Multiple Sclerosis

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1. INTRODUCTION

The term "demyelinating disease" is conventionally given to a group of diseases of the central nervous system (CNS) characterized by focal or diffuse areas of demyelination and neuroglial sclerosis, usually unrelated to tracts and fiber systems. Demyelination is of two types, the so-called primary demyelination and secondary demyelination. The primary demyelinating diseases are multiple sclerosis (MS), Schilder's diffuse cerebral sclerosis, post- and parainfectious encephalomyelitis, postvaccinal and vaccine encephalomyelitis, acute necrotizing hemorrhagic leukoencephalitis, subacute combined degeneration, central pontine myelinoclastosis, Marchiafava–Bignani syndrome, and progressive multifocal leukoencephalopathy. A characteristic finding in primary demyelination is that the myelin sheets disintegrate initially, leaving the axon intact.

In secondary demyelination (Wallerian degeneration)¹ e.g., caused by cutting a nerve, this lesion will be followed by a fragmentation of myelin on the first day followed on the third or fourth day by swellings in the sheet. Between the seventh and the tenth day numerous spherules and fatty droplets appear, and at the end of the second week, a large part of the myelin has been removed by phagocytosis. In Wallerian degeneration, the peripheral part of the neuron degenerates.

The most widespread demyelinating disease is multiple sclerosis (i.e., disseminated sclerosis). This disease was originally described by Cruveillier² and Carswell.³ Supplementary descriptions were later made by Valentiner⁴ and Rindfleisch.⁵ However, Charcot⁶ was the first to recognize that the pathological findings were related to demyelination without destruction of axons. The areas of demyelination were given the term "*sclérose en plaques*." Many plaque areas are visible by the naked eye in sections of the brain and medulla. They appear as gray, slightly depressed areas, asymmetrically and sharply outlined. In an advanced stage of the disease, the brain may show shrinkage of the cerebral hemispheres with widening of the sulci and slight or moderate en-

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Fig. 1. Typical sites of lesions in multiple sclerosis. The plaque areas are numbered from 1 to 12. Plaques occur especially around the ventricles. (From Fog.²¹)

largement of the ventricles. On touching the tissue in simple palpation, it is obvious that the MS brain is more solid than the normal autopsy brain. The plaques may occur all over the brain, but in advanced stages of the disease especially, the wall of the lateral ventricles may contain numerous plaques. In the brainstem and spinal cord, favored sites are around iter Sylvius, under the floor of the fourth ventricle, and especially in the spinal cord. Characteristic lesions are below the pial surface and extend inwards as hemispherical or conical areas (Fig. 1).

Clinically, the disease is characterized by symptoms varying in time and place.^{7,8} Thus, retrobulbar neuritis, i.e., blindness of one eye lasting a few weeks, may be one of the initial symptoms.^{9,10} Of patients with retrobulbar neuritis, 30% develop MS, and 30% probably develops retrobulbar neuritis following MS. Other symptoms may be paresthesias of days or weeks in duration, vertigo, or paresis of muscular groups, thus producing speech and gait disturbances, neuralgia, spasticity, disturbed function of urinary bladder and defecation, and, finally, lack of coordination of muscular groups. The clinical course may be unpredictable and can be of different types.¹¹ Electrophysiologically, the demyelination may hamper nerve conduction in specific CNS pathways including those examined by visual evoked potentials, somatosensory responses, and auditory evoked potentials.¹²⁻¹⁴ Assay of functions of these pathways may contribute to tracing the topographic localization of the plaques.

Fig. 2. A central vessel with ramifications where plaque has occurred (confirmed by histological studies).

2. HISTOLOGICAL FINDINGS

Under low magnification, the outlines of most plaques appear sharply defined from the white matter. Ludwin¹⁵ indicates that there are two types of plaques, i.e., the active and the chronic plaque. The chronic plaque is sharply demarcated from the white matter. Here, the density of neurons is slightly diminished, and macrophages and oligodendrocytes are nearly lacking.¹⁶ The active plaque represents the early lesion in MS; here, numerous lipid-laden

Fig. 3. A drawing showing the early plaque formation growing along the venules. In these areas, lymphocytes are accumulating. (From Adams.^{21a})

Fig. 4. An infiltrate of lymphocytes extending out of a plaque along the course of a vein (Dawson's finger) (stain, hematoxylin and eosin; magnification $\times 400$). (From Adams.^{21a})

macrophages are encountered. With higher magnification, some myelin sheaths are seen to extend into the demyelinated areas, creating a border zone about 50–100 μm in width.

In the spinal cord, partially demyelinated areas are common. The demyelinated areas may be common. These areas may be visualized by means of the aniline blue method or by means of the reduced ammoniacal silver technique.¹⁷ Often the axons are intact; thus, King¹⁸ demonstrated by studying 125 cerebral plaques that only 10% showed severe loss of axons. However, axons do not pass intact through a plaque. By 1903, Bielschowsky¹⁷ had noticed evidence of regeneration of axons in plaques. Itoyama *et al.*¹⁹ have even shown evidence of remyelination of axons in the spinal cord by Schwann cells. Silver impregnation²⁰ has traced the accumulation of reticulin fibers in neuroglia.

Fig. 5. Perivenous cuff formed almost exclusively of lymphocytes in an area of normal myelin in active MS (stain, hematoxylin and eosin; magnification $\times 100$). (From Adams.^{21a})

From an anatomic point of view, it is interesting that most plaques are localized around venules and seem to grow and spread along the vascular system²¹ (Figs. 2,3). In early plaques, lymphocytes accumulate perivenously as “perivenous cuffs” (Figs. 4–6). These perivenous lymphocytes most probably accumulate prior to plaque formation (Fig. 5).

Attempts to draw conclusions about the etiology of MS on the basis of the histological findings have been hampered by the failure to demonstrate patterns of demyelination in MS resembling those found in experimental animal models, whether toxic, viral, or autoimmune in nature.^{22–25} Thus the so-called “vesicular” demyelination found in experimental allergic encephalomyelitis (EAE)²⁶ has only been demonstrated in one autopsy brain 4 hr after death.²⁷

Furthermore, virus inclusion bodies (e.g., measles virus, paramyxovirus, virus 6/94, multiple-sclerosis-associated agent, simian SV-5-virus, and coron-

Fig. 6. The incidence and prevalence of multiple sclerosis in white South African ethnic groups (including immigrants from England). It is obvious that in all age groups above 15 years, the incidence and prevalence are much higher among immigrants than among native-born South Africans. (From Dean.⁴⁸)

avirus) have rarely been traced, and such reports are contradicted by other laboratories.²⁸⁻³⁹ The viruslike bodies found have never been confirmed by reinvestigations in other laboratories, but it may very well be that in MS as in the Theiler virus infection (associated with demyelination) of the rat brain, the virus is only present in the brain tissue in minor amounts.⁴⁰

In the border zones of plaques, there seems to be an accumulation of microglia cells, and the astrocytes have swollen cell bodies and are binucleated. Here, the demyelination is associated with liberation of lysosomal enzymes, especially proteinases.⁴¹ The proteolytic enzymes may well be liberated within the myelin sheath in early stages of the degeneration, resulting in disruption of lipids bound to trypsin-digestible proteins. It is probable that the increased proteolytic activity in the degenerating nerves is not mainly a result of increased cellular secretion but of liberation or activation of enzymes preexisting in or around the normal nerve, and only at later stages of the demyelination may the increased proteolytic activity be related to liberation from macrophages and microglial cells.⁴² *In vitro* studies have furthermore revealed that soluble enzymes of leukocytes are able to degradate basic protein and Wolfgram protein, both of which are structural proteins of the myelin sheath.⁴³ The degeneration of the myelin sheath proteins is associated with a change in the lipid composition in the plaque areas. Thus already in 1945, Rossiter⁴⁴ demonstrated increased esterified cholesterol.

As is mentioned below, in EAE and MS, macrophages and subpopulations of lymphocytes accumulate in the perivascular space. Here they form lymphoid accumulations and lymphaticlike capillaries, probably channeling specific antibodies into the brain.⁴⁵

Fig. 7. Worldwide distribution of MS according to high- (solid), medium- (dotted), and low- (diagonal dashed) risk areas as of 1974. The high-risk area of Europe may reach the head of the Adriatic Sea in the Balkans. (From Kurtzke.⁵³)

3. EPIDEMIOLOGIC STUDIES

3.1. Topographic Studies

Several authors⁴⁶⁻⁵² have demonstrated that the prevalence of the disease among immigrants to an area is dependent on the age at which they migrate. Thus, Englishmen migrating to South Africa have nearly the same prevalence and incidence of multiple sclerosis in South Africa as in England if the individuals are above 15 years of age at the time of migration (Fig. 6). Similar studies have been performed by Leibowitz,⁴⁷ who studied immigrants in Israel originating from Arabic countries compared to those coming from central Europe. However, if the age at immigration is below 15 years, the incidence decreases to a lower level. These differences are explained by the theory that MS patients are infected by an unknown agent (virus?) in childhood and because of a very long incubation period, the disease does not become apparent until after adulthood. Clearly, the disease has the highest incidence and prevalence in industrial countries in the northern latitudes corresponding to central Europe and the northern parts of the United States⁵³ (Fig. 7). These are areas of high hygienic standards. In this respect, there are many similarities between multiple sclerosis and poliomyelitis.

Recently, two separate studies performed by Kurtzke and Hyllested⁵⁴ and by Poskanzer *et al.*⁵⁵ gave further support to the infectious theory. Thus, Kurtzke and Hyllested⁵⁴ demonstrated that the incidence of MS on the Faroe

Fig. 8. Distribution of all cases of MS among native-born resident Faroese according to calendar year of clinical onset, 1920 to 1977. Resident population for the Faroes is also noted. (From Kurtzke.⁵³)

Islands sharply rose to a peak value 4–5 years after these islands were occupied by English troops during World War II, and the epidemic curve declined 3–4 years after the English troops had left the Faroes after second World War (Figs. 8,9). Furthermore, a retrospective study⁵⁵ on the social connections between MS patients living on the Orkney and Shetland Islands demonstrated significantly high incidences of either previous personal contact or common contact persons. These data are supported by Gudmundsson,⁵⁶ who, on Iceland, found accumulation of MS in three local areas. Finally, however, the data by Isager *et al.*⁵⁷ seem to indicate that, opposite to what may be the case for Hodgkin's disease, MS does not seem to be related to increased school contact.

3.2. Genetic Implications in Multiple Sclerosis

In MS patients of the western European populations, certain tissue types seem to occur with a significantly higher rate than in the normal population, especially the tissue types HL-B7 and DW2 and, to a smaller extent, HL-A3.^{58,59} However, in other parts of the world, other HL types may be related to MS. Thus, in Canada, Pathy *et al.*⁶⁰ found MS related to HL-A7 and W-18. Compared to other HL types related to diseases such as ankylosing spondylitis, where the HL types at risk have 140 times more risk for disease than other HL types,⁶¹ in MS, the risk is only about two times (Table I). The hypothesis has been put forward that the association between MS and HL types may result from a gene linked to the HLA complex.⁶² Haile *et al.*⁶³ have conducted a

Fig. 9. Residences of MS patients during World War II (large solid circles) superimposed on locations of British troops (dotted areas) during the 1940-1945 occupation of the Faroe Islands. (From Kurtzke.⁵³)

Table I
Linkages between HL Antigens and Various Syndromes^a

Disease/syndrome	HL-A antigen	Occurrence of HL types in patients (%)	Occurrence of HL types in controls (%)	Relative risk
Spondylitis ankylopoetica	HL-A27	90	7	141.0
Reiters syndrome	HL-A27	76	6	46.6
Acute uveitis anterior	HL-A27	55	8	16.7
Myasthenia gravis	HL-A8	52	24	4.6
Multiple sclerosis	HL-A3	36	25	1.7
	HL-A7	36	25	1.5
Acute lymphocytic leukemia	HL-A2	63	37	1.7
	4c(W5)	25	16	1.6
Hodgkin's disease	HL-A1	39	32	1.3
	HL-A8	26	22	1.3
Ragweed hayfever	HL-A7	50	19	4.0

^a The table demonstrates a comparison of the occurrence of certain HL antigens in patients with different diseases and in a control population (expressed as percent). From these data the relative risk for development of the diseases can be calculated. From Lewin.^{61a}

formal linkage analysis in 40 families. If one assumes an autosomal dominant model of inheritance, the results are supported by a linkage when the presumed penetrance of the MS gene is low. These data are in part supported by Drachman *et al.*,⁶⁴ who studied the HLA types in families with more than two MS cases among first-degree relatives. No HL-A chromosome or type had any direct causal relationship to MS susceptibility, but indirect association could not be ruled out.

3.3. Epidemiologic Studies on the Virus Antibody Titers in MS

Sigurdsson⁶⁵ introduced the term "slow virus disease" to refer to a disease characterized by

1. A protracted period of latency (for months or years) after the virus has entered the body.
2. The latency period being followed by clinical symptoms. The disease often develops to a progressive fatal outcome.
3. The infection may often affect a single species and even often one specific organ.

Since several animal diseases such as scrapie and visna in sheep, mouse hepatitis caused by a corona virus (JHM), and canine distemper are caused by slow virus infections of the CNS, clinically active MS has been suggested to be a slow virus disease. Several authors⁶⁶ have tried by epidemiologic means to trace a probable virus as the etiologic agent. In 1962, Adams and Imagawa⁶⁷ demonstrated that groups of MS patients showed increased serum antibody titers against measles virus. Several studies have confirmed this finding⁶⁸⁻⁷¹ (Figs. 10,11).

Fig. 10. The measles antibody titers in multiple sclerosis patients, their relatives, and the control population. Abscissa, reciprocal value of serum dilution; ordinate, number of patients in the respective groups. It is obvious that the MS group includes a group with increased measles antibody titers. (From Ammitzbøll and Clausen.⁶⁹)

Fig. 11. Plot of the frequency of measles titer ≥ 128 versus mean age. The regression line for the groups without MS has the slope -0.52 ± 0.09 . MS, MS patients; Ch, children of MS patients; Si, siblings of MS patients; Pa, parents of MS patients; C < 30, control < 30 years old; C $\geq$ 30, control $\geq$ 30 years old. (From Ammitzbøll and Clausen.⁶⁹)

At the moment it is not clear if the high measles antibody titers in MS are an epiphenomenon or directly related to the disease. Thus, on one hand, annealing experiments⁷² have given evidence that in MS brains restricted areas of RNA express a measles virus genome, whereas on the other hand, Ammitzbøll *et al.*⁷³ were not able by lysolecithin fusion of cells from MS patients with Vero cells to demonstrate virus particles, e.g., measles virus. Furthermore Jersild *et al.*^{58,59} demonstrated that among MS patients those with HL-A types prone for MS (HL-A3, A7, and/or W18) exhibited higher measles antibody titers than patients with other HL-A types. These studies have in part been confirmed by Whitaker *et al.*⁷⁴ who, furthermore, excluded any other relationship of HL-1 types and serum antibody titers for rubella, herpes simplex, cytomegalovirus, and parainfluenza virus. Since canine distemper virus (CDV) has antigens in common with measles virus, and since it may well be that the increased incidence of MS on the Faroe Islands during World War II was related to canine distemper, epidemiologists have compared incidences of CD and MS and the CDV titers in MS and non-MS patients; however, no connection has been established.^{75,76}

Other viruses have also been implicated in MS, i.e., vaccinia, herpes, parainfluenza,⁷⁷⁻⁷⁹ (cf. Section 4) and even bacterias and rickettsia.⁸⁰ In regard to herpes virus, Martin⁸¹ has put forward the theory that MS could be caused by a herpes type 2 infection in an adult who did not have any childhood infection with herpes type 1. The overlapping antigenic specificities of the two viruses lead to antibodies against type 1 in part protecting against type 2 infection. The material on which the above-mentioned studies were made are too small for a safe conclusion to be drawn as to a relationship.⁸²

4. BIOCHEMICAL AND PATHOLOGICAL FINDINGS IN TISSUE AND BIOLOGICAL FLUIDS

4.1. Changes in Nervous Tissue

Since the pathological changes in MS include both demyelination and changes in the cellular composition in the plaques and border zone areas, biochemical abnormalities have been found in lipid, protein, and carbohydrate composition.

Changes in lipids have been reported both in plaque areas and so-called macroscopically normal white matter of MS patients. Thus, a characteristic finding is the decrease in myelin lipids and increase in the cholesterol ester concentration (cf. survey in ref. 82). This accumulation may be a mechanism for complexing the fatty acids liberated from the polar lipids degraded during the demyelination, thus preventing the free fatty acids from precipitating in the plaque area as calcium soaps. The cholesterol esters formed⁸⁴ are digested and transported by microglia and macrophages. Similarly, the digestion of the myelin sheath is followed by a decrease in the amount of proteins. Generally, in MS there is an increased fragility of cerebral lysosomes, giving rise to increased autolysis.⁸⁵ Recently, Yahara *et al.*,⁸⁶ by a careful dissection and separation

Table II
Comparison of Fatty Acid Composition of Normal and MS Myelin^a

	Total myelin	PC	PE	PS	Sph	Cerebroside II
C 14:0			I > II			
C 16:0					I > II**	
C 16:1	I < II		I > II			
C 18:0				I < II	I > II	I > II†
C 18:1			I > II			
C 20:4		I > II*	I < II			
C 22:6			I < II			
C 24:0	I < II*					
C 25:1					I < II	
C 24:1						I < II
C 26:1						I < II

^a Where nothing is mentioned, the groups are significantly different ($t > t_{0.995}$). * $P < 0.01$; ** $P < 0.025$; † $P < 0.05$. I, fatty acid percentage of normal myelin; II, fatty acid percentage of MS myelin. PC, phosphatidylcholin; PE, phosphatidyl ethanolamine; PS, phosphatidylserin; Sph: Sphingomyelin. From Clausen and Hansen.⁹³

of plaque areas from periplaque and apparently normal white matter, compared the lipid composition in these zones. It was possible to demonstrate an increase in the cerebroside and sulfatide content from the central plaque area to the normal-appearing white matter.

In 1953, Swank⁸⁷ suggested a close epidemiologic correlation between MS and the dietary intake of saturated fat and/or dairy products (cf. also ref. 86). This suggestion led workers to focus on changes in the fatty acid content of CNS and blood, especially with regard to the content of essential fatty acids (EFA).⁸⁹ Thus, Cumings *et al.*,⁹⁰ Gerstl *et al.*,⁹¹ and Thompson⁹² demonstrated in "normal white matter" of MS patients that the fatty acid composition was characterized by increased saturation.

Clausen and Hansen⁹³ found unrelated changes in saturation in different phospho- and glycolipid fractions of MS brains, thus arguing that in MS tissue there are no signs of general EFA deficiency (Table II). On the other hand, Clausen and Møller⁹⁴ found an increased susceptibility of rats to development of experimental allergic encephalomyelitis in EFA-deficient animals compared to normal control animals. These facts may be related to the disputed findings of a decreased content of linoleic acid in MS serum lipids,⁹⁵ which have not been confirmed by other workers⁹⁶⁻¹⁰⁰ or have been reported to be unspecific because similar changes are found in other neurological diseases and serious illnesses.^{101,102}

The controversy about the significance of changes in the linoleate level in MS has been resolved by the demonstration of a low level of glutathione peroxidase activity in MS. Glutathione peroxidase (GSH-Px) eliminates the peroxides formed by peroxidation of polyunsaturated fatty acids. Therefore, low levels of GSH-Px may give rise to peroxidation of linoleate. Glutathione peroxidase is a selenium-containing enzyme. Therefore, in areas of the world with low dietary intake of selenium causing low GSH-Px activity, low linoleate levels

may be seen. Clausen (unpublished data) has also noticed that the GSH-Px activity changes with the disease activity of MS (Section 4.3.2).

Myelin can be isolated from human brains by gradient ultracentrifugation of homogenate, usually in 0.32 M sucrose.¹⁰³ Discontinuous centrifugation in gradients increasing from 0.32 M sucrose has revealed the myelin to be distributed into different subfractions from 0.5 to 0.9 M sucrose.^{103,104} Because of this, and because of lysosomal digestion and different cellular distribution in MS tissue, even in macroscopically normal appearing matter, where microplaques may be situated, the myelin and its subfractions isolated from MS brains may be partially dismantled and may even contain nonmyelin particulate fractions. It is unclear to what extent myelin fractions isolated from MS brains thus represent an anatomically pure fraction. This may explain the conflicting results obtained in earlier studies. Thus, Cumings and Goodwin¹⁰² found a decrease in the cerebroside/sulfatide ratio. On the other hand, Clausen and Hansen⁹³ and Woelk and Borri¹⁰⁵ found a decreased sulfatide content (expressed per dry weight) and an increase in the cerebroside/sulfatide ratio of MS myelin. Phosphatidylserine and -inositol have also been found to be decreased.^{93,105} Since the myelin of MS brains originates from nonplaque areas, these findings argue for biochemical abnormalities even in nonplaque areas.^{86,106} The myelin, furthermore, shows an abnormal distribution with regard to its buoyant densities in sucrose gradients.^{107,108} Thus, Yu *et al.*¹⁰⁸ state that the "pathological" myelin in MS is less dense than normal myelin. They even found evidence from their findings on the P₀-protein in the upper layer from MS spinal cord that "remyelinated" myelin is present in MS. Thus, it must be concluded that MS myelin shows an abnormal structural density.¹⁰⁹

The finding by Riekkinen *et al.*¹¹⁰ of a lack of basic protein in MS myelin has not been corroborated by Wolfgram and Tourtelotte.¹¹¹ Eng *et al.*,¹¹² or Wolfgram.¹⁰⁶ Thus, apart from changes in the buoyant densities of MS myelin, most authors conclude that the MS myelin from normal-appearing white matter of MS brain may be quite normal, at least in its protein pattern. The lipid changes found mainly concern fatty acid chain length and probably changes in the proportion of cerebrosides to sulfatides.

With regard to changes in CNS proteins in MS, Riekkinen *et al.*¹¹³ found increased proteinase activities in border zones to plaques, probably explaining the decreased content here of myelin basic protein.¹¹⁴ No changes were found in other myelin proteins.

Although, until now, no conclusive answers have been drawn by histological studies as to whether a microbiological agent is involved in the pathogenesis of MS, early immunochemical studies revealed circulating complement-fixing antibodies in MS serum against an antigen extractable with ethanol.¹¹⁵ Although conflicting results have later been accumulated,¹¹⁶ this has stimulated further work on the question of whether "MS-specific antigens" are present in MS autopsy brains. Thus, it has been possible by means of salting out procedures, column ion exchange, and sepharose affinity chromatography to isolate from MS brains a glycoprotein fraction in which two glycoprotein antigens could be traced by crossed immunoelectrophoresis. Neither of the two

Fig. 12. Crossed immunoelectrophoresis of a glycoprotein fraction isolated from a MS brain. The fraction was obtained from the cytosol ($100,000 g_{\max}$ 1 hr supernatant) by stepwise molecular filtration, DEAE column chromatography, followed by con A sepharose column affinity chromatography (for details see refs. 101–103). The glycoprotein fraction was then subjected to crossed immunoelectrophoresis [40 μ g of the glycoprotein fraction was applied in hole B in 1% buffered agarose (0.05 M sodium veronal buffer, pH 8.6)] and electrophoresed from left to right. Then, the electrophoretic fraction was electrophoresed perpendicularly to the first dimension into an antibody-containing gel. The antibody was manufactured by immunizing rabbits with the cytosol fraction of MS brain tissue. The antibody was absorbed with normal brain cytosol fraction (for details see refs. 101–104). Two precipitates appear corresponding to two antigens that are specific for MS brain tissue.

glycoprotein antigens was present in control brains.^{117–120} A third antigen was further traced in the MS cytosol fraction (Fig. 12, Table III).

A blinded preparative study on 42 brains¹²⁰ revealed these antigens only to be present in MS and not in non-MS brains. In MS autopsy brains, the antigens were traced in 80% of all samples studied and in 100% of all spinal cord MS samples studied (Table IV).

Immunoglobulin G isolated from MS serum furthermore seems to contain antibody specificity against the “MS-specific antigens”¹²⁰ and to the cytosol proteins of MS brains but not to other particulate fractions of MS brains.^{121,122}

Table III
Quantitation of the Yields of Crude Glycoprotein Fraction (G) and Glycoprotein Fraction 2 (G2) Isolated from Multiple Sclerosis (MS) and Control (K) Brain Cytosol (60 mg Protein, Approximately 3 g Brain Wet Weight)^a

Brain type	No. of brains used	Total G isolated (mg protein) (Mean $\pm$ S.D.)	Total G2 isolated (μ g protein) (Mean $\pm$ S.D.)
MS	7	2.50 $\pm$ 0.32	262.5 $\pm$ 53.8
K	9	2.04 $\pm$ 0.22	141.3 $\pm$ 30.7
		$P < 0.025$	$P < 0.001$
<i>Percent recovery from</i>			
MS cytosol		4.2	0.44
K cytosol		3.4	0.24
<i>Percent of G2 isolated from</i>			
MSG			10.5
KG			6.9

^a P values are calculated for Student's t test. G was isolated by means of Con A-Sepharose column and G2 by DEAE cellulose column chromatography of G. From Rastogi and Clausen.¹¹⁹

Chemically, two of these three antigens may be characterized as glycoproteins since they have affinity for Con A. They have molecular weights above 100,000, and the third of them may be formed in measles-infected Vero cells.¹¹⁸ Furthermore, these antigens are able to specifically to stimulate MS (but not non-MS) lymphocytes in the active Rosette method similarly to what has been demonstrated in experimental allergic encephalomyelitis, where Hashim *et al.*¹²³ demonstrated that the basic protein increases the number of rosette-forming T cells. It may well be the MS-specific antigens that cause crude brain extracts of MS brains to increase the number of active rosetting T cells from the peripheral blood of MS patients.¹²⁴⁻¹²⁷ This reaction seems specific for MS lymphocytes, although lymphocytes from patients with rheumatoid arthritis and systemic lupus erythematosus are also significantly stimulated.

The MS-specific antigens are able to induce a significantly higher loss of neutral proteinase from MS leukocytes than from control lymphocytes, which

Table IV
Presence of MS-Specific CNS Antigens (MSG 2) in CNS Sections^a

Diagnosis	Number of patients		Sections containing MSG2/total number of sections		
	Total	With MSG2	Cerebral hemispheres	Brainstem	Spinal cord
Multiple Sclerosis	23	18	13/23	3/6	6/6
Other Neurologic Diseases (OND)	12	0	0/10	0/2	0/2
Controls	10	0	0/10	0/1	0/0

^a From Rastogi *et al.*¹²⁰

are able to degrade myelin proteins¹²⁸ (Fig. 13). The supernatant of stimulated leukocytes hampers the uptake of thymidine of MS lymphocytes significantly more than non-MS lymphocytes.¹²⁹ The identity and origin of the MS specific proteins are, however, still unknown.

4.2. Changes in the Cerebrospinal Fluid

In 1942, Kabat *et al.*¹³⁰ demonstrated by means of free electrophoresis that γ -globulins were elevated in the CSF of MS patients. Later, by means of agar-agarose and polyacrylamide (PAGE) electrophoresis,¹³¹⁻¹³⁷ it has been shown that the increase affects discrete intervals of mobilities in the γ areas, i.e., that the γ -globulins appear as oligoclonal bands representing products of few lymphocyte clones (B cells) (Figs. 14,15). In contrast to the findings in subacute sclerosing encephalitis (SSPE), these bands do not represent antibodies against one type of virus (measles virus in SSPE) but contain antibodies against measles, rubella, vaccinia virus, and/or bacterias that differ from MS patient to MS patient^{135,138,140} corresponding to the increased viral antibody titers in CSF of MS patients.⁷⁰ The oligoclonal bands have also been traced in plaques, but the bands of different plaques from the same patient do not correspond.¹⁴¹ The oligoclonal immunoglobulins are synthesized by lymphocytes (B cells) that have migrated to CNS.¹⁴² Recent data collected on the immunologic mechanisms involved in experimental allergic encephalomyelitis (EAE) may explain at least some of the findings mentioned above. Thus, EAE is an autoimmune disease (considered to be an animal model of MS) provoked by challenging experimental animals with myelin basic protein (BP) or by peptide remnants of BP treated with proteinases.^{123,124}

During the development of EAE, lymphocytes of peripheral blood and lymph nodes migrate to CNS. They are thus localized in the perivascular and perineural space as well as in CNS tissue. Recent studies by Traugott *et al.*¹⁴⁵ have documented that B and T lymphocytes in EAE have different localizations. Thus, the B cells were found in the perivascular space, whereas the T cells were traced to the CNS tissue. This different distribution may mean that the B cells do not communicate with subsets of T cells, and, locally, they are challenged by different viruses to which the patient has previously been infected and which probably are activated for unknown reasons. Because the B cells are not communicating with T cells, the B cells may uncontrollably synthesize individual clones of immunoglobulins of different immunologic specificity. This is in agreement with the findings of Ammitzbøll, Clausen, and Fog¹⁴⁶ that oligoclonal bands occur preferentially in the CSF of MS patients with high measles virus titers. Kam-Hansen¹⁴⁷ demonstrated that among the cells of CSF in MS, the T cells are proportionally higher and the B cells lower than in blood. Furthermore, she found a lower number of active T cells in CSF compared to blood in MS. Since, as described above, an uneven distribution and lack of communication among different subsets of lymphocytes may well occur in MS, the biological meaning of the abnormal lymphocyte distribution in CSF of MS patients may be difficult to evaluate. Also, other facts may influence the proportions among subsets of lymphocytes. Thus, Coyle *et al.*¹⁴⁸ found immune

Fig. 13. The glycoprotein fractions of cytosol from MS and control brains (MSG2 and KG2, respectively) were isolated as indicated in Fig. 12. Left: Percent loss of intracellular neutral proteinase from leukocytes of an MS patient incubated with various amounts of MSG2 and KG2 for 4 hr *in vitro*. Abscissa, nanograms protein MSG2 or KG2; ordinate, percent loss of neutral proteinase activity. Right: Lysosomal neutral proteinase activity of leukocytes of a normal subject after incubation with KG2 and MSG2 in PBS for various periods of time. Abscissa, incubation period; ordinate, neutral proteinase activity.

Fig. 14. Oligoclonal bands. Photo of agar-gel microelectrophoresis of 100-fold concentrated CSFs from a patient with lymphocytic meningitis and from a patient with multiple sclerosis. A: Case H.F. no. 633, lymphocytic meningitis. B: Case A.A. no. 3439, multiple sclerosis. A volume of 10 μ l was applied in the trough.

complexes in CSF from 50% of all MS patients associated with a relative decrease in the number of T- γ cells (suppressor cells) (*vide infra*). The lack of communication among different subsets of lymphocytes in CNS may also explain the finding of oligoclonal bands in cerebrovascular diseases.¹⁴⁹

The spinal fluid is formed by the plexus choroideus and as an effusion from the surface of the brain. Therefore, the demyelination process in MS gives rise to liberation of lipids into CSF. In normal CSF, the concentrations of lipids and lipoproteins are very low. Thus, the level of serum β -lipoprotein is in the range of 0.010 to 0.062 mg/100 ml.¹⁵⁰ In the demyelination process, the concentration of β -lipoprotein is raised, probably because of a decreased blood-brain barrier. Compared to the normal CSF, the MS CSF has a raised glycolipid/lecithin ratio, probably as a result of liberation into CSF of cerebroside from the CNS.¹⁵¹

As an alternative to the theory that MS may be caused by a microbiological agent, several authors have put forward the theory that MS, like EAE, is an autoimmune disease caused by serum or spinal fluid autoantibodies against myelin basic protein, myelin lipids, e.g., gangliosides, or muscular or oligodendroglial antigens.¹⁵²⁻¹⁵⁷ Since membrane antigens, basic protein, and several other antigens such as the staphylococcal antigen A bind to the Fc moiety and not to the antibody site of immunoglobulins, such studies cannot demonstrate any specificity before work with the (Fab)₂ piece has proven the spec-

Fig. 15. This figure demonstrates the specific intrathecal synthesis of IgG in multiple sclerosis by relating the IgG index, i.e., the ratio of concentration of IgG in spinal fluid and serum to the corresponding ratio for albumin:

$$\frac{(\text{CSF IgG})/(\text{serum IgG})}{(\text{CSF albumin})/(\text{serum albumin})} = \text{IgG Index}$$

to the albumin coefficient (concentration of albumin of spinal fluid to that of serum). The albumin coefficient reflects the increased permeability of the blood-brain barrier, which may cause a passive diffusion of IgG from serum to spinal fluid compartment. This factor is included and compensated for in the IgG index. Obviously, the MS group demonstrates a significantly raised IgG index, arguing for specific intrathecal IgG globulin synthesis.

ificity of the reaction.¹⁵⁹ Using the (Fab)₂ piece, Traugott and Raine¹⁵³ demonstrated that the autoantibodies to the basic protein in the spinal fluid in MS are an unspecific phenomenon, since other patients also have these autoantibodies. On the other hand, by means of the complement fixation test, Ryberg¹⁵⁸ was able to trace anti-brain antibodies of IgG type in both MS serum and CSF (92% of all MS patients studied). The titers of these antibodies correlated with the malignancy and disability of the disease.

Recently, Gerhard *et al.*¹⁵⁸ and Nagelkerken *et al.*¹⁵⁹ elucidated the oligoclonal bands in CSF from MS patients by means of anti-idiotypic antibodies. The levels of these antibodies have been demonstrated to be 8–15 times higher in CSF than in serum. Gerhard *et al.*¹⁵⁸ further demonstrated that the anti-idiotypic antibodies have specificity towards Theiler murine encephalomyelitis virus. Only more extensive studies can evaluate the implications of these findings.

4.3. Changes in Blood

Although MS is a disease of the central nervous system, several biochemical and immunologic parameters are changed outside the CNS. These changes involve the formed elements of blood, lipids, proteins (enzymes), and trace elements.

Broman¹⁶¹ demonstrated by means of supravital staining that there is an open communication between the blood compartment and the plaque areas. This may explain some of the changes traced in blood of MS patients.

The extracerebral changes in MS have been shown to be either changes in immunoregulatory mechanisms or changes in membrane function. At the present state of the research, it is not possible to evaluate if these changes are epiphenomena or directly related to the etiology.

4.3.1. Changes in Immunoregulatory Mechanisms

Studies on the proportion of total number of T cells to that of B cells in MS compared to normal controls and related to phase and type of multiple sclerosis have not given consistent results.^{147,148,162} As already mentioned in Section 4.2, the immunoregulatory control of B cell secretion of antibodies may be lacking in MS as a result of different topographic localization of B cells and T cells in MS (T_μ, T_γ, and T_k cells). Thus, in phases of acute exacerbations of MS, Arnason and Antel¹⁶³ demonstrated reduced concanavalin-A-induced suppressor cell activity. The specificity of these changes is difficult to evaluate because the immunologic abnormalities related to the demyelinating process occur in CNS as in EAE.¹⁶⁴ Thus, the lymphocytes from the blood in MS may migrate to the CNS, and the remaining circulating cells of the blood may thus only reflect an epiphenomenon. In acute attacks of MS, most authors^{163–168} have traced a deficient suppressor function (increase in T_γ cells).

These data are, however, difficult to explain from data demonstrating a decreased reactivity of lymphocytes towards measles virus antigens^{169,170} and other virus antigens.¹⁷¹ Neighbor and Bloom¹⁷² even demonstrated a reduced

virus (measles)-dependent lymphocyte suppressor and interferon production in MS. The T γ cells are, however, labile and, when exposed to immunocomplexes, redistribute their receptors by forming receptors for the μ chain of IgM, whereby the T γ cells are transformed to T μ cells.¹⁷³ Therefore, since Goust *et al.*¹⁷⁴ traced immunocomplexes in 14 out of 19 MS patients, the abnormal proportion of T γ to T μ cells in MS may simply reflect circulating immunocomplexes, and this may explain why Huddlestone and Oldstone¹⁷⁵ only traced in MS a transient decrease in suppressor cells during disease exacerbations.

In contrast to these findings, Dore-Duffy *et al.*¹⁷⁶ demonstrated an increased adherence of MS blood lymphocytes to epithelial cells persistently infected with measles virus. Frey *et al.*¹⁷⁷ and Kinnman and Link¹⁷⁸ could, however, not confirm this phenomenon as specific for MS. Furthermore, the reactivity of blood lymphocytes towards a crude brain extract and towards MS-specific brain antigens (*vide supra*) is increased when lymphocyte reactivity is assayed by means of the active rosette method of Fudenberg *et al.*^{124–127} The lymphocytes rosetting with sheep red blood cells within a few minutes of reaction are those T cells involved in the delayed hypersensitivity—the so-called active T cells. This increased activity was, however, lower in the group of patients with relapses.¹²⁷ At the moment, it is not clear how to explain the discrepancy between the deficient suppressor function activity and the increased number of active T cells. Gangliosides liberated from CNS may influence the T-cell reactions.¹⁷⁹

Studies on a possible autosensitization of lymphocytes in MS patients to myelin basic protein or its fragments as well as studies on the presence of cytotoxic antibodies against the basic protein or glia cells or on the possibility that this protein induces secretion of interferon or leukocyte or macrophage migration inhibition or enhancement factors have given conflicting results.^{179–184}

The communications among different types of leukocytes are mediated by secretion products. Among these are proteinases. Since, as stated above, the plaques are in open communication with the blood compartment, the proteinases probably involved in the demyelination are liberated, e.g., from macrophages to the blood, where they are partially complexed to α_2 -macroglobulin (causing a decreased electrophoretic mobility of serum α_2 M in MS¹⁸⁴). The α_2 -M-bound proteinases in MS contain a fraction with an acidic isoelectric point of 4.2.¹⁸⁶ At the moment, it is not clear which role that factor plays in either the demyelination or the immunoregulation. Since proteinases may be secreted like lymphokines from lymphocytes and macrophages and may regulate the function of subsets of lymphocytes (and leukocytes), the liberation of proteinases in MS brains may well contribute to the above-mentioned abnormal distribution of lymphocyte subpopulation.

4.3.2. Changes in Membranes

Early in MS research (*vide supra*), it was suggested that the disease was related to a high intake of saturated fat and/or a deficiency in essential fatty acids (EFA).^{87,187,188} These changes in fatty acid composition, although not

specific for MS,^{96,101} may lead to changes in membrane fluidity, “sludging” of the red cells with increased fragility of these cells,¹⁸⁹ and increased agglutinability of thrombocytes.⁸⁹ Indeed, Prosiegel *et al.*,¹⁹⁰ in a careful study of thrombocyte agglutinability in MS and control patients that took into account dietary and pharmacological factors, have recently demonstrated that in acute exacerbations of MS the thrombocytes reveal an increased agglutinability induced by ATP and serotonin. At the moment, it is not possible to decide if this finding is an epiphenomenon or directly related to the etiology. However, the increased agglutinability may be related to an increased peroxidation activity in MS as a result of decreased activity of glutathione peroxidase.^{191–193} The increased peroxidation rate in MS may also explain the decreased levels of EFA. Glutathione peroxidase is a selenium-containing enzyme. In agreement with the fact that Jensen *et al.*¹⁹⁴ found changes in serum selenium levels in MS that were proportional to the lowered GSH-Px activity of MS lymphocytes, the low GSH-Px levels found in MS lymphocytes were also traced in granulocytes and erythrocytes of MS patients.¹⁹⁴ In MS patients with low GSH-Px activities of hematogenous cells and low serum selenium levels as well as low linoleate levels in the plasma membranes of peripheral lymphocytes, antioxidative medication with 3 mg selenium (as sodium selenite) combined with 2 g vitamin C and 500 mg vitamin E per day completely normalized the above mentioned biochemical abnormalities. At the moment, it is unclear if this normalization process is associated with clinical improvement (J. Clausen, preliminary results). The low level of glutathione peroxidase may cause a low rate of prostaglandin formation,¹⁹⁵ since the 15-hydroperoxyprostaglandin R₂ is decomposed by GSH peroxidase; thus, the low level of decomposition of peroxystaglandin intermediates may induce platelet aggregation.¹⁹⁶

5. TREATMENT OF MULTIPLE SCLEROSIS

Neither preventive measures nor curative treatments of MS are available. The evaluation of clinical effects of treatment of a disease such as MS with a more or less progressive development and with spontaneous remissions is difficult and should always follow a strictly defined line.^{197,198} From what has been mentioned above about theories about the pathogenesis of MS, clinical trails to stop or retard the disease have concerned:

1. Immunologic intervention.
2. Intervention with the proteolytic degradation of myelin.
3. Dietary treatments.
4. Neuropharmacological treatment.

5.1. Immunologic Intervention

Immunologic intervention has implied use of ACTH, corticosteroid hormones, IgG, cyclophosphamide, transfer factor treatment, and plasmapheresis.^{199,202}

Steroid hormones and ACTH seem to restore clinical performance during acute exacerbations but do not influence the major clinical course, even if intrathecal application is used.²⁰³ Neither the transfer factor²⁰⁴ nor cyclophosphamide seems to be able to arrest the disease, although a recent study seems to indicate that the transfer factor can retard cases with moderate activity.²⁰⁵ However, studies on a limited number of MS patients of the effect of plasmapheresis seem to indicate a beneficial effect on a selected group of patients refractory to prednisone (cases with acute severe exacerbations). At the moment, the clinical data are too small to give any safe conclusions.

Initial studies of the beneficial effect of human immunoglobulins¹⁹⁹ on a limited number of MS patients seem to indicate improvement of clinical scores that measure physical symptoms.

5.2. Intervention with the Proteolytic Degradation of Myelin

As mentioned above (cf. also ref. 124), the demyelination in EAE and MS may be caused by the activation and liberation *in situ* of proteinases. Experimentally, Boehme *et al.*^{206,207} and Sibley *et al.*²⁰⁸ were able to suppress EAE by means of proteinase inhibitors. Therefore, it may be relevant to use such inhibitors in MS treatment.

Only few studies have been performed in this field. Amaducci *et al.*²⁰⁹ elucidated the effect of ϵ -aminocaproic acid (EACA), a proteinase inhibitor, i.e., inhibitor of the conversion of plasminogen to plasmin in a short-term open clinical trial. Small probable improvements in clinical scores were noticed.

5.3. Dietary Treatment

Originally, Swank^{187,188} treated MS patients with a low-fat diet in long-term experiments. Clinical improvement but no arrest of the disease has been quoted. Following demonstration of low EFA levels in MS serum, three double-blind studies of the effect of dietary linoleic acids on the clinical course of MS have been performed. In two double-blind studies,^{210,211} a significant beneficial effect was found on severity and duration of relapses, whereas a third study²¹² was unable to demonstrate any effect. Dworkin,²¹³ however, has reinvestigated all three studies and drawn attention to the fact that in all three studies the beneficial effect of linoleic acid is found mainly in patients with illness of short duration and/or low disability.

The question about the eventual beneficial effect of dietary treatment of MS patients with essential fatty acids should, however, be reinvestigated from the point of view of whether the low linoleate level is related to increased autoperoxidation of essential fatty acids as a result of low glutathione peroxidase and selenium levels in MS.^{191–194} An antiperoxidative treatment such as that indicated above with selenium (as sodium selenite), vitamin C, and vitamin E may more easily normalize low essential fatty acid levels (J. Clausen, unpublished data).

5.4. Neuropharmacological Treatment

This treatment is only symptomatic and concerns treatment of spasticity, urinary bladder function, and defecation. These treatments are not the topic of the present communication, but the reader is referred to classical pharmacological textbooks.

6. CONCLUSION

Multiple sclerosis is a demyelination disease characterized by a perivenous demyelination in areas of the brain where lymphocytes and macrophages also initially accumulate. Most authors believe it to be an inflammatory disease, probably of viral origin. Most abnormalities demonstrated are nonspecific. Thus, the finding of oligoclonal bands in the spinal fluid is nonspecific since this is also found in cerebrovascular diseases, and the variation in pattern of oligoclonal bands in MS tissue (CNS) also argues that this finding is nonspecific. Further, the demonstration that CSF oligoclonal bands in MS contain polyspecific antibodies against different viruses and even bacteria argue for either the nonspecificity of this finding or lack of immunologic communication between subsets of lymphocytes in CNS. Changes in subsets of lymphocytes in serum and CSF in MS may either be explained by a redistribution phenomenon with migration of specific subsets to CNS or by redistribution of lymphocyte receptors as a result of their exposure to immunocomplexes identified in both MS serum and CSF.

The finding of autoantibodies in MS to myelin proteins and lipids is also nonspecific, since these antibodies are found in other neurological diseases as well.

The pathogenic role and specificity of the so-called MS-specific antigens found in CNS of MS patients need more studies; the same applies to both the increased level of measles virus antibodies in biological fluids of MS patients and the annealing experiments that identified a measles virus genome in MS CNS.

The changes in fatty acid composition of cellular membranes and the low levels of essential fatty acids of serum lipids in MS as well as the increased peroxidation rate may well be epiphenomena, but, on the other hand, they argue for systemic cellular changes in MS.

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