favouring each hypothesis, and it is in fact quite possible that both mechanisms may have a contributing role. Our purpose in this presentation will be to discuss only the infectious hypothesis, and more particularly the possibility of a virus aetiology. However, whether or not one or a combination of viruses is implicated in the pathology of MS, it appears that immunopathological events must be considered to explain the clinical evolution of the disease.

Arguments in Favour of a Viral Aetiology

These consist of epidemiological, clinical, and biological studies, and analogies between MS and other human and animal models of probable or certain viral origin.

EPIDEMIOLOGICAL AND CLINICAL STUDIES

Epidemiology

The geographical distribution of MS has been delimited into certain zones of high and low risk. Studies of people who have moved from one zone to another have suggested that if an infection is responsible, it would occur before the age of 20, and have an incubation period lasting several years (Acheson, 1968).

Study of the high risk zones has revealed no genetic, climatic, nutritional, or toxic factors, or any vector or animal reservoir, which could explain the geographical distribution of the disease, although the existence of a genetic host susceptibility is probable in the rare familial cases. The agent must be only weakly contagious, since there has been no

increase in the number of cases in the low risk zones during the last 50 years.

It is possible that one or more known viruses of general distribution infect people in whom MS manifests itself only as a consequence of an unusual immunological response (Sibley and Foley, 1965), leading to a geographical distribution more limited than that of the virus itself. Or, MS could be caused by an as yet unknown virus.

Clinical studies

Patients with MS can be grouped into three clinical categories. In the majority of cases, after an initial period of relapses and remissions, the disease shows a steady progression to death. In other cases, after one or two attacks (from which the diagnosis is made) the disease either stabilizes for life or eventually disappears. And some cases are from the outset steadily progressive to death. Although the occurrence of these latter two forms might favour a purely infectious aetiology, the clinical course differing by virtue of variability between the virulence of the agent and host susceptibility, the much more frequent form of remitting followed by progressive disease suggests two different pathological mechanisms, of which the first could be infectious and the second an immunological response set in motion by the infection.

Pette (1968) has pointed out that some neurological signs resembling MS can be seen in measles encephalitis, eg, optic neuritis, nystagmus, or dizziness, or after measles vaccinations (Milovanovic and Katz, cited by Pette, 1968). Nevertheless, no statistical study has revealed an increased frequency of measles in the past history of MS patients (Ross, Lenman, and Rutter, 1965; Panelius, 1969; Wilhelm, 1970). Apart from the special problem of subacute sclerosing panencephalitis, no human or animal disease of known viral origin has a clinical evolution similar to MS, and it may also be mentioned that relapses following different kinds of vaccinations

(Miller, Cendrowski, and Shapira, 1967) suggest an immunological process in the disease.

Biological Studies

Elevation of CSF gamma globulin levels without associated changes in serum proteins, discovered by Kabat in 1942, constitute a striking and frequent concomitant of the disease. Extensive experience over a 10-year period at the Salpêtrière (Castaigne, Lhermitte, Schuller, and Loridan, 1971a; Castaigne, Lhermitte, Schuller, Rouques, and Loridan, 1971b; Castaigne, Lhermitte, Schuller, Delasnerie, Deloche, and Dumas, 1972; Schuller, 1972) has yielded the following observations: (1) increased CSF gamma globulins are found in most MS patients (Table I).

Total No. of Cases	Number of Cases with Raised			No. of Cases with Oligoclonal Gamma Globulin Pattern
	Total Protein	Gamma Globulins	Ratio of Gamma Globulins	
166	63 (37.9%)	130 (78%)	124 (74%)	62 (37.5%)

Table I Profile of total proteins and gamma globulins in the CSF of patients with MS

(2) The quantity of gamma globulin is the same whether the measurements are made chemically or electrophoretically (Table II). (3) The increase in

Total Protein (mg %)	Multiple Sclerosis (166 cases)	Controls	
		Neurological (166 cases)	Normal (28 cases)
	52.0 ± 16.58	46.5 ± 13.0	40.8 ± 6.29
Chemical estimation			
Absolute value	11.9 ± 8.30	2.8 ± 1.38	2.1 ± 0.57
% TP	21.6	5.7	5.1
Electrophoretic estimation			
Absolute value	11.7 ± 7.98	5.0 ± 2.28	3.9 ± 1.33
% TP	21.4	10.5	9.5

Table II Means and standard deviations of gamma globulin in the CSF of MS and control patients

gamma globulins is responsible for the increase in total protein, clearly seen when the elevation is expressed as a ratio. (4) Elevation of gamma globulins increases as the number of lymphocytes in the cerebrospinal fluid increases (but it must be added that in old cases, ie, of more than 10 years' duration, gamma globulins persist while the lymphocytes disappear) (Fig. 1). (5) Gamma globulin levels vary according to the phase of the disease (Table

Fig. Disseminated sclerosis cerebrospinal fluid. Total proteins and gamma globulin level compared to number of cells

Measurement Technique (mg %)	Acute Attack (28 cases)	Subacute Attack (35 cases)	Remission (44 cases)	Stationary Phase (39 cases)
Chemical	15.7	13.5	10.1	8.8
Electrophoretic	14.6	13.5	10.5	8.5

Table III Mean CSF gamma globulin levels during different clinical phases of MS (166 cases)

III). (6) The electrophoretic pattern of gamma globulins is often oligoclonal (Table I).

This oligoclonal protein pattern (Laterre, Calla-waert, Heremans, and Sfaello, 1970), that is to say, the occurrence of two or more different gamma globulin peaks on electrophoresis, has been found in more than one third of the MS patients. It is found in as high frequency only in patients with trypanosomiasis, neurosyphilis, and SSPE (Table IV), but is not limited to these few diseases, being found also in such diseases of the central nervous system as diverse as encephalomyelo-meningitis, polyradiculo-

Disease	No. of Cases
MS	12 11 (41%)
Neurosyphilis	78 (41%)
SSPE	23 (65%)
Trypanosomiasis	6 (100%)

Table IV Gamma globulin: oligoclonal patterns in CSF¹

¹From a study in La Salpêtrière done between 1967 and 1971 based in 4620 CSF electrophoreses (Castaigne *et al*, 1972).

neuritis, bacterial meningitis, and tumours. However, apart from tumours, this type of protein pattern appears to occur only in illnesses of infectious or immunological aetiology.

That these gamma globulins are antibodies is certain. Their origin during the development of MS is disputed: they may originate in the serum and pass through an altered blood-brain barrier, or be locally produced by immunocompetent cells which have migrated into the central nervous system. Local production seems assured in at least those cases of MS in which only gamma globulin levels are elevated in the cerebrospinal fluid, without any corresponding increase in total proteins. In any case, the antigen or antigens corresponding to these antibody proteins continues to be the subject of intensive research in several laboratories.

COMPARISON WITH OTHER VIRAL DISEASES OF MAN OR ANIMALS

Multiple sclerosis satisfies three of the four criteria that Sigurdsson (1954) proposed for a slow virus infection (or in current usage, a slow virus disease) (Johnson, 1970). These criteria are (1) a long incubation period lasting months to years; (2) a limited range of susceptible hosts; (3) restriction of pathology to a single organic system; and (4) slow inexorable progression of the disease to death, with virus detectable in the affected organ at death.

The fourth criterion is not encountered since, although usually slow, MS only exceptionally shows continuous progression to death, and as yet no virus has been isolated from MS brain.

Since Sigurdsson's original proposal, the list of slow virus diseases has considerably expanded and we shall now discuss a number of them with reference to MS.

The spongiform encephalopathies

This group of diseases comprises kuru and Creutzfeldt-Jakob disease in man, and scrapie and mink encephalopathy in animals (Gibbs and Gajdusek, 1970). They all have similar clinical, anatomical, and biological characteristics. The infectious agents have some rather special properties that set them apart from other known viruses, and in fact their viral nature has been questioned. Up to the present they have been transmitted *in vivo* but not *in vitro*, and viral particles have not been seen in diseased tissue by electron microscopy. They all progress without remission relentlessly to death, are limited largely to the grey matter, and are unaccompanied by inflammatory signs or any detectable immunological response of the host. Thus, this group of diseases does not appear at first sight to serve as a useful model for multiple sclerosis.

Subacute sclerosing panencephalitis (SSPE) and canine distemper

Measles virus has been isolated repeatedly from the cerebral tissues of SSPE patients (Payne, Baublis, and Itabashi, 1969; Horta-Barbosa, Fuccillo, London, Jabbour, Zeman, and Sever, 1969; Katz, Oyanagi, and Koprowski, 1969), and a second 'papova like' virus has also been seen (Koprowski, Barbanti Brodano, and Katz, 1970). From the brains of dogs with canine distemper, a virus closely related to measles has been isolated (distemper virus), but no other types of virus particles have been seen. The neurological lesions of canine distemper in its subacute encephalitic form are so similar to those of SSPE that it seems likely they share a common physiopathological mechanism. Subacute sclerosing panencephalitis involves a hyperimmune state to measles virus. The CSF protein pattern is qualitatively very similar to that of MS, but quantitatively of great magnitude. In SSPE as in MS, several forms of clinical evolution have been described: some cases progress rapidly to death, others show partial remissions between relapses, a few cases regress or stabilize for more than 10 years, and complete recovery has even been described (Cobb and Morgan-Hughes, 1968). This disease could thus represent a useful model for the study of multiple sclerosis.

Progressive multifocal leucoencephalopathy (PML)

This is a steadily progressive, fatal demyelinating disease. Virus particles resembling those of a papova virus have been seen under the electron microscope (Howatson, Nagai, and ZuRhein, 1965; ZuRhein and Chou, 1965). More recently a virus related to SV40 has been isolated in tissue culture (Padgett, Walker, ZuRhein, Eckroade, and Dessel, 1971) (Weiner, 1972) from brain tissue of two patients, in one of whom serum antibodies to SV40 were found. One may speculate that demyelination results from viral-induced destruction of myelin-forming cells. However, no CSF protein disturbance similar to that of MS has been found in this condition and it thus appears that the origin of the demyelination may be different.

Visna virus

This RNA virus, which produces syncytia, and is also capable of transforming cells in tissue culture (Takemoto and Stone, 1971), has been isolated from several organs, including the brain of diseased sheep. Like measles virus, visna virus can be either pneumotropic or neurotropic (maedi or Montana sheep disease is the pneumotropic form), and specific antibodies circulate in the serum but do not prevent viral multiplication. The widespread viral multiplication of visna virus is to be distinguished from

the limited virus distribution in SSPE, where measles virus has been recovered only from nervous tissue and lymph nodes (Dietzman, Horta-Barbosa, Krebs, Madden, Fuccillo, and Sever, 1972). Also in visna, foci of perivascular lymphocytic infiltration occur in nervous tissue before demyelination (Sigurdson, Palsson, and Grinson, 1957).

This virus, which buds from the cytoplasmic membrane in non-nervous tissue culture, when grown in cultured brain cells shows also some larger forms which have no visible core and are not infectious. According to Harter and Choppin (1967), syncytial activity results from a direct effect of the virus on the cell membrane and does not require viral multiplication and these authors suggest a similar mechanism for demyelination.

Postinfectious encephalopathies (Harter and Choppin, 1971)

These disorders, which sometimes affect myelin in man or animal, are rare complications of common childhood viral infections, including RNA virus infections (measles, mumps, influenza, and rubella), DNA virus infections (varicella, vaccinia, variola, and herpes zoster), and after JHM virus infection in the mouse. All these viruses contain lipoprotein envelopes, and all except the pox-virus group carry a part of the cellular membrane in their envelopes in the course of maturation. However, neither characteristic is exclusive to these viruses. Arboviruses have a lipoprotein envelope and herpes simplex virus has an envelope derived from cellular membrane, but neither virus causes 'postinfectious encephalopathy'. Some of these viruses are capable of altering cellular membranes in forming syncytia and can also multiply in the oligodendroglia cells which produce myelin (measles, mumps). Two different theories have been proposed to explain the pathophysiology of these postinfectious encephalopathies: (1) direct destruction of myelin by the virus, with immunization of the host by cellular debris carried into the circulation either in the viral envelope or in macrophages; or (2) persistent non-cytopathogenic infection of the cell membrane with the viral antigens attracting and fixing antibody-complement complexes or sensitized lymphocytes. An experimental study on mumps encephalitis in hamsters has served further to complicate this problem, since absolutely no relationship was found to exist between perivascular inflammation and the distribution of virus in the brain or meninges (Johnson, 1968).

Comparative Neuropathology

To attempt comparisons of the neuropathology seen

in these diseases is a hazardous enterprise for anyone not specialized in this discipline, especially as in many cases the nosological demarcations are not sharp, and exceptional forms always exist. Moreover, the microbiologist tends to accord less importance than the neuropathologist to the distribution of lesions within the central nervous system, necessary to nosological precision, and more importance to the histological appearances. Notwithstanding these cautions, we offer the following observations.

A cytopathic effect is absent in MS, but then it is not always seen even in known cytopathic viral encephalitis (SSPE—rabies), and some neurotropic viruses are not cytopathic (mumps).

Perivascular inflammatory signs may be found in the acute form of MS which are very similar to those of EAE and viral encephalitis. Their absence does not exclude a viral origin since they disappear in experimental animals submitted to immunosuppressive therapy (Webb and Smith, 1966).

Demyelination may occur following either an inflammatory phenomenon (EAE, postinfectious encephalitis, SSPE, MS), or a possible transformation of glial cells (PML). Both phenomena could have a viral origin.

Oncogenic viruses seem to be related to some demyelinating diseases (PML, Visna, SSPE), and Shein (1970) has obtained transformation of glial cells *in vitro* by SV40 and polyoma virus. It has been suggested that in scrapie and kuru the glial and astrocytic proliferation could be due to cellular transformation (Webb and Smith, 1966). Spongiosis in the spongiform encephalopathies varies according to the species inoculated (Beck, Daniel, Gajdusek, and Gibbs, 1970). Moreover, administration of an immunosuppressive agent can produce experimental spongiosis in an animal infected with an arbor virus (Zlotnik, Smith, Grant, and Peacock, 1970). Thus, it is possible to find related features in those diseases in which both immunological and oncogenic mechanisms could be operating (Field, 1969).

Attempts to Demonstrate a Viral Pathogen

Attempts to demonstrate a viral agent have been guided by the general principles of microbial study of the infectious diseases, that is, to search for an agent in brain tissue by its cytopathic effect, by visualizing particles by electron microscopy, antigenic localization by immunofluorescence, inoculation of ground brain suspensions into tissue cultures or animals, and by immunological study of the disease, including search for specific antibodies and the demonstration of a delayed hypersensitivity phenomenon.

SEARCH FOR AN AGENT¹

No inclusion bodies nor any viral particles have yet been seen in tissue from MS patients examined by light or electron microscopy (Field and Raine, 1964, cited by Field, 1969; Perier and Gregoire, 1965; Rinne, 1968). Immunofluorescence studies have so far been limited to searches for and localization of immunoglobulins, rather than to the detection of homologous or specific viral antigens (Simpson, Tourtelotte, Kokmen, Parker, and Itabashi, 1969; Lumsden, 1971; Tourtelotte, 1971).

We shall not attempt here to review the many efforts to pass MS to animals, which include the isolation (Margulis, Soloviev, and Shubladze, 1946; Dick, McKeown, and Wilson, 1958) of a strain of rabies virus from MS brain, but will simply call attention to two lines of recent investigation.

Gibbs and Gajdusek (personal communication) have inoculated chimpanzees with plaque-containing MS brain tissue without any positive results to date, possibly because of virus antibody complexing. In fact, some attempts to transmit SSPE into animals have also failed (Adels, Gajdusek, Gibbs, Albrecht, and Rogers, 1968), and inoculations of brain tissue culture or brain material after treatment to destroy the antigen-antibody complexes are under current study. After inoculation of MS brain tissue into sheep Palsson, Pattison, and Field (1965) and Field (1966) reported a syndrome reminiscent of scrapie in sheep and in mice respectively. These findings could not be confirmed (Dick, and McAlister, 1965). It was, however, rather surprising to find a syndrome as different clinically and pathologically as scrapie following inoculation of MS brain, and one must be extremely cautious in interpreting the results of Palsson and of Field, for, as pointed out by Dick, McAlister, McKeown, and Campbell (1965), it is difficult to exclude possible contamination of the MS tissue in Field's experiments, or activation of a latent scrapie-like virus in the sheep inoculated by Palsson. Field (1969) discussed various possibilities and remarked that astroglial proliferation precedes demyelination in MS, as Charcot had observed in 1872. Osetowska (1959) also emphasized the relationship between glial proliferation and demyelination in subacute sclerosing panencephalitis.

IMMUNOLOGICAL STUDIES: SEARCH FOR VIRAL ANTIBODIES IN THE SERUM AND CEREBROSPINAL FLUID

In 1962 Adams and Imagawa showed that MS

¹Since this symposium, Ter Meulen *et al* reported the isolation of a virus related to parainfluenza I group from tissue cultures of brain cells in MS (1972). No antibodies against parainfluenza I to V have been found using neutralization tests in CSF of MS patients (Brown and Cathala, 1971). However, neutralization test was not shown to be sensitive enough in measles antibodies studied in the CSF of MS to detect differences with CFS controls.

patients had higher levels of serum measles antibodies than controls, and that CSF measles antibodies were often detectable in MS patients but not in control patients, a line of investigation that was further stimulated by the implication of measles virus in SSPE (Payne *et al*, 1969; Katz *et al*, 1969; Horta-Barbosa *et al*, 1969). A number of studies have since been made on measles antibodies in MS patients, using a variety of techniques.

Haemagglutination inhibition tests (Reed, Sever, Kurtzke, and Kurland, 1964; Clarke, Dane, and Dick, 1965; Just, Rieder, and Ritzel, 1967; Panelius, 1969; Adams, Brooks, Fisher, and Tyler, 1970; Chateau, 1970; Henson, Brody, Sever, Dyken, and Cannon, 1970) (Table V) have not shown a con-

Titre Level of Several Authors Used for Comparison	Multiple Sclerosis		Controls	
	Percentage Positive	No. Tested	Percentage Positive	No. Tested
Reed/Sever (1964) ≥ 1/64	24	33	12 group I 3 group II	33 33
Crums (1964, in Reed/Sever) ≥ 1/80	28	14	26 group I 18 group II	31 33
Clarke (1965) ≥ 1/80	77	26	29	52
Just (1967) ≥ 1/64	54	61	46	175
Panelius (1969) > 1/160	24	135	10	136
Adams (1970) ≥ 1/64	47	133	17 group I 24 group II	65 107
Henson (1970) Chateau (1970) ≥ 1/32	'Significant' but data not in tabulated form 35	28	27 group I 49 group II	37 71

Table V Measles haemagglutination-inhibiting antibody in the serum of multiple sclerosis patients and controls

sistently significant difference between MS patients and controls in individual series; however, when considered together, one can see a clear tendency for higher serum titres to occur in the MS patients.

Complement fixation tests (Table VI) (Adams and Imagawa, 1962; Ross, 1962; Sibley and Foley, 1963; Reed *et al*, 1964; Pette and Kuwert, 1965; Adams, 1967; Panelius, 1969) have more often detected significant differences, but these remain fairly small.

Neutralization tests (Table VII) (Adams and Imagawa, 1962; Reed *et al*, 1964; Adams *et al*, 1970), which are the techniques least often employed, have also yielded the smallest differences. These equivocal results are in contrast with the clear differentiation seen when the levels of measles antibody in SSPE patients are compared with controls (Table VIII).

Titre Level of Several Authors Used for Comparison	Multiple Sclerosis		Controls	
	Percentage Positive	No. Tested	Percentage Positive	No. Tested
Adams/Imagawa (1962) > 1/64	31	109	12	94
Sibley/Foley (1963) > 1/20	15	72	8	81
Pette/Kuwert (1964) > 1/20	36	70	18 group I 21 group II	139 409
Reed/Sever (1964) > 1/32	18	33	9 group I 9 group III	32 33
Crumbs (1964) (in Reed and Sever) > 1/20	7	14	3 group I 0 group II	31 33
Ross (1965) > 1/32	11	81	6	81
Adams (1967) > 1/64	27	128 ¹	8	122 ¹
Panelius (1969) > 1/16	19	59	7	57
Henson (1970)	Significant but data not in tabulated form			

Table VI Measles complement-fixing antibody in the serum of multiple sclerosis patients and controls

¹Figures include subjects studied in original report (Adams/Imagawa, 1962).

Titre Level of Several Authors Used for Comparison	Multiple Sclerosis		Controls	
	Percentage Positive	No. Tested	Percentage Positive	No. Tested
Adams/Imagawa (1962) > 1/16	36	107	14	96
Reed/Sever (1964) > 1/16	72	33	63 group I 75 group II	33 33
Adams (1967) > 1/16	37	132	13	110

Table VII Measles neutralizing antibody in the serum of multiple sclerosis patients and controls

Author	Technique Used		
	HI	Neut	CF
Adams/Imagawa (1962)	¹	Significant	Significant
Sibley/Foley (1963)	—	—	Significant
Pette/Kuwert (1964)	—	—	Significant
Reed/Sever (1964)	Significant	Not significant	Significant
Crumbs (1964) in Reed/Sever	Not significant	—	Not significant
Ross (1965)	—	—	Not significant
Clarke (1965)	Significant	—	—
Just (1967)	Not significant	—	—
Adams (1967)	—	Significant	Significant
Panelius (1969)	Significant	—	Significant
Adams (1970)	Significant	—	—
Henson (1970)	Significant	—	Significant
Chateau (1970) (thesis)	Not significant	—	—

Table VIII Significance of higher serum measles antibody titre in multiple sclerosis patients than in controls

¹Test not used

In some studies antibodies to several other viruses have also been found to be raised in MS patients (herpes zoster, herpes simplex, influenza type C, mumps, parainfluenza III (Ross, Lenman, and Rutter, 1965; Ross, Lenman, and Melville, 1969; Brody, Sever, and Henson, 1971; Millar, Fraser, Haine, Connolly, Shirodaria, and Hadden, 1971). Is the difference in the antibody levels in patients and controls in these studies dependent on the controls used? Henson and Brody (Henson *et al.*, 1970; Brody *et al.*, 1971; Brody, Sever, Edgar, and McNew, 1972) tried to answer this question by carefully matching MS cases with control subjects chosen from siblings and school friends of the patients. The results of one part of this study (in Indiana) showed that the level of serum measles antibodies was higher in both the MS and sibling groups than among friends of the patients. In the other part of the study (in Washington) the MS group and female siblings had higher levels than male siblings or friends. Thus, differences in measles antibody levels between MS patients and non-sibling controls appear to be valid and show familial and sex-linked phenomena.

Ross *et al.* (1969), correctly regarding optic neuritis as a possible early form of MS, compared the serum antibody titres to measles virus in 17 cases of optic neuritis with a group of MS patients, and found a lower measles antibody titre in the optic neuritis group. This result could be interpreted as favouring a rise of antibody levels to measles in the course of the illnesses: however, she was not able to show any trend towards higher antibody levels during the evolution of MS in several cases which she followed.

Other techniques for the detection of serum measles antibodies have also been used. Immuno-adherence tests (Caspary, Chambers, and Field, 1969) showed a positive difference in favour of multiple sclerosis. Gel precipitation tests by Panelius, Salmi, and Halonen (1970) showed common measles antigen precipitation bands between MS and subacute sclerosing panencephalitis. Platelet aggregation tests by the same authors (Panelius *et al.*, 1948) showed a positive result for a single measles antigen. Millar *et al.* (1971), using immunofluorescence, found measles specific IgM antibodies in four of 43 MS sera, as well as two instances of mumps IgM antibodies. In one of the measles-positive sera and one of the mumps-positive sera, the IgM antibodies persisted for more than two years. One case among the 43 controls had measles IgM antibody. These observations are very interesting as they suggest the persistence of measles virus as an infective agent continuously multiplying in the host. It recalls the demonstration that measles virus grows for extended periods in the lymph nodes

of the monkey after experimental measles infection (Enders Ruckles) as well as in man in the course of SSPE (Dietzman *et al*, 1972). Measles antibodies in the cerebrospinal fluid (Adams and Imagawa, 1962; Sibley and Foley, 1963; Reed *et al*, 1964; Pette and Kuwert, 1965; Adams, 1967) have also been sought for in MS patients (Table IX). Results have varied, and other virus antibodies such as influenza type C (Reed *et al*, 1964) have also been shown to be more frequent in MS than in control patients.

Author	Multiple Sclerosis			Controls		
	CF	Neut	HI	CF	Neut	HI
% positive	71	52	1	0	0	—
No. tested	35	35	—	56	48	—
Sibley/Foley (1963)						
% positive	13	—	—	12	—	—
No. tested	53	—	—	93	—	—
Pette/Kuwert (1964)						
% positive	4	—	—	0	—	—
No. tested	28	—	—	22	—	—
Reed/Sever (1964)						
% positive	18	55	30	12	46	15
No. tested	33	33	33	33	33	33
Adams (1967)						
% positive	38	15	—	8	0	—
No. tested	21	27	—	24	31	—

Table IX Measles antibodies in the CSF of patients with multiple sclerosis and controls

The results of all of these studies suggest that the presence of IgG measles antibodies in the cerebrospinal fluid would be the single most encouraging biological phenomenon favouring measles involvement in the aetiology of MS. Since the amount of antibody in the CSF could be very low in consequence of minimal viral multiplication, we attacked the problem of antibody detection by testing a large series of sensitive mixed haemadsorption (HAD) techniques of Fagraeus (Brown, Cathala, Gajdusek, and Gibbs, 1971).

Using five different techniques to minimize the possibility of overlooking any selectivity in the production of antibodies, we found a significant difference in the titres of antibodies to measles virus, but not to other myxo- or paramyxoviruses in MS patients and controls. The mixed haemadsorption technique showed that 79% of the MS patients were positive as against 47% of the controls. This technique and the complement-fixation test have yielded the most highly significant difference in antibody levels between MS and control patients (Table X). It must be mentioned that after natural measles antibodies in cerebrospinal fluid are not found in all specimens, even when serum antibody levels are high and the method of detection used is the sensitive

	Technique				
	Neut	CF	HI	FA ¹	H-D ²
Multiple Sclerosis					
Positive (%)	13	39	11	12	79
No. tested	116	61	119	89	119
Controls					
Positive (%)	10	10	4	4	47
No. tested	169	61	112	95	112
Significance ³	N.S.	P < 0.001	P < 0.05	P < 0.05	P < 0.001

Table X Measles CSF antibodies in multiple sclerosis and control patients tested by five different techniques

¹Fluorescent antibody

²Mixed haemadsorption

³P < 0.001 for combined antibody tests (permutation test)

	MS Patients		Control Patients	
	Antibody Positive (10)	Antibody Negative (22)	Antibody Positive ² (3)	Antibody Negative (51)
Gamma globulins (mg %)	16.3 ³	3.9	23.8 ⁴	8.7
Total protein (mg %)	64.4		125.6	

Table XI Relation between the CSF gamma globulin and measles antibody levels in high titre specimens¹

¹Titre $\geq 1:10$ for one or more of the following tests, N, CF, HI, or FA

²Consisting of one case each of SSPE, total protein, 128 mg%, gamma globulin, 35 mg%; polyradiculoneuritis, total protein, 130 mg%, gamma globulin, 18 mg%; subdural neurinoma, total protein, 130 mg%, gamma globulin, 17 mg%

³P < 0.001 (compared to antibody-negative specimens)

⁴Statistical analysis not meaningful due to small number of specimens

HAD test (Castaigne, Cathala, Chateau, Schuller, Colomb, Baylet, Girard, and Dumas, 1972) (Table XIII). An overall correlation existed between the gamma globulin and measles antibody levels in the cerebrospinal fluid of MS patients, and the mean value of gamma globulin was highest in the group of patients with the highest antibody titres (Table XI). The three control cases with high measles antibody titres had SSPE, polyradiculoneuritis, and a subdural. It should be noted that the gamma globulin levels in many of the antibody-negative control patients were actually higher than the mean gamma globulin value in the highly positive MS patients, and in particular that some cases of MS showed a measles titre as high as those seen in SSPE (Table XII). A comparison between serum and cerebrospinal fluid measles antibody titres was made in 50 subjects (33 MS, 17 controls). Most of the subjects with positive cerebrospinal fluid titres also had high serum titres, but no statistically significant correlation was found, and equally high serum titres were associated with a number of negative specimens of cerebrospinal fluid.

Diagnosis	CSF Protein		Reciprocal Antibody Titre				
	Total Protein (mg %)	Gamma Globulin (mg %)	N	CF	HI	FA	HAd
Multiple sclerosis	38	3	10	4	10	< 5	80
Multiple sclerosis	43	10	1	2	10	< 5	40
Multiple sclerosis	71	9	< 1	4	< 5	10	40
Multiple sclerosis	78	21	< 1	4	10	< 5	160
Multiple sclerosis	74	24	1	2	10	5	640
Multiple sclerosis	51	23	< 1	< 4	< 5	10	20
Multiple sclerosis	65	23	5	< 4	20	5	80
Multiple sclerosis	96	27	1	< 4	20	5	320
Multiple sclerosis	92	16	2	< 4	40	5	320
Multiple sclerosis	36	7	< 1	< 4	20	5	20
Polyradiculoneuritis	130	18	10	< 4	40	5	640
Cervical subdural neurinoma	119	17	5	< 4	20	10	320
Subacute sclerosing panencephalitis	128	35	10	2	10	40	40

Table XII Patients with highest CSF measles antibody titres¹

¹Titre 1 : 10 for any conventional (N, CF, HI, FA) antibody

We believe that we have shown clearly that both the frequency and the titres of cerebrospinal fluid measles antibody is higher in MS than in control patients. The results suggest a relationship between cerebrospinal fluid measles antibody and gamma globulin, but the correlation is not absolute and does not allow us to conclude that the raised gamma globulin level in cerebrospinal fluid is due to the component of measles antibody. Certainly a part of it could be, and thus be independent of serum levels. In this connexion, we mention that from a study of 31 patients in various stages of MS, the majority of measles-positive specimens of cerebrospinal fluid came from patients with rapidly evolving symptoms of severe disease. We have shown earlier that the highest gamma globulin levels were also seen in patients with rapidly evolving disease.

Study of delayed hypersensitivity to measles virus antigens

Sever and Kurtzke (1969) and Knowles and Saunders (1970), using skin tests or tests of lymphocyte transformation, found no evidence of delayed type sensitivity to measles antigen in multiple sclerosis. On the contrary, the results of Reinert, Moulia, Goust, Harpey, and Cathala (1971) in SSPE patients suggested a state of tolerance in that disease, which would be in accord with the hypotheses of Burnett (1968).

Immunofluorescence Studies of MS Plaques

Tourtelotte (1972) has for a long time insisted upon the importance of gamma globulin associated with plaques. In SSPE, Ter Meulen, Enders Ruckle, Muller, and Joppich (1969) have demonstrated IgG and complement in lympho-plasmacytic infiltrates, and in the cytoplasm and axones of nerve cells. After separating the antigen-antibody complex by

Serum Titre (HI)	Frequency of Positive CSF					
	Measles Patients		MS Patients		Control Patients	
	No. Tested	Positive (%)	No. Tested	Positive (%)	No. Tested	Positive ¹ (%)
10-160	7	HI HAD 0 43	27	HI HAD 11 81	14	HI HAD 7 57
≥ 320	5	0 80	5	20 100	2	0 50

Table XIII Comparison of measles antibodies in sera and CSF of patients convalescent from measles, MS patients, and neurological controls

¹Positive control patients: one each of stroke, ophthalmic zoster, behcet, rickettsial, meningo-encephalitis, medullary compression, tumour, polyradiculoneuritis, parathyroid adenoma, myelitis of unknown aetiology

chemical methods he showed measles-specific antigen in nerve cells. He concluded that myelin destruction is provoked by the association of those antibodies with the antigen in infected cells. Membrane alteration by a complement-antibody complex has already been demonstrated *in vitro* (Mannweiler, 1969) in a chronically measles-infected strain of Hela cells. Thus, SSPE could well have a double origin: viral, possibly a 'modified' strain of measles virus (Horta-Barbosa *et al*, 1969; Ter Meulen *et al*, 1969) with or without an associated oncogenic virus, together with or followed by an altered immune host state.

Immunofluorescent study of MS plaques (Simpson *et al*, 1969; Lumsden, 1971; Tourtelotte, 1971) has also demonstrated IgG, IgM, and complement. According to Lumsden, the antimyelin activity of antibody is certain and it is well known that such antibodies present in the serum of MS patients can damage myelin *in vitro* (Bornstein and Appel, 1956; Lumsden, 1971; Tourtelotte, 1971). The immunological nature of plaque formation seems highly likely, and the example of SSPE suggests that at least

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the initial phases of MS might involve one or more viruses among which a virus similar or identical to measles virus could play the predominant role.

Summary

We have reviewed in this paper the current evidence in favour of a viral aetiology of disseminated sclerosis. Epidemiological data are consistent with an infection occurring before the age of 20, and having an incubation period of several years. However, the usual clinical course of an early relapsing phase followed by a later steady progression is difficult to explain solely on the basis of an infective process, and raises the possibility of an infection stimulating an abnormal immune response. Indeed, the frequently striking elevation of gamma globulins in cerebrospinal fluid, often in an oligoclonal pattern, and the presence of IgG, IgM, and complement in plaques, indicate an important immunological involvement in the disease.

Search for a viral agent responsible for initiating these events has so far proved inconclusive. No virus particles or inclusion bodies have been seen, and virus isolations have been rare and unconfirmed. However, a number of studies of serum and cerebrospinal fluid for virus antibodies have pointed to a possible role of measles virus in at least some cases of MS, and the recent proof of measles virus involvement in the evolution of SSPE, with which MS shares some clinical, pathological, and immunological features, provides further encouragement for this thesis. There are also some points of similarity between MS and certain other 'slow virus diseases', most notably visna in sheep, and PML and the 'post-infectious encephalopathy' group in man.

Studies of brain tissue from early acute cases, including initial efforts to dissociate possible antigen-antibody complexes, and cultivation or passage in the presence of immunosuppressive agents, or transforming viruses, should offer hopeful future approaches to this disease.

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