# Multiple Sclerosis

## Present and Future

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# Multiple Sclerosis
## Present and Future

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Proceedings of an International Workshop on Multiple Sclerosis, held August 29–
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PREFACE


     This book is based on the papers delivered at an International
Workshop on Multiple Sclerosis held in Erice, Italy from 29th August
to 1st September 1983.  The Meeting was organized with great
efficiency in the delightful setting of the Centro di Cultura
Scientifica Ettore Mahorana, by its Director, Antonino Zichichi,
and was directed by the Editors of the Proceedings and by Professor
C. Alvisi of the University of Bologna.

     The emphasis was deliberately on the contributions of laboratory
science to the understanding of multiple sclerosis, its etiology,
pathogenesis, diagnosis and treatment.  Where so much is unknown,
disagreement was expected and indeed welcome, and it is regrettable
that it was not possible to publish verbatim the often animated and
always interesting discussion.

     Differing views on the relevance of experimental allergic
encephalomyelitis to multiple sclerosis were expressed by Dr.
Wisniewski, Professor Seitelberger and Professor Alvord, who graphi-
cally illustrated his changing beliefs over the years.  Professor
Seitelberger laid much greater emphasis on remyelination in remission
in multiple sclerosis than had previously been the accepted view.
The disorder of both humoral and cellular immunity in multiple scler-
osis was discussed by Dr. Roos and Professor Link.  Dr. Gilden
described how recombinative techniques might be applied to the detec-
tion of a virus in multiple sclerosis.  The continued inability to
detect any antigen to match the oligoclonal IgG bands in the CSF in
multiple sclerosis patients was disappointing and there was an
increasing tendency to regard the bands as "nonsense" antibodies.
Dr. Latov spoke of the myelin-associated glycoprotein that might be
the initial point of attack for the demyelinating process.

     Professor Field reiterated challenging views on the laboratory
and clinical aspects of multiple sclerosis that have not found wide
acceptance.

The difficulties of epidemiological studies were emphasized by Professor Granieri and colleagues, with particular reference to interesting findings of the unexpectedly high incidence in southern Italy.

Diagnostic techniques with particular reference to the patho-physiology of multiple sclerosis and also to its treatment were discussed by Dr. Kolar and Dr. Schauf and the topic of evoked potentials in this context was also reviewed.

Finally, Dr. Gonsette presented a most comprehensive account of treatment of MS based on study of the immunological disorder.  It was possible to detect that a number of the authors he cited had tended to confuse evidence of immunosuppression with evidence of clinical benefit, and it was evident that much more precise methods of manipulating the immunological system were required.

G. Scarlato and
W.B. Matthews

# CONTENTS

PATHOGENESIS OF PERIVENOUS AND DEMYELINATING
ENCEPHALOMYELITIS AND ITS RELEVANCE FOR
MULTIPLE SCLEROSIS RESEARCH*

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INTRODUCTION

The inflammatory demyelinating plaque in the brain and spinal
cord is the hallmark of multiple sclerosis (MS) pathology (Charcot,
1868; Marburg, 1906; Dawson, 1915). The pathology consists of pri-
mary demyelination (Marburg, 1906) and chronic perivenous inflam-
mation, mainly located around larger veins and venules (Seitelberger,
1970). It was noted at the beginning of this century that plaques
show a high variability of structural changes, i.e., from extensive
cellular infiltration to few cells present, various degrees of axonal
and oligodendroglia loss, and various degrees of remyelination,
gliosis and connective tissue reaction. These observations lead to a
large number of different, sometimes even contradictory pathogenetic
theories of this disease (Dawson, 1915; Lassmann, 1983).

Experimental allergic encephalomyelitis (EAE), an autoimmune
disease of animals induced by challenge with central nervous system
(CNS) antigen, had been considered as an experimental model of MS
since the earliest descriptions by Rivers et al., (1933). Because
the goal of many of these experiments was to determine which com-
ponent of the brain was responsible for the demyelinating encephalo-
myelitis, there was a great need for a method which would shorten the
time and procedure required to induce the disease. This was achieved
when Freund and McDermott (1942) introduced a new technique of immu-
nization by emulsifying antigens in various adjuvants. Using this
technique (Kies and Alvord, 1959; Roboz and Henderson, 1959; Laatsch
et al., 1962) it was possible to demonstrate that the antigen respon-
sible for EAE was myelin basic protein (MBP). Introduction of
Freund's ajuvant (CFA) accelerated the search for finding the antigen

responsible for EAE; however, it changed the clinical course and the
morphology of the disease.  In contrast to the previous chronic, low
mortality demyelinating encephalomyelitis, EAE became an acute mono-
phasic disease.  The monophasic nonprogressive course of EAE in
contrast to progressive and multiphasic course of multiple sclerosis
(MS) is one of the main reasons that many investigators do not con-
sider EAE as a model of MS.  However, in recent years reproducible
small animal models of chronic EAE have become available (Stone and
Lerner, 1965; Wisniewski and Keith, 1977; Massanari, 1980; Lublin et
al., 1981; Brown et al., 1982).  These small animal models cover the
full spectrum of MS pathology (Wisniewski et al., 1982; Lassmann,
1983) and allow the study of the pathogenesis of chronic inflammatory
demyelinating plaque formation.  The present review will focus on new
data obtained from these models which may help us understand the
mechanisms leading to perivenous and demyelinating encephalomyelitis
in humans and animals.


## Antigen Recognition in the CNS by Sensitized Lymphocytes

     EAE is induced by peripheral sensitization with CNS antigens.
Evidence suggests that the disease is initiated by a cellular immune
reaction directed against MBP (Waksman and Morrison, 1951; Kies et
al., 1960).  However, it is not clear how the sensitized T cells,
present in the circulation of EAE animals cross the blood brain
barrier (BBB) and recognize an antigen(s) which is located in the
major dense lines of the myelin sheath.  One of the possible mechan-
isms explaining this phenomenon is that there appears to be a low
rate of physiological exchange of lymphocytes between the CNS and the
circulation, which allows the entry of the sensitized T cells.  The
presence of a small number of mononuclear cells (Oemichin, 1983) in
the CSF of normal individuals gives some support to this possibility.
However, comparing the large number of lymphocytes in the circulation
with the small number of hematogenous cells in the normal brain it
seems unlikely that sensitized T cells in EAE reach the brain as a
result of normal turnover of lymphocytes between the brain and the
blood.  Another possibility is that sensitized T cells recognize the
antigen at the level of the endothelium of cerebral vessels.  In a
recent study concerning experimental subacute sclerosing panence-
phalitis (SSPE) we have shown that virus antigen is present in the
cytoplasm and on the luminal surface of endothelial cells of cerebral
vessels in infected animals (Wisniewski et al., 1983a,b).  This viral
antigen may be the target of the initial inflammatory reaction in
this disease.  In EAE, recognition of the antigen at the level of the
endothelium of cerebral vessels is only possible if the following
requirements are fulfilled: 1) the presence of a soluble pool of MBP
in the extracellular space of the brain; 2) the transport of MBP
through the endothelial barrier of cerebral vessels; and 3) the
presentation of MBP together with histocompatibility antigens on the
luminal surface of cerebral vessels.  Recently we have shown that MBP

injected into the CSF of normal rats is transported through the endothelial barrier of meningeal vessels, and is then bound to the luminal surface of endothelial cells of meningeal veins (Vass et al., 1983). Furthermore the presence of immune response associated (Ia) antigen on the surface of cerebral endothelial cells has been documented in normal as well as EAE animals (Sobel, 1983). These results indicate that MBP, when liberated in the CNS tissue may be transported through the BBB and presented on the luminal surface of cerebral vessel endothelium. The question remains unsettled, whether the release of MBP in normal animals is sufficient to allow recognition of this antigen on the endothelial surface. The presence of an MBP serum factor in normal and EAE animals has been described by Paterson and Day (1979), which may originate from MBP turnover in the CNS tissue. Furthermore it has to be considered, that minor traumatic events in the CNS tissue may liberate small amounts of MBP. This could explain the high incidence of lesions in EAE and MS in areas of the brain and cord with a high probability of minor mechanic strain, like tearing and shearing forces (Oppenheimer, 1978; Lassmann, 1983).

## Factors Governing the Development of Acute and Chronic Disease

Clinico-pathological studies using various doses of antigens suggest that the induction of a chronic type of EAE depends upon a delicate balance between the antigen dose and the host response (Lassmann and Wisniewski, 1979; Wisniewski and Madrid, 1983; Lassmann, 1983). For example, sensitization of young guinea pigs with 25 mg spinal cord tissue and 1 mg Mycobacterium tuberculosis leads to severe and fatal acute EAE. Doubling the encephalitogenic dose in the same animals reduces the acute mortality and leads to a high incidence of chronic EAE. Further increase of the dose leads to further reduction of mortality in acute EAE, but also decreases the severity of chronic EAE. Challenging adult animals with a similar dose of encephalitogen as necessary for the induction of chronic relapsing EAE in juvenile animals results in high mortality during acute EAE (Lassmann and Wisniewski, 1979). However, chronic progressive EAE may be induced in adult guinea pigs with a very high dose of encephalitogen (Wisniewski and Madrid, 1983). Thus the induction of chronic EAE may be due to suppression of acute EAE with high doses of antigen, which however are not high enough to induce full high zone tolerance.

## Continuous Antigenic Stimulation – A Requirement for Chronic Disease Activity

The factors responsible for the establishment of chronic progressive or recurrent disease in MS pathogenesis are unresolved. Observations based on both humans and animals suggest the necessity

of a peristent peripheral antigenic stimulation for development of
chronic inflammation and demyelination (Wisniewski et al., 1982).
These observations were confirmed by Tabira et al., (1983) who, after
removal of the inoculum by leg amputation, observed arrest of chronic
EAE.  Exactly how a peripheral deposit of neuroantigen acts on the
maintenance of the disease process in chronic EAE is at present
unknown.  It is possible that a balance between release and local
degradation of the neuroantigen is established through the action of
local factors at the site of injection.  If at one point in time a
mitogenic dose of neuroantigen reaches the draining lymph nodes, then
proliferation of specific neuroantigen-sensitized cells would occur
and a clinical relapse would follow.  A recent report describing the
induction of relapsing EAE in mice after five-six months of adoptive
transfer of syngeneic lymph node cells from MBP-sensitized animals
would appear to contradict the prerequisite of continuous or discon-
tinuous antigenic stimulation for the induction of chronic relapsing
EAE (Mokhtarian et al., 1983).  The mechanism of induction of chronic
disease by adoptive transfer is as yet unclear.

In this regard, interesting observations have been made in
humans during the study of neurological complications after rabies
vaccination (Uchimura and Shiraki, 1957), and as a result of thera-
peutic trials with brain tissue (Seitelberger et al., 1967).  In both
situations, an inflammatory demyelinating disease of the CNS, closely
resembling acute MS, was induced during the course of injections.
However, the disease dissipated weeks to months after the last in-
jection of brain tissue (Haruhara, 1958).  There is also no evidence
that such pathological processes of the brain as inflammation, tumor,
trauma or stroke release autoantigens sufficient to develop peri-
venous or demyelinating encephalomyelitis.  These observations
suggest that in humans autosensitization leading to demyelinating
disease does not occur.  It is possible that in humans, like in
experimental animals, discontinuous or continuous peripheral anti-
genic stimulation may be necessary for maintenance of a chronic
inflammatory demyelinating disease.


## The Humoral Immune Response in EAE and its Relation to Disease Activity

One of the important findings in MS is the increased level of
IgG in CSF (Tourtellotte, 1972).  Comparison of IgG to albumin ratios
in CSF and blood (CSF IgG index) indicates that IgG is synthesized
within the CNS in MS (Ganrot and Laurell, 1974; Olsson and
Pettersson, 1976).  A number of investigators (Mehta et al., 1981;
Karcher et al., 1982) have shown that guinea pigs with acute and
relapsing EAE have increased concentrations of IgG in their CSF or in
neutral pH extracts of brain tissue.  Employing the CSF IgG index,
some investigators (Glynn et al., 1982; Suckling et al., 1983) were
unable to determine whether IgG synthesis took place within the CNS

of relapsing EAE animals.  BBB damage which accompanies active demye-
linating lesions was probably a contributing factor in the difficulty
in determining whether IgG was synthesized within the CNS or entered
the brain as a result of BBB changes.  In contrast, recent studies
(Karcher et al., 1983; Olsson et al., 1983) showed that animals which
were sacrificed after 125 days, and demonstrated mild clinical signs
and symptoms as well as few active lesions had evidence of intra-
thecal synthesis of IgG.  Immunofluorescence and electron microscopic
studies (Grundke-Iqbal et al., 1980; Madrid et al., 1981) have shown
the presence of plasma cells within the parenchyma in relapsing EAE
animals supporting the notion that part of the IgG may be synthesized
within the CNS.

A number of investigators using electrophoresis or isoelectric
focusing demonstrated the presence of oligoclonal IgG bands in CSF
from 85 to 90% in patients with MS (Johnson et al., 1977; Laurenzi et
al., 1980; Lowenthal, 1979; Mattson et al., 1981; Mehta et al.,
1981).  Evidence of bands in serum, CSF and CNS extracts in relapsing
EAE was also found (Mehta et al., 1981; Olsson et al., 1982; Karcher
et al., 1983; Glynn et al., 1982).  Recent studies have shown that in
chronic relapsing EAE animals, the oligoclonal IgG band patterns were
identical in CSF and serum (Mehta et al., 1983).  These results
suggest that the plasma cells present within and outside the CNS are
of the same type.  In contrast, about 50% of MS sera did not show
oligoclonal bands and those which showed bands had patterns either
partially similar or completely different from those seen in matching
CSF (Mehta et al., 1982).  The reason for the discrepancy at present
is not clear, however, it could be they are a response to (a) anti-
gen(s) present within the CNS or (b) they could be masked by poly-
clonal IgG.  Thus, it seems that in MS, the CSF oligoclonal IgG
represents a response to antigen(s) present within the CNS, whereas
in serum the bands are probably masked by polyclonal IgG or presence
of immune complexes.  The chronic EAE animals have peripheral de-
posits of CNS antigens.  Therefore, the pattern of oligoclonal bands
in the brain extracts, CSF and serum is similar.  In MS the location
of the antigens driving the disease are not known.

The specificity of the major oligoclonal IgG in MS is not known.
However, in most MS patients, evidence of the intrathecal IgG syn-
thesis to a number of viruses (Vartdal et al., 1980; Salmi et al.,
1983) as well as the presence of anti-brain antibodies in their CSF
(Ryberg, 1978) has been reported.  It is possible that the major
oligoclonal IgG bands are not directed against any specific set of
antigens but represent a general activation of B-cell clones during
attacks of MS (Paterson and Whitacre, 1981).  A similar situation may
exist in the sera of relapsing EAE animals, since a majority of
oligoclonal bands in sera of chronic relapsing EAE animals were not
directed against CNS antigens (Glynn et al., 1982).  Although a
number of studies showed antibody to MBP in CSF and sera, and anti-
body to glycolipid and myelin in sera (Schwerer et al., 1983), it is

not clear if the activity is associated with oligoclonal or poly-
clonal IgG.

In summary, increased IgG levels and oligoclonal bands are
present in both chronic EAE and MS.  Moreover, they do not seem to
change during remissions and exacerbations in both MS and relapsing
EAE.  In chronic-EAE the oligoclonal bands (in spite of the fact that
CNS antigens were used for sensitization) are not directed solely to
CNS antigens.  Without the knowledge of the sensitizing antigen in
chronic EAE, analysis of oligoclonal brand and the levels of IgG, in
spite of existing deposits of these antigens, did not tell us that
the disease was initiated and driven by antigens of CNS origin.  If
we cannot determine the antigens to which the animal is sensitized
using a powerful adjuvant, strong encephalitogenic antigens and
sensitive immunologic techniques, the possibility of determining the
driving antigens for the initial attack and relapse in MS using the
same techniques, in our opinion is hopeless.  Data from animal
studies have shown that of many CSF oligoclonal bands, only a few can
be absorbed with CNS antigens (Glynn et al., 1982).  It is not clear
whether these non-CNS antibodies play any role in the pathogenesis of
tissue damage.  THeoretically all anti-brain antibodies (see mech-
anism of tissue damage) and antibodies directed toward foreign anti-
gens residing in the CNS can contribute to the process of demyelin-
ation, oligodendroglia and axonal loss.  However, available method-
ologies have not allowed the determination of antibodies which may be
important during a new attack of the disease.


The Cellular Immune Response in Chronic EAE and its Relation to
Disease Activity

Cell-mediated immune (CMI) mechanisms are known to be largely
responsible for allograft rejection, microbial immunity of the tuber-
culin type as well as the recognition of a variety of simple chemical
and possibly tumor antigens.  The role of CMI in human autoimmune
disease is less clearly established, but sufficient evidence suggests
an important component and even central role of CMI in experimental
autoimmune thyroiditis, orchitis, myasthenia gravis, and EAE (Neuwelt
and Clark, 1978).

There are numerous studies of CMI in animals with acute EAE.
Relatively few studies have investigated CMI in a chronic relapsing
model.  Traugott et al., (1978) have reported a decrease in circulat-
ing early T cells (using rosetting techniques) associated with both
the onset of disease and with subsequent relapses.  It is not clear,
however, whether MBP-reactive T cells are responsible for relapses in
chronic EAE.

Our results indicate that a peripheral T cell response to MBP
correlates with the onset of the first attack of chronic EAE.  This

time course parallels that of skin-reactivity to MBP (Shaw, 1965) and
that of acute circulating T cells (Traugott, 1978) in acute EAE.
Neither the T cell response, as measured by lymphocyte transformation
to MBP, nor serum MBP-antibody level seem to correlate with relapses
and remissions in chronic EAE (Schwerer et al., 1983).  Thus, the
involvement of MBP in the precipitation of relapses remains unclear.
On the other hand, as discussed above, CNS-antigens other than MBP
might be responsible for relapses and demyelination in chronic EAE.
CMI studies do not conclusively show that immune reactions against
MBP or other CNS-antigens play a role in the pathogenesis of MS.

Most studies of CMI in EAE have used lymphocytes obtained from
extraneural sites.  By studying the properties and functions of
inflammatory cells within the CNS lesion, one would expect to better
understand the relationship of lymphoid cells to disease activity.
One such study by Lublin and Maurer (1981) was aimed at showing the
differences in the immune response of lymphocytes from the CNS of
guinea pigs with acute EAE compared to peripheral blood lymphocytes
from these animals.  They reported no proliferative response when the
CNS lymphocytes were cultured with MBP.  THere are several possible
explanations for this lack of response.  There may be factors (sup-
pressor macrophages or lymphocytes) which render the isolated cells
unresponsive to MBP (see discussion below).  Also myelin fragments
from the brain tissue may saturate the receptors for MBP.  It may
also be possible that too few MBP responsive T cells were isolated by
this technique.

Our laboratory has also studied (Schuller-Levis et al., 1983)
inflammatory cells isolated from the CNS of guinea pigs with chronic
EAE.  Similar to the results of Lublin and Maurer (1981), preliminary
findings show a minimal response of these lymphocytes to MBP, again
showing no evidence for the relationship of disease activity and CMI
under these conditions.

With the standard proliferation assay, up to now it has not been
possible to detect a significant proliferative response to MBP from
both peripheral and brain lymphocytes in chronic EAE models.  The
studies of Ben-Nun and Lando (1983) in an acute EAE mouse model have
shown that the removal of the adherent cell fraction from the immun-
ized cell population and its replacement with fresh naive accessory
cells could reveal a significant proliferative response to MBP.
THese findings indicate that MBP responsive T cells may be present
during the course of chronic EAE, but are in an unresponsiveness
state under the conditions employed.  The reasons for the unrespon-
siveness to MBP requires further studies and may be important in
understanding the course of chronic EAE.  MBP antigen excess with
subsequent high dose tolerance and activation of T cell suppression
also has to be seriously considered.  T cell activation is known to
require syngeneic Ia products plus antigen (Schevach and Rosenthal,
1973; Benacerraf and McDevitt, 1972).  The T cell recognizes the Ia

product and the MBP epitope. Studies with synthetic polypeptide
antigens (Kapp et al., 1975; Benaceraff and McDevitt, 1975) have
shown that polypeptide antigens, free from Ia containing cells, will
activate T suppressor mechanisms. If the same principle holds for
MBP or other polypeptides possibly involved in chronic EAE, similar
mechanisms may be implicated. The finding of fresh Ia bearing cells
restoring unresponsiveness suggests that an equilibrium exists bet-
ween the accessory cell Ia saturated or unsaturated with MBP and the
T cell with its Ia and MBP receptors. These data suggest that the
state of responsiveness or unresponsiveness of the T cell may be
important in the pathogenesis of EAE. Future studies, rather than
concentrating on the standard assessment of CMI during the course of
EAE should focus attention on the reasons behind the unresponsive
state of the T cell.

The abnormalities of cell mediated immunity in MS have been well
reviewed by Waksman (1981) and McFarlin and McFarland (1982). Total
T and B cells vary little with the long term course of the disease.
However, at the time of acute exacerbation a slight fall in T cells
has been shown by the use of rosetting techniques and monoclonal
antibodies (Lisak, 1975; Reinherz, 1980). Functional changes have
also been reported in peripheral T cells with regard to respons-
iveness to mitogens. Also, in one study natural killer (NK) cells
and NK precursors induced by interferon were deficient in a third or
more of the MS patients (Bloom, 1980). Peripheral blood leukocytes
have been shown to have a marked increase in antibody-dependent
cellular cytotoxicity (Merrill 1982). There are also many other
changes in CMI reported in MS. However, most of these subtle changes
are not clearly related to clinical disease activity, and may also
occur in other neurological diseases and normal individuals.

As indicated above, T and B cells and macrophages do play an
important role in the pathogenesis of MS, although changes in these
cells do not always correlate with disease activity. Problems emerge
in evaluating CMI data due to the high variability of the disease
from patient-to-patient, lack of longitudinal studies, different
methods of evaluation, problems of quantifying in vitro assays, and
variation of therapy. The changes on CMI produced by steroids alone
cannot be ignored. Most investigations of CMI in MS have been done
using peripheral lymphoid cells, because of the difficulty in obtain-
ing CNS lymphocytes. It is important to emphasize that CMI changes
in the peripheral lymphoid system are subtle and that studies of
lymphocyte abnormalities in the CNS could be more promising. How-
ever, thus far, alterations in CMI have not clearly identified the
primary event (autoimmune, virus) which leads to the development of
MS nor have these changes correlated closely with disease activity.

## Inflammatory and Demyelinating Antigens

Sensitization studies with various fractions of CNS tissue
indicate that independent antigens may be responsible for inflam-
mation and demyelination (Figure 1).  Sensitization with purified MBP
may lead to acute or chronic encephalomyelitis.  This disease however
is predominantly inflammatory with perivenous distribution and little
demyelinating activity (Pannitch and Ciccone, 1981, Madrid et al.,
1982).  Recent studies show that there are other CNS antigens like
lipophilin and proteolipid apoprotein (Hashim et al., 1980; Madrid et
al., 1982; Cambi et al., 1983) which can also induce perivenous
encephalomyelitis.  A combined sensitization with MBP and antigens
like galactocerebroside or sialogalactosylceramide (GM4) leads to the
formation of large confluent demyelinating plaques (Madrid et al.,
1983).  The concept of two classes of antigen needed for induction of
demyelinating plaques is consistent with our previous observations
(Madrid et al., 1981; Wisniewski et al., 1982) that polyantigenic
stimulation was most effective in the development of extensive
demyelination.


## Mechanisms of Tissue Damage

Antibody dependent cell mediated cytotoxicity (ADCC) and by-
stander demyelination have been proposed (Wisniewski and Bloom 1975;
Wisniewski, 1977; Brosnan et al., 1977) as a leading mechanism re-
sponsible for tissue damage and plaque formation in inflammatory
demyelinating diseases.  These as well as other observations on the
role of macrophage activation products, complement and direct cell-
ular attack were recently reviewed (Wisniewski and Lassmann, 1983).
In this section some of the new information not included in these
reviews is discussed.

Recent data by Trotter and Smith (1983) have demonstrated that
peritoneal macrophages secrete phospholipases and that macrophage
homogenates degrade myelin _in vitro_.  Opsonization of myelin resulted
in a 50% to 300% increase in the uptake of myelin by monolayers of
macrophages.

There is also recent evidence for a direct cellular destruction
of oligodendrocytes _in vitro_ by T lymphocytes sensitized against
galactocerebrosides (Niediek and Lehman, 1982).  However, there is
overall little evidence for direct cellular attack in the absence of
humoral factors.

Several recent experiments have focused on the role of serum
factors and complement in demyelination.  In sera from rabbits with
white matter-induced-EAE, Grundke-Iqbal et al., (1981) have demon-
strated that in addition to IgG complement-dependent factors non-IgG
proteins are responsible for _in vitro_ demyelination.  In addition,

INFLAMMATORY
(ENCEPHALITOGENIC) ANTIGENS                    DEMYELINATING ANTIGENS

MBP                                            Galactocerebroside

Proteolipid Proteins                           Ganglioside $GM_4$

Lipophilin                                     Exogenous Antigens?

Exogenous Antigens
(Virus, Bacteria, Vaccine)

Perivenous
Encephalomyelitis

Inflammatory
Demyelination
with Plaque
Formation

No Disease
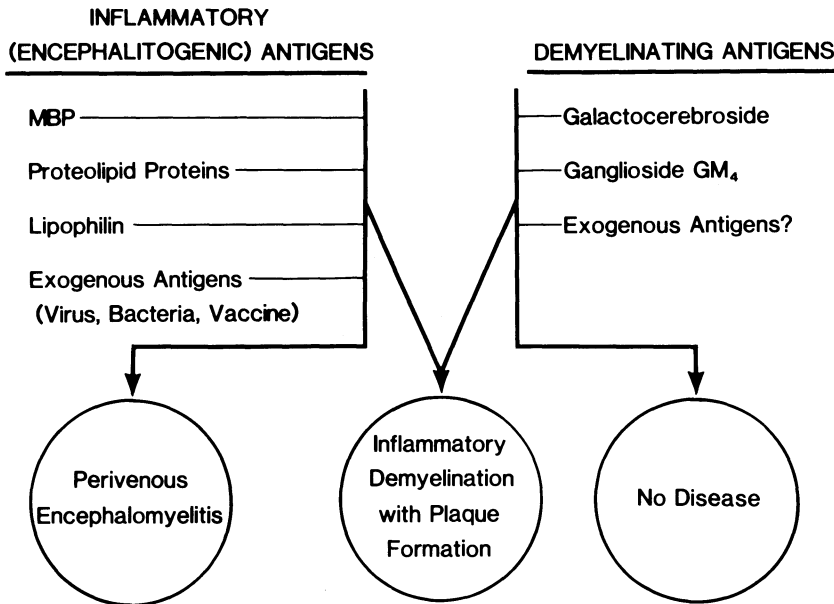
Fig. 1.   Inflammatory and demyelinating antigens and their role in
          pathogenesis of perivenous and demyelinating encephalo-
          myelitis.

data indicate that, unlike acute EAE, in MS most of the serum demye-
linating activity is due to non-immunoglobulin factors, which are
possibly complement dependent, and that serum immunoglobulins play a
minor role in demyelination in vitro (Bornstein and Grundke-Iqbal,
1982).

     The exact interaction of cells and serum factors at the site of
inflammation which lead to demyelination remains unknown; indeed,
understanding this process may eventually lead to modification of
tissue damage by pharmacologic agents.

Multiple Sclerosis as an Autoimmune Disease

     Usually in the immune system some lymphocytes are present which
are capable of recognizing autoantigen (Weckerle et al., 1974).  Thus
in normal individuals a low level of autoreactivity against CNS
antigens like MBP, cerebroside and ganglioside is frequently present
(Yung et al., 1973; Diessner et al., 1974; Dore-Duffy et al., 1980;
Frick, 1982).  The manifestation of an autoimmune disorder appears to
be prohibited by immunoregulatory mechanisms.  Recent immunological
studies in MS have shown an apparent defect in immune regulation
(Waksman, 1981; Weiner and Hauser, 1982), most importantly a decrease

of suppressor cells in the blood and CSF of patients during activity of the disease (Antel et al., 1979). It is interesting that in a recent study all MS cases with active disease demonstrated a high cytotoxicity of T-lymphocytes directed against MBP, encephalitogenic peptide, cerebroside, as well as gangliosides (Frick, 1982). However, earlier studies, using different test systems, did not show good evidence for autoimmunity in MS (Weiner and Hauser, 1982). Furthermore, treatment trials with MBP in MS thus far have not revealed clear-cut positive results (Salk et al., 1980). The pathogenetic role of MBP and other autoantigens in MS is therefore still controversial.

## The Role of Hetero-Antigens in the Etiology of MS

Although, as discussed before, autoimmunity appears to play an important role in the pathogenesis of inflammatory demyelinating lesions, several aspects of MS and related inflammatory demyelinating diseases are difficult to explain on the basis of autoimmunity alone. One important aspect deals with the induction and maintenance of the disease process. As discussed above in chronic EAE the disease is arrested within a few weeks after loss or removal of the sensitizing antigen from the inoculation site (Wisniewski et al., 1982; Tabira et al., 1982). A similar continuous peripheral antigenic stimulation may also be required for MS. In humans, a disease closely resembling acute MS (Marburg, 1906) is induced by active sensitization of patients with brain tissue (Uchimura and Shiraki, 1957; Seitelberger et al., 1958). However, in all described cases the disease process ceased a few months after the last injection of antigen. Furthermore, there is at present no evidence available that damage to CNS tissue with liberation of CNS antigens may by itself induce a self perpetuating autoimmune disease directed against myelin or oligodendroglia.

Indirect epidemiological evidence suggests that MS may be induced by an exogenous agent. At this point it should be noted that the many attempts to demonstrate the presence of virus in the brain of MS patients, using tissue culture and/or morphological techniques (EM, immunohistochemistry) have failed to find virus. However, absence of evidence is not evidence of absence, and there are nucleic acid hybridization studies which suggest that virus material may be present in the CNS of MS patients (Weiner et al., 1973; Simon, 1982).

There are several ways in which exogenous agents may be involved in the etiology and pathogenesis of MS. A virus may, by infecting lymphoid cells, interfere with immune regulation. This may allow the action of autoreactive clones of lymphoid cells directed against CNS antigens. It is difficult to understand however how selective auto-sensitization against CNS tissue can occur. One of the theoretical possibilities is that components of self, especially Ia product, are

involved in T cell recognition and that different Ia plus different environmental antigens may result in organ specific attack in individuals of selected histocompatibility.  Viruses may also induce an autoimmune reaction against CNS antigens by cross-reactivity or by transient infection of oligodendrocytes.  However, currently there is no evidence for cross-reactivity of viral and brain antigens and no evidence that autoimmune disease can be established as a result of oligodendroglia infection.  Finally, it is possible that the genomic infection of the CNS by the virus may result in an inflammatory demyelinating disease morphologically indistinguishable from that resulting from autosensitization.  This possibility is under current study in several laboratories.

SUMMARY

In a summary statement of the Report and Recommendations of the National Commission on Multiple Sclerosis published in 1974 it was said "One obstacle in the path of investigators seeking the cause of and cures and treatments for multiple sclerosis is the fact that the disease occurs, so far as is yet known, only in man", and "what MS researchers need for the ultimate benefit of patients is a susceptible animal – in laboratory language an animal model.... So far, either the appropriate animal has not been selected or a correct method of inducing the disease has not been found."  At present, the closest approach to an animal model of MS is EAE to which many species of laboratory animals are susceptible.  Approximately $1,000,000 a year is being spent on EAE research, primarily in the hope that it will provide clues to multiple sclerosis.  However, there is controversy among investigators as to the value of this model.  Advocates of the EAE model point to its resemblence to MS in one important respect: it results in loss of myelin in the CNS – the amount varying with the species of animal used.  EAE can be induced at will in a susceptible strain of animals by the injection of any one of a number of antigenic proteins; it can be almost as readily switched off by an injection of the same material that induced the disease.  Critics of EAE point to the important way in which it differs from the human disease: when it is allowed to run its course, EAE is not marked by the unpredictable cycles of exacerbation and remission so characteristic of MS.  The National Advisory Commission took a "judgment reserved" position in regard to the value of this experimental model of MS.

A Satellite Conference on EAE was held July 16-19, 1983 in Seattle, Washington, and the question asked by Dr. E. C. Alvord was, "How good a model of MS is EAE today?"  Dr. E. C. Alvord, one of the most productive contributors in the studies of EAE, during the welcoming remarks showed his graph illustrating, since 1944, the ups and downs regarding suitability of EAE as a model of MS.  He stated, "I am 80% convinced that EAE is the best model of MS.  Which way are you going to move me?"

In our opinion recent data does not call for an up or down shift of the graph but rather for a change of the graph. From the studies of the autoimmune chronic relapsing and/or chronic progressive EAE, it appears that the clinical course of the disease, immunology, electrophysiology, and pathology is similar to that seen in MS (Table 1). Therefore, for the study of the pathogenesis of MS the pure autoimmune chronic relapsing and progressive model of EAE in guinea pigs and rats should score almost 100% on Dr. Alvord's graph. However, there is growing evidence that the initiating antigen in MS is not MBP or any other antigen(s) of CNS origin. For example, none of the lesions releasing a lot of autoantigens such as trauma, stroke and tumors of the brain, lead to MS-like pathology. Therefore, from an etiological point of view MS and chronic EAE are diseases caused by very different antigens.

What are some of the lessons that have been elucidated from the autoimmune acute and chronic relapsing and/or progressive EAE models:

1.  CNS tissue contains both inflammatory and demyelinating antigens.
2.  Histocompatibility antigens and various levels of tolerance govern the susceptibility and development of acute and/or chronic relapsing or progressive autoimmune EAE.
3.  Continuous or discontinuous antigenic stimulation is needed for the development of chronic relapsing or progressive EAE.
4.  There is no evidence that naturally occurring pathological processes in the brain (inflammation, tumor, mechanical trauma, stroke) lead to the release of autoantigens sufficient to initiate or to continue an autoimmune perivenous or demyelinating encephalomyelitis.
5.  Presence of anti-brain antibodies, (whatever the reason for their appearance) in the presence of macrophages may lead to demyelination, oligodendroglia and axonal loss.
6.  In chronic EAE current humoral and cell mediated immunologic diagnostic techniques have not allowed us to determine the antigens to which the animal was sensitized. In practical terms it means that in chronic EAE, immunological methods, which in infectious diseases can help to identify the infectious agent and determine the activity of the disease, did not clearly identify the antigen(s) responsible for initiation of the disease. This conclusion is disturbing because current immunological techniques are unable during the chronic stage of autoimmune EAE to determine the antigens which were used for sensitization and of which deposits still are present at the site of injection. If this is true in experimental situations it is probably also true in MS. Therefore with immunological technologies discussed above, it is unlikely that either the antigens responsible for initiating MS nor the antigens responsible for relapses in MS will be determined.

Table 1.  Similarities of the Chronic Relapsing EAE Model to MS.

| | |
|---|---|
| Clinically | Chronic relapsing progressive course. |
| Pathology | Large (macroscopically visible) demyelinated plaques with comparable topographical distribution. <br> Inflammation (mononuclear cells). <br> Primary demyelination. <br> Oligodendrocyte loss. <br> Reactive gliosis. <br> Simultaneous presence of plaques of different age. <br> Plaque growth by either confluence of perivenous lesions or radial growth in preexisting plaques. |
| Immunology | Involvement of both cellular and humoral immune systems in the pathogenesis of the disease. |
| Electrophysiology | Dominance of delayed conduction velocity in VEP. <br> Some reduction in amplitudes. |
| Other | Critical age of dependency. <br> Genetic susceptibility. |

### Should the Inducing Antigen(s) (Etiology) of MS be Identified Today; How Effectively could the DIsease be Treated?

1.  If MS is a pure autoimmune disease, no specific (i.e., desensitization) therapy is effective in other known autoimmune diseases (e.g., thyroiditis).  A broad spectrum of immunosuppressive and anti-inflammatory therapies was currently in use.

2.  If MS is caused by exogenous (hetero) antigen(s), e.g., persistent or genomic viral infection, today there is no specific antiviral therapy.  Experience with SSPE using antimeasles antibodies or vaccine during the advanced stage of disease does not appear to be effective.

3.  If MS is a result of a combination of the autoimmune and exoantigen(s) hypothesis, again, there is no effective therapy.

Historically, knowledge of the cause and/or pathogenesis of a given disease leads to cure or a rational therapy.  Experience with slow infections caused by well known conventional viruses like SSPE indicate that so far a therapeutic strategy to treat the patient once the signs and symptoms of the disease occur.  However, if vaccination programs are successful then chances of eradicating the disease are good.  Even a superficial review of the treatment regimes of autoimmune diseases indicate that we did not succeed in development of

any specific therapy.  Why did it happen?  The reason is the lack of
basic information about the immune system mechanism rejecting or
allowing infectious agents or any other antigen(s) to interact with
host cells (histocompatibility antigens, mechanism of low and high
tolerance, role of interferons and interferon like molecules etc).
Our poor understanding of the mechanism of viral genomic and
persistent infections is another factor which does not allow the
development of successful antiviral therapy.

       Recognizing that not enough is known about the basic processes
which lead to the development of MS, what can be done to improve the
chances of effectively treating MS patients?  On the basis of the
data discussed in this review, we think it is possible to identify
areas of the most promising research in MS rather than leave it to a
chance phenomenon – which appears to be done by some of the MS re-
search funding agencies.  While discussion on specific priorities of
research in MS is beyond the scope of this review, we do elaborate
these issues in a paper in preparation: "Current Trends in MS Re-
search – Areas of Priorities."

\*       To Mrs. Alma Sagan, the best fighter of the MS malady and all
        those heros who fight back the disease, this paper is dedicated.
\*\*      Dr. Hans Lassmann is a visiting scientist from the Neurological
        Institute, University of Vienna, Vienna, Austria.


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REFERENCES

Alvord, E. C., 1983, Historical perspective and challenge:  How good
        a model of Multiple Sclerosis is experimental allergic en-
        cephalomyelitis today?  Abstract from the Satellite Meeting of
        the International Society of Neurochemistry, Seattle,
        Washington.
Antel, J. P., Arnasan, B. G. W., and Medof, M.E., 1979, Suppressor
        cell function in multiple sclerosis: correlation with clinical
        disease activity, Ann.Neurol., 5:338.
Benacerraf, B., and McDevitt, H., 1972, Histompatibility-linked
        immune response genes, Science  175:273.
Ben-Nun, A., and Lando, Z., 1983, Detection of autoimmune cells
        proliferating to myelin basic protein and selection of T cell
        lines that mediate experimental autoimmune encephalomyelitis
        (EAE) in mice, J.Immunol., 130:1205.
Bloom, B., 1980, Immunological changes in multiple sclerosis, Nature
        (London) 287:275.

Bornstein, M. B., and Grundke-Iqbal, I., 1982, Circulating
      meylinotoxic and neuroelectric blocking factors in
      demyelinative disorders, in: "Clinics in Immunology and
      Allergy," W. B. Waksman, eds., W. B. Saunders Co., Ltd.,
      Philadelphia, Vol. 2, p.297.
Brown, A., McFarlin, D. E., and Raine, C. S., 1982, Chronologic
      neuropathology of relapsing experimental allergic encephalo-
      myelitis in the mouse, Lab.Invest, 46:171.
Cambi, F., Lees, M. B., Williams, R. M., and Macklin, W. B., 1983,
      Chronic EAE produced by bovine protolipid apoprotein.  Immuno-
      logic studies in rabbits, Ann.Neurol., 13:303.
Charcot, J. M., 1868, Histologie de la sclerose en plaque, Gazette
      hopital (Paris) 41:554.
Dawson, J. W., 1915, The histology of disseminated sclerosis,
      Trans.Royal.Soc.Edinb., 50(3):517.
Diessner, H., Neumann, V., and Schmitdt, R. M., 1974, Zur Einwirkung
      von J-125 markiertem encephalitogenen Progein auf periphere
      Blutylmphozyten, Schweiz.Arch.Neurol., 114:57.
Dore-Duffy, P., Goertz, V., and Rothman, B. L., 1980, Lymphocyte
      adherence to myelinated tissue in multiple sclerosis,
      J.Clin.Invest., 66:843.
Freund, J., and McDermott, K., 1942, Sensitization to horse serum by
      means of adjuvants, Proc.Soc.Exp.Biol. & Med., 49:548.
Frick, E., 1982, Cell mediated toxicity by peripheral blood
      lympocytes against basic protein of myelin, encephalitogenic
      peptide, cerebrosides and gangliosides in multiple sclerosis,
      J.Neurol.Sci., 57:55.
Ganrot, K., and Laurell, C. B., 1974, Measurement of IgG and albumin
      content of cerebrospinal fluid, and its interpretation, Clin.
      Chem., 2:571.
Glynn, P., Weedon, D., Edwards, J., Suckling, A. J., and Cuzner, M.
      L., 1982, Humoral immunity in chronic relapsing experimental
      autoimmune encephalomyelitis, J.Neurol.Sci., 57:369.
Grundke-Iqbal, I., and Bornstein, M. B., 1980, Multiple sclerosis:
      Serum gamma globulin and demyelination in organ culture,
      Neurol., 30:749.
Grundke-Iqbal, I., Lassmann, H., and Wisniewski, H. M., 1980,
      Immunohistochemical studies in chronic relapsing experimental
      allergic encephalomyelitis, Arch.Neurol., 37:651.
Grundke-Iqbal, I., Raine, C. S., Johnson, A. B., Brosnan, C. F., and
      Bornstein, M. B., 1981, Experimental allergic encephalo-
      myelitis: Characterization of serum factors causing demye-
      lination and swelling of myelin, J.Neurol.Sci., 50:63.
Haruhara, C., 1958, Clinical observations on encephalitis and its
      residual symptoms caused by anti-rabies treatment, Seishin
      Shinkeigaku Zasshi, Psychiatria et Neurologia Japonica,
      58:355.
Hashim, G. A., Wood, D. D., and Moscarello, M. A., 1980, Myelin
      lipophilin-induced experimental allergic encephalomyelitis in
      guinea pigs, in: "Neurochemistry and Clinical Neurology," L.

Battistin, G. A. Hashim and A. Lajtha, eds., A. R. Liss, New York, p.21.

Johnson, K. P., Arrigo, S. C., Nelson, B. J., and Ginsberg, A., 1977, Agarose electrophoresis of cerebrospinal fluid in multiple sclerosis. A simplified method for demonstrating cerebrospinal fluid oligoclonal immunoglobulin bands, Neurology., 27:273.

Kapp, J., Pierce, C., and Benacerraf, B., 1975, Genetic control of immune responses in vivo. IV. Experimental conditions for the development of helper T cell activity specific for the terpolymer L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$(GAT) in nonresponder mice, J.Exp.Med., 142:50.

Karcher, D., Lassmann, H., Lowenthal, A., Kitz K., and Wisniewksi, H. M., 1982, Antibodies - restricted heterogeneity in serum and cerebrospinal fluid of chronic relapsing experimental allergic encephalomyelitis, J.Neuroimmunol., 2:93.

Kies, M. W., and Alvord, E. C., Jr., 1959, "Allergic Encephalomyelitis," Charles C. Thomas, Springfield, Illinois.

Kies, M. W., Murphey, J. B., and Alvord, E. C., 1960, Fractionation of guinea pig brain proteins with encephilitogenic activity, Fed.Proc., 19:207.

Lassmann, H., 1983, A Comparative Neuropathology of Chronic Experimental Allergic Encephalomyelitis and Multiple Sclerosis, Neurology Series, Vol. 25, Springer, Berlin-Heidelberg-New York.

Lassmann, H., Karcher, D., Kitz, K., Lowenthal, A., and Verslegers, W., 1983, Humoral immune response in guinea pigs at different stages of EAE. Abstract from the Satellite Meeting of the International Society of Neurochem., Seattle, Washington.

Lassmann, H., Stemberger, H., Kitz, K., and Wisniewski, H. M., 1983, In vivo demyelinating activity of sera from animals with chronic experimental allergic encephalomyelitis: Antibody nature of the demyelinating factor and the role of complement, J.Neurol.Sci., 59:123.

Lassmann, H., and Wisniewski, H. M., 1979, Chronic relapsing experimental allergic encephalomyelitis. Clinicopathological comparison with multiple sclerosis, Arch.Neurol., 36:490.

Lassmann, H., and Wisniewski, H. M., 1979, Chronic relapsing experimental allergic encephalomyelitis: Effect of age at the time of sensitization on clinical course and pathology, Acta Neuropathol., 47:111.

Laatsch, R. H., Kies, M. W., Gordon, S., and Alvord, E. C., Jr., 1962, The encephalomyelitic activity of myelin isolated by ultracentrifugation, J.Exp.Med., 115:777.

Laurenzi, M. A., Mavra, M., Kam-Hansen, S., and Link, H., 1980, Oligoclonal IgG and free light chains in multiple sclerosis demonstrated by thin-layer polyacrylamide gel isoelectric focusing and immunofixation, Ann.Neurol., 8:241.

Lisak, R., Levinson, A., Zweiman, B., and Abdou, N., 1975, T and B lymphocytes in multiple sclerosis, Clin.Exp.Immunol., 22:30.

Lowenthal, A., 1979, Restricted heterogeneity of the IgG in neurology, in: "Humoral Immunity in Neurological Diseases," D. Karcher, A. Lowenthal, A. D. Strosberg, eds., Plenum Press, New York, pp. 281-288.

Lublin, F. D., and Maurer, P. H., 1980, Separation and properties of lymphocytes obtained from the central nervous system, J.Clin. Lab.Immunol., 4:195.

Madrid, R. E., Wisniewski, H. M., Hashim, G. A., Moscarello, M. A., and Wood, D. D., 1982, Lipophilin-induced experimental allergic encephalomyelitis in guinea pigs, J.Neurosci.Res., 7:203.

Madrid, R. E., Wisniewski, H. M., Iqbal, K., Pullarkat, R. K., and Lassmann, H., 1981, Relapsing experimental allergic encephalomyelitis induced with isolated myelin lipids, J.Neurol.Sci., 50:399.

Madrid, R. E., Yu, R. K., Deshmukh, D., and Wisniewski, H. M., 1983, Chronic relapsing and progressive EAE induced by supraoptimal immunization with MBP and galactocerebroside, J.Neuropath Exp.Neurol., 42:358.

Marburg, O., 1906, Die sogenannte "akute multiple sclerose." Jahrb.f.Psych., 27:211.

Massanari, R. M., 1980, A latent-relapsing neuroautoimmune disease in Syrian hamsters, Clin.Immunol.Immunopath., 16:211.

Mattson, D. H., Roos, R. P., and Arnason, B. G. W., 1981, Comparison of agar gel electrophoresis and isoelectric focusing in multiple sclerosis and subacute sclerosing panencephalitis, Ann. Neurol., 9:34.

McFarlin, D. E., and McFarland, H. F., 1982, Multiple sclerosis, New Engl.J.Med., 309:1183.

Mehta, P. D., Lassmann, H., and Wisniewski, H. M., 1981, Immunologic studies of chronic relapsing EAE in guinea pigs - Similarities to multiple sclerosis, J.Immunol., 127:334.

Mehta, P. D., Mehta, S. P., and Patrick, B. A., 1982, Identification of light chain type bands in CSF and serum oligoclonal IgG from patients with multiple sclerosis, J.Neuroimmunol., 2:119.

Mehta, P. D., Patrick, B. A., and Wisniewski, H. M., 1981, Isoelectric focusing and immunofixation of cerebrospinal fluid and serum in multiple sclerosis (MS), J.Clin.Lab.Immunol., 6:17.

Mehta, P. D., Patrick, B. A., and Wisniewski, H. M., 1983, Oligoclonal IgG bands in chronic relapsing EAE and MS. Abstract from the Satellite Meeting of the International Society of Neurochem., Seattle, Washington.

Merrill, J., Wahlin, B., Siden, A., and Perlmann, P., 1982, Elevated direct and IgM enhanced ADCC activity in multiple sclerosis patients, J.Immunol., 128:1728.

Mokhtarian, F., McFarlin, D. E., and Raine, C. S., 1983, Induction of chronic relapsing experimental allergic encephalomyelitis by adoptive transfer of lymph node cells, J.Neuropath.Exp. Neurol., 42:359 (abstr).

Neuwelt, E. A., and Clark, W. K., 1978, "Clinical Aspects of Neuro-
    immunology," Williams and Wilkins Co., Baltimore.
Olsson, T., Henriksson, A., Link, M., and Kristensson, K., 1983,
    Humoral immune response in chronic relapsing experimental
    allergic encephalomyelitis in guinea pigs.  Abstract from the
    Satellite Meeting of the International Society of Neurochem.,
    Seattle, Washington.
Olsson, T., Kristensson, K., Leijon, G., and Link, H., 1982,
    Demonstration of serum IgG antibodies against myeling during
    the course of relapsing experimental allergic encephalomye-
    litis in guinea pigs, J.Neurol.Sci., 54:359.
Olsson, J., and Pettersson, B., 1975, A comparison between agar gel
    electrophoresis and CSF serum quotients of IgG and albumin in
    neurological diseases, Acta Neurol.Scandinav., 53:308.
Omechein, M., 1983, Inflammatory cells in the central nervous system:
    An integrated concept based on recent research in pathology,
    immunology and forensic medicine, in: "Progress in Neuropatho-
    logy," H. M. Zimmerman, ed., 5:277.
Oppenheimer, D. R., 1978, The cervical cord in multiple sclerosis,
    Neuropath.Appl.Neurobiol., 4:151.
Paterson, P. Y., and Day, E., 1979, Neuroimmunologic disease:
    Experimental and clinical aspects, Hosp.Pract., 14:49.
Paterson, P. Y., and Whitacre, C. C., 1981, The enigma of oligoclonal
    immunoglobulin G in cerebrospinal fluid from multiple sclero-
    sis patients, Immunol.Today 2:111.
Reinherz, E., Weiner, H., Hauser, S., Cohen, J., Distaso, J., and
    Schlossman, S., 1980, Loss of suppressor T cells in active
    multiple sclerosis: Analysis with monoclonal antibodies,
    N.Engl.J.Med., 303:125.
Rivers, T. M., Sprint, D. H., and Berry, G. P., 1935,
    Encephalomyelitis accompanied by myelin destruction experi-
    mentally produced in monkeys, J.Exp.Med., 61:689.
Roboz, E., and Henderson, N., 1959, Preparation and properties of
    water-soluble proteins from bovine cord with "allergic" en-
    cephalomyelitic activity, in: "Allergic Encephalomyelitis," M.
    W. Kies and E. C. Alvord, Jr., eds., Charles C. Thomas,
    Springfield, Illinois, pp.281-292.
Ryberg, B., 1978, Multiple specification of antibrain antibodies in
    multiple sclerosis and chronic meylopathy, J.Neurol.Sci.,
    38:357.
Salmi, A., Rennanen, M., Ilonen, J., Panelius, M., 1983, Intrathecal
    antibody synthesis to virus antigens in multiple sclerosis,
    Clin.Exp.Immunol., 52:241.
Salt, I., Westall, F. C., Romine, J. S., and Wiederholt, W. C., 1980,
    Studies on myelin and preliminary report on immunologic
    observations, in: "Progress in Multiple Sclerosis Research,"
    H.J. Bauer and S. Poser, eds., Springer, Berlin, p. 419.
Schuller-Levis, G., Clausen, J., Schwerer, B., Madrid, R. E., and
    Wisniewski, H. M., 1983, Dynamics of cellular immunity during
    CR-EAE in guinea pigs.  Abstract from the Satellite Meeting of
    the International Society of Neurochem., Seattle, Washington.

Schwerer, B., Egghart, M., Kitz, K., Lassmann, H., and Bernheimer, H., 1983, Serum antibodies against central nervous system (CNS) antigens in chronic relapsing experimental allergic encephalomyelitis (A-EAE), in: "Protides of the Biological Fluids," H. Peeters, ed., Pergamon Press, Oxford, pp.193-196.

Schwerer, B., Schuller-Levis, G. B., Mehta, P. D., Madrid, R. E., and Wisniewski, H. M., 1983, Cellular and humoral immune response to MBP during the course of chronic relapsing EAE. Abstract from the Satellate Meeting of the International Society of Neurochem., Seattle, Washington.

Seitelberger, F., 1967, Autoimmunologische aspekte der entmarkungsenzephalitiden, Nervenarzt 38:525.

Shaw, C. M., Alvord, E. C., Kaku, J., and Kies, M. W., 1965, Correlation of experimental allergic encephalomyelitis with delayed-type skin sensitivity to specific homologous encephalitogen, Ann.N.Y.Acad.Sci., 122:318.

Shevach, E., and Rosenthal, A., 1973, Function of macrophages in recognition by guinea pig T-lymphocytes. II. Role of the macrophages in the regulation of genetic control of the immune response, J.Exptl.Med., 138:1213.

Sobel, R. A., Blanchette, B. W., and Colvin, R. B., 1983, Pre-inflammatory expression of fibronectin (Fn) and Ia in acute experimental allergic encephalomyelitis (EAE): An active role for endothelial cells (EC) in the immune response detected by quantitative immunoperoxidase studies using monoclonal antibodies (MAb). Abstract from the Satellite Meeting of the International Society of Neurochem., Seattle, Washington.

Stone, S. H., and Lerner, E. M., 1965, Chronic disseminated allergic encephalomyelitis in guinea pigs, Ann.N.Y.Acad.Sci., 122:227.

Suckling, A. J., Reiber, H., Kirby, J. A., and Humsby, M. G., 1983, Chronic relapsing experimental encephalomyelitis: Immunological and blood-cerebrospinal fluid - barrier dependent changes in the CSF, J.Neuroimmunol., 4:35.

Tabira, T., Stoyama, Y., and Kuroiwa, Y., 1983, Is continuous antigenic stimulation at the immunized loci necessary for chronic relapsing EAE? Abstract from the Satellite Meeting of the International Society of Neurochem., Seattle, Washington.

Tourtellotte, W. W., 1976, Interaction of local central nervous system immunity and systemic immunity in the spread of multiple sclerosis demyelination, in: "Multiple Sclerosis: Immunology, Virology and Ultrastructure," G. Wolfgang, G. W. Ellison, J. G. Stevens, J. M. Andrews, eds., Academic Press, New York, pp.285-332.

Traugott, U., Stone, S., and Raine, C. S., 1978, Experimental allergic encephalomyelitis - Migration of early T cells from the circulation into the central nervous system, J.Neurol. Sci., 36:55.

Trotter, J., and Smith, M. E., 1983, Macrophage-mediated demyelination: the role of lipases and antibody. Abstract

from the Satellite Meeting of the International Society of
Neurochemistry, Seattle, Washington.

Uchimura, I., and Shiraki, H., 1957, A contribution to the class-
ification and the pathogenesis of demyelinating encephalo-
myelitis, J.Neuropath.Exp.Neurol., 16:139.

Vartdal, F., Vandvik, B., and Norrby, E., 1980, Viral and bacterial
antibody response in multiple sclerosis, Ann.Neurol., 8:248.

Voss, K., Lassmann H., Kitz K., Wisniewski, H. M., and Iqbal, K.,
1983, Ultracytochemical distribution of myelin basic protein
after injection into the cerebrospinal fluid: Evidence for
transport through the blood brain barrier and binding to the
luminal surface of cerebral veins, J.Neurol.Sci., in press.

Waksman, B., 1981, Current trends in multiple sclerosis research,
Immunol.Today 2:87.

Waksman, B. H., and Morrison, L. R., 1951, Tuberculin-type
sensitivity to spinal cord antigen in rabbits with isoallergic
encephalomyelitis, J.Immunol., 66:421.

Weiner, H. L., and Hauser, S. L., 1982, Neuroimmunology I: Immuno-
regulation in neurological disease, Ann.Neurol., 11:437.

Wekerle, H., Cohen, I. R., and Feldman, H., 1974, Selbst-Toleranz und
Autoimmunitat, Dtsch.med.Wschr., 99:1734.

Wisniewski, H. M., 1977, Immunopathology of demyelination in
autoimmune diseases and virus infection, Br.Med.Bull., 33:54.

Wisniewski, H. M., Brown, H. R., and Thormar, H., 1983a, Pathogenesis
of viral encephalitis: Demonstration of viral antigen(s) in
the brain endothelium, Acta Neuropathol. 60:107.

Wisniewski, H. M., and Keith, A. B., 1977, Chronic relapsing EAE an
experimental model of multiple sclerosis, Ann.Neurol., 1:144.

Wisniewski, H. M., and Lassmann, H., 1983b, Etiology and pathogenesis
of monophasic and relapsing inflammatory demyelination – human
and experimental, Acta Neuropathol., Suppl.IX., pp. 21-29.

Wisniewski, H. M., Lassmann, H., Brosnan, C. F., Mehta, P. D.,
Lidsky, A. A., and Madrid, R. E., 1982, Multiple sclerosis:
Immunological and experimental aspects, in: "Recent Advances
in Clinical Neurology," W. B. Matthews and G. H. Glaser, eds,
Churchill Livingstone, Edinburgh, No. 3, pp. 95-125.

Wisniewski, H. M., and Madrid, R. E., 1983, Chronic progressive
experimental allergic encephalomyelitis in adult guinea pigs,
J.Neuropath.Exp.Neurol., 42:243.

Yung, L. L. L., Diener, E., McPherson, T. A., Barton, M. A., and
Hyde, H. A., 1973, Antigen-binding by lymphocytes in normal
man and guinea pig to human encephalitogenic protein,
J.Immunol., 110:1383.

# COMPARATIVE NEUROPATHOLOGY AND PATHOGENETIC ASPECTS OF CHRONIC RELAPSING EXPERIMENTAL ENCEPHALOMYELITIS AND MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a disease well known clinically for more than 200 years. The first detailed description of its pathology has been provided by Charcot in Paris in the late 19th Century. SInce that time many research efforts have been devoted to clarify the etiopathogenesis of MS and to find some effective treatment, yet so far without convincing success.

The disease MS had been named from its characteristic neuro-pathological finding, the sclerotic plaque. The plaque, however, already is a late product of the underlying pathological process, apparently representing the final deleterious stage of the clinical disease, manifested in the CNS. All steps of this final phase of the process are supposed to have their corresponding morphological correlates in the target organ, the CNS. Therefore it is surprising that since the classical neuropathological descriptions, which after Charcot were presented among others by Marburg in Vienna, Hallervorden in Berlin, to mention 2 of the most prominent workers, only very few original studies of MS have been performed. That is regrettable because in the past decades not only have several potent morphological methods been developed but also numerous relevant experimental studies on inflammatory demyelination were done. Thus, there is a certain deficit above all in comparative investigations of human and experimental demyelination and some stagnation of disease-oriented basic research in this important field of neurology. Due to the recent development of reproducible chronic models of EAE (Stone and Lerner 1965, Wisniewski and Keith 1977, Massanari 1980, Lublin 1982) a new challenging situation arose: The far reaching conformity of the clinical and pathological findings in cr-EAE and MS makes it possible to draw exact comparisons between the series of tissue events, their quality and time course, and to identify the position

of the individual changes related to the coordinates of the complex
disease process.

Before discussing some selected results of our recent extensive
comparative studies I would like to sketch the pathological aspects
of human MS and its position among the demyelinating disease.

The neuropathological diagnosis of MS is based on the presence
of focal lesions presenting two different alterations, namely chronic
inflammatory changes associated with elective demyelination, i.e.
loss of myelin sheaths without axonal damage.  This essential com-
bined lesion pattern of the foci is in itself variable concerning
intensity, cellular composition of the perivenous and perivenular
inflammation, size and topography of demyelination, and affection of
oligodendroglia.  These primary changes have also partial influence
upon the orthological reactions of the central nervous tissue fol-
lowing the lesion especially regarding astroglial reparative pro-
liferation with scar formation and remyelination depending on the
preservation of oligodendrocytes.  The state of a focal MS-lesion
encountered will also depend on its individual age, determining the
intensity of myelin degradation and inflammation but also of remy-
elination and scar formation.  Furthermore it is of utmost importance
that the lesions in MS are significantly different dependent on their
development in different phases (early or late) of the disease along
its chronic course: that stage-dependent quality of MS-foci must be
strictly distinguished from the histological age of an individual
plaque.

From the above findings it can be concluded that there is be-
sides the variable dualistic structure of the fundamental histo-
logical alterations in every case of MS a large intraindividual
variability of the lesions and between single cases a remarkable
interindividual variability.  These circumstances make it conceivable
that several subtypes of MS, also considering clinical features, may
exist:

1) typical relapsing – remittent
2) chronic progressive
3) subacute ponto-cerebellar (Redlich)
4) acute (Marburg)

There is furthermore the group of inflammatory demyelinating diseases
among which MS is the most prominent but often not exactly distin-
guishable example.

1) acute hemmorrhagic leukoencephalitis
2) acute disseminated encephalomyelitis
3) inflammatory diffuse sclerosis (Schilder)
4) encephalitis concentrica (Balo)
5) neuromyelitis optica (Devic)
6) myelitis necroticans

Finally the differential diagnosis of MS against the monophasic postvaccinal and parainfectious diseases is sometimes difficult, particularly because transitional cases with MS are described in the literature.

We feel that all variations and overlappings, especially the inherent metamorphosis of the individual disease, have not been duly respected in the past and that therefore a rehearsal of MS neuropathology from the basis of the present knowledge is strictly indicated. Before that a short review of the neuropathology of cr-EAE will be given.

A good animal model of MS should fulfill the following criteria (Wisniewski et al., 1982): Clinically: Course in relapses and remissions, symptoms of multifocal central nervous lesions. Neurophysiologically: Oscillations of conduction velocity and amplitude. Neuropathologically: Primary inflammatory demyelination with foci in different stages. Neuroimmunologically: Oligoclonal bands of IgG in the CSF, IgG-synthesis in the CNS and participation of immunoregulatory cells.

As mentioned before cr-EAE appears to be the most close experimental model of MS available at present. The extensive neuropathological analysis of this model has demonstrated that it can simulate all stages and variable forms of human MS and all the different inflammatory demyelinating diseases of MS-type (Lassmann 1983). This potentiality depends above all upon the time interval between sensitization procedure and sampling of the animals.

In the following we will report on the most relevant findings of our neuropathological analysis of cr-EAE and then present some results of comparative study between this model and human MS.

In the experiment of cr-EAE the initial disease occurs 10-20 days after sensitization in the form of acute perivenous inflammation without demyelination or in severely affected animals in the form of an acute hemorrhagic leukoencephalitis (Lassmann and Wisniewski 1979a). There is not typical focal demyelination or plaque formation. Animals with active disease during the subacute stage of the disease (20-40 days after sensitization) show the classical picture of acute perivenous leukoencephalitis with extensive perivenous mononuclear cell infiltration and sleeve-like perivenous demyelination (Lassmann et al., 1980).

The first relapse with focal and substantial demyelination appears 40-60 days after sensitization. With the onset of initial clinical symptoms of the relapse in the respective area an invasion of mononuclear cells from the perivenous inflammatory spaces into the white substance can be seen (Lassmann and Wisniewski 1978). Many of these inflammatory cells are T-lymphocytes (Traugott et al., 1982).

Immediately after that the myelin sheaths are fragmented to so-called
"myelin balls" and "myelin droplets" (Lassmann and Wisniewski 1979b).
These myelin balls present histochemical qualities similar to the
intact myelin sheaths concerning the myelin lipids, that means they
are Luxol fast blue – and Marchi-positive.  Myelin degradation in the
fragments starts 10-14 days later with the appearance of sudano-
philic, PAS-positive degradation products which like the myelin balls
are taken up by phagocytes.  Simultaneously also a glial reaction in
the form of increase in size and number of astrocytes appears.  The
duration of the degradation phase depends on the size and localiz-
ation of the focus.  Superficial foci may be cleared of degradation
debris already 14 days after the focus formation.  In foci located in
the depth of the white matter the degradation may last up to several
months.

In the acute and early chronic stages CSF findings indicate a
transient blood-brain barrier damage which correlates with disease
activity.  Intrathecal immunoglobulin synthesis is not apparent from
the CSF protein profiles.  The late chronic stage (100-400 days after
sensitization) more closely resembles chronic MS.  The inflammatory
response in the CNS, although invariably present, is much less severe
than in earlier stages, even in actively demyelinating lesions.
Numerous demyelinated sclerotic or partially remyelinated lesions are
found throughout the neuraxis in similar topographical distribution
as in human MS.  The majority of the lesions are inactive or re-
paired, only few actively demyelinating plaques may be found in
animals sampled during a relapse of the disease.  CSF protein shows a
partial or complete repair of blood-brain-barrier function and evi-
dence for intrathecal immunoglobulin synthesis (Mehta et al., 1981,
Clynn et al., 1982, Karcher et al., 1982, Lassmann et al., 1983).

With regard to the modality of the myelin sheath decomposition
the following processes can be distinguished:

1.  Direct attack of phagocytes against the myelin sheath in the form
    of "myelin stripping" (Lampert 1965).  This change mostly starts
    from the paranodal area; it is accompanied, however, by the
    disintegration of the total internodium.
2.  Another process is the so-called "vesicular disruption of myelin"
    which is encountered in variable intensity (Lampert 1965, Dal
    Canto et al., 1975).  This type seems to be a pathogenetically
    independent process.
3.  The low incidence of myelin stripping and vesicular disruption of
    meylin in active cr-EAE plaques, however, indicates that other,
    less characteristic alterations of the myelin sheath may
    represent initial stages of demyelination too (Lassmann 1983).

Myelin degradation is unanimously thought to be performed by
hematogenic macrophages (Kosunen et al., 1963, Lampert and Carpenter
1965).  Local glial cells, however, may participate as one can find

so-called "fixed degradation" in astro- and probably oligodendrocytes
(lassmann 1983).  This type of "Abbau" seems to be more prominent in
foci of the chronic phase and less prominent in the acute phase of
the disease.

With regard to the shape and formation of the demyelinating foci
one can distinguish the perivenous demyelinating seams, typical
plaques, plaques with peripheral (radial) extension zones and super-
ficially located so-called "Mantel-Herde" (Lassmann 1983).  These
types of foci do not occur irregularly but according to the respec-
tive varying pathogenetic situation along the course of the experi-
mental disease.  The "Mantel-Herde", e.g. seem to be conditioned by
the increased pathogenicity of CSF, probably related to extensive
seeding of inflammatory cells in the meninges (Lassmann et al.,
(1981a) and on the ventricular surface (Lassmann et al., 1981b).

The distribution and topography of foci in cr-EAE has been
studied in relation to the perivenous inflammatory changes in the pia
arachnoid of spinal cord.  It could be demonstrated that spot-like
dense cuffs of hematogenic cells appear around the postcapillary
radial venules draining the spinal cord: so-called "Stich-Venen".
Respective areas of the spinal cord are predilection sites for demy-
elinated plaques (Lassmann et al., 1981a).

Role of Oligodendroglia

As the myelin sheaths sensu stricto are parts of the oligoden-
droglial cells, the possibility of a pathogenic effect to the meylin
sheaths via the oligodendroglia has long been discussed.  The sol-
ution of the problem is difficult because identification of oligoden-
droglia in the active demyelinating focus is not easy and often
impossible even if using elective staining methods and electron
microscopy.  From the neuropathological standpoint, however, a
primary affection of oligodendroglia is not probable because histo-
logical alterations which precede de-myelination cannot be seen.
Furthermore the demyelination pattern in diseases due to primary
affections of oligodendroglia, e. g. viral encephalitis with in-
clusion bodies and Progressive Multifocal Leukoencephalopathy (PML)
differs essentially from the focal demyelination in EAE and in MS.
Finally the satellite oligodendroglia cells in the grey matter are
always completely unaffected.

In foci of the experimental disease the oligodendroglia are
reduced to variable degrees.  The highest rate of oligodendroglia
loss is generally found in late chronic foci in which correspondingly
remyelination is slight whereas in more acute foci loss of oligoden-
droglia cells is moderate and therefore an intense remyelination can
be seen (Lassmann et al., 1980, Lassmann 1983).  These foci cor-
respond to the so-called shadow-plaques which therefore are not foci

of incomplete demyelination but represent foci with intensive remy-
elination. This may possibly be due to an immunopathological cause
based on the increase of antimyelin antibodies during the chronic
stage of the disease (Schwerer et al., 1983, Lassmann et al., 1983).
Such antibodies may not only be involved in demyelination and oligo-
dendroglia destruction (Bornstein and Appel 1961, Raine and Bornstein
1970, Lassmann et al., 1981c) but may also inhibit remyelination
(Seil et al., 1973, Bornstein and Raine 1970). It is this phase of
the disease which produces the typical myelin-free sclerotic plaques
rich in glial fibers. There occurs also the peripheral type of
remyelination by invading Schwann cells from the spinal nerve roots
into demyelinating plaques of the spinal cord (Raine et al., 1978).


MULTIPLE SCLEROSIS

The Inflammatory reaction

      In multiple sclerosis (MS) perivenous inflammatory infiltrates
composed of lymphocytes, plasma cells, large mononuclear cells and
macrophages are regularly present (Marburg 1936). In an extensive
study on autopsy cases of MS (Guseo and Jellinger 1975) inflammatory
infiltrates were found in 60% of total cases and in 74% of actively
demyelinating cases. MS cases without perivenous inflammation were
either inactive (burned out) or were pre-treated with immunosup-
pressive therapy. Thus from neuropathology there is so far no hard
evidence for the occurrence of actively demyelinating lesions in the
absence of inflammation although it cannot be definitely excluded in
chronic MS. It is, however, even more difficult to prove the primary
nature of inflammation, i.e. that inflammation always precedes demy-
elination and is not a secondary alteration due to tissue damage.
The main support for the primary nature of inflammation in MS comes
from the study of acute MS cases. In these cases a continuous trans-
ition of the classical perivenous inflammatory lesions of acute
disseminated luekoencephalitis type into typical MS plaques is regu-
larly found (Marburg 1904, Pette 1928, Seitelberger 1973, Krücke
1973). The intensity of the inflammatory reaction is very high in
acute MS whereas in chronic MS inflammation is much less pronounced
even in active plaques. The number of inflammatory cells, however,
in chronic inactive MS cases is still rather high (Prineas and Wright
1978). The number of inflammatory cells withing chronic MS plaques
varies from case to case and even from plaque to plaque within the
same case (Guseo and Jellinger 1975). Concerning distribution of
inflammatory cells in acute MS the infiltrates are arranged around
all segments of the venous vascular tree (Seitelberger 1973). In
chronic MS inflammation is focal, predominantly affecting large
drainage veins (Seitelberger 1973). The inflammatory cells are
generally localized in a perivenous position. In the demyelinating
lesions lymphoctyes and plasma cells are present only in small
numbers. The high cellularity of active MS plaques is mainly due to

phagocytes (Hallervorden 1940, Guseo and Jellinger 1975. The presence of T-cells in MS plaques has been shown histochemically (Traugott et al., 1983).


## Vascular Pathology

Compared with cr-EAE vascular pathology in MS is not as prominent. It is still unresolved, however, to what extent occlusion or damage to small vessels which sometimes occurs in MS plaques (Siemerling and Raeke 1914) may contribute to the lesional growth (Putnam 1935). The intensity of chronic vascular changes like perivenous and pericapillary fibrosis is variable from case to case. These alterations are found mainly in the same areas where pericapillary accumulation of phagocytes is pronounced in active lesions (Lassmann 1983).


## Demyelination and Myelin Degradation

Elective demyelination is the most characteristic event in the pathology of MS. In MS the identification of initial structural changes leading to demyelination is rather difficult. The most frequent alteration of myelin in lesions which were believed to be active is splitting of myelin lamellae and the formation of small intramyelinic vesicles (Perier and Gregoire 1965, Suzuki et al., 1969, de Preux and Mair 1974, Kirk 1979). Recently also a lesion called micropinocytosis vermiformis (Prineas and Connell 1978) has been described as the initial alteration. It should, however, be stressed that the presence of sudanophilic myelin degradation products used as a marker of lesional activity in the above mentioned studies is not a good marker for initial lesions. Based on the experience of the study of the sequences of events in cr-EAE, the pattern of demyelination in cases of acute and chronic MS from the collection of the Neurological Institute has recently been investigated (Lassmann 1983). In acute MS the most frequent initial alteration in all investigated lesions was extensive vesicular disruption of myelin. This transformation either involved the whole myelin sheath or was localized in the inner or outer loops of the sheath, especially near the node of Ranvier. In the same areas invasion of phagocytes or their cell processes into the myelin sheath was frequently noted corresponding to myelin stripping. Intramyelinic phagocytes were found either between the axon and the myelin sheath or between myelin lamellae. In the vicinity of completely or partially demyelinated fibers numerous phagocytes were observed containing fragments of the myelin sheaths. Active plaques in chronic MS differed from the demyelinating lesions in acute MS in so far as the intensity of perivenous inflammatory reaction together with the hypercellularity of actively demyelinating areas was less pronounced and the invasion of phagocytes into the myelin sheaths was rare.

Initial stages of demyelination in these cases included partial
vesicular disruption of myelin sheaths.  The main structural change,
however, consisted of intramyelinic edema with massive swelling of
the sheaths, widening of the node of Ranvier and paranodal asym-
metrical thinning and removal of myelin sheaths.

Further myelin degradation in MS follows a standard histo-
logical, histochemical and ultrastructural pattern corresponding to
that found in other inflammatory demyelinating diseases or that of
diseases with secondary destruction of myelin sheaths (Seitelberger
1960).  Myelin degradation starts with a stage of physical disinte-
gration of the myelin sheath followed by the chemical degradation of
myelin lipids, resulting in the appearance of sudanophilic and later
of PAS-positive material.  Sudanophilic lipids seem to be present
within the lesions up to several months after plaque formation.  The
abundance of PAS-positive ultrastructurally pleomorphic degradation
products (Prineas 1975) cannot be taken as characteristic for early
MS plaques but is also found in other demyelinating conditions
(Seitelberger 1960, Hauw and Escourolle 1977, Lassmann et al., 1978).
The accumulation of debris-containing phagocytes in the pericapillary
region is more pronounced in acute MS than in active lesions of
chronic MS.  A proportion of debris is digested in local cells such
as astroglia (Marburg 1906).

Thus it can be stated that the patterns of demyelination and
myelin degradation are found to be very similar in cr-EAE and MS,
provided active lesions are identified in MS on the basis of myelin
degradation products.

Oligodendroglia

The interpretation of oligodendrocyte alterations in MS is of
special importance for the pathogenetic interpretation of demyelin-
ating lesions and especially regarding the question of whether the
disease process is primarily directed against myelin or whether
demyelination follows alterations or destruction of oligodendrocytes.
In the literature there are divergent opinions about the fate of
oligodendrocytes in MS (McAlpine et al., 1955, Ibrahim and Adams
1963, Lumsden 1970, Itoyama et al., 1980, Raine et al., 1981).  Our
own studies in accordance to the results obtained by Ibrahim and
Adams (1963, 1965) indicate that there are differences in the extent
of oligodendroglia destruction between small lesions of acute and
rapidly progressive chronic MS compared with the typical large
plaques of chronic MS (Lassmann 1983).  In a general pattern oligo-
dendrocytes are most effectively destroyed in the large lesions of
chronic MS although even in that situation a plaque completely free
of oligodendroglia-like cells is rare.  In acute and rapidly pro-
gressive chronic MS cells resembling oligodendroglia may be present
in active completely demyelinated plaques in numbers comparable to

those in normal periplaque white matter.  Furthermore remyelination
in these plaques is pronounced, occurring within a few days to weeks
after formation of the lesion.  Therefore MS does not seem to be a
primary disorder of oligodendrocytes.  This is further supported by
the pattern of demyelination reported above suggesting that myelin is
the main target in the pathogenesis of the disease.  Oligodendrocytes
as a functional unit with myelin sheaths may degenerate to a variable
extent during active demyelination.  Alternatively the immune re-
action in different MS patients may be variable, directed either
against myelin antigens alone or in other cases against antigens
shared by myelin and oligodendroglia (Lassmann 1983).


Remyelination

     Due to the difficulty in the identification of remyelination in
tissue obtained from autopsy, this important repair mechanism had not
been recognized and respected enough in the past.  In this connection
it is interesting that already Marburg (1906) in a detailed and
sequential study of plaque formation in acute MS had suggested that
shadow plaques in MS can be explained on the basis of remyelination.
In recent electron microscopic studies the presence of remyelination
at the margins of MS plaques has been documented (Suzuki et al.,
1969, Prineas and Connell 1979).  Remyelination as a repair mechanism
of whole plaques, however, has been questioned up to now.  The study
of the dynamics of remyelination in cr-EAE, however, helped to under-
stand the factors regulating the extent of remyelination also in the
human disease.  The phenomenon of remyelination in MS is mostly
connected with the occurrence of so-called shadow plaques.  The
occurrence of these had been explained by a concept of incomplete
demyelination (Lumsden 1970).  At present it is, however, indicated
from our results that the responsible mechanisms of the appearance of
shadow plaques is remyelination (Lassmann 1983).  In acute MS shadow
lesions are especially frequent.  In chronic MS cases shadow plaques
although present in variable numbers are rare compared with acute MS.
From studies of chronic MS cases with short duration the sequence of
events leading to the formation of shadow plaques is the following:
Already during the sudanophilic stage of myelin degradation, simul-
taneously with the onset of fibrillary sclerosis, small clusters of
thinly myelinated nerve fibres appear.  Later, areas of myelin pallor
with uniform distribution of thinly myelinated fibers are present.
Shadow plaques finally are characterized by a decreased density of
myelinated fibers and by myelin sheaths which are still thinner than
those of the surrounding white matter.  The presence of sclerotic
plaques without detectable myelin loss in some MS cases indicates
that even complete remyelination may occur.  Thus in general lesions
in cases with short clinical history, especially acute MS cases,
remyelinate more effectively than chronic cases.  Remyelination may
start rapidly after plaque formation.  With chronicity of the disease
not only the extent but also the velocity of remyelination decreases

in newly formed lesions. The rapid and complete clinical recovery of patients after the first exacerbations of the disease may be partly due to rapid and extensive remyelination.


## Sclerosis

The formation of a dense glial scar in MS is one of the most characteristic aspects of its pathology. Glial scar formation is a general repair mechanism in the central nervous system. There had been, however, a discussion whether sclerosis in MS is an event involved in the pathogenesis of the disease or whether it merely represents a sequel of tissue destruction (Müller 1904, Prinease and Raine 1976, McKeown and Allen 1978). Studies especially from acute MS demonstrated that sclerosis is clearly a secondary event following demyelination and was not found earlier than 14 days after plaque formation (Pette 1928). The first change is intense proliferation and increase in size of astrocytes with often bizarre size and forms and several nuclei (Peters 1935). In active lesions of chronic MS cases, however, sclerosis was found to be a very early feature in plaque formation and frequently simultaneously with active demyelination. It should be mentioned that there is some indication that inflammatory pathogenetic factors such as lymphocyte products or liberated debris may activate astrocytes and thus influence the density of glial scar formation (Fontana et al., 1980). On the other hand glia activation may possibly contribute to the pathogenesis of the demyelinating lesions via proteolytic enzymes (McKeown and Allen 1978).


## Neuronal and Axonal Pathology

It is well known that in MS reduction of axons occur and nerve cell changes can be found which in general are interpreted as secondary alterations due to tissue damage. In MS the degree of axonal loss is variable from plaque to plaque also within a single case. In most cases axonal loss appears to correspond to the intensity of the inflammatory reaction and demyelination during the active phase of the lesions. However, considering exceptional cases with severe axonal and neuronal damage it cannot be excluded that in some cases the immune reaction may be directed not only against myelin/oligodendrocytes but also against nerve cell antigens (Fraenkel and Jakob 1913).


## Peripheral Nervous System Pathology

MS is generally believed to be an exclusive central nervous system disease (Lumsden 1970). However, some cases have been described of indisputable plaque-like inflammatory demyelination in the

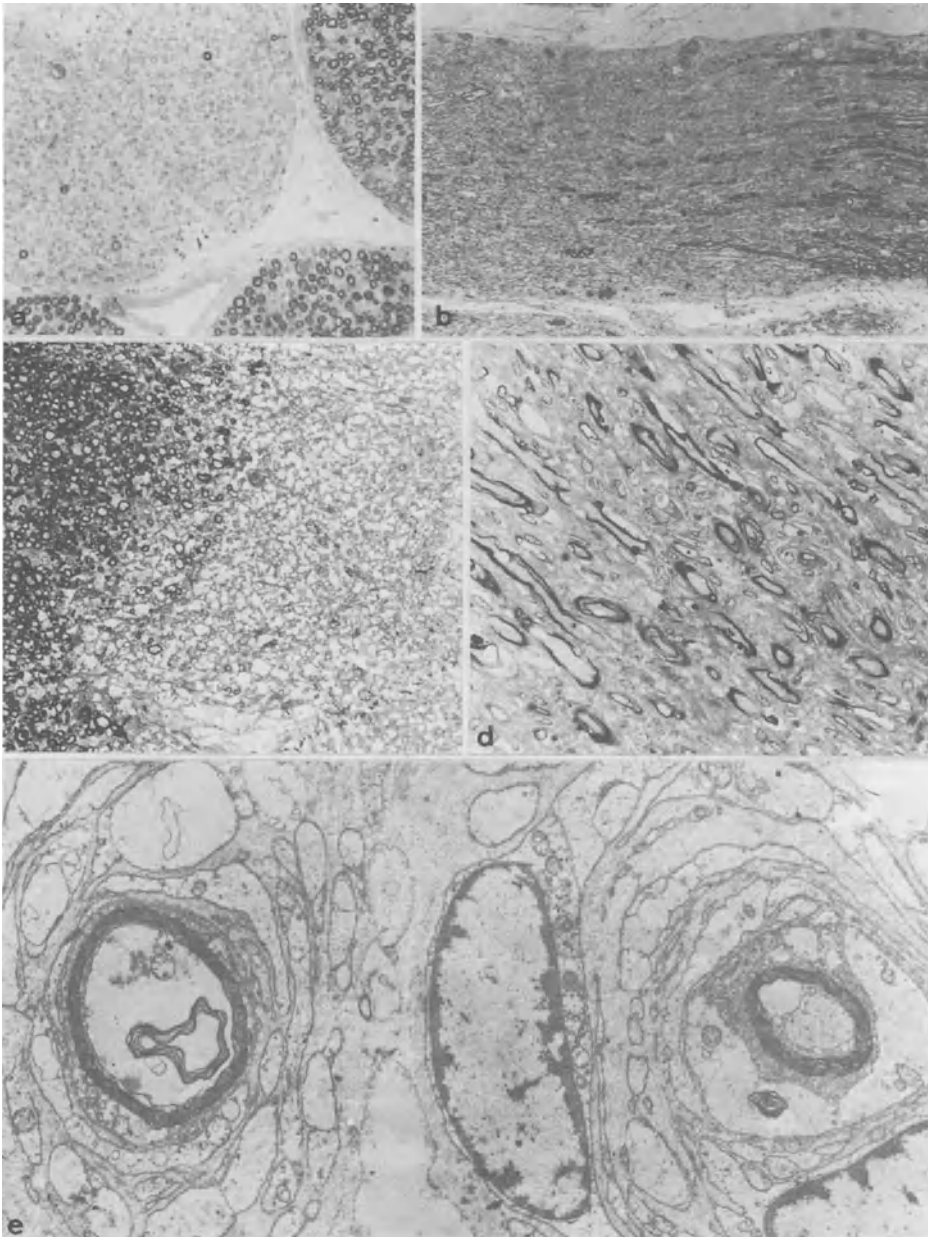Fig. 1.   a,b: 26 years old woman; acute MS; clinical history and
          neuropathological findings published elsewhere (Lassmann et
          al., 1981d).   a: Nerve root of cauda equina; thin myelin
          sheaths representing remyelination.   Toluidine blue, x 150.
          b: Cervical spinal root; plaque-like demyelination and
          remyelination in the peripheral portion of the root.

Fig. 1.  Continued

> Toluidine blue, x 150.  c,d,e: 34 years old man: chronic
> MS.  c: Border zone of an old plaque in the optic nerve.
> d: Olfactory nerve: decreased density of myelinated fibers;
> onion bulbs.  Toluidine blue, x 630.  e: Olfactory nerve;
> onion bulb formation. EM x 4 000.

peripheral nerves and roots, although the incidence of peripheral
nervous system (PNS) involvement in classical chronic MS seems to be
very low (Jellinger 1969).  Of course changes like secondary nerve
damage due to malnutrition, vitamin deficiency etc. have to be ex-
cluded.  Inflammatory demyelination in the PNS, however, is frequent
in Marburg's type of acute MS (Marburg 1906, Pette 1928, Lassmann et
al., 1981d).  As in EAE demyelination has been noted mainly in the
spinal roots.  The spectrum of alterations include inflammation,
primary demyelination, plaque-like distribution of the lesions,
remyelination and the formation of onion bulbs.  The factors respons-
ible for the cross reaction between CNS and PNS myelin in human
diseases are not yet known.

## Mechanisms of Plaque Growth

In cases of acute MS the main type found is the confluence of
adjacent perivenous lesions.  Radial plaque growth is generally found
in active cases of chronic MS.  It must be emphasized, however, that
confluent perivenous lesions may also occur in chronic MS and radial
plaque growth in acute MS cases.

## Lesional Topography in the CNS

The distribution of lesions in the CNS has been intensively
studied for may years.  The pattern of demyelination and lesional
distribution varies during chronicity of the disease.  In acute
perivenous leukoecephalitis perivenous inflammatory infiltrates and
perivenous sleeves of demyelination are disseminated and may be found
at any location in the CNS (Seitelberger 1973).  In contrast in
chronic MS demyelinated plaques are focal, oriented towards larger
vessels and localized mainly in certain predilection sites in the CNS
(Steiner 1931), Seitelberger 1973).  In chronic MS the relationship
of large demyelinated plaques to the distribution of large veins is
clearly established and is especially well documented in the spinal
cord (Fog 1950).  Other factors e.g. the mechanical strain on the
vasculature may help to precipitate a lesion at a given place
(Oppenheimer 1978).  In addition there is another plaque type in
chronic Ms which is related to the inner and outer surfaces of CNS.
The most classical lesion of this type is the so-called "Mantel-Herd"

in chronic MS (Steiner 1931).  Other predilection sites are the
lateral angles of the lateral ventricles, the so-called "Wetter-
winkel", the cerebellar peduncles, the cortical-subcortical areas,
the lateral surface of the spinal cord and the optic nerves.  In
acute MS, however, periventricular lesions are rare and, if present,
small and not related to the ventricular surface.  It may be inter-
esting that in acute MS cases the predilection sites for lesions in
typical chronic MS are apparently not more frequently involved than
other regions of the brain.  It should be noted that in MS there is
an inverse relationship between involvement of the brain and spinal
cord which is best exemplified in cases of spinal MS and Devic's
disease, where the involvement of the brain is minimal with the
exception of the optic nerves (Seitelberger 1982).

Many of these pathohistological aspects of MS have been extens-
ively described and well documented during the past 100 years.  It
was, however, difficult to interpret these individual aspects of MS
pathology in their temporal and sequential order of plaque develop-
ment.  The close similarity of structural aspects of inflammatory
demyelinating lesions in cr-EAE and MS, however, has helped to re-
arrange the structural details of demyelinating lesions in MS into a
sequential concept of plaque formation.

Furthermore the comparative study of the neuropathology of
cr-EAE and MS has led to a different conceptual approach in the
interpretation of the pathology in these diseases.  Although being
aware of the broad spectrum of inter- and intraindividual variability
of the structure of MS-plaques, most neuropathologists primarily
tried to extrapolate the features of the lesions common to all MS-
cases.  Pathohistological and pathogenetic studies in cr-EAE, how-
ever, indicate that multiple immunopathogenetic mechanisms directed
against multiple CNS-antigens, different from animal to animal, are
involved in the formation of the lesions (Wisniewski et al., 1982,
Lassmann 1983, Wisniewski and Lassmann 1983).  It thus appears es-
pecially necessary to reemphasize a fact known in neuropathology
since the beginning of this disease:  Besides the obligatory alter-
ations in MS (i.e. chronic perivenous inflammation, plaque-like
demyelination and reactive gliosis) involvement of other structures
of the CNS is regularly found although variable from case to case and
from lesion to lesion.  This, however, at the same time is a warning
not to draw far reaching pathogenetic conclusions from sophisticated
studies on a single MS-plaque or a single MS-case.

REFERENCES

Bornstein, M. B., and Appel, S. H., 1961, The application of tissue
    culture to the study of experimental "allergic" encephalomyel-
    itis. I. Patterns of demyelination, J. Neuropathol.Exp.
    Neurol., 20:141.

Bornstein, M. B., and Raine, C. S., 1970, Experimental allergic
        encephalomyelitis: antiserum inhibition of myelination in
        vitro, Lab.Invest., 23:536.
Charcot, J. M., 1868, Histologie de la sclerose en plaque, Gaz
        Hopital, 41:554.
Dal Canto, M. C., Wisniewski, H. M., and Johnson, A. B., 1975,
        Vesicular disruption of myelin in autoimmune demyelination,
        J.neurol.Sci., 24:313.
De Preux, J., and Mair, W. G. P., 1974, Ultrastructure of optic nerve
        in Schilder's disease, Devic's disease and disseminated
        sclerosis, Acta Neuropath., 30:225.
Fog, T., 1950, Topographic distribution of plaques in the spinal cord
        in multiple sclerosis, Arch.Neurol., 63:382.
Fontana, A., Grieder, A., and Arrenbrecht, St., 1980, In vitro
        stimulation of glia cells by a lymphocyte produced factor,
        J.neurol.Sci., 46:55.
Fraenkel, M., and Jakob, A., 1913, Zur Pathologie der multiplen
        Sklerose mit besonderer Berücksichtigung der akuten Formen,
        Z.Neurol., 14:565.
Glynn, P., Weedon, D., and Edwards, J., 1982, Humoral immunity in
        chronic relapsing experimental allergic encephalomyelitis.
        The major oligoclonal bands are antibodies to mycobacteria,
        J.neurol.Sci., 57:369.
Guseo, A., and Jellinger, K., 1975, The significance of perivascular
        nfiltrations in multiple sclerosis, Neurol., 211:51.
Hallervorden, J., 1940, Die zentralen Entmarkungs-erkrankungen,
        tsch.Z.Nervenheilk., 150:201.
Hauw, J. J., and Escourolle, R., 1977, Filaments and multilamellated
        cytoplasmic inclusions in progressive multifocal leukoencepha-
        lopathy, Acta Neuropath., 37:263.
Ibrahim, M. Z. M., and Adams, C. W. M., 1963, The relationship
        between enzyme activity and neuroglia in plaques of multiple
        sclerosis, J.Neurol.Neurosurg.Psychiat., 26:101.
Ibrahim, M. Z. M., and Adams, C. W. M., 1965, The relationship
        between enzyme activity and neuroglia in early plaques of
        multiple sclerosis, J.Path.Bacteriol., 90:239.
Itoyama, Y., Sternberger, N. H., and Webster, H. de F., 1980,
        Immunocytochemical observations on the distribution of myelin
        associated glycoprotein and myelin basic protein in multiple
        sclerosis lesions, Ann.Neurol., 7:167.
Jellinger, K., 1969, Einige morphologische Aspekte der multiplen
        Sklerose, Wien.Z.Nervenheilk., Suppl.II:12.
Karcher, D., Lassmann, H., and Lowenthal, A., 1982, Antibodies-
        restricted heterogeneity in serum and cerebrospinal fluid of
        chronic relapsing experimental allergic encephalomyelitis,
        J.Neuroimmunol., 2:93.
Kirk, J., 1979, The fine structure of the CNS in multiple sclerosis.
        II. Vesicular demyelination in a acute case, Neuropathol.Appl.
        Neurobiol., 5:289.

Kosunen, T. V., Waksman, B. H., and Samuelsson, K., 1963, Radioauto-
      graphic study of cellular mechanisms in delayed hypersensitiv-
      ity.II. Experimental allergic encephalomyelitis in the rat,
      J.Neuropathol.Exp.Neurol., 22:367.
Krücke, W. 1973, On the histopathology of acute hemorrhagic
      leucoencephalitis, acute disseminated encephalitis and con-
      centric sclerosis, Int. Symposium on aetiology and patho-
      genesis of the demyelinating diseases, Kyoto, pp.11.
Lampert, P. W., 1965, Demyelination and remyelination in experimental
      allergic encephalomyelitis, J.Neuropathol.Exp.Neurol., 24:371.
Lampert, P. W., and Carpenter, S., 1965, Electron microscopic studies
      on the vascular permeability and the mechanisms of demyelin-
      ation in experimental allergic encephalomyelitis,
      J.Neuropathol.Exp.Neurol., 24:11.
Lassmann, H., 1983, Comparative neuropathology of chronic experi-
      mental allergic encephalomyelitis and multiple sclerosis,
      Neurology Series Vol.25, Springer, Berlin, Heidelberg, New
      York, Tokyo.
Lassmann, H., and Wisniewski, H. M., 1978, Chronic relapsing EAE.
      Time course of neurological symptoms and pathology, Acta
      Neuropathol., 43:35.
Lassmann, H., and Wisniewski, H. M., 1979a, Chronic relapsing
      experimental allergic encephalomyelitis.  Effect of age at the
      time of sensitization on clinical course and pathology, Acta
      Neuropathol., 47:111.
Lassmann, H., and Wisniewski, H. M., 1979b, Chronic relapsing
      experimental allergic encephalomyelitis: Morphological se-
      quence of myelin degradation, Brain Res., 169:357.
Lassmann, H., Ammerer, H. P., and Kulnig, W., 1978, Ultrastructural
      sequence of myelin degradation. I. Wallerian degeneration of
      the rat optic nerve, Acta Neuropathol., 44:91.
Lassmann, H., Kitz, K., and Wisniewski, H. M., 1980, Structural
      variability of demyelinating lesions in different models of
      subacute and chronic experimental allergic encephalomyelitis,
      Acta Neuropathol., 51:191.
Lassmann, H., Kitz, K., and Wisniewski, H. M., 1981a, Histogenesis of
      demyelinating lesions in the spinal cord of guinea pigs with
      chronic relapsing experimental allergic encephalomyelitis,
      J.neurol.Sci., 50:109.
Lassmann, H., Kitz, K., and Wisniewski, H. M., 1981b, The development
      of periventricular lesions in chronic relapsing experimental
      allergic encephalomyelitis, Neuropathol.Appl.Neurobiol., 7:1.
Lassmann, H., Kitz, K., and Wisniewski, H. M., 1981c, In vivo effect
      of sera from animals with chronic relapsing experimental
      allergic encephalomyelitis on central and peripheral myelin,
      Acta Neuropathol., 55:297.
Lassmann, H., Budka, H., and Schnaberth, G., 1981d, Inflammatory
      demyelinating polyradiculitis in a patient with multiple
      sclerosis, Arch.Neurol., 38:99.

Lassmann, H., Karcher, D., Kitz, K., Lowenthal, A., and Verslegers,
        W., 1983a, Humoral immune response in guinea pigs of different
        stages of EAE, in: "Experimental Allergic Encephalomyelitis: A
        Good Model for Multiple Sclerosis," E. C. Alvord, and M. W.
        Kies, eds., A. Liss Inc. New York (in press).
Lassmann, H., Suchanek, G., Kitz, K., Stemberger, H., Schweret, B.,
        and Bernheimer, H., 1983b, Antibodies in the pathogenesis, of
        demyelination in chronic relapsing EAE, in: "Experimental
        Allergic Encephalomyelitis: A Good Model for Multiple
        Sclerosis," E. C. Alvord, M. W. Kies, and A. J. Suckling,
        eds., A. Liss. Inc. New York, (in press).
Lublin, F. D., 1982, Delayed relapsing experimental allergic
        encephalomyelitis in mice, J.neurol.Sci., 57:105.
Lumsden, C. E., 1979, The neuropathology of multiple sclerosis, in:
        "Handbook of Clinical Neurology," P. I. Vinken, and G. W.
        Bruyn, eds., Elsevier, New York, vol.9, p.217.
Marburg, O., 1906, Die sogenannte "akute Multiple Sklerose,"
        ahrb.Psychiatrie, 27:211.
Marburg, O., 1936, Multiple Sklerose, in: "Handbuch der Neurologie,"
        O. Bumke, and O. Foerster, eds., Springer, Berlin, vol. 13/12,
Massanari, R. M., 1980, A latent relapsing neuroautoimmune disease in
        syrian hamsters, Clin.Immunol.Immunopathol., 16:211.
McAlpine, D., Compston, N. D., and Lumsden, C. E., 1955, "Multiple
        Sclerosis," Livingston, Edinburgh.
McKeown, S. R., and Allen, I. V., 1978, The cellular origin of
        lysosomal enzymes in the plaque in multiple sclerosis: a
        combined histological and histochemical study,
        Neuropath.Appl.Neurobiol., 4:471.
Mehta, P. D., Lassmann, H., and Wisniewski, H. M., 1981,
        Immunological studies of chronic relapsing EAE in guinea pigs:
        similarities to multiple sclerosis, J.Immunol., 127:334.
Müller, E., 1904, "Die multiple Sklerose des Gehirns und
        Rückenmarks," Fischer, Jena.
Oppenheimer, D. R., 1978, The cervical cord in multiple sclerosis,
        Neuropathol.Appl.Neurobiol., 4:151.
Perier, O., and Gregoire, A., 1965, Electron microscopic features of
        multiple sclerosis lesions, Brain, 88:937.
Peters, G., 1935, Zur Frage der Beziehungen zwischen der
        disseminierten nicht eitrigen Enzephalomyelitis und der
        multiplen Sklerose, Z.gesamt Neurol.Psychiatr., 153:356.
Pette, H., 1928, Über die Pathogenese der multiplen Sklerose,
        Dtsch.Z.Nervenheilk., 105:76.
Prineas, J. W., 1975, Pathology of the early lesions in multiple
        sclerosis, Hum.Pathol., 6:531.
Prineas, J. W., and Connell, F., 1978, The fine structure of
        chronically active multiple sclerosis plaques, Neurology,
        28:68.
Prinease, J. W., and Connell, F., 1979, Remyelination in multiple
        sclerosis, Ann.Neurol., 5:22.

Prinease, J. W., and Raine, C. S., 1976, Electron microscopy and
    immuneperoxidase studies in early multiple sclerosis lesions,
    Neurology, 26:29.
Prinease, J. W., and Wright, R. G., 1978, Macrophages, lymphocates
    and plasma cells in the perivascular compartment in chronic
    multiple sclerosis, Lab.Invest., 38:409.
Putnam, T. J., 1935, Studies in multiple sclerosis. IV. Encephalitis
    and sclerotic plaques produced by venular obstruction, Arch.
    Neurol., 33:929.
Raine, C. S., and Bornstein, M. B., 1970, Experimental allergic
    encephalomyelitis: and ultrastructural study of experimental
    demyelination in vitor, J.Neuropathol.Exp.Neurol., 29:177.
Raine, C. S., Traugott, U., and Stone, S. H., 1978, Glial bridges and
    Schwann cell migration during chronic demyelination in the
    CNS, J.Neurocytol., 7:541.
Raine, C. S. Scheinberg, L., and Waltz, J. M., 1981, Multiple
    sclerosis: oligodendroglia survival and proliferation,
    Lab.Invest., 45:534.
Schwerer, B., Egghart, M., and Kitz, K., 1983, Serum antibodies
    against central nervous system (CNS) antigens in chronic
    relapsing experimental allergic encephalomyelitis (crEAE), in:
    "Protides of the Biological Fluids," H. Peeters, ed., Pergamon
    Press, Oxford, p. 1983.
Seil, F. J., Rauch, H. C., and Einstein, E. R., 1973, Myelination
    inhibition factor: its absence in sera from subhuman primates
    sensitized with myelin basic protein, J.Immunol., 111:96.
Seitelberger, F., 1960, Histochemistry of demyelinating diseases
    proper including allergic encephalomyelitis and Pelizäus-
    Merzbacher's disease, in: "Modern Scientific Aspects of Neur-
    ology," J. N. Cummings, ed., Arnold, London.
Seitelberger, F., 1973, Pathology of multiple sclerosis,
    Ann.Clin.Res., 5:337.
Seitelberger, F., 1982, Comparative neuropathology of oriental and
    western multiple sclerosis cases, in: "Multiple Sclerosis East
    and West," Y. Kuroiwa, and L. T. Kurland, eds., Kyushu Uni-
    versity Press, Fukuoka.
Siemerling, E., and Raecke, E., 1914, Beitrag zur Klinik und
    Pathologie der multiplen Sklerose mit besonderer
    Berücksichtigung ihrer Pathogenese, Arch.Psychiat.Nervenkr.,
    53:385.
Steiner, G., 1931, "Krankheitserreger und Gewebsbefund bei multipler
    Sklerose," Springer, Berlin.
Stone, S. H., and Lerner, E. M., 1965, Chronic disseminated allergic
    encephalomyelitis in guinea pigs, Ann.N.Y.Acad.Sci., 122:227.
Suzuki, K., Andrews, J. M., and Waltz, J. M., 1969,
    Ultrastructural studies of multiple sclerosis, Lab.Invest.,
    20:444.
Traugott, U., Shevach, E., and Chiba, J., 1982, Chronic relapsing
    experimental allergic encephalomyelitis:  Identification and
    dynamics of T and B cells within the central nervous system,
    Cell Immunol., 68:261.

Traugott, U, Reinherz, E., and Raine, C. S., 1983, Multiple
      sclerosis: Distribution of T-cell subsets within active
      chronic lesions, Science, 219:308.
Wisniewski, H. M., and Keith, A. B., 1977, CHronic relapsing
      experimental allergic encephalomyelitis – and experimental
      model of multiple sclerosis, Ann.Neurol., 1:144.
Wisniewski, H. M., and Lassmann, H., 1983, Etiology and pathogeneis
      of monophasic and relapsing inflammatory demyelination – human
      and experimental, Acta.Neuropathol., Suppl.9:21.
Wisniewski, H. M., Lassmann, H., and Brosnan, C. F., 1982,
      Multiple sclerosis: immunological and experimental aspects,
      in: "Recent Advances in Clinical Neurology," W. B. Mathews,
      and G. H. Glaser, eds, Churchill–Livingstone, London, vol.3,
      p.95.

# WHAT EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

# TEACHES US ABOUT MULTIPLE SCLEROSIS

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After 37 years of study of experimental allergic encephalomye-
litis (EAE) and multiple sclerosis (MS) I obviously know more about
these conditions than I did when I began, but two questions arise:
1) Could I have predicted where I am now sufficiently clearly to have
convinced any granting agency to support my research for these 37
years, and 2) from my vantage point can I predict how and when the
puzzle will be solved?  Of course, the answer to both questions is no
- the first, emphatically no; the second, more questioningly so.  I
have the impression I am looking over a photographer's shoulder,
watching a picture being developed, with the more heavily exposed
portions appearing first, apparently randomly scattered and only
slowly coalescing to provide some details while the whole picture is
still not recognizable.

In such a situation I can only tell you where I see us now,
where I think we can progress rapidly and possibly where the big
holes remain.

The year 1983 is too important an anniversary to allow it to
pass without specific comment: the centennial of Pasteur's invention
of rabies vaccine, the semi-centennial of River's proof that the
"neuroparalytic accidents" of this rabies vaccine were due to the
neural contaminants, and the quarter-centennial of Kies's extraction
of myelin basic protein (BP) and proof that this was the specifically
responsible neural antigen.

There are those purists who would wait another couple of years
for the true centennial, but the pace of Pasteur's laboratory dis-
coveries 100 years ago is difficult to appreciate today.  In the 5
years centered around 1883 Louis Pasteur and his collaborators

(Chamberland, Roux and Thuillier) succeeded not only in cultivating
but also in subduing the virus of rabies, in a wide variety of ex-
perimental animals as well as in humans, thereby setting the stage
for the next century of research on what we now know as EAE.

The first 50 years of studies of the "neuroparalytic accidents"
of rabies vaccine were the slowest since only a few investigators,
such as Koritschoner and Schweinburg (1925) and Miyagawa and Ishii
(1926), came up with what we now know to be essentially the right
answer.  It remained for Rivers and his collaborators (Schwentker,
Sprunt and Berry) to show not only that the complication was due to a
neural contaminant but also that demyelination was a prominent fea-
ture.  That they mentioned Schilder's disease, not MS, is one of
those ironies of fate.

The next 10 years must have been the most frustrating, since the
concept that many demyelinating diseases might be due to an allergy
comparable to that inducing EAE could hardly be further refined with
such a tedious experimental design requiring so many injections over
such a long period of time.  Not until Jules Freund developed his
adjuvants 40 years ago could the experimental mode be manipulated.

If the pace of the first 60 years was slow and frustrating, the
pace of the last 40 years has certainly been accelerating and the
results increasingly productive.  In the decade between the meeting
of the Association for Research in Nervous and mental Diseases on MS
and our first symposium on EAE, that is, the decade including most of
the 1950's, modest advances occurred, especially in identifying BP,
but the really significant advances have occurred in the past 25
years, as illustrated in the graph below, recording my impressions of
the ups and downs of the possible relationships between EAE and MS.
As you can see, I am today about 80% convinced that EAE is a most
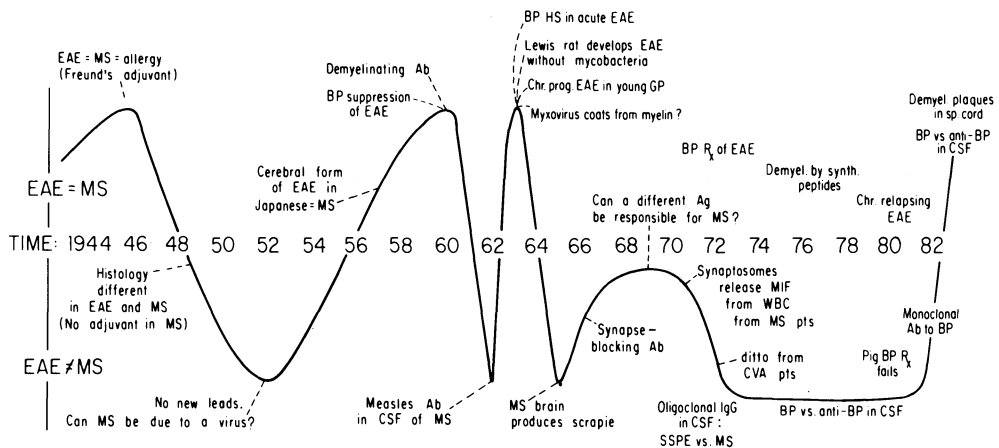appropriate model of MS.



Fig. 1.

At our recent symposium on "How Good a Model of MS is EAE Today?" in Seattle last July some 65 papers were presented and will soon be published by Alan R. Liss, Inc., N. Y. and I will draw on some of these papers-in-press to round out the story.

The 4 major areas of our own current research are:

1    the definition of the onset of EAE by clinical, immunochemical and electrophysiological changes (Alvord et al., 1983a)
2    a comparison of cerebrospinal fluid changes in EAE and MS (Alvord et al., 1983b)
3    a morphologic comparison of 3 models of chronic remitting-relapsing EAE with MS (Shaw and Alvord, 1983)
4    an analysis of antigenic determinants in myelin basic protein by suing monoclonal antibodies (Hruby et al., 1983).

Time does not permit me to summarize each of these. Instead, since Dr. Wisniewski and Dr. Seitelberger have already presented their views on the relationships between EAE and MS, perhaps the best approach for me to take will be to outline what I have seen, paying special attention to where I disagree with each of the previous speakers. I will make 7 points:

First of all, I would emphasize that ordinary EAE looks different in each species that I am familiar with: guinea pig, rat, rabbit, monkey and man. That is not to say that there are not many similarities which allow us to produce and diagnose EAE, but there are significant differences which make comparisons with MS difficult (Table 1).

So, in Table 1 are some minor differences which I would call to the attention of Drs. Wisniewski and Seitelberger. These differences may be only quantitative or technical, but I cannot speak of EAE without specifying the animal and the protocol.

Second, I would emphasize that chronic-relapsing EAE has been produced in each of 3 species (guinea pig, rat and monkey) with quite different protocols. It is not yet fair to speak of only one mechanism producing demyelination. Let me describe each of these:

One of the advantages of being a pathologist is the ability to compare, at least at one point in time, the morphologic appearances of diseases in humans and experimental animals. During the past decade it has been my privilege to be able to study dozens of cases of multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE) in hundreds of monkeys, both in its untreated and usually acute form (Shaw and Alvord, 1976) and in its treated and healed forms (Alvord et al., 1979a), as well as in its suboptimally treated and usually chronic remitting-relapsing form (Alvord et al., 1983a,b). In addition, two new forms of chronic remitting-relapsing

Table 1.   A Comparison of Some Histologic Features of EAE in
           Different Species with MS.

|  | EAE | | | |
| --- | --- | --- | --- | --- |
|  | guinea pig | rat | monkey | MS |
| 1. Acute: perivascular lymphocytes | ++ | ++ | ++ | + |
| perivascular demyelination | 0-± | 0 | ± | ++ |
| 2. Hyperacute: hemorrhage, necrosis | 0 | ++* | ++* | ++ |
| 3. Chronic: demyelination | ++ | ++ | ++ | +++ |
| sudanophilic lipids | 0 | | ++ | +++ |
| swollen axons | +++ | ++ | 0± | 0-± |
| gliosis | 0 | | + | +++ |
| necrosis | 0 | | + | ± |

* with pertussis
** with or without pertussis


EAE have been produced in guinea pigs and rats (Driscoll et al.,
1982).  These 3 experimental models suggest that pure demyelination
can be produced, most likely as an intermediate degree of damage
between forces that can produce necrosis at one extreme and non-
destructive inflammation at the other.

The monkey model, as described by Alvord et al., (1983a,b),
involves Macaca fascicularis monkeys sensitized to homologous
(monkey) myelin basic protein (BP) in Freund's complete adjuvants
(CFA) and treated suboptimally with BP and corticosteroids in saline
in osmotic minipumps implanted subcutaneously.

The guinea pig model, as described by Driscoll et al., (1982),
uses adult strain 13 guinea pigs actively challenged with homologous
(guinea pig) spinal cord in CFA 1 day after receiving homologous
BP-sensitized strain 13 lymph node cells which had been cultured for
24 hours in the presence of BP and an additional nonspecific stimulus
(a mixed lymphocyte reaction) provided by normal strain 2 peritoneal
exudate cells.  These cells were given in suboptimal numbers; i.e.,
at 10-20% of the numbers required for passive transfer of acutely
fatal EAE (Driscoll et al., 1979; 1982).  Half of the animals were
completely protected but half developed chronic varieties of EAE:
progressive-fatal, remitting-relapsing or chronic-stable.  The
animals were sacrificed at intervals from 1 to 6 months.

The rat model of Namikawa (unpublished) follows the technique of Richert et al., (1981a,b) and uses Lewis rats actively challenged with homologous (rat) spinal cord in CFA.  THis induces acute EAE followed by recovery.  At 30 days the rats received passive transfer of guinea pig BP-sensitized rat lymph node and spleen cells, which induced a second attack of EAE followed by recovery.  Similar passive transfers were given at 50 and 70 days, but each attack of EAE was followed by slower and less complete recovery.  The animals were sacrificed at 90 days.

In general, each animal was sacrificed under nembutal anesthesia and perfused with fixative (10% formalin with neutral buffer).  Representative blocks of brain and spinal cord were stained for myelin with gallocyanin-Darrow red or luxol fast blue, periodic acid-Schiff and hematoxylin and other stains as indicated.

Ordinary untreated EAE in the monkey is very frequently hyper-acute, with hemorrhagic necrosis and extensive polymorphonuclear leucocytic exudate in the brain more than the spinal cord (Shaw and Alvord, 1976).  Treatment with BP and an auxiliary factor (penicillin for M. mulatta and steroid for M. fascicularis) for a week or two can be completely successful (Shaw et al., 1976; Alvord et al., 1979), but cessation of treatment as soon as recovery has occurred (usually within a few days) allows another attack of EAE to occur, so that most of our monkeys have at least 2 and some 3 or even 4 attacks of EAE over a course of 2 to 3 months.  The lesions in these chronic remitting-relapsing monkeys are very frequently demyelinating, the edges rather sharply defined, and only the center of the lesions shows evidence of old necrosis with loss of axons.  The demyelinated axons are not noticeably swollen.  Sudanophilic myelin breakdown products are prominent in frozen sections stained with oil-red-O.  Plump reactive astrocytes are also prominent within the lesion.

Ordinary EAE in guinea pigs is typically predominantly inflam-matory with perivenous lymphocytes in both brain and spinal cord.  Demyelination is scanty but definite in about 15% of unselected animals, especially in animals showing disease of short incubation period and duration (Alvord et al., 1975).  In the chronic guinea pig, however, there is relatively little inflammation and the demye-lination is extensive with long subpial plaques scattered throughout the length of the spinal cord.  Macrophages loaded with myelin debris are prominent, throughout the lesions during the first 1 to 2 months and at the edges of the lesion as it heals during the 3rd and 4th months.  No sudanophilia has been seen in frozen sections stained with oil-red-O.  The axons are swollen but remain intact.  Reactive astrocytes, if present, do not stain with Holzer's stain; indeed, the extreme swelling of the axons suggests that there is little if any space to be accounted for by astrocytic reaction.  Swollen myelin sheaths and balls of myelin are prominent at the edges, within other-wise normal-appearing white matter, without obvious cellular

infiltrate, in all states except the completely healed.  This pattern
suggests a radial expansion of the demyelinating process.

Ordinary EAE in Lewis rats is also typically inflammatory, with
perivenous lymphocytes predominantly in the spinal cord, especially
caudally in the gray matter.  In the present animals with repeated
attacks induced by repeated passive transfer of BP-sensitized cells,
however, the lesions closely resemble those in the chronic guinea
pigs, with long subpial patches of extensive demyelination.  Macro-
phages contain abundant myelin debris.  The axons are intact, only
slightly separated by the macrophages, swollen only a little if at
all.  Reactive astrocytes are not prominent.

Each of the 3 experimental models has its own characteristic
features which make each recognizably different.  The common features
include repeated attacks of EAE (in the monkey and guinea pig models
deliberately induced by suboptimal treatments or numbers of cells)
and striking demyelination (remarkably pure in the guinea pig and
rat, still with central necrosis in the monkey).  Two of the models
(guinea pig and rat) use whole spinal cord in CFA as an active chal-
lenge in addition to BP-sensitized cells, but one (monkey) uses BP
only.  Thus, although other antigens may be important, BP can suf-
fice.  Here is a major point where I disagree with Dr. Wisniewski:
how many antigens are necessary to produce chronic relapsing EAE?

More specifically, is BP the whole antigen for EAE?  We know
that it can induce ordinary EAE and protect against ordinary EAE even
when EAE is induced with whole CNS (Alvord et al., 1965).  We know
that suboptimal treatments of monkeys that have received no antigen
other than BP can induce relapsing EAE with relatively prominent
demyelination (Shaw and Alvord, 1983).  The demyelination is not so
pure as we see it in the guinea pig or rat, where whole CNS is also
used, and where for the moment I have to admit the real possibility
of another antigen.  So, on this point I can as yet only partly
disagree with Dr. Wisniewski, but I would point out that BP-sensitive
cells capable of transferring ordinary EAE persist in such chronic
guinea pigs for months (Driscoll et al., 1982), as though the part-
iculate BP-complex in whole CNS persists at the site of innoculation
longer than BP does in solution (Tabira et al., 1983).  There may be,
therefore, again merely quantitative, rather than qualitative,
factors.  As for galactocerebroside being the antigen inducing demye-
linating antibody, Seil and Agrawal (1983) have pointed out that it
evokes antibodies that cause peripheral as well as central myelin to
lyse and that a chloroform-methanol insoluble "protein" may be re-
sponsible for the specific CNS demyelinating antibody.

For my third point, I would like to return to the pathogenesis
of EAE:  How does the sensitized lymphocyte recognize the target?  BP
is now generally considered to be on the inside of the oligodendrog-

lial membrane, thus protected by the outer part of this cell's mem-
brane, to say nothing of the glial foot plates, basement membrane and
endothelial cells that separate the CNS from the circulating
leucocytes.

Dr. Wisniewski would have us believe that local leakage of BP
from myelin (as part of the metabolic cycle that allows BP to escape
into the CSF) occurs through venules into the blood and that the
spotty presence of BP on the endothelial surface of these venules
attracts the sensitized lymphocytes to that particular focus.  If
there is BP in the blood – and I remain unconvinced that there is –
why would it not desensitize the lymphocytes?  No, I'd prefer to see
the lymphocytes randomly migrating into and through cells, liberating
lymphokines whenever they see the antigen to which they are sensi-
tized, the lymphokines recruiting 90% of the cells in the inflamma-
tory exudate.

To summarize at this point, let me say that I recognize a spec-
trum of clinical and histological reaction in these different
species, which can be understood by oversimplifying and identifying
them as hyperacute, acute and chronic.  As I proposed 20 years ago,
the simplest hypothesis that I see is that these represent changes in
the balance of power between two major forces, hypersensitivity and
immunity in the older terminology, helper and suppressor cells in the
newer terminology.  The major question, however, is whether these two
forces represent qualitative or merely quantitative differences, as
Knight (1982) has proposed.  At the moment I am biased by the obser-
vations of Driscoll et al., (1982), who could convert passive trans-
fer of EAE to passive transfer of protection against EAE merely by
transferring fewer cells.

Similarly, I see demyelination as part of a quantitatively
defined spectrum of damage intermediate between necrosis and simple
inflammation, much as Hurst (1944) maintained many years ago.

This general conclusion then leads me to my fourth point and
forces me to re-evaluate the definition of MS.  In retrospect, the
classical definition of MS as consisting of relatively large areas of
relatively pure demyelination occurring as multiple attacks over time
and space must represent a wastebasket, catching all the possible
variations on the theme, and including a spectrum from acute dissemi-
nated encephalomyelitis and acute hemorrhagic-necrotic encephalomye-
litis, including many cases of Balo's (Courville, 1970) and Devic's
syndromes (Cloys and Netsky, 1970), through acute MS to relapsing MS
and chronic MS.  In the human species, in contrast to the other
species studied experimentally, the most common reaction in most
parts of the world is what we recognize statistically as "MS"; but in
certain parts of the world, such as Japan and South America, the most
common reaction is Devic's syndrome (Shiraki, 1968).

   In my opinion, the most important question we have to face up to
is whether MS is a disease per se or a syndrome.  And as I have
indicated above, I believe we are dealing with a syndrome, in which
the several equivalents of the experimental protocols in the various
species are operating upon the genetically non-homogeneous human
populations to produce one or another portion of the clinical-
histological spectrum.

   For my fifth point and derived from this background, let me
continue with some other observations which may have important impli-
cations for further investigations and interpretations, especially
with regard to the question whether BP is the right antigen for MS.

   Our studies of monoclonal antibodies to BP (Hruby et al., 1983)
have reinforced the observations of many investigators that BP has
many antigenic as well as many encephalitogenic determinants.  In
general, the encephalitogenic sites are species-specific, each re-
cipient developing EAE following sensitization to a different se-
quence of amino-acid residues (Alvord, 1983).  Although sequences as
short as 6 residues may be encephalitogenic (Nagai, 1978), longer
sequences are more effective, and perhaps as many as 20 may be
necessary to mimic the whole BP when compared on a molar basis
(Alvord, 1983).  Some of these determinants are located in relatively
invariant regions of the molecule, others in hypervariable regions,
so that I see no rule which would permit a prediction of which
region(s) may be encephalitogenic for humans.  Indeed, based on the
different susceptibilities of inbred strains of mice (Chou et al.,
1983) and guinea pigs (Kies and Driscoll, 1982), I suspect that the
genetically non-homogeneous humans may react to several different
determinants.

   The encephalitogenic sites so far defined can be summarized in
the following diagram:


```
                              SJL mice,
     PL mice         rabbits  (rats), monkeys?     rabbits
      ↑                ↑         ↑                    ↑
  1-----20----40----60----80----100----120----140----160----178
              ↓                ↓                    ↓
            (rats),         str. 3              rhesus
            monkeys?    ↓   guinea              monkeys
                            pigs
                      Lewis
                      rats,
                      monkeys?,
                  str. 2 guinea pigs?
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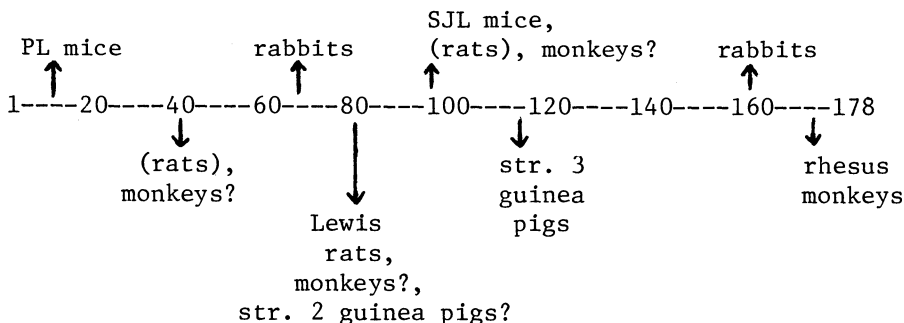

   The parentheses indicate minor determinants, the ?'s indicate
the lack of precise localization.

The other determinants, i.e., antibody-binding sites, are at least as interesting in their own right. Those so far identified by monoclonal antibodies are listed in Table 2.

As an aside, I should point out an interesting corollary of these differences, namely, that some antibody-binding determinants are more affected by fixation than others, and that some myelinated fibers appear to require better fixation than is provided by simple 10% formalin (4% formaldehyde, neutral buffered). For example, the deep horizontal fibers of the molecular layer of the cerebellum do not stain with monoclonal antibody-immunoperoxidase or even chemically unrelated common myelin sheath stains (such as luxol fast blue or gallocyanin) unless the specimen is fixed in formaldehyde containing metallic ions (copper or mercury), as in Susa ($HgCl_2$) or Bouin-Hollande-sublimate (Cu acetate). I do not yet know the implications of this observation for MS, but I suspect 1) that abnormal myelin will be subject to similar requirements for a fixative which is better than the classical formaldehyde and 2) that better stains may modify our diagnostic opinions!

Sixth, is BP the right antigen for MS? We know that pig BP failed as a treatment of MS (Romine and Salk, 1983); but, by analogy with the species-restrictions of some of our monoclonal antibodies and with the lack of encephalitogenicity of bovine BP for Lewis rats, I suspect that the human encephalitogenic determinant(s) may not be present in pig BP (i.e., that the homologous sequence is not the same in pig as in human BP). We know that BP in the CSF is not specific for MS and that anti-BP antibodies in the CSF are not specific for MS (Alvord et al., 1983b), but we wonder if we are testing for the right antigenic determinant. We know that the demyelinating factor in human sera is not specific for MS, that it might not even be an antibody, and that it may represent an epiphenomenon not at all related to the demyelinating antibodies of whole-CNS-induced EAE (Bradbury and Suckling, 1983).

Table 2.  Monoclonal Antibody-Reactive Sites in BP.

| Residues* | Class of MAb | Degree of Species-Restriction |
|-----------|--------------|-------------------------------|
| 1-14 | rat IgG | 0? |
| 90-99 | rat IgG | + |
| 86-100 | mouse IgM | 0? |
| 114-124 | rat IgG | ++ |
| 131-140 | mouse IgG, IgM | ++++ |

* numbering based on a hypothetical molecule with 178 positions including Phe-Phe @ 44-45 and 91-92; Trp @ 118; rat-14kDa deletion of 120-161 (Martenson, 1983).

So, we have some powerful arguments against BP being the right
antigen for MS.

Seventh, and last, do we have as powerful arguments against EAE
as the best model for MS? Here the evidence is more suggestive that
we are increasingly gaining experimental control of the mechanisms
producing demyelination. It is of interest to compare these lesions
with those in humans with MS, where several forms have been
described: 1) phagocytosis of normal-appearing myelin (Alvord, 1977;
Prineas and Connell, 1978), 2) phagocytosis of abnormal appearing
myelin (Zimmerman and Netsky, 1950), 3) confluence of small peri-
vascular lesions (Prineas, unpublished) and 4) large "pre-plaques"
identified by accumulations of IgG-laden macrophages (Prineas, un-
published). The third is the rarest but bears the closest resem-
blance to ordinary EAE in practically all animals. The first is the
most debated, since it implies a primary attack by macrophages,
stripping off otherwise normal myelin. The second is also debated,
since it implies a primarily noncellular (? viral, ? antibody, ?
enzyme) damage to myelin; it is quite easily seen in the present
guinea pig model but not in the rat or monkey. Of the 4 patterns
that have been described in MS, we have seen 2 in EAE: 1) a conflu-
ence of small perivascular lesions and 2) a radial expansion with
phagocytosis of abnormal myelin. The radial expansion with phago-
cytosis of normal myelin and the large "pre-plaque" accumulation of
IgG-laden macrophages have not yet been reproduced. .

Although a review of my first publication (Alvord and Stevenson,
1950) makes it clear that some of the chronic lesions were accepted
then as parts of the spectrum of ordinary EAE in guinea pigs, it is
now obvious that different aspects of these lesions are coming under
more strict experimental control. In particular, remarkably pure
demyelination can be produced.

Thus, I return to the concept that MS is a syndrome, with mul-
tiple demyelinating mechanisms, some of which can be mimicked by EAE.
But the most frustrating point is that we need more information about
the early lesions of MS: does it involve leucocytes or not? We
could eliminate a number of hypotheses if we knew the answer to this
simple question. In EAE we are reasonably confident that leucocytes
are primarily involved, but in MS we are not so sure. We can
occasionally see lesions in frozen sections stained with oil-red-O
and hematoxylin (Alvord, 1977), lesions which by analogy with strokes
can be only several hours to a few days old and are still evolving,
but is every active lesion of this type, with macrophages phagocytos-
ing normal-appearing myelin? Since other investigators describe
other lesions as being active, I conclude that we do not know the
whole story.

In conclusion, then while we know the etiology, pathogenesis,
prevention and treatment of EAE, we do not know any of these aspects

of MS.  Even so, I am still 80% convinced that EAE is the best model
we have.


## Acknowledgment

## REFERENCES

Alvord, E. C., Jr., 1965, Pathogenesis of experimental allergic
     encephalomyelitis:  Introductory remarks, Ann.N.Y. Acad.Sci.,
     122:245-255.
Alvord, E. C., Jr., 1977, Demyelination in experimental allergic
     encephalomyelitis and multiple sclerosis, in: "Slow Virus
     Infections of the Central Nervous System, Investigational
     Approaches to Etiology and Pathogenesis of These Diseases,"  V.
     ter Meulen, and M. Katz, eds., Springer-Verlag, New York, pp.
     166-185.
Alvord, E. C., Jr., 1983, Species-restricted encephalitogenic
     determinants, in: "Is EAE a Good Model for MS?", E. C. Alvord,
     Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss, New
     York.
Alvord, E. C., Jr., and Stevenson, L. D., 1950, Experimental
     production of encephalomyelitis in guinea pigs, Res.Publ.Assoc.
     Res.Nerv.Ment.Dis., 28:99-112.
Alvord, E. C., Jr., Shaw, C. M. Hruby, S., and Kies, M. W., 1965,
     Encephalitogen-induced encephalomyelitis:  Prevention, suppress-
     ion and therapy, Ann.N.Y. Acad.Sci., 122:333-345.
Alvord, E. C., Jr., Shaw, C. M., Hruby, S., Peterson, R., and Harvey,
     F. H., 1975, Correlation of delayed skin hypersensitivity and
     experimental allergic encephalomyelitis induced by synthetic
     peptides, in: "The Nervous System", D. B. Tower, ed., Raven
     Press, New York, pp. 647-653.
Alvord, E. C., Jr., Shaw, C. M., and Hruby, S., 1979, Myelin basic
     protein treatment of experimental allergic encephalomyelitis in
     monkeys, Ann.Neurol., 6:469-473.
Alvord, E. C., Jr., Shaw, C. M., Hruby, S., Sires, L. R., and Slimp,
     J. C., 1983a, The onset of experimental allergic encephalomye-
     litis as defined by clinical, electro-physiological and immuno-
     chemical changes, in: "Is EAE a Good Model for MS?", E. C.
     Alvord, Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss,
     New York.
Alvord, E. C., Jr., Hruby, S., Shaw, C. M., and Slimp, J., 1983b,
     Myelin basic protein and its antibodies in the cerebrospinal
     fluid in experimental allergic encephalomyelitis, multiple
     sclerosis and other diseases, in: "Is EAE a Good Model for MS?",

E. C. Alvord, Jr., M. W. Kies and A. J. Suckling eds., Alan R. Liss, New York.

Bradbury, K., and Suckling, A. J., 1983, The nature of factors in chronic relapsing EAE and MS sera which induce myelino-toxicity and cellular changes in organ culture, in: "Is EAE a Good Model for MS?", E. C. Alvord, Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss, New York.

Chou, C.-H. J., Shapira, R., and Fritz, R. B., 1983, Further delineation of encephalitogenic determinant for PL/J and (SJLxPL)F1 mice, in: "Is EAE a Good Model for MS?", E. C. Alvord, Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss, New York.

Cloys, D. E., and Netsky, M. G., 1970, Neuromyelitis optics, in: "Handbook of Clinical Neurology," P. J. Vinken, and G. W. Bruyn, eds., North-Holland Publishing Company, Amsterdam, 9:426–436.

Courville, C. B., 1970, Concentric sclerosis, in: "Handbook of Clinical Neurology," P. J. Vinken, and G. W. Bruyn, eds., North Holland Publishing Company, Amsterdam, 9:437–451.

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr., 1979, Enhanced transfer of experimental allergic encephalomyelitis with strain 13 guinea pig lymph node cells: Requirement for culture with specific antigen and allogeneic peritoneal exudate cells. J.Immunol., 125:1817–1822.

Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr., 1982, Suppression of acute experimental allergic encephalomyelitis in guinea pigs by prior transfer of suboptimal numbers of EAE-effector cells: Induction of chronic EAE in whole tissue-sensitized guinea pigs, J.Immunol., 128:635–638.

Hruby, S., Alvord, E. C., Jr., Martenson, R. E., Deibler, G. E., Hickey, W. F., and Gonatas, N. K., 1983, Epitopes in myelin basic protein reactive with monoclonal antibodies, in: "Is EAE a Good Model for MS?", E. C. Alvord, Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss, New York.

Hurst, E. W., 1944, A review of some recent observations on demyelination, Brain, 67:103–124.

Kies, M. W., and Alvord, E. C., Jr., eds., 1959, "Allergic' Encephalomyelitis," Charles C. Thomas, Springfield.

Kies, M. W., and Driscoll, B. F., 1983, Immunologic reactivity of myelin basic protein (BP) in inbred guinea pigs. Protides of the Biological Fluids, 30:115–118.

Knight, S. C., 1982, Control of lymphocyte stimulation in vitro: 'Help' and 'suppression' in the light of lymphoid population dynamics, J.Immunol.Meth., 50:R51–R63.

Koritschoner, R., and Schweinburg, F., 1925, Klinische und experimentelle Boebachtugen über Lähmungen nach Wutschutzimpfung, Zts f. Immunitätsf, 42:217–282.

Martenson, R. E., 1983, Myelin basic protein speciation, in: "Is EAE a Good Model for MS?", E. C. Alvord, Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss, New York.

Miyagawa Y, and Ishii, S., 1926, On the influence of the constituents
    of central nerve cells parenterally injected on the living
    organism, Jap.J.Exp.Med. (Sci. Reports Govt. Inst. Inf. Dis.,
    Tokyo Imp. Univ.), 5:331-370.
Nagai, Y., Akiyama, K., Suzuki, Kusumoto, S., Ikuta, F., and Takeda,
    S., 1978, Minimum structural requirements for encephalitogen and
    for adjuvant in the induction of experimental allergic
    encephalomyelitis, Cell.Immunol., 35:158-167.
Pasteur, L., 1885, Méthode pour prévenir la rage aprés morsure,
    Comptes rendus des séances de l'academie des sciences,
    101:765-774.
Pasteur, L., Chamberland, Roux, Thuillier, 1881, Sur la rage, Comptes
    rendus des séances de l'académie des sciences, 92:1259-1260.
Prineas, J. W., and Connell, F., 1978, The fine structure of
    chronically active multiple sclerosis plaques, Neurology,
    28(Suppl):68-75.
Richert, J. R., Kies, M. W., Alvord, E. C., Jr., 1981a, Enhanced
    transfer of experimental allergic encephalomyelitis with Lewis
    rat lymph node cells, J.Neuroimmunol., 1:195-203.
Richert, J. R., Driscoll, B. F., Kies, M. W., and ALvord, E. C., Jr.,
    1981b, Experimental allergic encephalomyelitis: Activation of
    myelin basic protein-sensitized spleen cells by specific antigen
    in culture, Cell.Immunol., 59:42-53.
Rivers, T. M., and Schwentker, F. F., 1935, Encephalomyelitis
    accompanied by myelin destruction experimentally produced in
    monkeys, J.Exp.Med., 61:689-701.
Rivers, T. M., and Schwentker, F. F., 1935, Encephalomyelitis
    accompanied by myelin destruction experimentally produced in
    monkeys, J.Exp.Med., 61:689-701.
Romine, J. S., and Salk, J., 1983, A study of myelin basic protein
    as a therapeutic probe in patients with multiple sclerosis, in:
    "Multiple Sclerosis," J. F. Hallpike, C. W. M. Adams, and W. W.
    Tourtellotte, eds., University Press, Cambridge, pp.621-630.
Seil, F. J., and Agrawal, H. C., 1983, Serum antimyelin factors in
    experimental allergic encephalomyelitis and multiple sclerosis,
    in: "Is EAE a Good Model for MS?", E. C. Alvord, Jr., M. W.
    Kies, and A. J. Suckling, eds., Alan R. Liss, New York.
Shaw, C. M., and Alvord, E. C., Jr., 1976, Treatment of experimental
    allergic encephalomyelitis in monkeys, II. Histopathological
    studies, in: "The Aetiology and Pathogenesis of the Demyelinat-
    ing Diseases," H. Shiraki, T. Yonezawa, and Y. Kuroiwa, eds.,
    Japan Science Press, Tokyo, pp. 377-395.
Shaw, C. M., and ALvord, E. C., Jr., 1983, A morphologic comparison
    of three experimental models of experimental allergic encephalo-
    myelitis with multiple sclerosis, in: "Is EAE a Good Model for
    MS?", E. C. Alvord, Jr., M. W. Kies, and A. J. Suckling, eds.,
    Alan R. Liss, New York.
Shaw, C. M., Alvord, E. C., Jr., and Hruby, S., 1976, Treatment of
    experimental allergic encephalomyelitis in monkeys. I.
    Clinical studies, in: "The aetiology and Pathogenesis of the

Demyelinating Diseases," H. Shiraki, T. Yonezawa, and Y. Kuroiwa, Japan Science Press, Tokyo, pp. 367-376.

Shiraki, H., 1968, The comparative study of rabies postvaccinal encephalomyelitis and demyelinating encephalomyelitides of unknown origin, with special reference to the Japanese cases, in: "The Central Nervous System, Some Experimental Models of Neurological Diseases," O. T. Bailey, ed., The Williams & Wilkins Company, Baltimore, pp. 87-123.

Tabira, T., Itoyama, Y., and Kuroiwa, Y., 1983, The role of locally retained antigens in chronic relapsing experimental allergic encephalomyelitis in guinea pigs, in: "Is EAE a Good Model for MS?", E. C. Alvord, Jr., M. W. Kies, and A. J. Suckling, eds., Alan R. Liss, New York.

Zimmerman, H. M., and Netsky, M. G., 1950, The pathology of multiple sclerosis, Res.Publ.Assoc.Res.Nerv.Ment.Dis., 28:271-312.

ANTIGEN AND ANTIBODY STUDIES IN DEMYELINATING DISEASE

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INTRODUCTION

   A potential clue to our understanding of the pathogenesis of multiple sclerosis (MS) is the presence in MS cerebrospinal fluid (CSF) and brain eluates of heterogeneously distributed IgG, i.e. oligoclonal IgG bands.  The first description of abnormal IgG bands in MS was by Lowenthal (1960) who observed that CSF IgG from MS had "restricted heterogeneity" when analyzed by agar gel electrophoresis (AGE).  Laterre (1965) referred to these bands as "oligoclonal" in order to emphasize the derivation of these bands from several clones of antibody synthesizing cells.  The monoclonality of the bands has been supported by evidence that individual bands possess a single light chain type (Link and Laurenzi, 1978; Mattson et al., 1982a) and single idiotype (Ebers et al., 1979; Nagelkerken et al., 1980; Gerhard et al., 1981; Tachovsky et al., 1982).

   In order to elucidate the MS humoral IgG response, we have found it valuable to compare the characteristics of the CSF IgG pattern in MS with the pattern found in a persistent central nervous system (CNS) infection, subacute sclerosing panencephalitis (SSPE), and an autoimmune CNS disease, experimental allergic encephalomyelitis (EAE).  Our investigations, as reported here, show that the MS humoral immune response is clearly different from that seen in SSPE patients and indicates a different fundamental pathogenesis of disease.  Our studies suggest that perhaps much or all of the MS IgG is not related to a specific etiologic agent or autoimmunogen, i.e. it is "nonsense" antibody.  Our unsuccessful search to date for a specific antigenic target for the MS IgG may result from the presence of nonsense antibody.

ANTIBODY STUDIES

## IEF Technical Aspects

Our initial studies compared the techniques of AGE and iso-
electric focusing (IEF).  The separation of proteins by AGE is on the
basis of net charge, size and shape, while IEF separation only
depends on the isoelectric point (pI) of the protein.  IEF has super-
ior resolution compared to AGE, but it is more difficult to inter-
pret.  A main difficulty in interpretation stems from a background
pattern of uniform markings seen even when normal serum or CSF is
focused.  These normal markings presumably result at least partly
from "mini-steps" in a pH gradient which is not completely homo-
geneous and which reflects a finite number of ampholines.  (Maxey and
Palmer, 1975).

In order to increase our sensitivity and allow for immunochemi-
cal identification of the IgG, we originally used an immunoperoxidase
overlay technique (Mattson et al., 1980a).  More recently, we have
employed an extremely sensitive silver staining method (Roos and
Lichter, 1983) (Figure 1) which can be combined with an immunofix-
ation step.  The ability to resolve as little as 80 ng of IgG in
specimens has enabled us to analyze unconcentrated CSF and brain
eluates.

## IEF Banding

Clinical Correlates. In our original studies (Mattson et al.,
1981), we found that 88% and 100% of CSF from MS and SSPE patients
had oligoclonal IgG bands respectively.  In MS, but not SSPE, the
number of bands detected by IEF correlated with increasing disease
duration, CSF IgG and CNS IgG synthesis.  Each patient's pattern was
unique.  Although the overall banding pattern remained fairly stable
over time, some changes in band number and relative intensities did
occur.

Banding Pattern.  Densitometric scanning of IEF gels enabled us
to make the following distinctions between MS, SSPE and normal CSF
IgG IEF patterns: SSPE CSF oligoclonal bands were more peaked and
prominent than MS bands; MS CSF had more "background" polyclonal IgG
than normal CSF suggesting a local synthesis of polyclonal (as well
as oligoclonal) IgG; SSPE CSF had less "background" polyclonal IgG
than MS or even than normal CSF indicating that a large proportion of
SSPE IgG was oligoclonal in distribution.

Light Chain Composition.  We studied the light chain composition
of MS and SSPE CSF IgG in order to verify the monoclonality of in-
dividual CSF bands and to contrast the findings in the two diseases
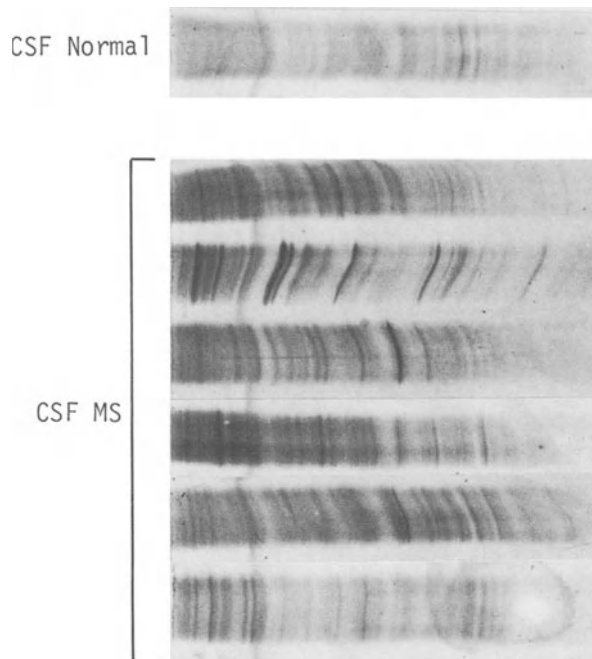(Mattson et al., 1982b).  When studied by IEF followed by an immuno-

Fig. 1.  IEF patterns in normal and MS CSF run on LKB Ampholine PAG
         plate gels, pH 3.5-9.5 and silver stained (Roos and Lichter,
         1983).  In this and all subsequent gels, only the cathodal
         end of the gel is shown.  Bands corresponding to IgG are
         present in the MS specimens.

chemical overlay technique many of the bands in MS and SSPE CSF were
found to contain only one light chain type, indicating their mono-
clonality.  MS bands tended to be κ predominant, whereas SSPE bands
had a more normal κ/λ ratio.  The reason for the greater predominance
of κ light chains in MS CSF is not certain, but may relate to the
nature of the antigen or the age of the individual when the clone is
generated.  MS antigens may tend to generate κ predominant antibody
responses, as reported in other antibody responses (Barundun et al.,
1976).  SSPE individuals usually have a primary measles infection
early in life when κ/λ ratios are known to be lower than in adults
(Barundun et al., 1976).

    Occassional bands stained for λ light chain, but not IgG heavy
chains; these bands presumably represent free light chains since IgM
does not enter the polyacrylamide gels we used and since IgA focuses
in another area of the gel.  The presence of free λ light chains has
been noticed by others in MS (Laurenzi et al., 1979) but their patho-
genesis remains obscure, especially since there is an overall κ/λ

predominance of the IgG bands.  Occasional IgG bands stained for both κ and λ light chains, probably because of a limited pH gradient set up by the finite number of ampholines resulted in cofocusing of different bands.

In summary, our investigations demonstrated a general similarity between the light chain composition of SSPE and MS IgG bands, except for the more frequent predominance of IgG λ bands in MS than in SSPE.

### Nonsense Antibody

In our investigations of MS and SSPE IgG bands, we compared the band patterns of serum, CSF and eluates from different areas of the brain from the same individual (Mattson et al., 1980b); Mattson et al., 1982a).  In the case of MS, patterns from different regions and compartments from a single individual had some bands in common, but also different unique bands (Figures 2, 3); these results were recently reproduced by others (Mehta et al., 1982).  In contrast, SSPE band patterns from different compartments of a single individual were extremely similar (Figure 4), suggesting a difference in the pathogenesis of IgG bands in the two diseases.  In SSPE, there appears to be a common response of identical plasma cell clones to the same antigen, measles virus, in all the compartments.  The difference in patterns seen in MS indicates that different plasma cell clones are present in these different areas; most likely these clones are responding to different antigens.  The band that are unique to one compartment may represent nonsense antibody, i.e. IgG that is not relevant to disease pathogenesis; the bands in common in different regions may be directed against a relevant antigenic target.
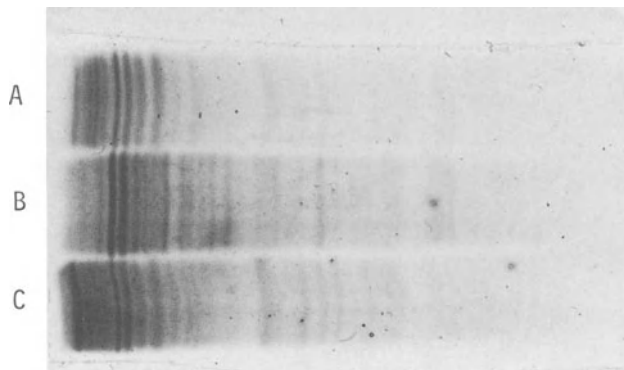


Fig. 2.  IEF with immunoperoxidase anti-IgG overlay staining of brain eluates from MS case 1.  A) acid eluate of pooled white matter; B) acid eluate of plaque 1; C) acid eluate of plaque 2.  For details regarding the procedure see Mattson et al., 1980b, 1982a.

There are several other lines of data that we and others have presented which support the presence of nonsense antibody in MS. Our finding that MS CSF IEF band number correlates with disease duration (Mattson et al., 1981) suggests that, with time, antibody-synthesizing cell clones accummulate in the CNS, producing more bands. We know from studies with cultured mononuclear cells stimulated by pokeweed mitogen (Oger et al., 1981) that this non-specific stimulation of a limited number of B cell clones can produce an oligoclonal response. Some of the oligoclonal bands may blend together into the increased polyclonal IgG of MS CSF that we observed on IEF gels (Mattson et al., 1981). Norrby et al. (1974) reported that individual MS patients can have antibody to multiple viruses locally produced in the CNS. Activity of these varied antibodies can be localized to a small part of the oligoclonal CSF IgG bands as well as the polyclonal IgG with a sensitive electroimmunofixation method (Rostrom et al., 1981; Vartdal and Vandvik, 1982).

What determines which antibodies comprise the MS IgG response? Perhaps antigens which frequently invade the brain, such as measles virus, generate plasma cell clones which reside in the CNS for extended periods and occassionally become active as part of an antibody response directed against another target. In this connection, one might ask why SSPE does not display nonsense IgG. Evidence suggests that all of the SSPE IgG bands comprise antibodies that are only directed against measles virus: all the bands have activity against measles virus and can be absorbed out by measles virus, SSPE IgG banding patterns from different compartments are similar; IEF band
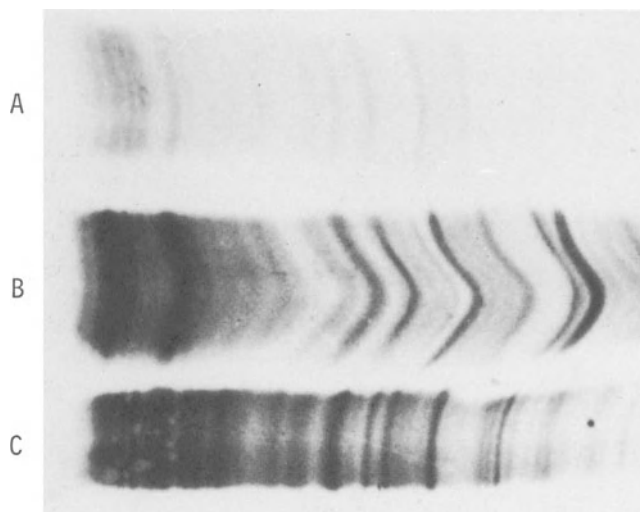


Fig. 3.   IEF with immunoperoxidase anti-IgG overlay staining of MS specimens from case 2.   A) acid eluates of pooled white matter; B) neutral saline eluate of pooled white matter; C) CSF.
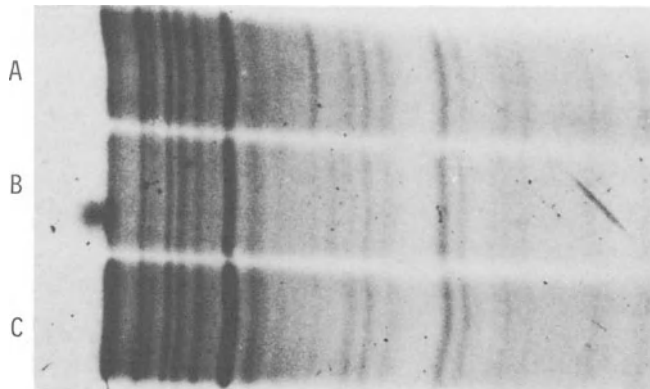
Fig. 4.   IEF with immunoperoxidase anti-IgG overlay staining of brain
          eluates from SSPE case.  A) acid eluate of region 1; B) acid
          eluate of region 2; C) acid eluate of region 3.

number does not correlate with increased CNS IgG synthesis in SSPE
(as it does in MS) implying that with continuing hyperimmunization
all SSPE oligoclonal IgG production goes into the same set of bands
with anti-measles antibody.  Perhaps the extreme intensity and chron-
icity of the measles virus antigenic stimulation leads to this "non-
nonsense" antibody response in SSPE.

    There is substantial precedent for the generation of a non-
specific antibody production associated with a specific one.  One of
the most dramatic examples of this phenomenon was described in mumps
meningitis (Vandvik et al., 1982); ten mumps meningitis patients had
oligclonal IgG bands directed against mumps virus, but four had, in
addition, oligclonal bands directed against measles, herpes and/or
rubella virus appearing in variable association and order.  In
several experimental systems a nonsense antibody response has been
found to antedate or even exceed a specific antibody response (see
Mattson et al., 1982a for references).  The important question that
now remains is whether all of the MS oligoclonal IgG response is
nonsense antibody - a result of B and T cell immune dysregulation -
or whether there remains a portion which is directed against the
relevant etiologic agent or autoimmunogen.

Oligoclonal IgG Bands in EAE

    We initiated studies on EAE in order to determine whether bands
were present in autoimmune diseases and additionally whether these
bands might represent nonsense antibody.  Our studies, performed in
collaboration with Drs. Caroline Whitacre, Philip Paterson and Eugene

Day have involved acute EAE in the rabbit (Whitacre et al., 1981; Whitacre et al., 1982). Because hemoglobin bands focus near the pI or rabbit IgG, we chose to use AGE for our studies. Rabbits immunized with whole CNS tissue or myelin basic protein (MBP) displayed IgG bands in sera and CSF. Slices of the agar gels from electrophoresed serum and CSF of rabbits immunized with MBP were eluted and assayed for anti-MBP activity. There was no localization of MBP antibody activity to band-containing fractions. In addition, absorption with MBP did not change the banding pattern. In one experiment absorption with the BCG component of Freund's complete adjuvant also did not change the pattern.

Bands have also been described in sera and CSF from guinea pigs with chronic relapsing EAE (CREAE) (Glynn et al., 1982; Karcher et al, 1982). Absorption with M. tuberculosis removed most of the IgG bands. Although animals had evidence by immunoblotting of antibody against varied CNS polypeptides, including MBP, absorption with CNS homogenates did not change the banding pattern (Glynn et al., 1982). It may have been valuable to have specifically localized the M. tuberculosis antibody activity to the bands more precisely, for example, by elution and, in addition, to have ruled our non-specific absorption of IgG to the M. tuberculosis. My suspicion is that many of the bands in CREAE are, as reported, directed against M. tuberculosis, but that a smaller proportion of the bands are also directed against CNS antigens.

Although controversy exists as to the major antigenic target of bands found in EAE, the presence of M. tuberculosis antibody and the lack of correspondence between the bands and an autoimmunogenic neural antigen lend support to the concept that oligoclonal IgG may be irrelevant to the pathogenetic autoimmunogen, i.e. it may be nonsense antibody. As Glynn et al. (1982) hypothesize, "it may be that, by analogy, the oligoclonal IgG in an MS patient represents a summation of humoral immune responses to infectious agents, which in certain individuals, can act as immunological adjuvants.".

## Antigen Studies

Although it is clear that some of the polyclonal and oligoclonal IgG is nonsense antibody, our working hypothesis has been that at least a portion of the oligoclonal "orphan" IgG (i.e., antibody looking for an antigenic target) has a relevant etiologic agent or autoimmunogen as its target.

## Absorption Studies

Our first attempts at antigen identification utilized absorption techniques. We predicted that absorption with most antigens would cause an unavoidable absorption of protein in general; we therefore

monitored the specificity of absorption with an antigen by demon-
strating the following: a decrease in the IgG/albumin ratio of the
specimen (indicating a preferential decline in the IgG over albumin);
a loss in pre-absorption IgG bands by IEF; a recovery of the IgG
bands from the absorbed pellet by elution.

We found it useful to verify the effectiveness of the technique
by first studying SSPE CSF IgG.  A decline in the IgG/albumin ratio
was observed following absorption of SSPE CSF IgG with a pelleted
measles virus preparation, but not with control Vero cells, or con-
trol brain homogenates of MBP; when SSPE CSF was tested against MS
brain homogenates, the IgG/albumin ratio also decreased, perhaps
because a generally large overall amount of protein was absorbed
(possibly due to electrostatic interactions).  Following absorption
with a sufficiently large amount of virus the IgG/albumin ratio fell
from .84 to .39, oligoclonal IgG bands were specifically lost from
the IEF pattern and bands representing 10% of the original IgG were
recovered by elution (Figure 5).

MS CSF specimens were then separately absorbed with a battery of
putative antigens including: saline and acid washed control brain,
saline and acid washed MS brain, MBP, crude sheep myelin, sheep
purified oligodendrocytes, Vero cells, rat C6 glioma cells, human
medulloblastoma cells, human red blood cells, human mononuclear
cells, and torpedo electric organ.  The data was difficult to inter-
pret because several antigens, especially the brain, myelin and
electric organ caused a decrease in the Ig/G albumin ratio.  The
changed ratio probably reflected the generally large loss of protein
from these absorptions and/or binding to Fc receptors.  Analysis of
post-absorption IEF gels did not demonstrate any preferential loss of
oligoclonal bands.

Because of difficulty in interpreting the MS absorption experi-
ments, we decided to change our approach.  It was also apparent that
the presence of a large amount of nonsense antibody would make
absorption with a relevant antigen difficult to detect.  If one
assumes that the IgG bound to MS brain (and released by acid elution)
is specifically bound, then one should be able to "stick" the eluted
IgG back to the brain it was eluted from.  In collaboration with Dr.
Joel Oger, we monitored the disappearance of IgG from (neutralized)
acid eluates of the brain when recombined with the eluted brain.  In
order to maximize the effectiveness of absorption and to avoid anti-
body excess, we used iodinated IgG.  As a first check on our system,
we detected substantial binding of iodinated SSPE brain eluted IgG to
measles virus and not Vero cells.  Unfortunately, we were unable to
detect binding of the SSPE IgG back to the brain from which it was
eluted.  The reasons for this failure may be related to denaturation
of the brain measles virus antigen during elution or to insufficient
antigen in the brain preparation.  The lack of success with this
approach in SSPE deterred us from further absorption experiments
using MS brain IgG.
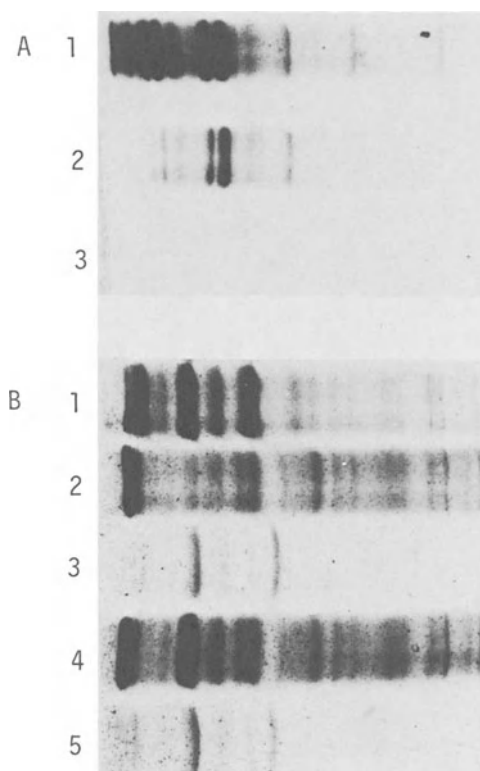
Fig. 5.  Absorption of SSPE CSF oligoclonal IgG bands by measles
         virus.  IEF pattern (with Coomassie Blue staining) of: (A)
         SSPE #1:  CSF before absorption with measles virus (1), CSF
         after absorption with measles virus (2), the acid eluate of
         the measles pellet (3); (B) SSPE #2: CSF before absorption
         with measles virus (1), CSF (700 µl) after two absorptions
         with measles virus (2), CSF (1400 µl) after two absorptions
         with measles virus (4), and the acid eluates of the measles
         pellets in each case (3 and 5, respectively).


## Immunoblot Studies

     Latov et al. (1981) effectively used immunoblotting as a means
of detecting the antigenic target for what must now be considered a
new autoimmune, human demyelinating disease, benign monoclonal gammo-
pathy with neuropathy (BMGN).  In the immunoblotting procedure,
proteins containing putative antigenic targets are subject to sodium
dodecyl sulfate-polyacrylamide gel electrophoresis, transferred
(blotted) to nitrocellulose paper by electroelution, overlaid with
the IgG in question and then identified with an immunochemical stain.
Latov et al. (1981) demonstrated that the IgM of BMGN patients
immunoblots myelin associated glycoprotein (MAG).

Fig. 6.   Western blot in which purified myelin from MS case 1 (MS 1) was electrophoresed on a 12.5%
SDS-polyacrylamide gel and then electroeluted on to nitorcellulose paper.  After
"quenching" of remaining binding sites with albumin, the nitrocellulose was overlaid with
sera or CSF (as listed below), washed, and then overlaid with peroxidase conjugated anti-
human IgM, washed and then developed with 3, 3'-diaminobenzidine tetrahydrochloride and
hydrogen peroxide.  Lane A is an amido black stain of the blotted proteins; the other
lanes were overlaid with the following specimens: (1) normal (nl) 1 serum, (2) MS 2
serum, (3) MS 3 CSF, (5) nl 2 serum, (6) MS 1 brain eluate, (7) MS
1 serum, (8) nl 3 serum, (9) MS 5 serum, (10) MS 5 CSF, (11) MS 6 serum, (12) MS 6
CSF, (13) MS 7 CSF, (14) BMGN serum.

We have been employing this technique in order to delineate the antigenic target of MS immunoglobulin. We have used optic nerve, spinal cord, control and MS white matter, control and MS purified myelin and purified sheep oligodendrocytes (from Dr. Sara Szuchet) as antigens. We have tested these tissues with various combinations of sera and CSF from patients with optic neuritis, transverse myelitis, post-infectious encephalomyelitis and MS; IgG and IgM antibodies were screened for. Frequently, especially in overloaded gels, there was mild staining of occasional bands when either normal or abnormal sera or CSF was applied. An example of definite positive staining can be seen in Figure 5 which demonstrates MAG protein staining in a case of BMGN (Stefansson et al., 1983), and an absence of MAG protein antibody in MS CSF, sera and brain eluates. Occassional specimens had a band of immunoreactivity against CNS material, as displayed by an optic neuritis CSF in Figure 6, but generally there was little evidence of consistent reactivity. Negative immunoblot studies using MS CNS material screened against MS CSF and brain eluates have been reported by others (Newcombe et al., 1982).

Why has immunoblotting been unsuccessful? If all the MS immuno-globulin is nonsense antibody all attempts to elucidate a patho-genetic antigen will obviously fail. Our working hypothesis at present has been, however, that some portion of the MS immunoglobulin is relevant antibody. This relevant antibody may nevertheless be very scarce compared to a large amount of nonsense antibody. It may also be that MS serum and CSF contain far more nonsense antibody that brain eluates - certainly the banding patterns of MS serum, CSF and brain eluates are remarkably different (Mattson et al., 1982a; Figure 3). Perhaps eluates from MS post-mortem brain have changed over time and no longer contain antibodies that were once relevant to the disease pathogenesis. Another issue relates to the antigen we are screening for. The antigen we electrophorese may not be the correct one, or it may not be concentrated enough or it might not solubilize well or it may be denatured by SDS with a consequent loss of anti-geneity. It is disquieting to realize that had we not known that acetylcholine receptor was the antigenic target of myasthenia gravis serum, we might have had a long, fruitless search screening human muscle and even endplates given the relative insolubility of the receptor and its low abundance.

CONCLUSIONS

Our studies have emphasized the differences in the MS and SSPE humoral immune response. It is clear that the MS IgG response is not as specifically directed as that seen in SSPE. The elusive antigen of the MS "orphan" antibody must await new technologies and appro-aches that will allow us to discriminate a reaction of a relevant specific antibody against a background of non-specific B cell responses.

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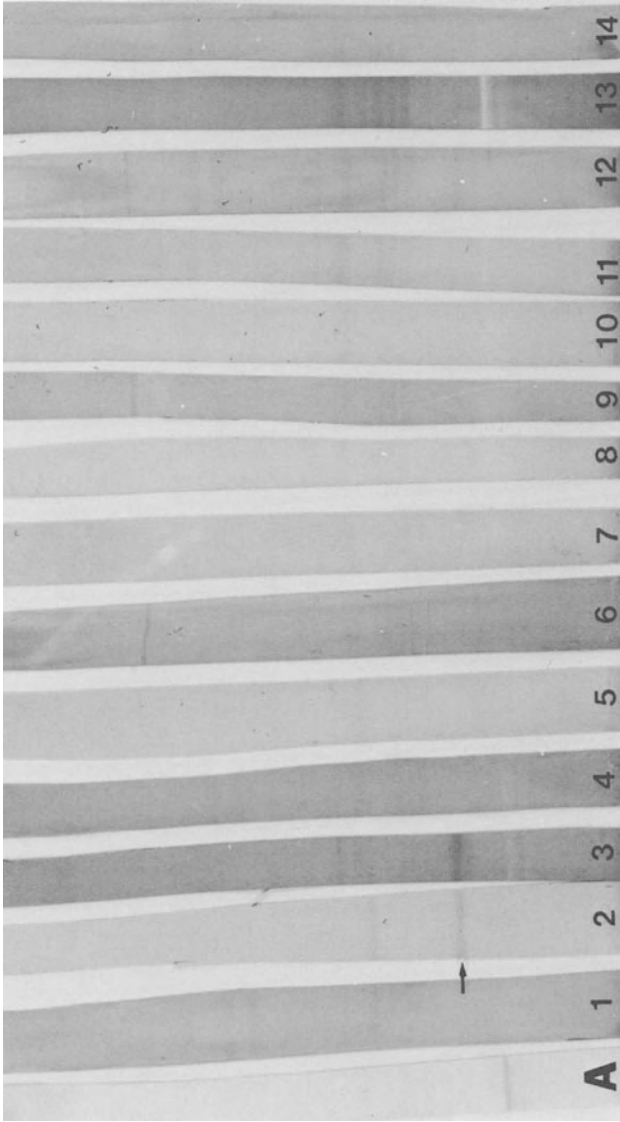


Fig. 7. Western blot in which solubilized optic nerve proteins were electrophoresed on an SDS-polyacrylamide gradient (7–25%) gel and then processed similarly to Figure 6 except peroxidase conjugated anti-human IgG rather than IgM was used. Lane A is an amido black stain of the blotted proteins. The other lanes were overlaid with the following specimens: (1) nl 1 serum, (2) optic neuritis (ON) 1 CSF, (3) ON 1 serum, (4) nl 2 serum, (5) control CSF, (6) nl 3 serum, (7) ON 3 CSF, (8) ON 4 CSF, (9) nl 3 serum, (10) ON 5 CSF, (11) ON 5 serum, (12) nl 5 serum, (13) MS CSF (with optic atrophy), (14) nl 6 serum. The arrow points to a low molecular weight band stained in lane 2 and less so in lane 3. Other bands are vaguely seen and appear in some normals as well as abnormal specimens.

## REFERENCES

Barundun, S., Skragill, R., and Morell, A., (1976), Imbalance of the
    $\kappa/\lambda$ of human immunoglobulins, in: "F. H. Bach and R. A. Good
    eds., Clinical Immunobiology, Academic Press, New York, N.Y.,

Ebers, G. C., Zabriski, J. B., and Kunkel, H. G., (1979), Oligoclonal
    immunoglobulins in subacute sclerosing panencephalitis and
    multiple sclerosis: A study of idiotypic determinants, Clin.
    Exp.Immunol., 35:67-75.

Gerhard, W., Taylor, A., Wroblewska, Z., et al. (1981), Analysis of
    a predominant immunoglobulin population in the crebrospinal
    fluid of a multiple sclerosis patient by means of an anti-
    idiotypic hybridoma antibody, Proc.Natl.Acad.Sci., USA,
    78:3225-3229.

Glynn, P., Weedon, D., Edwards, J., Suckling, A. J., and Cuzner, M.
    L., 1982, Humoral immunity in chronic relapsing experimental
    autoimmune encephalomyelitis: the major oligoclonal IgG bands
    are antibodies to mycobacteria, J.Neurol.Sci., 57:369-384.

Karcher, D., Lassmann, H., Lowenthal, A., Kitz, K., and Wisniewski,
    H. M., 1982, Antibodies-restricted heterogeneity in serum and
    cerebrospinal fluid of chronic relapsing experimental allergic
    encephalomyelitis, J.Neuroimmunol., 2:93-106.

Laterre, E. C., Les proteines du liquide cephalorachidien a l'etat
    normal et pathologique, Brussels, Arscia, Paris, Maloine.

Latov, N., Braun, P. E., Gross, R. B., Sherman, W. H., Penn, A. S.,
    and Chess, L., 1981, Plasma cell dyscrasia and peripheral
    neuropathy: identification of the myelin antigens that react
    with human paraproteins, P.N.A.S. 78:7139-7142.

Laurenzi, M. A., Maria, M., Kam-Hansen, S., and Link, H., Oligoclonal
    IgG and free light chains in multiple sclerosis demonstrated by
    thin-layer polyacrylamide gel isoelectric focusing and immuno-
    fixation, Ann.Neurol., 8:241-247.

Link, H., and Laurenzi, M. A., 1979, Immunoglobulin class and light
    chain type of oligoclonal bands in CSF in multiple sclerosis
    determined by agarose gel electrophoresis and immunofixation.,
    Ann.Neurol., 6:107-110.

Lowenthal, A., van Sande, M., and Karcher, D., 1960, The differential
    diagnosis of neurologic diseases by fractionating electro-
    phoretically the CSF gammaglobulins., J.Neurochem., 6:51-56.

Mattson, D. H., Roos, R. P., Hopper, J. E., and Arnason, B. G. W.,
    1982, Light chain composition of CSF oligoclonal IgG bands in
    multiple sclerosis and subacute sclerosing panencephalitis,
    J.Neuroimmunol., 3:63-76.
Mattson, D. H., Roos, R. P., and Arnason, B. G. W., 1980, Isoelectric
    focusing of IgG eluted from multiple sclerosis and subacute
    sclerosing panencephalitis brains, Nature, 287:335-337.
Mattson, D. H., Roos, R. P., and Arnason, B. G. W., 1982, Oligoclonal
    IgG in multiple sclerosis and subacute sclerosing pan-
    encephalitis brains, J.Neuroimmunol., 2:261-276.
Mattson, D. H., Roos, R. P., and Arnason, B. G. W., 1980,
    Immunoperoxidase staining of cerebrospinal fluid IgG in iso-
    electric focusing gels: a sensitive new technique, J.Neurosci.
    Meth., 3:67-75.
Mattson, D. H., Roos, R. P., and Arnason, B. G. W., 1981, Comparison
    of agar gel electrophoresis and isoelectric focusing in multi-
    ple sclerosis and subacute sclerosing panencephalitis, Ann.
    Neurol., 9:34-41.
Maxey, C. R., and Palmer, M. R., 1975, The behavior of gelatin
    preparations in the acidic region of the pH gradient, in:
    "Isoelectric Focusing", J. P. Arbuthnott and J. A. Beeley, eds,
    Butterworths, Woburn, Ma; pp.261-269.
Mehta, P. D., Miller, J. A., and Tourtellotte, W. W., 1982,
    Oligoclonal IgG bands in plaques from multiple sclerosis
    brains, Neurology 32:372-376.
Nagelkerken, L. M., Aalberse, R. C., VanWalbeek, H. K. V., et al.,
    1980, Preparation of antisera directed against the idiotype(s)
    of immunoglobulin G from the cerebrospinal fluid of patients
    with multiple sclerosis, J.Immunol., 125:384-389.
Newcombe, J., Glynn, P., and Cuzner, M. L., 1982, The immunological
    identification of brain on cellulose nitrate in human demye-
    linating disease, J.Neurochem., 38:267-274.
Newcombe, J., Glynn, P., and Cuzner, M. L., 1983,  Analysis by
    transfer electrophoresis of reactivity of IgG with brain pro-
    teins in multiple sclerosis, J.Neurochem., 39:1192-1194.
Norrby, E., Link, H., Olsson, J.-E., Panelius, M., Salmi, A., and
    Vandvik, B, 1974, Comparison of antibodies against different
    viruses in cerebrospinal fluid and serum samples from patients
    with multiple sclerosis, Infect.Immun., 10:688-694.
Oger, J. J.-F., Mattson, D., Roos, R., Antel, J., and Arnason, B. G.
    W., 1981, Isoelectric focusing of IgG secreted by human peri-
    pheral lymphocytes in multiple sclerosis and controls, Neuro-
    logy., 21:144.
Roos, R., and Lichter, M., Silver staining of cerebrospinal fluid IgG
    in isoelectric focusing gels, J.Neuroscience Methods, In press.
Rostrom, B., Link, H., Laurenzi, M. A., Kam-Hansen, S., Norrby, E.,
    and Wahren, B., 1981, Viral antibody activity of oligoclonal
    and polyclonal immunoglobulins synthesized within the central
    nervous system in multiple sclerosis, Ann.Neurol., 9:569-574.

Siemes, H., Siegart, M., Hanefeld, F., Kolmel, H. W., and Paul, F.,
    1977, Oligoclonal gamma-globulin banding of cerebrospinal fluid
    in patients with subacute sclerosing panencephalitis, J.Neurol.
    Sci., 32:395-409
Stefansson, K., Marton, L., Antel, J. P., Wollmann, R. L., Roos, R.
    P., Chejfec, C., and Arnason, B. G. W., 1983, Neuropathy accom-
    panying IgMλ monoclonal gammopathy, Acta.Neuropathologica,
    59:255-000.
Tachovsky, T. G., Sandberg-Wolheim, M., and Baird, L. G., 1982, CSF
    igG characterization of antidiotype antibodies produced against
    MS CSF and detection of cross reactive idiotypes in several MS
    CSF, J.Immunol., 129:764-770.
Vandvik, B., Nilsen, R. E., Vartdal, F., and Norrby, E., Mumps
    meningitis, specific and non-specific antibody responses in the
    central nervous system, Acta Neurol. Scand., 65:468-487.
Vartdal, F., and Vandvik, B., 1982, Multiple sclerosis:
    electrofocused "bands" of oligoclonal CSF IgG do not carry
    antibody activity agains measles, varicella-zoster or rota-
    viruses, J.Neurol.Sci., 54:99-107.
Whitacre, C. C., Mattson, D. H., Day, E. D., Peterson, D. J.,
    Paterson, P. Y., Roos, R. P., and Arnason, B. G. W., 1982,
    Oligoclonal IgG in rabbits with experimental allergic ence-
    phalomyelitis: non-reactivity of the bands with sensitizing
    neural antigens, Neurochem.Res., 7:1209-1221.
Whitacre, C. C., Mattson, D. H., Paterson, P. Y., Roos, R. P.,
    Peterson, D. J., and Arnason, B. G. W., 1981, Cerebrospinal
    fluid and serum oligoclonal IgG bands in rabbits with experi-
    mental allergic encephalomyelitis, Neurochem.Res., 6:87-96.

# STUDIES ON B-LYMPHOCYTE FUNCTION IN

# MULTIPLE SCLEROSIS

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## INTRODUCTION

Among abnormalities reported in multiple sclerosis (MS) and
considered to reflect derangements of the immune system, those of
B-lymphocyte function within the CNS-CSF compartment are generally
accepted as the most consistent and clear-cut.  These abnormalities
have recently been extensively reviewed (Trotter and Brooks, 1980;
Brooks et al., 1983; Walsh and Tourtellotte, 1983; Walsh et al.,
1983).  They include elevation of the CSF-IgG/total protein ratio
(Kabat et al., 1948) and the CSF-IgG index, equal to (CSF-IgG/serum-
IgG): (CSF-albumin/serum-albumin) (Tibbling et al., 1977), accent-
uated predominance in CSF of IgGl (Palmer and Minard, 1976) and of
IgG kappa (Link and Zettervall, 1970), and oligoclonal distribution
of IgG on separation of CSF by electrophoresis or isoelectric focus-
ing on suitable media such as agar or agarose, in about 90%.  MS
patients heterozygous to Glm (1) and Glm (3) allotypes of IgGl showed
greatly increased concentrations in the CSF of Glm[1] in comparison
with patients with other neurological disorders indicating that, in
the heterozygous MS patient, most plasma cells in the CNS-CSF com-
partment preferentially synthesize Glm (1) IgGl molecules (Salier et
al., 1981).  Furthermore, unexpected Gm allotypes may be found in CSF
but not serum from an occasional MS patient (Salier et al., 1983).  A
majority of patients with MS display evidence for intrathecal syn-
thesis of IgG class antibodies against various viruses, especially
measles (for review see Norrby, 1978).  Simultaneous intrathecal
production of antibodies against 11 different viral antigens in
individual MS patients has been reported (Salmi et al., 1983).
Furthermore, synthesis may occur within the CNS-CSF compartment of
antibodies against bacteria (Vartdal et al., 1980) and various CNS

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epitopes (for review see Roström, 1981; Walsh et al., 1983).

Recently, elevated CSF-IgM index reflecting intrathecal IgM production has been reported by others (Sindic et al., 1982) and by us (Forsberg et al., 1983) in about 60% of patients with MS (Figure 1). A parallel may be drawn with the situation in neurosyphilis where a prolonged intrathecal IgM-response has been described (Strandberg-Pedersen et al., 1982), which may normalize several months after successful treatment of the disease. The antibody specificity(ies) of locally produced IgM in MS remain to be elucidated.

The above abnormalities are all consistent with B-lymphocyte hyperfunction and the consequence of increased concentrations of <u>free immunoglobulins</u> (Igs) within the CNS-CSF compartment. Whether aberrations of free Ig measured in the form of these variables are really representive for existing derangements of B-lymphocyte functions is, however, far from clear. This might be elucidated by studying Igs in supernatants of cultivated CSF mononuclear cells. In such a way, " a complete repertoir" or Ig-producing cells from CSF compartment could be obtained.
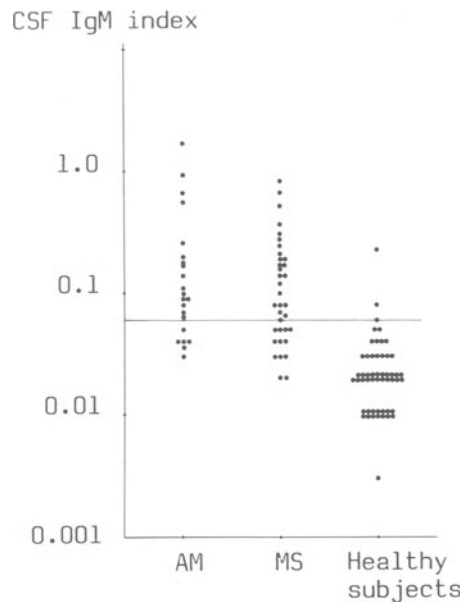


Fig. 1. CSF-IgM index (logarithmic scale) for reference group of 51 "healthy subjects", i.e. individuals mostly with tension headache and with normal findings at neurological and routine CSF examinations, and for 35 patients with multiple sclerosis (MS) and 22 with aseptic meningoencephalitis (AM). Our upper reference limit (mean + 2SD) for CSF-IgM index of 0.06 is drawn.

The concentrations in CSF of individual Igs as well as antibodies can be considered to be determined by an afferent and an efferent axis.

| Afferent axis | Efferent axis |
| --- | --- |
| Transudation via blood-brain barrier | Metabolism |
| Local production within CNS and meninges | Differences in half-life (IgG 25 days; IgA 6 days; IgM 5 days) (Hood et al., 1978) |
| Local synthesis by CSF lymphocytes | Proteolysis |
| | Consumption at target |

Local synthesis by lymphocytes present in the CSF is probably quantitatively of low-grade importance in comparison with the two other mechanisms constituting the afferent axis. The mechanisms of the efferent side have hitherto mostly been neglected in discussions of Ig concentrations in CSF. Greatly elevated activities of the peptidase leucyl-B-naphthylamide (Rinne and Riekkinen, 1968) and of acid and neutral proteinases (Rinne and Riekkinen, 1968; Cuzner et al., 1979) have been reported in CSF from patients with MS. To what extent these abnormalities influence Ig levels in CSF is not known. The hitherto unknown relation between pentamers and monomers of IgM in CSF should have influence on IgM concentrations in CSF. Little is known about consumption of antibodies at targets but it may be anticipated that his mechanism has substantial influence on concentrations in CSF of Igs of various classes and subclasses, and of antibody specificities.

Concentrations of free Igs in water-soluble CNS extracts are probably influenced by similar mechanisms. Consumption at target can be exemplified by our observation that water-soluble CNS extracts obtained from guinea pigs with chronic relapsing experimental allergic encephalomyelitis (r-EAE) did not contain measurable amounts of IgG antibodies against myelin when examined by a sensitive enzyme-linked immunosorbent assay (ELISA) while the same assay revealed high concentrations of such antibodies in supernatants from short-term (18 h) cultures of CNS and meningeal lymphocytes obtained from the same animals (Olsson et al., manuscript in preparation).

It can be anticipated that studies performed directly on CSF-lymphocytes (CSF-L) in comparison with peripheral blood lymphocytes (PBL) from the same individual will give information in addition to that available from determination of concentrations of free Igs or antibodies in CSF. In this paper, we present results from our lab-

oratory regarding enumeration of B-lymphocytes in CSF and the peripheral blood; enumeration of Ig-secreting cells in CSF and peripheral blood by applying an indirect haemolytic plaque-forming cell (PFC) assay; and analysis of concentrations of Igs secreted into supernatants obtained from long-term (7 days) cultures of CSF-L and PBL obtained from patients with MS and from controls. Our results indicate that enumeration of PFC and determination of concentrations of Igs - and probably of antibodies - in lymphocyte culture supernatants reflect B-lymphocyte hyperfunction more appropriately than concentrations of free Igs in body fluids.


B-LYMPHOCYTES IN CSF AND PERIPHERAL BLOOD IN MULTIPLE SCLEROSIS

On stimulation, B-lymphocytes are transformed from the resting state to plasma cells which secrete Ig belonging to different classes and subclasses, and carrying various antibody specificities. B-lymphocytes have markers consisting of Ig molecules on the surface membrane, while plasma cells lack such markers.

The topic on B-lymphocyte proportions in CSF and peripheral blood in MS and neurological disorders has been carefully reviewed (Kam-Hansen, 1980; Brooks et al., 1983; Walsh et al., 1983). Great controversy exists on the presence of consistent differences in lymphocyte populations, including B-cells, in patients with MS. We found significantly ($p < 0.001$; Student's t-test) lower mean B-lymphocyte value in peripheral blood from a group of 18 MS patients compared to patients with mumps meningitis as well as healthy controls (Figure 2). All patients with MS had a pleocytosis and oligoclonal IgG in the CSF, and 14 of the patients had elevated CSF- IgG index. The mean B-lymphocyte value was slightly lower in CSF compared to peripheral blood in the MS patient group (Kam-Hansen et al., 1978). These data are consistent with our subsequent finding in MS patients (vide infra) of increased numbers, especially in CSF but also in peripheral blood, of Ig-secreting cells lacking surface Ig and thus no longer identifiable as B-lymphocytes. It must, on the other hand, be emphasized that quantitation of B- and of total T-lymphocytes comprises a very gross assessment of lymphocyte populations, and more sophisticated tests are warranted to detect effects of possible derangements in immune regulation in MS.


IMMUNOGLOBULIN-SECRETING CELLS IN CSF AND PERIPHERAL
BLOOD IN MULTIPLE SCLEROSIS AND CONTROLS

Enumeration of mature B-lymphocytes, i.e. plasma cells secreting Ig, in CSF and peripheral blood from patients with MS has given us a completely different picture than mere quantitation of B-lymphocytes. Enumeration of Ig-secreting cells may be carried out by an indirect haemolytic PFC assay which is based on the capacity of one single
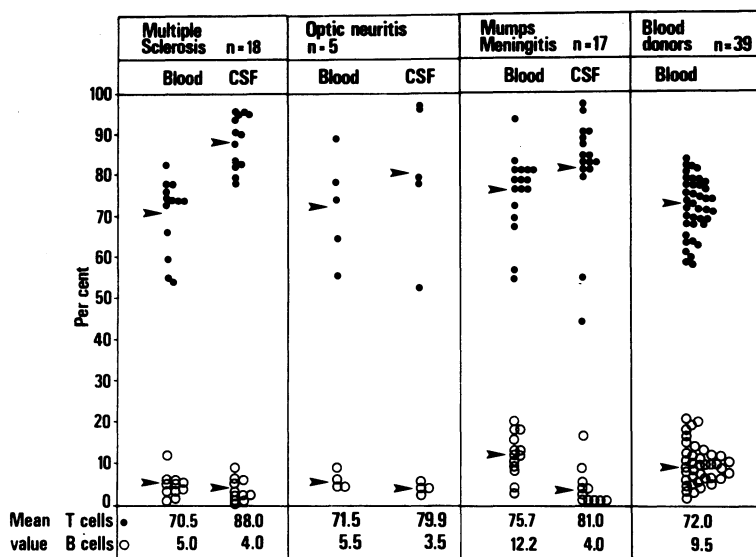
Fig. 2.  Percentages of B cells (o) and T cells (●) in blood and
         cerebrospinal fluid (CSF) from patients with multiple
         sclerosis, optic neuritis and mumps meningitis, and in blood
         only from blood donors.  Arrows depict mean values.

plasma cell to produce and secrete Ig molecules of a certain class
and antibody specificity.  In this assay (Figure 3), secretion is
detected in a system comprising sheep red blood cells (SRBC) coated
with protein-A which binds Fc part of antibody molecule directed
against heavy chains of the human Ig class to be detected.  The
antibody molecules bind Ig secreted by plasma cells.  In presence of
complement, lysis of SRBC is induced and this haemolysis is then used
as indicator of secretion.  Lymphocytes are prepared from peripheral
blood by Ficoll-Isopaque centrifugation, and from CSF by centri-
fugation at 200 x g for 10 min at 4°C.  The indirect haemolytic PFC
assay has been adopted in our laboratory for the study of 20 x $10^3$
lymphocytes, in exceptional cases even 10 x $10^3$ lymphocytes
(Henriksson et al., 1981).  This makes it possible to examine CSF
specimens with slight pleocytosis or even normal mononuclear cell
counts.  Lymphocytes, protein-A-coated SRBC and monospecific anti-
serum are mixed together with complement and agar, and poured on a
Petri dish.  After incubation at 37°C for 6 h, haemolytic plaques are
counted visually.  Each plaque corresponds to one Ig-secreting cell.
In order to establish a safe marginal, the occurrence of one or two
plaques was judged as negative.

     In peripheral blood from healthy subjects, we found a predomin-
ance of IgA-producing cells, followed by cells producing IgG and IgM
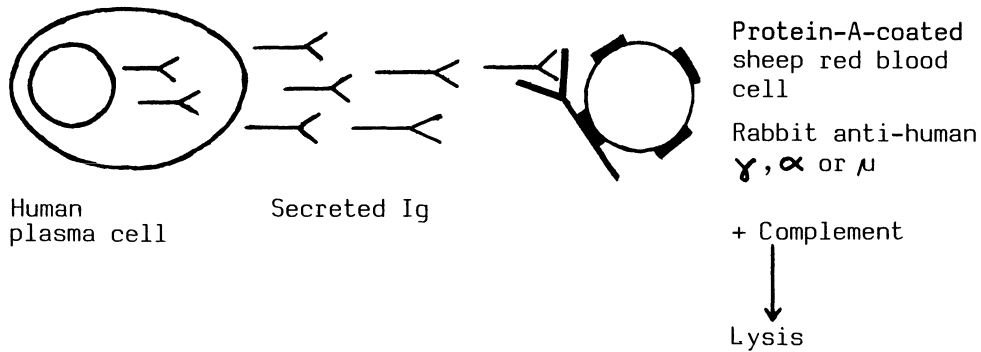(Figure 4).  In normal CSF, it is difficult to enumerate Ig-producing

Fig. 3.   Principles of indirect haemolytic plaque-forming cell
          (PFC) assay.

cells because of the cell paucity.  In a study of four individuals
with tension headache, no Ig-producing cells per 10 x $10^3$ lymphocytes
used in the test were detectable in the CSF of these presumably
healthy individuals.  For these reasons, we consider the presence of
more than two plaques on examination of 20 x $10^3$ CSF lymphocytes as
evidence of B-lymphocyte activation in the CSF compartment.

Enumeration of PFC in CSF and peripheral blood from 11 patients
with MS (Figure 4) revealed that 10 of the patients had higher
numbers of IgG-producing cells per 20 x $10^3$ lymphocytes in CSF than
in peripheral blood (Henriksson et al., 1981).  These are expected
results based on our previous knowledge of synthesis of free IgG
within the CNS in most patients with MS (see Introduction).  Inter-
estingly, 9 of the 11 patients had IgA-producing cells in CSF and 7
had IgM-producing cells, indicating intrathecal production also of
Igs belonging to these two classes in the majority of MS patients.
There were, however, no significant differences in these 11 MS
patients between CSF and peripheral blood for numbers of cells
secreting IgA or IgM.

To allow analysis of numbers of Ig-producing cells inter alia in
relation to clinical variables, the indirect haemolytic PFC assay was
applied on a larger material consisting of 37 ambulatory patients
with MS (Henriksson et al., manuscript in preparation).  None of
these patients had been treated with immunosuppressive or immuno-
stimulatory drugs for the last year, and the majority had never
received such medications.  All patients had oligoclonal IgG in CSF.

Among these 37 patients with MS, 89% had cells in CSF producing
IgG, 70% had cells producing IgM and 57% had cells producing IgA.  No
patient was found who was negative in CSF for Ig-producing cells.
Interestingly, four of the patients had in their CSF IgM-and/or
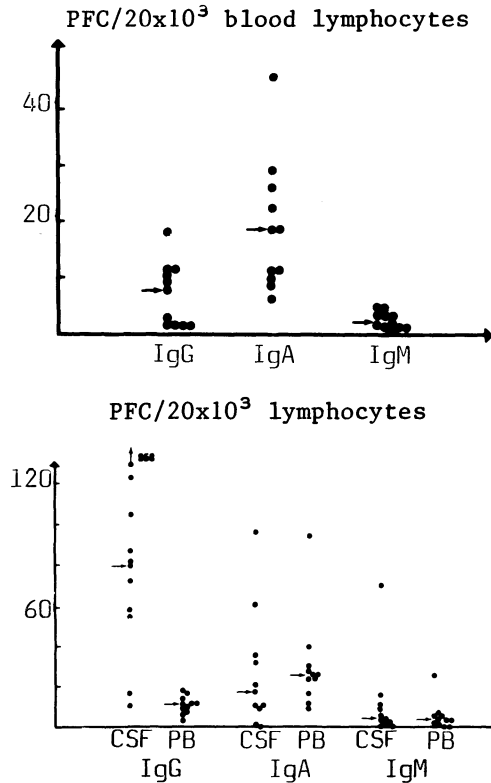IgA-producing cells but no cells producing IgG.

PFC/20x10$^3$ blood lymphocytes

Fig. 4.   Distribution of Ig-producing cells among 20 x 10$^3$
          lymphocytes from peripheral blood from 11 healthy controls
          (upper part) and from CSF and peripheral blood (PB) from 11
          patients with MS (lower part).   Arrows denote median values.

Our previous finding of much higher numbers of IgG-producing cells in CSF than peripheral blood was confirmed on this larger material of 37 patients with MS (Table 1).   In contrast, the number of IgA-producing cells was significantly lower in CSF, while the number of IgM-producing cells did not differ in CSF and peripheral blood.

Among these 37 patients with MS, IgG-producing cells predominated in CSF in 27, IgM-producing cells in 6, and IgA-producing cells in 3, while one patient had the same numbers of IgG- and IgA- producing cells in CSF (Table 2).   In contrast, IgA-producing cells predominated in peripheral blood in most of the patients, as in the healthy controls.   Among the 6 patients in whom IgM-producing cells predominated in CSF, two had active and four stable MS.   Among those two in whom IgM-secreting cells predominated in peripheral blood, one had active and one stable MS.

Table 1.  Absolute Numbers of Plaque Forming Cells per 20 x 10³ Lymphocytes in Peripheral Blood and CSF from 37 Patients with Multiple Sclerosis (MS) and After Subgrouping in Active and Stable MS, and 20 Patients with Acute Aseptic Meningo-Encephalitis (AM) Examined within 10 Days After onset, and in Peripheral Blood only from 27 Healthy Controls.

| Diagnosis and numbers of subjects | | IgG | | IgA | | IgM | | Sum of IgG + IgA + IgM | |
|---|---|---|---|---|---|---|---|---|---|
| | | Blood | CSF | Blood | CSF | Blood | CSF | Blood | CSF |
| MS (n=37) | Range | 2-26 | 0-858 | 0-13 | 0-94 | 0-27 | 0-307 | 7-169 | 6-989 |
| | Median value | 10 | 89 | 25 | 10 | 4 | 4 | 38 | 108 |
| Active MS (n=13) | Range | 2-19 | 0-858 | 8-95 | 0-60 | 0-27 | 0-77 | 12-141 | 15-989 |
| | Median value | 8 | 89 | 17 | 5 | 4 | 4 | 30 | 108 |
| Stable MS (n=24) | Range | 2-26 | 0-520 | 0-135 | 0-94 | 0-23 | 0-307 | 7-169 | 6-553 |
| | Median value | 10 | 91 | 25 | 10 | 4 | 3.5 | 40.5 | 110 |
| AM (n=20) | Range | 0-319 | 0-716 | 7-295 | 0-596 | 4-99 | 0-326 | 21-482 | 4-1508 |
| | Median value | 31 | 16 | 83 | 29 | 17 | 9 | 129 | 74 |
| Healthy controls (n=27) | Range | 2-20 | n.d. | 4-42 | n.d. | 1-7 | n.d. | 8-68 | n.d. |
| | Median value | 7 | | 12 | | 2 | | 21 | |

n.d. = not done

Table 2. Distribution of Predominating Class Specificity of Ig-Producing Cells in Peripheral Blood and CSF From 37 Patients with Multiple Sclerosis (MS) and After Subgrouping in Active and Stable MS, and 20 Patients with Acute Aseptic Meningo-Encephalitis (AM) Examined Within 10 Days After Onset, and in Peripheral Blood Only From 27 Healthy Controls.

| Diagnosis and numbers of Subjects | IgG | | IgA | | IgM | | IgG = IgA | | IgG = IgM | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | CSF | Blood | CSF | Blood | CSF | Blood | CSF | Blood | CSF |
| MS (n=37) | 3 | 27 | 32 | 3 | 2 | 6 | 0 | 1 | 0 | 0 |
| Active MS (n=13) | 1 | 11 | 11 | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| Stable MS (n=24) | 2 | 16 | 21 | 3 | 1 | 4 | 0 | 1 | 0 | 0 |
| AM (n=20) | 3 | 8 | 15 | 9 | 2 | 2 | 0 | 0 | 0 | 1 |
| Healthy controls | 2 | n.d. | 24 | n.d. | 0 | n.d. | 1 | n.d. | 0 | n.d. |

n.d. = not done

A positive correlation was found among the 37 MS patients bet-
ween the numbers of IgM-producing cells in CSF and the CSF-IgM index.
No such correlations were found for IgG or IgA.  For peripheral
blood, positive correlations were registered between serum IgG con-
centrations and numbers of IgG-producing cells, and also between
serum IgA concentrations and numbers of IgA-producing cells, while no
correlation was found concerning IgM.

Altogether 28 of the 37 MS patients had in their CSF cells
producing Ig of a certain class when the corresponding CSF-Ig index
was normal (Table 3).  These data confirmed that enumeration of
Ig-PFC is a more sensitive variable of the intrathecal immune status
than determination of free Ig concentrations, even when presented as
corresponding CSF-Ig index.

In peripheral blood, our 37 patients with MS had significantly
higher numbers of IgG-, IgA- and also IgM-producing cells compared
with the 27 health controls.  These findings indicate that MS is also
characterized by a systemic B-lymphocyte hyperactivity which is not
obvious when free serum Ig concentrations are determined.

It was possible to subgroup the 37 patients with MS into 13 with
active disease, i.e. clinical relapse or progressive disease for more
than 6 months duration, and the remaining 24 patients with stable
disease defined as no symptoms occurring over an interval extending
from 3 months before and 1.5 months after time of testing.  No sig-

Table 3.  Relation Between Numbers of Ig-Producing
          Plaque-Forming Cells (PFC) per 20 x $10^3$ CSF
          Lymphocytes and Corresponding CSF-Ig Index in
          37 Patients with Multiple Sclerosis

|  | Normal | Increased |
|---|---|---|
| CSF-IgG index (n=37) | | |
| IgG PFC | | |
|    Normal ($\leqslant 0.7$) | 0 | 5 |
|    Increased ($> 0.7$) | 4 | 28 |
| | | |
| CSF-IgA index (n=31) | | |
| IgA PFC | | |
|    Normal ($\leqslant 0.6$) | 8 | 22 |
|    Increased ($> 0.6$) | 0 | 1 |
| | | |
| CSF-IgM index (n=35) | | |
| IgM PFC | | |
|    Normal ($\leqslant 0.06$) | 10 | 4 |
|    Increased ($> 0.06$) | 5 | 16 |

nificant differences for Ig-producing cells were found between the
two groups (Table 1).  All  those abnormalities encountered for the
whole material of 37 patients were also found in both groups, i.e.
increased numbers of IgG-producing cells and decreased numbers of
IgA-producing cells in CSF, and no significant difference for IgM-
producing cells in CSF compared to peripheral blood.

Acute aseptic meningo-encephalitis (AM) is a benign self-
limiting viral infection of the CNS which may be accompanied by
elevated concentrations in CSF of IgG, IgA and/or IgM (Frydén et al.,
1978) and, in about a third of the cases, by oligoclonal IgG bands in
CSF (Laterre et al., 1970; Link and Müller, 1971).  The mononuclear
pleocytosis encountered in this group of patients makes them suitable
as controls for studies of intrathecal immune reactions in MS.  We
have found that in acute AM, as a reflection of generalized immune
system stimulation, there is a predominance of Ig-producing cells in
peripheral blood compared with in CSF (Table 1), although the reverse
was found in individual patients (Henriksson et al., 1981);  Forsberg
and Kam-Hansen, 1983).  Positive correlations were observed in the
acute stage of AM between numbers of Ig-producing cells in CSF of all
three classes and the corresponding CSF-Ig index values, while no
positive correlations were observed between the numbers of IgG-, IgA-
or IgM-producing cells in peripheral blood and the corresponding
serum Ig concentrations.  Since bone marrow - but not the blood - is
the major source of the Igs in serum (Hijmans and Schuit, 1972), no
such correlations are to be found in healthy subjects, while in
paraproteinemias positive correlations occurred.  The positive corre-
lations found in peripheral blood from our MS patients for IgG and
IgA may reflect a situation comparable to a low-grade IgG and IgA
paraproteinemia.

Several of the patients with AM had elevated numbers of Ig-
producing cells in the CSF in the presence of normal values of the
corresponding CSF - Ig index.  These observations also suggest that
enumeration of Ig-producing cells in CSF is a more sensitive indi-
cator of the immune status intrathecally than measurement of the free
Igs.  The sensitivity  of the indirect haemolytic PFC assay is also
corroborated by the finding that a few patients with AM during con-
valescence had normal leucocyte counts per microlitre of CSF, while
at the same time considerable proportions of the CSF cells were still
active Ig producers.

In conclusion, our studies applying an indirect haemolytic PFC
assay to enumerate Ig- (antibody-) producing cells in CSF in MS have
confirmed the occurrence of intrathecal B-lymphocyte hyperactivity:
Ig-producing cells were regularly demonstrable in MS-CSF, often in
high numbers and mostly secreting IgG; a pronounced intrathecal IgM
response was found, reflected by presence of IgM-producing cells in
70% of our 37 MS patients and, in fact, a predominance of IgM-produc-
ing cells in 6 (16%) of these patients; finally, 28 of these 37

patients had in CSF cells producing Igs of G, A or M class in pre-
sence of normal values of corresponding CSF-Ig index, confirming that
enumeration of Ig-producing cells in CSF is a more sensitive indic-
ator of the intrathecal immune status than measurements of concen-
trations of free Igs.  Regarding peripheral blood, systemic B-
lymphocyte hyperactivity has been demonstrated in MS in the form of
increased numbers of Ig-producing cells in presence of normal serum
concentrations of free Igs.

Future perspectives opened by application of an indirect haemo-
lytic PFC assay in MS include definition of the humoral immune re-
sponse on the IgG subclass level in more detail.  There may be not
only an increase of IgG1 but also derangements in proportions between
IgG or various subclasses having aggressive and protective effects,
respectively.  Modification of the assay to allow enumeration of
cells in CSF and peripheral blood producing specific antibodies has
high priority.  Finally, it should be of interest to examine numbers
of Ig-producing cells in peripheral blood and CNS in relation to
concentrations of free Igs in r-EAE.  In this experimental counter-
part to MS, our data point to several similarities when B-lymphocyte
function is considered (Table 4).

IN VITRO SYNTHESIS OF IMMUNOGLOBULINS BY CEREBROSPINAL FLUID AND
PERIPHERAL BLOOD CELLS IN MULTIPLE SCLEROSIS AND CONTROLS

CSF mononuclear cells from patients with MS have been shown to
have the capacity to produce IgG and also IgA when cultured in vitro

Table 4.  Similarities of B-Lymphocyte Function Between Multiple
          Sclerosis and Chronic Relapsing Experimental Allergic
          Encephalomyelitis (r-EAE).

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1.  Elevated concentrations of IgG and IgM in CSF and CNS

2.  Elevated concentrations of serum IgG antibodies against myelin
    and myelin basic protein

3.  Oligoclonal IgG present in serum, CSF and CNS

4.  Patterns of Oligoclonal IgG differ in various regions of
    individual CNS

5.  Oligoclonal IgG does not contain significant amounts of
    antibodies against myelin, myelin basic protein or
    galactocerebroside

6.  The major portion of oligoclonal IgG in CSF and CNS has not been
    defined with regard to antibody specificity

7.  No relations between Ig levels or oligoclonal IgG, and
    exacerbations or remissions

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(Sandberg-Wollheim, 1974). The synthesized IgG had an oligoclonal distribution and showed the same electrophoretic pattern as the IgG in the CSF. Blood lymphocytes from the same patients were found to synthesize an IgG in vitro that showed a completely different electro-phoretic pattern. The question about occurrence of oligoclonal IgG bands in MS patients' serum has later been elaborated with more sensitive protein separation techniques, mainly isoelectric focusing on polyacrylamide gels. Using subsequent immunofixation with mono-specific antisera, Laurenzi et al., (1980) showed that no less than 74% of MS patients displayed oligoclonal IgG bands simultaneously in CSF and in serum. Even though the bands had identical migration properties in CSF as in serum, those in serum were less in number and also weaker. These observations were interpreted as a result of intrathecal IgG production and subsequent transudation to serum, or occurrence of the same lymphocyte clones producing identical Ig inside and outside the blood-brain barrier. Contradictory to these findings, Mehta et al., (1982) reported oligoclonal bands in about 50% of MS sera with patterns either only partially similar or com-pletely different from those seen in matching CSF. Whether this is a consequence of isolation of serum-IgG carried out by these authors prior to isoelectrofocusing or really reflects extrathecal B-cell hyperfunction remains to be settled. The question might be resolved by repeating the experiments of Sandberg-Wollheim (1974), but with the use of more sensitive IgG separation and detection techniques such as isoelectric focusing followed by double antiserum-biotin-avidin-peroxidase staining (Olsson et al., 1983) of separated oligo-clonal IgG.

At the time when Sandberg-Wollheim (1974) performed her studies, less sensitive methods were available for e.g. quantification of Igs and this may be a reason why IgM was not demonstrable in the culture supernatants. Furthermore, a minimum of $0.25 \times 10^6$ CSF-L and $10^7$ PBL were needed for each in vitro culture.

On the basis of our findings of elevated CSF - IgM index and of IgM- and IgA-producing cells in CSF in a majority of patients with MS, reflecting intrathecal production of these Igs, we analyzed culture supernatants obtained from CSF -L and PBL cultivated for 7 days without and in presence of pokeweed mitogen (PWM), for concen-trations of Igs belonging to the three main classes (Henriksson et al., manuscript in preparation). PWM is a mitogen which stimulates B-cells, but also T-cells. The procedure of lymphocyte culturing and determination of Ig concentrations in culture supernatants is sum-marized in Figure 5.

The supernatants from unstimulated cultures of PBL from 11 healthy controls revealed the highest values for IgM concentrations, followed by IgA and IgG (Table 5). Cultures in presence of PWM resulted in increases of concentrations of Ig of all three classes in the supernatants, being about 4-fold for IgM, 13-fold for IgA and
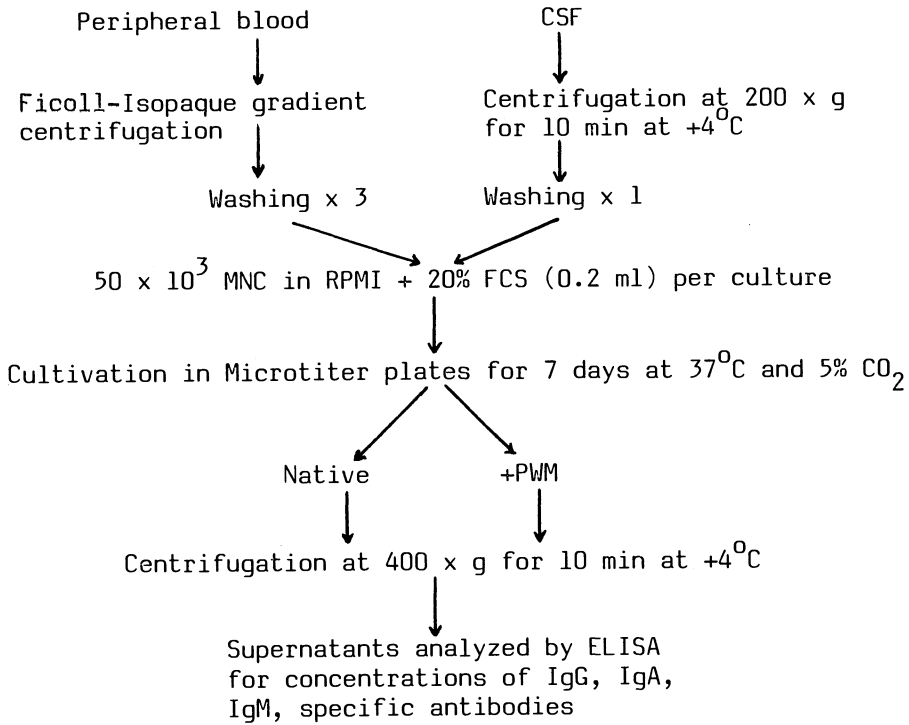
Peripheral blood                    CSF

Ficoll-Isopaque gradient            Centrifugation at 200 x g
centrifugation                      for 10 min at +4$^o$C

            Washing x 3             Washing x 1

50 x 10$^3$ MNC in RPMI + 20% FCS (0.2 ml) per culture

Cultivation in Microtiter plates for 7 days at 37$^o$C and 5% $CO_2$

            Native          +PWM

Centrifugation at 400 x g for 10 min at +4$^o$C

            Supernatants analyzed by ELISA
            for concentrations of IgG, IgA,
            IgM, specific antibodies

**Fig. 5.**  Scheme of procedure used for mononuclear cell (MNC)
            separation and cultivation, and determination of Ig con-
            centrations in culture supernatants.


33-fold for IgG.  The differences between values of PWM-stimulated
and unstimulated cultures can be considered to represent the net
potential of stimulated lymphocytes to produce Ig.  This difference
was highest for IgM, followed by IgA and IgG among the healthy
controls.

In 11 MS patients higher IgG concentrations were found in super-
natants from unstimulated PBL cultures when compared with the corres-
ponding values in healthy controls (Table 5).  For IgM and IgA, no
such differences were observed.  PWM induced significant increase of
concentrations of Ig belonging to all three classes, being about
15-fold for IgM, 13-fold for IgA and 9-fold for IgG.  The difference
between values of PWM-stimulated and unstimulated PBL cultures –
reflecting the net potential of stimulated lymphocytes to produce Ig
– was highest for IgM, followed by IgG and IgA (Table 5).

Goust et al., (1982), studying PBL only, have shown that PBL
from MS patients with active disease in 7 days cultures produced
significantly more IgG after stimulation with PWM in vitro than PBL

Table 5.  Median Values of Ig Concentrations in Supernatants from Unstimulated and PWM-Stimulated Cultures of Lymphocytes From Peripheral Blood and CSF from Patients with Multiple Sclerosis (MS) and Acute Aseptic Meningo-Encephalitis (AM), and from Peripheral Blood in Healthy Controls.

| Diagnosis and number of subjects | PWM | IgG | | IgA | | IgM | |
|---|---|---|---|---|---|---|---|
| | | Blood | CSF | Blood | CSF | Blood | CSF |
| MS (n=11) | - | 292 | 904 | 120 | 25 | 343 | 248 |
| | + | 2 672 | 743 | 1 567 | 31 | 5 283 | 48 |
| | Difference[1] | 2 004 | 9 | 1 307 | 5 | 4 316 | 4 |
| AM (n=11) | - | 163 | 85 | 84 | 20 | 517 | 51 |
| | + | 2 800 | 640 | 943 | 83 | 5 040 | 248 |
| | Difference[1] | 1 578 | 610 | 770 | 62 | 4 918 | 87 |
| Healthy subjects (n=11) | - | 40 | n.d. | 108 | n.d. | 333 | n.d. |
| | + | 1 326 | n.d. | 1 370 | n.d. | 1 454 | n.d. |
| | Difference[1] | 1 282 | | 1 343 | | 2 693 | |

[1]Difference means median value of (Ig concentration in supernatants from PWM-stimulated cultures - Ig concentration in corresponding supernatants from unstimulated cultures), equal to net potential of stimulated lymphocytes to produce Ig.
n.g. = nod done

from normal age-matched controls.  Our data are thus in agreement
with those of Goust et al., and represent an extension with regard to
IgA and IgM.

Supernatants of unstimulated CSF-L cultures from the MS patients
contained higher IgG concentrations in comparison with supernatants
of corresponding unstimulated PBL cultures.  Although much higher
concentrations of both IgA and IgM were found in supernatants from
unstimulated PBL than CSF-L suprnatants, illustrating the intensity
of intrathecal IgG response in MS.  As for PBL, IgM concentrations in
unstimulated CSF supernatants were higher than those of IgA.

In strong contrast to the situation for PBL, CSF-L from the MS
patients did not respond to PWM.  Thus, the concentrations in the
CSF-L supernatants of Ig belonging to all three classes remained
unaffected by PWM, which means that the strong IgG predominance from
unstimulated culture supernatants persisted.

Previously, low or absent proliferative response of MS-CSF-L to
PWM has been reported (Kam-Hansen et al., 1979).  Low or absent
responses of MS-CSF-L have also been obtained with two other mito-
gens, namely phytohaemagglutinin and concanavalin A.  One explanation
for this poor response to mitogens might be that the MS-CSF-L are
already maximally activated, according to one hitherto unproven
proposal because of viral infection of the lymphoid cells (Kam-
Hansen, 1980).

In 11 AM patients examined as controls, higher concentrations of
IgM and IgG were found in supernatants of unstimulated PBL when
compared with healthy subjects, and of IgM only when compared with MS
patients, while no such differences were registered for IgA.  PWM
induced increased concentrations of the Ig belonging to all three
classes in the PBL supernatants, being about 10-fold for IgM, 11-fold
for IgG and 17-fold for IgG.  THe difference between Ig concen-
trations in PWM-stimulated and unstimulated PBL cultures, reflecting
the net potential of stimulated lymphocytes to synthesize Ig, was
highest for IgM, followed by IgG and IgA, as in MS.  The values were
somewhat higher in MS for IgA and IgG.

Supernatants of unstimulated CSF lymphocytes displayed much
lower concentrations of the three Ig classes when compared with the
corresponding PBL.  This is in contrast to the situation in MS for
IgG which predominated markedly in CSF lymphocyte supernatants.  Even
the sum of concentrations of all three Igs in the CSF lymphocyte
supernatants of the AM patients was only about 20% of that in corres-
ponding PBL supernatants.  While the concentrations of IgM and IgA
were similar in unstimulated CSF lymphocyte supernatants of patients
with MS and AM, the IgG concentration was much lower in AM.

Significant increases of concentrations of the three Igs were

induced by PWM-stimulation of CSF lymphocytes in AM, being about 5-fold for IgM, 4-fold for IgA and 8-fold for IgG.  This is in sharp contrast to MS CSF-L where no significant increase in concentration could be demonstrated for any of the three Igs.  The difference between Ig concentrations in PWm-stimulated and unstimulated CSF lymphocyte cultures, i.e. the net potential of stimulated CSF lympho-cytes to produce Ig, was greatest for IgG, followed by IgM and IgA.

Summarizing these data from the studies on concentrations of IgG, IgA and IgM in lymphocyte culture supernatants, it has become clear that the IgG concentration in unstimulated MS CSF-L culture supernatants was about three times higher than in the corresponding PBL culture supernatants.  PWM induced no increase of IgG, nor of IgA or IgM in MS CSF-L supernatants, in contrast to the situation in AM. PBL from the MS patients responded to PWM as did PBL from healthy subjects and from patients with AM.  One explanation for the un-responsiveness of MS CSF-L might be that they are already maximally activated.  The results from the supernatant studies also confirmed that CSF mononuclear cells from most MS patients have the capacity to produce IgG as well as IgA and IgM, and that PBL are activated to a higher degree in MS than in healthy subjects regarding IgG production.


CONCLUDING REMARKS

MS is characterized by intrathecal activation of B-lymphocytes, the cause of which is still unsettled.  It might be the result of specific stimulation with a defined antigen, or a consequence of polyclonal triggering, or both.  The first alternative has not been excluded.  In vivo triggering of B-lymphocytes in MS patients, by as yet not defined polyclonal activators, might well explain the pro-duction of the IgG antibodies against different viruses and bacteria as well as of autoantibodies demonstrable in MS.  The proportion of these IgG antibodies in relation to the whole amount of intrathecally produced IgG is not known.  A possible T-cell defect with loss of negative immunoregulatory function in the form of deficiency of suppressor T-cells leading to a secondary B-lymphocyte hyperactivity has been in focus since the demonstration of reduction in number and function of suppressor T-cells in MS (Antel et al., 1979, Reinherz et al., 1980).

The recent observation that the humoral immune response in MS is not restricted to IgG, warrants more careful evaluation of the B-lymphocyte response than mere quantitation of free Igs.  Our results obtained with the indirect haemolytic PFC assay summarized in Table 6 have indeed demonstrated the occurrence in most MS patients of intra-thecal IgM- and IgA-production, and have also shown that systemic B-lymphocyte hyperactivity is a common finding in this disease.

Table 6.  B-cell Function in Multiple Sclerosis

| Variable | Blood IgG | Blood IgA | Blood IgM | CSF IgG | CSF IgA | CSF IgM |
|---|---|---|---|---|---|---|
| Conc. of free Ig | Normal | Normal | Normal | ↑↑ | ↑ | ↑↑ |
| Ig-producing cells | ↑ | ↑ | ↑ | ↑↑ | ↑ | ↑ |
| Ig conc. in lymphocyte culture super-natants | − ↑ | Normal | Normal | ↑↑ | ↓ | ↓ |
| PWM | + ↑ | Normal | ↑↑ | ↓↓ | ↓↓ | ↓↓ |

Analysis of IgG, IgA and IgM concentrations in supernatants from unstimulated lymphocyte cultures has confirmed that MS CSF-L have the capacity to produce Igs of these three classes, and IgG at a 3-fold higher rate than the corresponding PBL.  However, MS CSF-L lacked the capacity to respond to PWm with increased Ig production, in contrast to the corresponding PBL.  One explanation of the unresponsiveness of MS CSF-L might be that they are already maximally activated.

Future B-lymphocyte studies in MS employing inter alia similar techniques should concentrate on subclass definition of intrathecally produced IgG and of IgG antibodies, careful evaluation of the intrathecal IgM response with regard to antibody specificities, and characterization of antibodies in supernatants of CSF-L and corresponding PBL.

REFERENCES

Antel, J. P., Arnason, B. G. W., and Medof, M. E., 1979, Suppressor cell function in multiple sclerosis: correlation with clinical disease activity, Ann.Neurol., 5:338.

Brooks, B. R., Hirsch, R. L., and Coyle, P. K., 1983, Cellular and humoral immune responses in human cerebrospinal fluid, in: "Neurobiology of Cerebrospinal Fluid," Vol. 2, J. H. Wood, ed., Plenum Press, New York and London, pp. 263-329.

Cuzner, M. L., Davison, A. N., and Rudge, P., 1979, Preoteolytic enzyme activity of blood leukocytes and cerebrospinal fluid in multiple sclerosis, Ann.Neurol., 4:337.

Forsberg, P., and Kam-Hansen, S., 1983, Immunoglobulin-producing cells in blood and cerebrospinal fluid during the curse of aseptic meningoencephalitis, Scand.J.Immunol., 17:531.

Forsberg, P., henriksson, A., Link, H., and Ohman, S., 1983, Reference values for CSF-IgM, CSF-IgM/P-IgM ratio and IgM index, and its application on patients with multiple sclerosis and aseptic meningoencephalitis, Scand.J.Clin.Lab.Invest., (in press).

Frydén, A., Link, H., and Norrby, E., 1978, Cerebrospinal fluid and serum immunoglobulins and antibody titres in mumps meningitis and aseptic meningitis of other etiology, Infect.Immun., 21:851.

Goust, J.-M-, Hogan, E. L., and Arnaud, P., 1982, Abnormal regulation of IgG production in multiple sclerosis, Neurology, 32:228.

Henriksson, A., Kam-Hansen, S., and Andersson, R., 1981, Immunoglobulin-producing cells in CSF and blood from patients with multiple sclerosis and other inflammatory neurological diseases enumerated by protein-A plaque assay. J.Neuro immuno., 1:299.

Hijmans, W., and Schit, H. R. E., 1972, Immunofluorescene studies on immunoglobulins in the lymphoid cells of human peripheral blood, Clin.Exp.Immunol., 11:483.

Hood, L. E., Weissman, J., and Wood, W. B., 1978, "Immunology," The Benjamin/Cummings Publishing Co., Menlo Park, Ca., USA.

Kabat, E. A., Glusman, M., and Knaub, V., 1948, Quantitative estimation of the albumin and y-globulin in normal and patho- logical cerebrospinal fluid by immunochemical methods, Amer,J. Med., 4:653.

Kam-Hansen, S., Frydén, A., and Link, H., 1978, B and T cells in cerebrospinal fluid and blood in multiple sclerosis and acute mumps meningitis, Acta Neurol.Scand., 58:95.

Kam-Hansen, S., Link, H., Frydén, A., and Möller, E., 1979, Reduced in vitro response of CSF lymphocytes to mitogen stimulation in multiple sclerosis, Scand.J.Immunol., 10:161.

Kam-Hansen, S., 1980, Distribution and function of lymphocytes from the cerebrospinal fluid and blood in patients with multiple sclerosis, Acta Neurol.Scand., 62, Suppl. 62:1-81.

Laterre, E. C., Callewaert, A., Heremans, J. F., and Sfaello, Z., 1970, Electrophoretic morphology of gamma globulins in cerebro- spinal fluid of multiple sclerosis and other diseases of the nervous system, Neurology, 20:982.

Laurenzi, M. A., Mavra, M., Kam-Hansen, S., and Link, H., 1980, Oligoclonal IgG and free light chains in multiple sclerosis demonstrated by thin-layer polyacrylamide gel isoelectric focus- ing and immunofixation, Ann.Neurol., 8:241.

Link, H., and Zettervall, O., 1970, Multiple sclerosis: Disturbed kappa: lambda chain ratio of immunoglobulin G in cerebrospinal fluid, Clin.Exp.Immunol., 6:435.

Link, H., and Müller, R., 1971, Immunoglobulins in multiple sclerosis and infections of the nervous system, Arch.Neurol., 25:326.

Mehta, P. D., Mehta, S. P., and Patrick, B. A., 1982, Identification of light chain type bands in CSF and serum oligoclonal IgG from patients with multiple sclerosis, J.Neuroimmunol., 2:119.

Norrby, E., 1978, Viral antibodies in multiple sclerosis, Progr.Med.Virol., 24:1.

Olsson, T., Kostulas, V., and Link, H., 1983, Demonstration of
    oligoclonal IgG bands in unconcentrated CSF by agarose isoelec-
    tric focusing and antiserum-avidin-biotin-peroxidase labelling,
    J.Neurol.Neurosurg.Psych., submitted.
Palmer, D. L., and Minard, B. J., 1976, IgG subgroups in
    cerebrospinal fluid in multiple sclerosis, N.Engl.J.Med.,
    294:447.
Reinherz, E. L., Weiner, H. L., Hauser, S. L., Cohen, S. A. Distaso,
    J. A., and Schlossman, S. F., 1980, Loss of suppressor T cells
    in active multiple sclerosis.  Analysis with monoclonal anti-
    bodies, N.Engl.J.Med., 303:125.
Rinne, U. K., and Riekkinen, P. J., 1968, Esterase, peptidase and
    proteinase activities of human cerebrospinal fluid in multiple
    sclerosis, Acta.Neurol.Scand., 44:156.
Rostrōm, B., 1981, Specificity of antibodies in oligoclonal bands in
    patients with multiple sclerosis and cerebrovascular disease,
    Acta Neurol.Scand., 63, Suppl.86:1-84.
Salier, J. P., Goust, J. M., Pandey, J. P., and Fudenberg, H. H.,
    1981, Preferential synthesis of the Glm(1) allotype of IgGl in
    the central nervous system of multiple sclerosis patients,
    Science., 213:1400.
Salier, J. P., Goust, J. M., Link, H., Pandey, J. P., Daveau,
    Maryvonne, and Fudenberg, H. H., 1983, Latent immunoglobulin G
    (Gm) allotypes: occurrence in the cerebrospinal fluid in some
    neuropathological states, J. Immunogenetics., 10:311.
Salmi, A., Reunanen, M., Ilonen, J., and Panelius, M., 1983,
    Intrathecal antibody synthesis to virus antigens in multiple
    sclerosis, Clin.Exp.Immunol., 52:241.
Sandberg-Wollheim, M., 1974, Immunoglobulin synthesis in vitro by
    cerebrospinal fluid cells in patients with multiple sclerosis,
    Scand.J.Immunol., 3:717.
Sindic, C. J. M., Cambiaso, C. L., Depré, A., Laterre, E. C., and
    Masson, P. L., 1982, The concentrations of IgM in the cerebro-
    spinal fluid of neurological patients, J.Neurol.Sci., 55:339.
Strandberg-Pedersen, N., Kam-Hansen, S., Link, H., and Mavra, M.,
    1982, Specificity of immunoglobulins synthesized within the
    central nervous system in neurosyphilis, Acta.Path.Microbiol.
    Immunol.Scand.Sect.C., 90:97.
Tibbling, G., Link, H., and Ohman, S., 1977, Principles of albumin
    and IgG analysis in neurological disorders. I. Establishment of
    reference values, Scand.J.Clin.Lab.Invest., 37:385.
Trotter, J. L., and Brooks, B. J., 1980, Pathology of cerebrospinal
    fluid immunoglobulins, in: "Neurobiology of Cerebrospinal
    Fluid," Vol.1. J. H. Wood, ed., Plenum Press, New York and
    London, pp.465-486.
Walsh, M. J., and Tourtellotte, W. W., 1983, The cerebrospinal fluid
    in multiple sclerosis, in: "Multiple Sclerosis," J. F. Hallpike,
    C. W. M. Adams, and W. W. Tourtellotte, eds., Chapman and Hall,
    London, pp. 275-358.

Walsh, M. J., Tourtellotte, W. W., and Potvin, A. R., 1983, Central
    nervous system immunoglobulin synthesis in neurological disease,
    in: "Neurobiology of Cerebrospinal Fluid," Vol.2 J. H. Wood,
    ed., Plenum Press, New York and London, pp. 331-368.
Vartdal, F., Vandvik, B., and Norrby, E., 1980, Viral and bacterial
    antibody responses in multiple sclerosis, Ann.Neurol., 8:248.

# VARICELLA-ZOSTER VIRUS LATENCY IN THE NERVOUS SYSTEM

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## INTRODUCTION

The cause of multiple sclerosis (MS) is not known.  The main
hypotheses are that the disease is produced by a virus or is a virus-
triggered immunopathology.  Although evidence to support the theory
that MS is caused by a virus is only presumptive, there are four
compelling reasons to search for virus in MS tissue.  First, although
MS does not occur until adult life, epidemiologic studies suggest
that the causative agent is acquired in childhood.[1]  An exposure
period before or at the time of puberty followed by a long latent
period before the onset of the disease is known to occur with other
types of infection that lead to clinical CNS disease.[2]  Further-
more, a point-source epidemic of MS occurred on the Faroe Islands
from 1943-1960 during the British occupation;[3] thus MS on the
Faroes would appear to be transmissible and probably infectious.
Secondly, the cerebrospinal fluid (CSF) of patients with MS contains
oligoclonal bands and increased levels of immunoglobulin (Ig)G irres-
pective of the amount of total protein.  Only a few other diseases in
humans are associated with oligoclonal bands and increased CSF-IgG;
many are inflammatory and have also been shown to be infectious.[4]
Third, progressive multifocal leucoencephalopathy is a human demyeli-
nating disease in which a papovavirus produces a lytic infection of
oligodendrocytes.[5]  Fourth, numerous DNA and RNA viruses have been
shown to be capable of producing demyelinating disease in rodents
after experimental inoculation.[6]

Despite these findings, virus has not been detected in MS brain. There are no inclusion bodies nor have virus particles been detected by electron microscopy. Moreover, our examination of more than 20 MS brains by indirect immunofluorescence with multiple antiviral antisera has not revealed viral antigen. Growth of explanted MS brain cells in culture has not resulted in a viral cytopathic effect. Lysolecithin and polyethylene glycol-induced fusion of MS brain cells with a variety of indicator cells has also repeatedly failed to produce virus. Finally, analysis of MS brain cells experimentally infected with vesicular stomatitis virus did not result in the production of a virus pseudotype. Production of a non-neutralizing virus fraction would have suggested that a latent virus closely associated with cell membrane was present in the MS brain cells.

Because the classic methods to detect, isolate or rescue virus from MS tissue have failed, investigators are beginning to use the sensitive technique of nucleic acid hybridization to detect virus-specific gene sequences in tissue. Single-stranded nucleic acid "probes" that have been radioactively labelled to high specific activity are reacted in solution or with tissue (in situ hybridization) or in Southern blotting to detect complementary viral strands that will form double-stranded polynucleotides. To obtain probes that are "pure" (free of contaminating cellular or other DNA), the DNA fragments are cloned in plasmid or bacteriophage vectors.

This technique may have application in the search for virus genetic material in MS tissue. Probes prepared from a known virus or viral sequences van be prepared for hybridization with nucleic acid extracted from MS brain. The choice of virus probe should initially include each of those agents associated with the known human demyelinating disease, acute disseminated encephalomyelitis (ADEM). Thus measles, smallpox, rabies, influenza, mumps, varicellazoster virus (VZV), herpes simplex virus (HSV) and rubella are all candidates.

We have used nucleic acid hybridization techniques to detect VZV in human nervous system tissue. This virus is the causative agent of chickenpoz and shingles.[7] It is ubiquitious, acquired early in life and presumably becomes latest in dorsal root ganglia.[8] It is one of the most common causes of ADEM, particularly in the limited form of "cerebellar encephalitis" of childhood. Unless an individual has chickenpox or shingles just before death, it has not been possible to detect VZV in human ganglia. Tissue culture explant methods that have led to the successful isolation of HSV from human ganglia have not aided in the isolation of VZV from human dorsal root ganglia.[9]

The studies described below confirm for the first time that VZV is indeed latent in normal human dorsal root ganglia. Thus, there is a naturally occurring model system in humans with which to further study the biology of virus latency in the nervous system. As these

methodologies develop, probes for VZV genetic material in MS tissue
will also be made.


## METHODS

### Human Tissue, Virus and Cells

The trigeminal ganglion was obtained from a 53-year-old male who
drowned.  The sacral ganglion was removed from an 82-year-old male
who sustained myocardial infarction and developed sacral distribution
zoster immediately before death; VZV was isolated from his spinal
fluid.  Tissue was obtained 3 to 18 hours after death.  After nerve
roots were trimmed from the ganglia, they were washed three times
with Dulbecco's minimum essential medium and stored at -70°C until
DNA extraction.

The Web A (RIT) strain of VZV, originally isolated from the
vesicle of a patient with chickenpox, has been propagated in our
laboratory since 1977.[10]  VZV was serially passaged in the BSC-1
line of African green monkey kidney cells by transferring infected
cells into uninfected cultures at a ratio of 1:4.


### Preparation of DNA from Tissue, from Uninfected BSC-1
### Cells and from VZ Virions

The sacral ganglion was thoroughly Dounce-homogenized in 0.1 M
NaCl, 50mM EDTA and 10 mM Tris-HCl, pH 7.5 at 4°C.  The homogenized
tissue was made to contain 0.5% sodium dodecyl sulfate (SDS) and 0.5
mg/ml pronase (Calbiochem-Behring) and incubated for 3 hours at 37°C
with occasional agitation.  The DNA was then phenol chloroform-
extracted, ethanol-precipitated and resuspended in TE buffer (10 mM
Tris-HCl, pH 7.5, 1 mM EDTA).  The trigeminal ganglion was partially
Dounce-homogenized at 4°C and then digested with SDS-pronase for 18
to 24 hours at 37°C.  The tissue was then rehomogenized, incubated
for 3 hours at 37°C with an additional 250 µg/ml of pronase, and the
DNA was phenol chloroform-extracted, ethanol-precipitated and dis-
solved in TE buffer.  Uninfected BSC-1 cells were washed twice with
a solution of 0.2 M Tris-HCl, pH 7.5, 0.15 M NaCl, scraped into TE
buffer, and incubated for 3 hours at 37°C with SDS-pronase, phenol
chloroform-extracted, ethanol-precipitated and resuspended in TE
buffer.  VZ virions were prepared from infected BSC-1 cells according
to the method of Wroblewska et al.[11]  To extract viral DNA, the
virions were incubated for 3 hours at 37°C with SDS-pronase.  Viral
DNA was phenol chloroform-extracted, ethanol-precipitated and resus-
pended in TE buffer.  All extracted DNA, except that from virions,
was then incubated with pancreatic ribonuclease A (50 µg/ml) for 2
hours at 37°C, phenol chloroform-extracted, ethanol-precipitated and
dissolved in TE buffer.  The concentration of the DNA was determined

by measuring the absorbance at 260 nm (1 µg equal 0.02 $A_{260}$).
Approximately 300 µg of DNA was obtained from the trigeminal
ganglion, and 140 µg from the necrotic inflamed sacral ganglion.

## Preparation and Cloning of VZV-DNA

To clone VZV-DNA, viral DNA was extracted from VZV-infected
BSC-1 cells with 0.25% Triton X-100-0.2 M NaCl, and purified by
isopycnic centrifugation in CsCl as described by Gilden et al.[12]
Viral DNA was cloned in the plasmid pBR322 at the BamHI site, and the
plasmid was amplified and purified according to the methods described
by Maniatis et al.[13]

## Nick-translation of Cloned VZV-DNA

The cloned VZV-DNA was labelled with $\alpha$-$^{32}$P dATP and $\alpha$-$^{32}$P dCTP
(Amersham; 3000 Ci/mM) according to the nick-translation procedures
of Rigby[14] and Maniatis.[15]  The reaction mixture (50 µl) con-
tained 0.125 Ci of each labelled triphosphate, 200 ng DNA, nick-
translation buffer (50 mM Tris-HCl, pH 7.8 5 mM $MgCl_2$, 50 µg/ml
bovine serum albumin (BSA), 0.01 mM DL-dithiothreitol), 500 pg DNase
(Worthington), 1 mM dGTP, 1 mM dTTP, and 5 units of E. coli DNA
polymerase I (Boehringer-Mannheim).  Incubation was at 15°C for 90
minutes.  The reaction was terminated by the addition of 200 µl of
0.1 M NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.1% SDS containing
100 µg of sonicated salmon sperm DNA.  The solution was passed
through a Sephadex G-50 column, and the peak fractions containing the
radio-labelled DNA were pooled, ethanol-precipitated, centrifuged and
dissolved in 600 to 800 µl of TE buffer.  $^{32}$P-labelled DNA probes
with specific activities of 6-8 x $10^8$ cpm/µg of DNA were used for
DNA-DNA hybridization.

## Preparation of Blots

BamHI-cleaved DNA fragments were separated and transferred to
nitrocellulose filters according to the methods described by
Southern[16] and Wahl.[17]  Twenty to 40 units of the restriction
endonuclease BamHI were used to digest 10 to 20 µg of cell DNA in 50
mM NaCl, 7 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$ and 2 mM 2-mercapto-
ethanol.  After incubation for 2 hours at 37°C, an additional 10 to
20 units of enzyme were added and digestion was continued for 1 hour.
The reaction was stopped by the addition of 5 µl of a solution con-
taining bromophenol blue (0.005%), 30% sucrose and 50 mM EDTA.  DNA
fragments were separated in 0.6% agarose gels in Loening buffer (39
mM Tris-base, 34 mM sodium acetate, 2 mM EDTA, 0.18% acetic acid).
After electrophoresis, gels were stained with ethidium bromide.  Gels
were then blotted onto nitrocellulose paper according to the pro-
cedures described by Southern[16] and Wahl.[17]

## Hybridization

The hybridization technique was essentially that of Wahl et al.[17]  Nitrocellulose filters were pretreated at 50°C with 15 to 20 ml of 20% formamide, 0.1% SDS, 4X SSC (0.6 M NaCl, 60 mM sodium citrate), 5X Denhardt's solution (0.1% Ficoll, 0.1% polyvinyl-pyrolidine, 0.1% BSA), and 1 mM EDTA containing 250 μg/ml sonicated denatured salmon sperm DNA for approximately 18 hours.  Excess pre-hybridization solution was removed and hybridization was conducted at 50°C for 42 hours.  The hydridization solution consisted of 42% formamide, 10% dextran sulfate, 1X Denhardt's solution, 0.1% SDS, 5X SSC and 1 mM EDTA containing 100 μg/ml denatured sonicated salmon sperm DNA and heat-denatured probe ($1.2$-$1.6 \times 10^8$ cpm per filter). The filters were washed for 5 minutes at room temperature with 0.1X SSC and 0.1% SDS followed by four 15-minute washes with the same solution at 50°C and two 15-minute washes with 2X SSC and 0.1% SDS at 65°C.  Filters were dried, wrapped in Saran Wrap and autoradiographed using Kodak XAR-5 film in cassettes containing Dupont Cronex inten-sifying screens at -70°C for 3 hours to 7 days.

## RESULTS

A BamHI-cleaved VZV-DNA fragment was cloned into the plasmid vector pBR322.  The size of the VZV-DNA fragment is 9.7 kb, which corresponds to the C fragment produced by BamHI digestion of VZ virion DNA (Fig. 1) and maps at 0.16-0.25 map units.[18]  The cloned VZV-DNA fragment was labelled with $^{32}$P ($4$-$8 \times 10^8$ cpm/μg DNA) by nick-translation and hybridized to DNAs extracted from a single sacral ganglion removed from an 82-year-old male who had sacral distribution zoster immediately before death and from a normal trigeminal ganglion of a 53-year-old male who drowned.

Hybridization of the VZV-DNA fragment C to DNAs from sacral (lane 3) and trigeminal ganglia (lane 4) was observed.  However, no hybridization was detected when VZV-DNA fragment C was hybridized to cell DNA (lane 5) or to DNA extracted from herpes simplex virus (HSV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (not shown), thus indicating the presence of specific VZV-DNA sequences in human sensory ganglia.  Nick-translated $^{32}$P-labelled pBR322 also did not hybridize with DNA from the human ganglia.

## DISCUSSION

We have used DNA-DNA hybridization to demonstrate the presence of VZV-DNA sequences in human sensory ganglia.  The detection of VZV-DNA sequences in the sacral ganglion of a man with sacral distri-bution zoster immediately before death not only supports previous studies showing VZV antigen[19] and the isolation of VZV[20] from
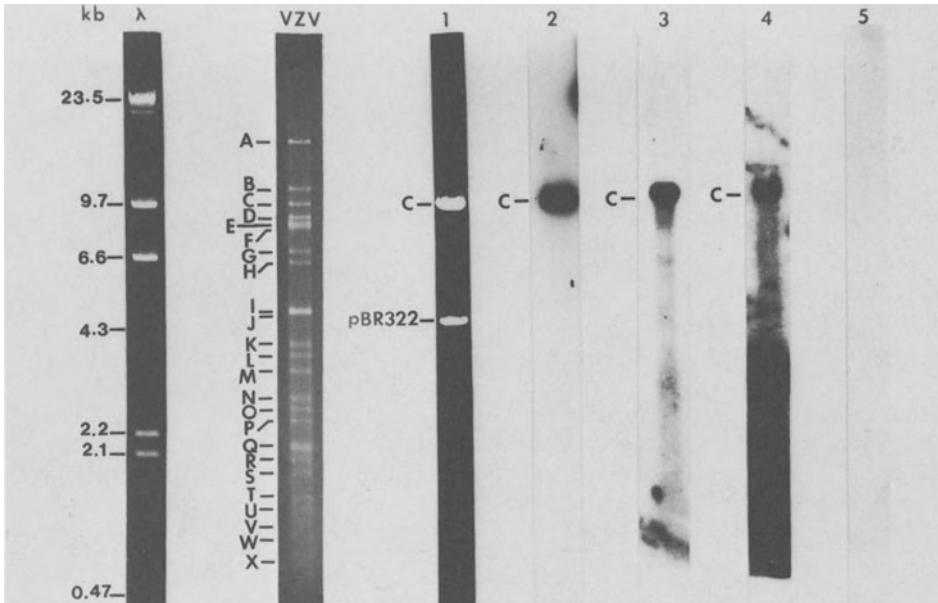
Fig. 1.  Detection of varicella-zoster virus (VZV) DNA sequences in
         human sensory ganglia.  VZV-DNA was cleaved with the BamHI
         restriction endonuclease and DNA fragments were cloned in
         the plasmid vector pBR322 as described in Methods.  VZV-DNA
         cloned fragment C (lane 1) was amplified in E. coli HB101
         and purified as described in Methods.  Viral DNA fragment C
         was then labelled with $^{32}$P and hybridized to BamHI-cleaved
         VZV-DNA (lane 2), sacral ganglion DNA (lane 3), trigeminal
         ganglion DNA (lane 4), and monkey kidney cell DNA (lane 5).
         Left lane shows the bacteriophage λ Hind III-cleaved DNA
         fragments used as a molecular size marker.  The VZV lane
         shows the pattern of DNA fragments produced by BamHI
         restriction endonuclease.  The bands marked C in lanes 3
         and 4 represent the presence of VZV-DNA sequences in human
         sensory ganglia.

acutely infected human ganglia, but also extends the techniques
available for detection of VZV in human tissue to the nucleic acid
level.  A common site for zoster development is on the face, and thus
the demonstration of VZV genetic material in a trigeminal ganglion is
not surprising.  More ganglia obtained from all levels of the human
neuraxis, including autonomic nervous system ganglia, must be tested
to determine the prevalence of VZV-DNA in ganglia.

    The studies described here indicate that pure well-characterized
probes prepared by recombinant DNA techniques can be used to identify
specific viral DNA sequences in normal nervous system tissue when the

classic virologic methods of co-cultivation and cell fusion have failed.  As more VZV-specific fragments become available, detection of viral sequences in the nervous system tissue will be enhanced. These probes will then be applied not only to further study the latent state of VZV in human ganglia, but also will be used to search for VZV sequences in MS tissue.

## Acknowledgements

## REFERENCES

1.  M. Alter, U. Leibowitz, and J. Speer, Risk of multiple sclerosis related to age at immigration to Israel, Arch.Neurol., 15:234-237 (1966).
2.  D. H. Gilden, Slow virus diseases of the CNS, Postgrad.Med., 73:99-118 (1983).
3.  J. F. Kurtzke and K. Hyllested, Multiple sclerosis in the Faroe Islands: I. Clinical and epidemiological features, Ann. Neurol., 5:6-21 (1978).
4.  J. R. Miller, A. M. Burke and C. T. Bever, Occurrence of oligoclonal bands in multiple sclerosis and other CNS diseases, Ann.Neurol., 13:53-58 (1982).
5.  L. P. Weiner, R. T. Johnson and R. M. Herndon, Viral infections and demyelinating diseases, New Eng.J.Med., 288:1103-1110 (1973).
6.  M. C. Dal Canto and S. G. Rabinowitz, Experimental models of virus-induced demyelination of the central nervous system, (Ann.Neurol., 11:109-127 (1981).
7.  B. D. Davis, R. Dulbecco, H. N. Eisen and H. S. Ginsberg, in: "Microbiology," Harper and Row, eds., Hagerstown, Maryland, 1068 (1980).
8.  R. E. Hope-Simpson, The nature of herpes zoster: a long-term study and a new hypothesis, Proc.Roy.Soc.Med., 58:9-20.
9.  S. A. Plotkin, S. Stein, M. Snyder and R. Immesoete, Attempts to recover varicella virus from ganglia, Ann.Neurol., 2:249 (1977).
10. D. H. Gilden, Z. Wroblewska, V. Kindt, K. G. Warren and J. S. Wolinsky, Varicella-zoster virus infection of human brain cells and ganglion cells in tissue culture, Arch.Virol., 56:105-117 (1978).

11. Z. Wroblewska, M. Devlin, K. Reilly, H. van Trieste, M. Wellish, and D. H. Gilden, The production of varicella zoster virus antiserum in laboratory animals, Arch.Virol., 74:233-238 (1982).

12. D. H. Gilden, Y. Shtram, A. Friedmann, M. Wellish, M. Devlin, A. Cohen, M. Fraser, and Y. Becker, Extraction of cell-associated varicella zoster virus with Triton X-100-NaCl, J.Virol.Meth., 4:263-276 (1982).

13. T. Maniatis, E. F. Fritsch and J. Sambrook, "Molecular cloning, A laboratory manual", Cold Spring Harbor Laboratory, 77-96 (1982).

14. P. W. J. Rigby, M. Dieckmann, C. Rhodes and P. Berg, Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I., J.Mol.Biol., 113:237-251 (1977).

15. T. Maniatis, A. Jeffrey and D. G. Kleid, Nucleotide sequence of the rightward operator of phage, Proc.Natl.Acad.Sci., 72:1184-1188 (1975).

16. E. M. Southern, Detection of specific sequences among DNA fragments separated by gel electrophoresis, J.Mol.Biol., 98:503-517 (1975).

17. G. M. Wahl, M. Stern and G. R. Stark, Efficient transfer of large DNA fragments from agarose gel to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate, Proc.Natl.Acad.Sci., 76:3683-3687 (1979).

18. J. R. Ecker and R. W. Hyman, Varicella zoster virus DNA exists as two isomers, Proc.Natl.Acad.Sci., 79:156-160 (1982).

19. M. M. Esiri and A. H. Tomlinson, Herpes zoster. Demonstration of virus in trigeminal nerve and ganglion by immunofluorescence and electron microscopy, J.Neurol.Sci., 15:35-48 (1972).

20. F. O. Bastian, A. S. Rabson, C. L. Yee and T. S. Tralka, Herpes-virus varicellae. Isolated from human dorsal ganglia, Arch.Pathol., 97:331-332 (1974).

DISCUSSION

Above all I would like to congratulate Dr. Gilden on his successful approach to viral pathology of the central nervous system (CNS) by application of the sophisticated nucleic acid hybridization technique. I feel that we can expect from this technique very important progress of knowledge in the near future.

With regard to the relevance of these results for etiopathogenesis of multiple sclerosis (MS), however, one has to say that the primary Varicella-Zoster-infections of CNS are very different in nature from MS. They are mostly monophasic diseases, only sometimes comparable to acute MS but never showing typical plaque-formation. They are also not linked to HLA-factors as MS. May I turn therefore with a few remarks to the problem of viral origin of MS and demyelination in general, as recently summarized by B. Waksman.

A viral infection could be responsible for MS in 3 ways:

1.    Direct infection and damage of oligodendrocytes would produce myelin breakdown and probably secondary inflammation.
2.    Antigens encoded by persistent virus genomic material might produce immunological response in the form of primary inflammation and secondary demyelination.
3.    A virus might induce an autoimmune response to some components of the oligodendroglia on the myelin sheath.

An animal model corresponding to the first possibility would be JHM virus and visna.  JHM mutants appear to infect oligodendrocytes and thus to produce demyelination with certain degree of inflammation.  An animal model for the second possibility is provided by Theiler's virus in mice.  In this model of persistence of viral genome there is continuous expression of viral antigens which is the prerequisite for production of an immunological disease.  With regard to the third possibility several new observations indicate that viruses infecting the CNS can induce autoimmunity against CNS (myelin) antigens.

If we turn to MS one has to state that a unique virus producing demyelination has not been isolated from neural tissue of MS-patients.  Apparent viral infections of oligodendrocytes as e.g. in PML are totally different in pathogenesis and pathomorphology.  Several reports of putative causal agents had been published which all appear non-specific.

By sensitive variants of the nucleid acid hybridization technique recently two common viruses had been demonstrated in CNS tissue.  Haase et al. found persistent measles virus genome in 6 or 12 MS-brains and 1 of 7 normal controls.  Frazier et al. demonstrated herpes simplex genome in 4 of 5 MS and 3 of 6 normals.  In some instances the complete viral genome was present, in others only part of the genome; both grey matter and white matter were involved.  So far no expression of antigens of measles or herpes has been found in MS-brains.  The fact that MS-patients present circulating antibodies against common viruses with frequently elevated titers appears to depend on the presence of HLA-A 3 or B 7-factors in the individual and not on the presence of MS.  On the other hand cell-mediated immunity against measles virus appears to be low or absent in MS-patients.  Responses of MS-patients to myelin or oligodendrocyte-antigens, in particular as an expression of the response to viral invasion of the CNS are not yet extensively studied.  The introduction of T-lymphocyte cloning techniques will allow a new and perhaps successful attack on the assessment of specific reactivities to viral and neural antigens.  De. Johnson recently in a study of measles cases of Lima has shown that myelin basic protein (MBP) appears early in the spinal fluid associated with pleocytosis as a direct evidence of tissue damage and that virus is present in the spinal fluid cells.

This may lead to rapid sensitization against neural antigens with lymphocytic reactivity in a high percentage of viral encephalitis cases. Therefore the hypothesis seems to be justified that auto-immunization as a consequence of early virus infections of the CNS in individuals with appropriate abnormalities of immune regulation may provide the basis of a chronic relapsing demyelinating disease.

# THE MYELIN ASSOCIATED GLYCOPROTEIN

# IN DEMYELINATING DISEASES

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## INTRODUCTION

The myelin associated glycoprotein (MAG) is an integral protein
of both central nervous system (CNS) and peripheral nervous system
(PNS) myelin.  Its molecular weight is approximately 100K Daltons
(Quarles, 1979; Figlewicz et al., 1981), and its concentration is
$2.7\mu g/mg$ protein in adult rat brain and $0.65\mu g/mg$ in sciatic nerve
(Johnson et al., 1982).  MAG is approximately one-third carbohydrate
by weight and contains fucose, mannose, galactose, N-acetylglucos-
amine and N-acetylneuraminic acid (Barbarash et al., 1981).

The localization of MAG, its structural arrangement within the
myelin sheath, and its function are currently under investigation.
Immunocytochemical studies with antibodies to MAG yield varying
results and partly depend on the antiserum, tissue and fixative used.
In the CNS, immunostaining of the periaxonal region (Sternberger et
al., 1979) or of compact myelin (Webster et al., 1983) is reported,
and in the PNS, immunostaining of periaxonal and paranodal regions
and of the Schmidt Lantermann clefts and outer mesaxon is observed
Trapp et al., 1982).  The mouse monoclonal antibody GEN-S8 immuno-
stains the periaxonal and outer regions of PNS myelin, whereas the
human anti-MAG M-proteins and the mouse monoclonal antibody anti-
HNK-1 (anti-Leu-7, Becton Dickenson) immunostain PNS myelin diffusely
(Abrams et al., 1982; Stefansson et al., 1983; Takatsu et al., sub-
mitted for publication; Nobile-Orazio et al., submitted for publi-
cation).  However, the M-proteins and anti-HNK-1 may react with
carbohydrate determinants in MAG which are shared by other PNS myelin
proteins and their immunostaining may not be specific for MAG
(Nobile-Orazio et al., submitted for publication).  It may be that
different determinants of MAG are differently oriented or exposed in

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CNS and PNS myelin and in different regions of the myelin sheath, or
that MAG is preferentially concentrated in certain myelin regions.
The function of MAG is unknown, but the antibodies that selectively
immunostain the periaxonal and outer regions of myelin may be recog-
nizing determinants which are bound and shielded in compacted myelin
but exposed on the unopposed periaxonal and outer myelin regions.
Alternatively, MAG may be involved in maintenance of the myelin-axon
junction (Sternberger et al., 1979; Trapp et al., 1982).

## MAG in Demyelinating Diseases of the Central Nervous System

In an immunocytochemical study of multiple sclerosis, there was
decreased immunostaining of MAG in white matter surrounding acute
lesions.  The same areas appeared normal when stained for myelin or
when immunostained for basic protein (Itoyama et al., 1980).  This
pattern of immunostaining was found to be specific for multiple
sclerosis and was not found in other demyelinating conditions in-
cluding in progressive multifocal leukoencephalopathy (Itoyama et
al., 1982), idiopathic polyneuritis (Schrober et al., 1981) and
experimental allergic encephalomyelitis (Itoyama et al., 1982).
These observations suggested that MAG may be affected early in demy-
elination and that it may be a primary target.

## MAG in Demyelinating Peripheral Neuropathy

In some patients with peripheral neuropathy there are IgM mono-
clonal antibodies or M-proteins that react with MAG (Latov, 1983;
Latov, et al., 1980; 1981; Braun et al., 1982; Mendell et al., 1982;
Steck et al., 1983; Stefansson et al., 1983; Saito et al., 1983;
Nobile-Orazio et al., 1983).  Morphological studies of affected sural
nerves show demyelination, widening of myelin lamellae, and depos-
ition of M-protein on the myelin sheaths (Nemni et al., 1983; Steck
et al., 1983; Stefansson et al., 1983; Takatsu et al., submitted for
publication).  Therapy directed at lowering the M-protein concen-
tration may be beneficial (Latov et al., 1980).  Studies using tissue
and species specificity, peptide mapping, deglycosylation, and com-
petitive binding assays, suggest that the M-proteins from different
patients all react with the same determinant of MAG, and that the
antigenic determinant contains carbohydrate residues (Shy et al.,
submitted for publication).

Patients with anti-MAG M-proteins suffer from neuropathy rather
than from CNS disease, but in two of four patients tested there were
abnormalities of the visual evoked responses suggestive of central
demyelination.  It is now known why the CNS is relatively spared but
the concentration of the M-proteins in brain may be kept low by the
blood-brain barrier, or in CNS myelin the reactive MAG determinant
may be shielded within the myelin sheath and binding of the M-protein
may be hindered.

In one patient studied, _in vitro_ production of anti-MAG M-protein was stimulated by OKT4+ T-helper cells and suppressed by OKT8+ suppressor T-cells (Latov et al., in preparation). The system may be analagous to a carrier/hapten system where the antigenic determinant is the hapten and the protein the carrier (Herzenberg et al., 1983). However, it is not known whether the carrier required for T-cell recognition is the MAG protein or another protein in PNS myelin or elsewhere sharing the antigenic determinant. This may be important as in some systems, the anti-hapten antibody response may be suppressed by repeated immunizations with the carrier protein alone (Herzenberg et al., 1983).

In preliminary experiments, passive transfer of serum from a patient with anti-MAG M-proteins induced focal demyelination in recipient cat sciatic nerve (Hays et al., 1983), but the mechanism of action or the relevance to the human disease is unclear. The immuno-regulatory mechanisms involved in elaboration of the M-protein, its role in the neuropathy, and the possible mechanism of action remain to be elucidated.

## MAG and Natural Killer Cells

It was recently reported that the mouse monoclonal antibody anti-HNK-1 (anti-Leu-7, Becton Dickenson) which reacts with some human natural killer (NK) cells, also binds to MAG (Heberman et al., 1979; Abo and Balch, 1981; Helfand et al., 1983). This could be important as immune reactivity to NK cells could secondarily involve myelin and cause demyelination, or since MAG is an integral myelin protein which binds to other myelin components, NK could also bind to these and cause demyelination. Binding of NK cells to myelin could occur in the absence of evidence for immune reactivity as tradition-ally demonstrated by specific antibody or proliferative response, but could be dependent on factors such as accessibility or alterations of the myelin membrane. NK cells could potentially also have a protec-tive effect as if infectious or immune agents were to bind to myelin _via_ receptors for MAG, NK cells could bind to these and prevent demyelination.

In recent experiments, no reactivity was detected between human peripheral blood lymphocytes and a human anti-MAG M-protein or three mouse monoclonal anti-MAG antibodies (Latov et al., submitted for publication). Also, in patients with anti-MAG M-proteins, the number of circulating HNK-1+ cells was the same as in controls. These suggest that the determinant which binds to anti-HNK-1 is different than that recognized by the human M-proteins, and that MAG and NK cells may have only the HNK-1+ determinant in common.

There is no evidence to date that NK cells or HNK-1+ lymphocytes are important in demyelination. The number of HNK-1+ cells in

patients with multiple sclerosis is the same as in normal subjects,
and studies of NK cell function in patients have yielded varying
results (Rice et al., 1983). However, lymphocytes cytotoxic to
oligodendroglia, myelin, or to cells modified with myelin antigens
have been described in multiple sclerosis (Berg and Kallen, 1964;
Eggers et al., 1981; Frick, 1982; Halpern et al., 1969; Hauw et al.,
1975), and NK cells may show considerable phenotypic and functional
heterogeneity (Hercend et al., 1983). The possible role of HNK-1+
cells in experimental demyelination and in demyelinating diseases of
the central and peripheral nervous system warrants further investi-
gation.

REFERENCES

Abo, T., and Balch, C. M., 1981, A differentiation antigen of human
        NK and K cells identified by a monoclonal antibody (HNK-1),
        J.Immunol., 127:1024-1029.
Abrams, G. M., Latov, N., Hays, A.P., Sherman, W. H., and Zimmerman,
        E. A., 1982, Immunocytochemical studies of human peripheral
        nerve with serum from patients with polyneuropathy and para-
        proteinemia, Neurology(NY), 32:821-826.
Barbarash, G. R., Figlewicz, D. A., and Quarles, R. H., 1981, Myelin
        associated glycoprotein: purification and partial characteriz-
        ation (abstract), Am.Soc.Neurochem., 12:165.
Berg, O., and Kallen, B., 1964, Effect of mononuclear blood cells
        from multiple sclerosis patients on neuroglia in tissue
        culture, J.Neuropath.Exptl.Neurol., 23:550-559.
Braun, P. E., Frail, D. E., and Latov, N., 1982, Myelin associated
        glycoprotein is the antigen for a monoclonal IgM in polyneuro-
        pathy, J.Neurochem., 39:1261-65.
Eggers, A. E., Tarmin, L., Plank, C. R., and Gamboa, E. T., 1981,
        Hyperactivity to myelin basic protein in multiple sclerosis,
        J.Neurol.Sci., 52:385-390.
Figlewicz, D. A., Quarles, R. H., Johnson, D., Barbarash, G. R., and
        Sternberger, N. H., 1981, Biochemical demonstration of the
        myelin associated glycoprotein in the peripheral nervous
        system, J.Neurochem., 37:749-758.
Frick, E., 1982, Cell mediated cytotoxicity by peripheral blood lym-
        phocytes against basic protein of myelin encephalitogenic
        peptide, cerebrosides, and gangliosides in multiple sclerosis,
        J.Neurol.Sci., 57:55-66.
Halpern, B., Bakouche, P., and Martial-Lasfargues, C., 1969, Destruc-
        tion des cellules nerveuses, cultivees in vitro par les lymph-
        ocytes de malades atteints de sclerose en plaques, Press.Med.,
        77:2103-2106.
Hauw, J. J., Berger B., and Escourolle, R., 1975, Etude de la cyto-
        toxicite lymphocytaire sanguine au cours de la sclerose en
        plaques, in: "Immunopathologie du Systeme Nerveus", M. E.
        Shuller, ed., Inserm, Paris.

Hays, A. P., Takatsu, M., Latov, N., and Sherman, W. H., 1983, Focal
    demyelination of cat sciatic nerve induced by intraneural
    injection of serum from patients with polyneuropathy and mono-
    clonal IgM reactive with myelin associated glycoprotein,
    (abstract), J.Neuropath.Exptl.Neurol., 42:349.
Helfand, S. T., McCarry, R., Eaton, L., and Roder, J. C., 1983, Human
    natural killer cells and myelin share a common antigen,
    (abstract), Neurology(NY), 33(suppl.2):107.
Herberman, R. B., DJeu, J. Y., Kay, D., Ortaldo, J. R., Riccardi, C.,
    Bonnard, G. D., Holden, H. T., Fagnani, R., Santoni, A., and
    Puccetti, P., 1979, Natural killer cells: characteristics and
    regulation of activity, Immunol.Rev., 44:43-70.
Hercend, T. Reinherz, E. L., Meuer, S., Schlossman, S. F., and Ritz,
    J., 1983, Phenotypic and functional heterogeneity of human
    cloned natural killer cell lines, Nature, 301:158-160.
Herzenberg, L. A., Tokuhisa, T., and Hayakawa, K., 1983, Epitope
    specific regulation, in: "Annual Review of Immunology, Vol.1",
    C. Garrison and H. Metzger, eds., Annual Review Inc., Ca.
Itoyama, Y., Sternberger, N. H., Webster, H. de F., Quarles, R. H.,
    Cohen, S. R., and Richardson, Jr., E. P., 1980, Immunocyto-
    chemical observations on the distribution of myelin-associated
    glycoprotein and myelin basic protein in multiple sclerosis
    lesions, Ann.Neurol., 7:167-177.
Itoyama, Y., and Webster, H. de F., 1982, Immunocytochemical study
    of myelin associated glycoprotein and basic protein in acute
    experimental allergic encephalomyelitis, J.Immunol., 3:351-
    364.
Itoyama, Y., Webster, H. de F., Sternberger, N. H., Richardson,
    E. P., Walker, D. L., Quarles, R. H., and Padgett, B. L.,
    1982, Distribution of papovavirus, myelin associated glyco-
    protein, and myelin basic protein in progressive multifocal
    leukoencephalopathy lesions, Ann.Neurol., 11:396-407.
Johnson, D., Quarles, R. H., and Brady, R. O., 1982, A radioimmuno-
    assay for the myelin associated glycoprotein, J.Neurochem.,
    39:1356-62.
Latov, N., 1983, Immunological abnormalities associated with chronic
    peripheral neuropathies: plasma cell dyscrasia and neuropathy,
    in: "First International Congress on Neuroimmunology", F.
    Spreafico and P. O. Behan, eds., Raven Press, N.Y. (in press).
Latov, N., Braun, P. E., Gross, R. B., Sherman, W. H., and Chess, L.,
    1981, Plasma cell dyscrasia and peripheral neuropathy;
    identification of the myelin antigens that react with human
    paraproteins, Proc.Natl.Acad.Sci.(U.S.A.), 78:7139-7142.
Latov, N., Godfrey, M., Nobile-Orazio, E., Messito, M. J., Perman,
    G., Abrams, J., Freddo, L., and Chess, L., Shared antigenicity
    between natural killer cells and the myelin associated glyco-
    protein, (submitted for publication).
Latov, N., Godfrey, M., Thomas, Y., Nobile-Orazio, E., Freddo, L.,
    Perman, G., Abrams, J., and Chess, L., In vitro production of
    anti-MAG M-proteins; regulation by T-cells, (in preparation).

Latov, N., Sherman, W. H., Nemni, R., Galassi, G., Shyong, J. S.,
    Penn, A. S., Chess, L., Olarte, M. R., Rowland, L. P., and
    Osserman, E. F., 1980, Plasma cell dyscrasia and peripheral
    neuropathy with a monoclonal antibody to peripheral nerve
    myelin, N.Engl.J.Med., 303:618-621.
Mendell, J. R., Sahenk, Z., Whitaker, J. N., Pittman, G., 1982, Mono-
    clonal IgM and peripheral neuropathy: studies on passive
    transfer and antibody characterization, (abstract), Neurology
    (N.Y.), 32(2):A183.
Nemni, R., Galassi, G., Latov, N., Sherman, W. H., Olarte, M. R., and
    Hays, A. P., 1983, Polyneuropathy in nonmalignant IgM plasma
    cell dyscrasia: a morphological study, Ann.Neurol., 14:43-54.
Nobile-Orazio, E., Hays, A. P., Latov, N., Perman, G., Golier, J.,
    Shy, M., and Freddo, L., Reactivity of mouse and human mono-
    clonal anti-MAG antibodies; antigenic specificity and immuno-
    fluorescence studies, (submitted for publication).
Nobile-Orazio, E., Vietorisz, T., Messito, M. J., Sherman, W. H., and
    Latov, N., 1983, Anti-MAG IgM antibodies in patients with
    neuropathy and IgM M-proteins. Detection by ELISA, Neurology
    (Cleveland), 33:939-942.
Quarles, R. H., 1979, Glycoproteins in myelin and myelin related mem-
    branes, in: "Complex Carbohydrates of Nervous Tissue", R. V.
    Margolis and R. K. Margolis, eds., Plenum Press, New York.
Rice, G. P. A., Casali, P., Merigan, T. C., and Oldstone, M. B. A.,
    1983, Natural killer cell activity in patients with multiple
    sclerosis given interferon, Ann.Neurol., 14:333-338.
Saito, T., Sherman, W. H., and Latov, N., 1983, Specificity and idio-
    type of M-proteins that react with MAG, J.Immunol., 130:2496-
    99.
Schrober, R., Itoyama, Y., Sternberger, N. H., Trapp, B. D.,
    Richardson, E. P., Asbury, A. K., Quarles, R. H., and Webster,
    H. de F., 1981, Immunocytochemical study of Po glycoprotein,
    P1 and P2 basic proteins, and myelin associated glycoprotein
    in lesions of idiopathic polyneuritis, Neuropath.Applied
    Neurobiol., 7:421-34.
Shy, M., Vietorisz, T., Nobile-Orazio, E., Freddo, L., and Latov, N.,
    Specificity of human anti-MAG M-proteins; peptide mapping,
    deglycosylation, and competitive binding studies, (in prepar-
    ation).
Steck, A. J., Murray, N., Meier, C., Page N., and Perruisseau, G.,
    1983, Demyelinating neuropathy and monoclonal IgM antibody to
    myelin associated glycoprotein, Neurology(NY), 33:19-23.
Steffanson, K., Marton, L., Antel, J. P., Wollmann, R. L., Roos,
    R. P., Chejfec, G., and Arnason, B. G. W., 1983, Neuropathy
    accompanying IgM monoclonal gammopathy, Acta Neuropath.
    (Berlin), 59:255-261.
Sternberger, N. H., Quarles, R. H., Itoyama, Y., Webster, H. de F.,
    1979, Myelin associated glycoprotein demonstrated immunocyto-
    chemically in myelin and myelin forming cells of developing
    rat, Proc.Natl.Acad.Sci.(USA), 76:1510-1514.

Takatsu, M., Hays, A. P., Latov, N., Abrams, G. M., Sherman, W. H., Nemni, R., Nobile-Orazio, E., Saito, T., and Freddo, L., Immunofluorescence study of patients with neuropathy and IgM M-proteins, (submitted for publication).

Trapp, B. D., and Quarles, R. H., 1982, Presence of the myelin assoc-iated glycoprotein correlates with alterations in the period-icity of peripheral myelin, J.Cell.Biol., 92:877-882.

Webster, H. de F., Palkovits, C. G., Stower, G. L., Farilla, J. T., Frail, D. E., and Braun, P. E., 1983, Myelin associated glyco-protein: electron microscopic immunocytochemical localization in compact developing and adult central nervous system myelin, J.Neurochem., 41:1469-1479.

DISCUSSION

Dr. Latov's contribution appears of great general relevance in immunopathological conditions. Recent evidence indicates a defect in immune regulation in MS patients. This mainly involves a defect of suppressor cells and of Natural Killer (NK) cells. An explanation of this phenomenon recently proposed by Arnason (Acta Neuropath. Suppl. IX) is the presence of shared antigenic determinants between T-cell sub-populations and myelin/oligodendroglia. In a recent study we were able to show that the monoclonal Leu 7 (HNK 1) antibody, a marker which recognizes NK-cells and a fraction of suppressor cells, also detects an antigen present in human central and peripheral myelin, in oligodendrocytes and in low concentration also in other components of the CNS (Schuller, Petrovic, Gebhart, Lassmann, Kraft and Rumpold; Nature: in press). Immunochemical evidence indicates, that this Leu 7 antibody recognizes an epitop of the Myelin Associ-ated Glycoprotein (MAG) (Helfand et al.; Neurology 33. Suppl. II, p.107). Thus it appears reasonable that an immune reaction against this myelin protein may simultaneously induce a defect in suppressor and NK-cells and result in imbalance of immune regulation. This may be a very important aspect of MS pathogenesis.

CEREBROSPINAL FLUID IMMUNOELECTROPHORETIC

FINDINGS IN MULTIPLE SCLEROSIS

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INTRODUCTION

In 1962, Laterre et al.,[1] and later Dencker[2] reported the presence of a second precipitation arc, in a parallel position to the precipitate of immunoglobulin G (IgG), in cerebrospinal fluid (CSF) immunoelectrophoresis (IE) of some patients with multiple sclerosis (MS). This doubling of the CSF IgG precipitate was not demonstrated in the corresponding serum specimens. It was later indicated by Laterre et al.,[3] that the duplication of the CSF IgG precipitate in certain CSF specimens of MS patients is due to an imbalance in the CSF $\kappa/\lambda$ light chain ratio.

In 1970, Link and Zettervall[4] found an increase in the CSF $\kappa/\lambda$ light chain ratio in six out of 11 MS patients in whom oliglclonal gammopathy was demonstrated in CSF agar-gel electrophoresis. It was subsequently recognized that besides individuals with increased concentration of CSF $\kappa$ chains, a relatively smaller proportion of MS patients may show increased concentration of CSF $\lambda$ light chains[5].

In this communication, we will demonstrate and discuss the incidence of doubling in the CSF IgG precipitate observed on CSF immunoelectrophoretic examination in 5,203 consecutive patients with various neuropsychiatric disorders.

MATERIALS AND METHODS

In 5,203 consecutive patients hospitalized at the Indiana University Hospitals, the modified Scheidegger's[6] microimmunoelectro-

phoresis with simultaneous examinations of concentrated CSF and the
corresponding serum specimen were performed as previously des-
cribed[7]. Antisera to human serum and to Fab fragments of IgG (Fab
IgG) (Behring Diagnostics, Somerville, NJ; Meloy Laboratories,
Springfield, VA) were used. In addition, antisera to κ and λ light
chains were applied in 983 patients in whom MS, inflammatory or
lymphoproliferative central nervous system (CNS) afflictions were
suspected. Electroimmunodiffusion technique[8] for examinations of
CSF IgG and albumin concentrations was applied.


RESULTS

    The incidence of doubling in the CSF precipitates of Fab IgG
and/or IgG is presented in Table 1. Approximately 80% of patients
with the CSF immunoelectrophoretic abnormalities studied were indi-
viduals with MS. Inflammatory CNS afflictions were diagnosed in an
additional 10% in this series. Table 2 shows the incidence of elon-
gation in κ and λ light chains and of doubling in the precipitates of
Fab IgG and/or IgG in individuals in whom CSF IgG concentrations were
simultaneously determined.

    It is obvious that elongation in the precipitate of CSF κ and λ
chains, particularly of κ chains, may be demonstrated on routine CSF
examination more frequently than doubling in the CSF precipitate of
Fab IgG and/or IgG. Doubling in the precipitation arcs of Fab IgG
and/or IgG, in absence of demonstrated elongation in the CSF precipi-
tate of light chains, was associated with highest incidence of in-


Table 1.  Doubling of the Precipitation Arc of the Fab Fragments of
          IgG and/or IgG

|                                      | Number of Patients | Percentage |
|--------------------------------------|--------------------|------------|
| Multiple Sclerosis                   | 92                 | 77.9       |
| Multiple Sclerosis – Suspected       | 5                  | 4.2        |
| Meningoencephalitis                  | 3                  | 2.5        |
| Guillain-Barre' Syndrome             | 3                  | 2.5        |
| Subacute Sclerosing Panencephalitis  | 2                  | 1.6        |
| Cerebrovascular Disease              | 2                  | 1.6        |
| Hydrocephalus                        | 2                  | 1.6        |
| Neurosyphilis                        | 1                  | 0.8        |
| Intracranial Vasculitis              | 1                  | 0.8        |
| Lymphoma                             | 1                  | 0.8        |
| Inadequate Medical Documentation     | 6                  | 5.0        |
| TOTAL                                | 118                | 99.3       |

Table 2. Cerebrospinal Fluid

| Immunoelectrophoresis | Number of Patients | Patients with Increased IgG Concentration (over 14% of Total Proteins) | Patients with Increased Total Proteins Over 50 mg% |
|---|---|---|---|
| Elongation of the precipitate of: | | | |
| Kappa light chains | 120 | 79 (65.8%) | 42 (35.0%) |
| Kappa and Lambda light chains | 60 | 29 (48.3%) | 26 (43.3%) |
| The Kappa light chains and doubling in the precipitation arc of the Fab fragments of IgG and/or IgG | 28 | 19 (67.8%) | 11 (39.2%) |
| The Kappa and Lambda light chains and doubling in the precipitation arc of the Fab fragments of IgG and/or IgG | 8 | 6 (75.0%) | 4 (50.0%) |
| Doubling of the precipitate of the Fab Fragments of IgG and/or IgG | 13 | 12 (92.3%) | 4 (30.7%) |

creased concentration in CSF IgG (92.3%) and with lowest occurrence
of elevated CSF total proteins (30.7%). In over 20% of the patients
in our series, doubling in the CSF precipitate was demonstrated only
by using the antiserum to Fab fragments of IgG. Doubling in the CSF
IgG precipitate may be demonstrated in some MS patients (Figure 1, A
#1) in the absence of corresponding abnormalities in the precipitate
of CSF Fab IgG. The CSF IgG precipitate may show elongation reflec-
ting imbalance in the concentration of CSF light chains (Figure 1, A
#2). Doubling in the CSF precipitates of Fab IgG and/or IgG may also
be found in the absence of increased concentration of CSF gamma
glycoproteins (Figure 1, B #3) which are frequently seen in patients
with MS. In MS patients, the most frequent immunoelectrophoretic CSF
abnormality found[7] is elongation of the precipitate of κ light
chains (Figure 1, C #4). Less frequently, elongation of the CSF
precipitates of λ light chains (Figure 1, E #5) compared to the
simultaneously obtained precipitation arc of κ light chains (Figure
1, D) may be found. In some MS patients, the precipitate of light
chains may form a second arc in the anodal segment (Figure 1, F #6).


DISCUSSION

        In our CSF diagnostic laboratories, we have performed CSF
immunoelectrophoresis using antisera to human serum and to Fab frag-
ments of IgG in over 15,000 patients with various neuropsychiatric
disorders. As was shown on reviewing CSF findings in 5,203 consecu-
tive patients in our series, doubling in the CSF precipitates of CSF
Fab and/or IgG was most frequently established in individuals with
MS. We agree with the conclusion of Laterre et al.,[3] that the
second precipitation arc, parallel to IgG, reflects increased concen-
tration and/or imbalance in CSF light chains.

        The precipitate of κ or λ chains may be seen in the CSF immuno-
electropherogram as a completely separated, parallel arc. It may
also cross the IgG or essentially the γ heavy chain precipitation arc
producing a splitting and/or elongation in the anodal or cathodal
segment of the IgG precipitate. The λ chain precipitate shows a
tendency to be more noticeably elongated in the anodal direction as
compared to the precipitation arc of the κ chain. In our patients
with MS, we were unable to demonstrate the deconfiguration in IgG
and/or γ heavy chain precipitate that is seen in serum and CSF
immunoelectrophoresis in patients with malignant lymphomas. It
indicates presence of paraproteins which reflect qualitative changes
in the γ heavy chain.

        Optimal results of CSF immunoelectrophoresis depend greatly on
the amount of CSF protein obtained following concentration of the CSF
specimen. In patients with doubling in the CSF precipitates of Fab
IgG and/or IgG, the immunoelectrophoretic abnormality may be demon-
strated even in instances where there is suboptimal quantity of the
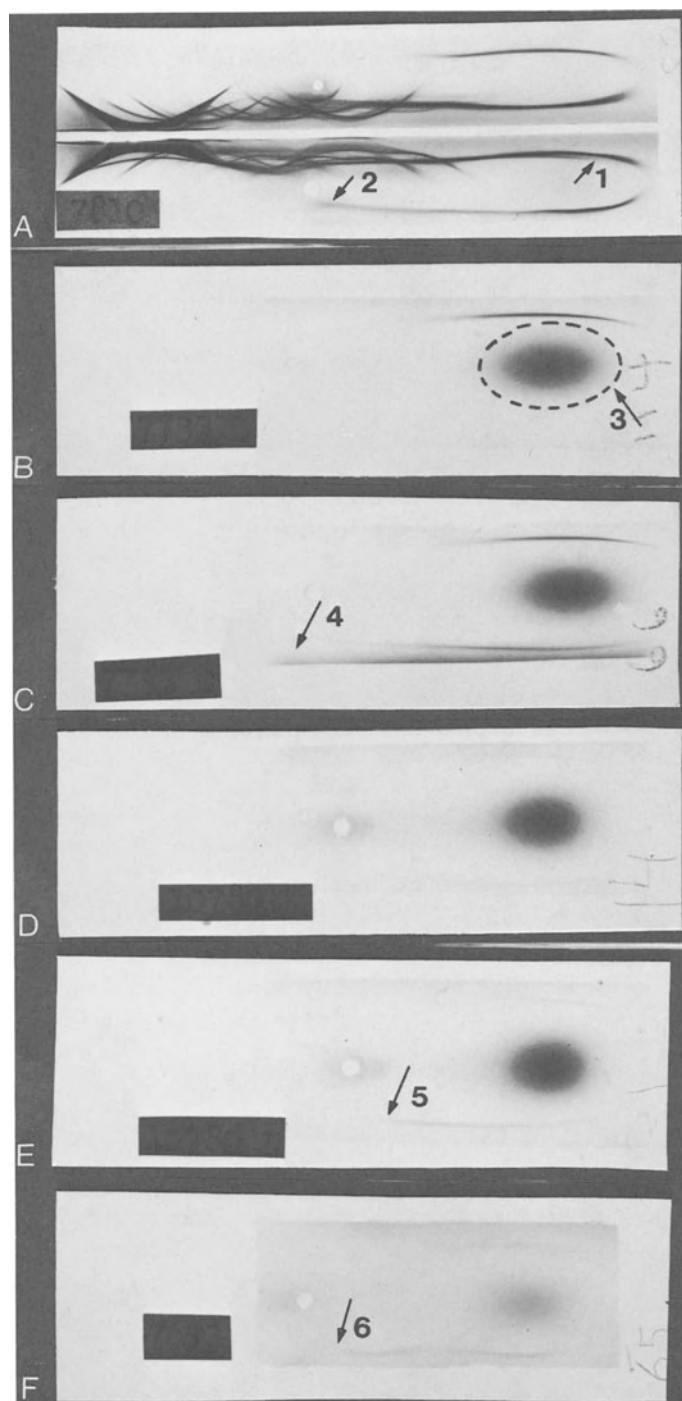CSF protein used for immunoelectrophoretic examination.

Fig. 1.   Cerebrospinal Fluid Immunoelectrophoretic Findings in
          Multiple Sclerosis.

As we reported previously[7] the most frequent CSF abnormalities found in our MS patients were reversed CSF γ/β globulin ratio ($\geq$ 1; 96.8%) and elongation in the precipitate of κ and/or λ light chains (95.7%). Doubling in the precipitate of Fab IgG and/or IgG was found only in 42.1% of the MS patients examined.

In our opinion, doubling in the CSF precipitate of Fab IgG and/or IgG and elongation in the precipitates of κ and/or λ light chains is an immunoelectrophoretic equivalent of oligoclonal gammopathy established on CSF protein electrophoresis.

As a routine CSF examination, CSF immunoelectrophoresis is particularly helpful in MS patients in whom the CSF protein electrophoresis reveals bands of questionable significance or in whom oligoclonal gammopathy is not demonstrated. In addition, CSF immunoelectrophoresis may reveal abnormalities suggestive of other pathologic mechanisms, as for example, alterations in the blood/CSF barrier structures and is therefore helpful in other differential diagnostic considerations.

## SUMMARY

On routine examination of CSF specimens in 5,203 consecutive patients with various neuropsychiatric afflictions, doubling in the precipitates of Fab fragments of IgG and/or IgG was established in CSF immunoelectrophoresis of 118 patients. In 92 instances (77.9%), definite, and in five (4.2%), probable or possible multiple sclerosis was diagnosed. Doubling in the precipitates of Fab fragments of IgG and/or IgG and elongation in the precipitates of κ and/or λ light chains seen on CSF immunoelectrophoretic examination is considered to be the equivalent of oligoclonal gammopathy. Immunoelectrophoretic abnormalities on CSF examination in MS patients may be established in individuals with only questionable or even absent bands in the CSF gamma globulin field and/or in subjects with normal CSF IgG concentration and/or normal CSF IgG/Albumin ratio.

## Acknowledgements

## REFERENCES

1.  E. C. Laterre, J. F. Heremans, and G. Demanet, La pathologie protéins du liquide céphalo-rachidien. Etude électrophorétique et immuno-électrophoretique (600 observations). Rev. Neurol., 107:500 (1962).

2.  S. J. Dencker, Immuno-electrophoretic investigation of cerebro-
    spinal fluid γ-globulins in multiple sclerosis, Act.Neurol.
    Scand., 40 (Suppl 10):57 (1964).
3.  E. C. Laterre, A. Callewaert, J. F. Heremans, and Z. Sfaello,
    Electrophoretic morphology of gamma globulins in cerebro-
    spinal fluid of multiple sclerosis and other diseases of the
    nervous system, Neurol.(Minneap.) 20:982 (1970).
4.  H. Link and O. Zettervall, Multiple sclerosis: disturbed kappa:
    lambda chain ratio of immunoglobulin G in cerebrospinal
    fluid, Clin.exp.Immunol., 6:435 (1970).
5.  O. Kolar and E. Anthony, Cerebrospinal fluid and serum light
    polypeptide chains in 160 patients with various nervous
    system disorders, Z.Neurol., 200:6 (1971).
6.  J. J. Scheidegger, Une micro-méthode de l'immunoélectrophorèse,
    Int.Arch.Allergy, 7:103 (1955).
7.  O. J. Kolar, P. H. Rice, F. H. Jones, R. J. Defalque, and J.
    Kincaid, Cerebrospinal fluid immunoelectrophoresis in
    multiple sclerosis, J.Neurol.Sci., 47:221 (1980).
8.  S. A. Schneck and H. H. Claman, CSF immunoglobulins in multiple
    sclerosis and other neurologic diseases, Arch.Neurol.(Chic.)
    20:132 (1969).

# MULTIPLE SCLEROSIS: AN ABIOTROPHY WITH

# HEURISTIC IMPLICATIONS

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"The normal process of acceptance of a scientific idea is
in four stages.
  (i)   this is worthless nonsense
 (ii)   this is an interesting, but perverse point of view
(iii)   this is true but quite unimportant
 (iv)   I have always said so."

                    J.B.S. Haldane, 1963, J.Genetics, 58:464

"Unless the lay public are taught to appreciate the poten-
tial significance of such symptoms as the temporary weak-
ness in a limb, transient dimness of vision, and diplopia
the latter part of Buzzard's (1897) criticism 'the full-
grown disease is frequently not recognized, the infant
disease practically never' must remain justified."

                    D.K. Adams et al., 1950, Brit.Med.J., ii:431

## INTRODUCTION

These words remain as cogent today as when penned; neither is it
widely appreciated that 0.5-1.0% of all MS patients are first
referred to a psychiatrist (personal communication from Dr. L.H.
Field). The existence of totally "silent" cases of MS is now well
established (Georgi, 1961; Vost et al., 1864; Ghatek et al., 1974;
Morariu and Klutzow, 1976; Castaigne et al., 1981). Georgi's series
is biggest and best known. He found amongst 15,644 autopsies carried
out at Basle Institute of Pathological Anatomy, 68 cases of anatom-
ically demonstrable MS, in 12 of which (18%) the disease had not been
at all suspected during life. Mackay and Hirano (1967) were of the

considered opinion that "perhaps clinically silent multiple sclerosis occurs in about one case for each four definitely diagnosed clinically".

Currently, 20% of cases are thus clinically undiagnosable, 2% being benign and 18% clinically silent and are lost to epidemiological and genetic studies - a problem which Myrianthopoulos (1970) discusses in depth.  Every neuropathologist of experience has examples of totally unexpected lesions indistinguishable from classical MS in his collection.

Subjects with "subclinical" MS, i.e. individuals in whom minor trivial discomfort such as transient pins and needles in an arm, heaviness in a leg - ?varicose veins, transient pain behind the eye with minor difficulty in reading print etc., who have never thought themselves to be in any way "ill", are common enough if sought and verified by laboratory testing.  But meralgia paraesthetica, somaesthetic migraine, unexplained neuritis, minor carpal and other "tunnel" syndromes may all closely mimic early MS as may, of course, "hysteria".  To anticipate:  there are many more MS subjects brought out by recently developed laboratory testing than present themselves clinically, just as there are many more sufferers from arthritic degenerative change of the knee joint than ever complain to their doctor.  It is here proposed that MS (like osteoarthritis) falls into the category of "Abiotrophic Disease" according to the concept associated with the name of Sir William Gowers (1902).  These diseases result from a poorly constituted tissue with insufficient "vitality" to last a full three score and ten years.  Consequently, it breaks down, apparently "spontaneously", earlier than do other better constituted tissues.  Such conditions would be expected to be primarily genetically determined, although secondary mechanisms may come into play in certain individuals once primary degeneration takes plane, e.g. an EAE mechanism in the case of MS.  Aggravating or precipitating factors for "spontaneous" myelin breakdown may be added, e.g. puerperium, stress, influenza, etc.  A genetic aetiology brings with it the possibility of establishing the biochemical basis of "lessworthy" nervous tissue (Curtius, 1933) and its rectification at a time when myelin is under active construction (between mid-fetal life and 5 years with great intensity and, thereafter, more slowly up to about 16 years) (Yakovlek and Lecours, 1967).

To enunciate clearly the thesis here presented:- Multiple Sclerosis belongs to the category of degenerative disease subsumed under the name of "abiotrophy".  Poorly constituted myelin breaks down in scattered regions of the nervous system well before the time of its customary life span.  All grades of poorly constituted myelin exist; minimal breakdowns will go unreported by ordinary people (and even by neuropathologists who look only for outstanding plaques).  This new concept develops from a consideration of the phenomenology of MS and new laboratory tests.  It brings new hope in the total management of the disease.

Pathogenesis of Multiple Sclerosis

In 1863 Rindfleisch observed that "in the most recently degener-
ated part of the white matter of the brain careful observation with
the naked eye showed a red dot or line (Strich), which corresponded
to a cross or obliquely cut engorged blood vessel".  The great
majority of studies have been made on the distribution of advanced
lesions by careful reconstruction of serial sections.  Plaques then
appear as "strings of pearls" strung out along small venules as they
converge to a trunk; or as "fingers" stretching along small veins.
Rindfleisch also noted that "the earliest basis (Grund) of the
disease is to be seen in changes in individual vessels and their
branches" - a close forerunner of the "perlschnurig" description to
follow decades later.  So clear and striking are Weigert-Pal stained
plaques with a central blood vessel, especially for teaching
purposes, that more and more beautiful photo-micrographs have been
reproduced in volume after volume devoted to the pathology of MS,
establishing the disease as primarily perivascular.  This view has
been reinforced by intensive study of EAE (an undoubted perivascular
disease) deriving especial impetus from well conceived and authori-
tative accounts by Waksman (1959) and others, and the wise caution
enjoined by Lumsden (1970) disregarded.  It must be said, at once,
that pathogenetically MS and EAE are quire different.  Of basic
significance is the absence of lymphocytes from the earliest stage of
demyelination in MS.  These cells play no part here in the initiation
of myelin breakdown.  Hassin (see below) and other careful observers
have not recorded participation of lymphocytes in earliest myelin
disintegration.

Mature plaques are "centered" on small veins.  The earliest
lesions are quite different.  There is in situ degeneration of
myelin; it simply disintegrates.  No doubt attaches to this.
Personal observation of the earliest changes in plaque formation
conforms with the vivid and accurate account given by Hassin (1922,
1937).  Hassin depicts (1922) (Figure 2) a longitudinal section of an
apparently healthy area stained by Bielchowsky's method and counter-
stained by the method of Alzheimer-Mann.  "The axons show no particu-
lar changes but the myelin sheath is tumified, forming a broad
swollen band around the well preserved axons.  In many places the
myelin appears fragmented, broken up into globules and droplets.
Under the oil immersion there can be observed, in the myelin, more or
less wide meshes giving the myelin a reticulated or fenestrated
appearance, as if it consists of numerous holes through which the
axons can be distinctly seen".  The distended meshwork of the myelin
may break down giving local cavities - the so-called "Lücken".  If
these early regions of changes are Marchi stained and counterstained
with Alzheimer-Mann, there are, in addition to the above changes,
numerous small black structureless droplets (Elzholz bodies) scat-
tered along the axon and intermingled with the fragments of broken
myelin.  The myelin fragments themselves may be laminated and may

stain brown (Marchi's globules). Hassin (loc. cit.) goes on to state
firmly that "swollen, fenestrated myelin, its fragmentation with
abundant formation of Elzholz bodies and Marchi globules in the
presence of a well preserved axon were found in all the thirteen
cases studied outside the patch proper in areas which, with ordinary
stains, appeared normal". There was no change in blood vessels
within these regions of early degeneration.

Hassin's description of the glial reaction is likewise clear and
accurate. Like others before him, he found "the myelin changes,
however mild, were always accompanied by marked proliferative changes
in the glial tissue which showed either increased fibers filling up
spaces between the parallel running swollen nerve fibers, or large
glial nuclei, rich in chromatin, invested by a visible, well devel-
oped membrane and abundantly supplied with cutoplasm". Hassin echoes
Anton and Wohlwill's (1912) observation that no significant
(nennenswerte) demyelination takes place without simultaneous, or
even prior, activation of the astroglia.

Detailed and accurate descriptions by German neuropathologists
bear out Hassin's description. Thus, Peters (1958) writes (p. 537),
"Swelling and enlargement (Quellung und Schwellung) of myelin sheaths
is for the most part (meist) the earliest visible sign of degener-
ation". Because of this swelling "the sheath develops a pale mesh-
like appearance". He quotes serial section work carried out by
Falkiewicz at the behest of Marburg, which showed clearly that the
shape and extent of a lesion was not dependent upon blood supply
and points out that clear vascular changes (ausgesprochene
Gefässveränderungen) are notably absent in very fresh lesions.
Figure 20 of Peters' account corresponds precisely with the author's
own preparations of very early lesions and with the description given
by Hassin.

As not uncommonly in the literature, some of the fundamental and
penetrating observations on the early pathogenesis of MS lesions go
back to Charcot (1872-73, pp. 187-190). He begins by pointing out
that not all the details of the origin of lesions are easily made out
in tissue fixed in chromic acid. Indeed, fresh material is advan-
tageous in some respects in that it allows of observations which
would certainly pass unnoticed in hardened material. Thus, the
former reveals the virtually constant existence of "globules et
granulations, d'apparence graisseuse ou médullaire" in considerable
numbers and these rapidly disappear when the preparation is allowed
to stand for some time in chromic acid. Such fatty globules can
sometimes be seen still attached to an axis cylinder already par-
tially denuded of myelin. Charcot goes on (p. 190) to draw the
distinction, which need not detain us here, between agglomerations of
fatty globules (corps granuleux) without nuclei visible (Fettkornchen
Agglomerate) and others with nuclei and cell membrane (Fettkornchen
Zellen).

The very well known and commonly reproduced illustration, Figure 10 (p. 191) of Charcot's (Figure 3 in DeJong, 1970, where is is, incidently, not altogether accurately labelled) illustrates the Virchow-Robin's space of a blood vessel distended by "goutelettes graisseuses volumineuses" and also "goutelettes graisseuses, groupés en petits amas disseminées, ça et là dans la preparation, en dehors des vaisseaux". Here we come close to Hassin's description. It is of interest to note that more than 100 years later Mackenzie and Wilson (1966) were also able to demonstrate free fatty globules in the brains of scrapie mice when fresh unfixed material, immediately after death, was embedded in 20% coloured gelatin broth and sections cut at once, on a cryostat. Such fat globules are not to be found in fixed material. These observations were readily confirmed by the author both in mouse and natural scrapie of sheep.

No better summary of the true state of affairs relating to the pathogenesis of MS can be given than the mature summary made by Hassin (1937). "Patches or plaques are not the essential feature of multiple sclerosis but merely one of its phases, a terminal condition."

"The essential feature of multiple sclerosis is the diffuseness of the degenerative process, which in the early stages occurs as foci of demyelination."

"Blood vessels play no part of the genesis of the changes in multiple sclerosis, in which the nerve fibers are attacked primarily and directly, without the intermediation of blood vessels."

Recently an attempt has been made to involve small blood vessels, notably by those who believe that vessel blockage by fat emboli, leading to minute hemorrhages, is at the root of MS lesions. Upon this supposition is based the use of Hyperbaric Oxygen Therapy. There is no doubt that very occasionally minute hemorrhages do occur and may even be accompanied by iron deposits indicating their ante-mortem formation. But they are rare, and Peters writes, "The regular occurrence and importance of such hemorrhages is not established by the majority of workers who have engaged on the study of MS path-ology". He quotes Steiner as saying "Occasional (gelegentliche) hemorrhages should be regarded as without significance (Bedeutungslose) and are apparently associated with the terminal state of the patient". Dawson (1916), whose personal observations are a landmark in British neuropathology, has been quoted by James (1982; 1983) as assigning aetiopathogenetic significance to capillary hemorrhages. Acknowledging the frequence with which Siemerling and Raecke (1911; 1914) reported hemorrhages, Dawson sought them with especial care. He reported "in close relation to the engorgement of the blood vessels, both within and without small hemorrhages have been found. These show, however, no changes, and were looked upon as probably the result of respiratory difficulties before death. The

vessel walls showed no changes which would explain the hemorrhages,
nor were there any signs of inflammation around them" (p. 650); and
continues (loc. cit.) "it is difficult to account for the origin of
the sclerotic areas in such primary changes. <u>The absence of an
histological evidence of changes in the vessel walls associated with
thrombosis or hemorrhages is quite incompatible with a primary vascu-
lar lesion in this sense</u>". The same agonal explanation was noted
above by Steiner.

Glial cells, both astro and oligodendroglia, may act as macro-
phages and become converted into gitter cells (Ferraro and Davidoff,
1928; Field, 1957), as Hassin also observed.

It is impossible to leave the question of astroglial activation
in early MS changes without paying passing tribute to the outstand-
ing, but long forgotten, contributions made by Rossolimo (1897), Strä
huber (1903) and others to glial studies in MS. They also recognized
the "pre-malignant" state of astroglia near acute lesions as brought
forward once more, many years later by Field et al (1962); Field
(1967), which explains the occasional association of MS with glioma
where there seems to be a gradual transition of enlarged astrocytes
into malignant cells (e.g. Scherer, 1938).

Once local myelophages are formed from glial cells they very
soon migrate to the nearest (never very distant) small vein so that
one has to search diligently to find a region of early primary de-
generation without small blood vessels at its margin already
surrounded by even a few macrophages stuffed with Sudan IV staining
fat. In addition a few lymphocytes and an occasional plasma cell may
be present. As myelophages encompass the venule, the number of
lymphocytes increases to produce a "cuffed vessel" with numerous
myelophages, which gradually becomes incorporated within the degener-
ated areas. Eccentric, at first, the blood vessel becomes more
central as it is engulfed within the abiotrophic region. By the time
an established plaque is seen there is a "central" venule and this is
the teaching handed down.

The recent revival of interest in the "normal" tissue described
above leads to a reappraisal of the whole nature of MS. It is not
primarily a perivascular disease at all. The extensive, stimulating
and highly influential work by Waksman (1959), which sees exper-
imental allergic disease as motivated primarily by lymphocytes
"targeted" on the organ concerned, is misleading when applied to the
beginnings of human MS. For MS is not primarily analogous with EAE
(despite the statement to this effect which so often begins scien-
tific articles and applications for Research Grants). Once again it
should be emphasized that the earliest changes in MS are found in the
total absence of lymphocytes.

After this description of the genesis of a lesion in MS the
contrast with EAE may be briefly made.  In the latter, lesions are
initially perivascular, characterized by increased permeability
(Lampert and Carpenter, 1965; Field and Raine, 1966), followed by
appearance of mononuclear cells.  If these break out, together with
exuded plasma, into the parenchyma, then demyelinative changes may
occur there, though a picture of actual attack of a mononuclear cell
on myelin is very rarely seen (Field and Raine, 1966).  On the other
hand the myelotoxic factor in plasma is found also in ALS (66% of
cases) (Bornstein and Appel, 1965; Hughes and Field, 1967).

MS is on the other hand a generalized disease of the central
nervous system.  Little attention has been paid to the "apparently
normal" white matter between discrete lesions.  Field (1967) drew
attention to widespread changes far beyond the attention-rivetting
plaque, especially activation and enlargement of astroglia cells, but
in doing so he was very far from the first.  Already for Charcot
(1872-3) enlargement of the interstitial elements was "incontest-
ablement" the earliest change in MS and he was followed by Müller
(1904), Anton and Wohlwill (1912), Dawson (1916) and Jacob (1969).
Doubts were cast upon the genuineness of glial changes because of the
possibility of minute lesions in the apparent white matter when
chemical analysis reported differences in this seemingly normal
tissue.  Most recently, however, it has come to be realized that MS
is a global disease of the CNS with focal intensification apparently
around small veins and more modern methods have been brought to bear
on the subject.  Arstila et al. (1973) reported abnormally then
myelin and an increase of astrocyte lysosomes together with increased
lysosomal enzymatic activity in biopsies from cerebral white matter
outside plaque regions.  In well controlled work, Allen and her
colleagues (1981, 1983) have extended this work and have come to
endorse the writer's long expressed opinion that "categorization of
MS material into plaque, peri-plaque and normal white matter may be
arbitrary and may limit our thinking on the pathogenesis of the
disease".  It certainly has; and, indeed, continues to do so and
hinders possible progress in disease prophylaxis (see below).


## The Multiple Sclerosis Diathesis

Although the "symptomatology and course of MS as described by
Charcot (1886) is so complete that subsequent literature may be
considered largely as differentiating only regarding type, frequency
and quantitation" (Fog, 1977), Charcot did make one fundamental
omission in failing to note familial clustering of the disease,
perhaps on account of the relatively small number of cases he saw
altogether.  He was indeed aware of "la famille neuropathologique",
and did not fail to ask about other diseases of the nervous system in
the patient's family, recording the amusing incident in his "Lecons
du Mardi à la Salpêtrière" in which in reply to his question "Je

voudrais savoir de vous enfin, s'il n'y a pas eu dans votre famille quelques personnes malades du système nerveux?, he drew the response "Il y a eu des poètes" (Charcot, 1892, p. 392). In the same volume (p. 179) Charcot declared "le clinicien n'a entre ses mains qu'un épisode, s'il veut se borner à l'étude du malade lei-même et n'embrasse pas l'histoire de la famille entière!"

## LABORATORY DIAGNOSIS OF MULTIPLE SCLEROSIS

The E-UFA (Field et al., 1977) and PGE$_2$ (Field and Joyce, 1977) tests are least demanding. But obsessional attention to detail and scrupulous preparation of glassware without the use of any detergent are prerequisites of success. Plastic material of any sort (e.g. as a saline bottle or tubes or disposable pipettes) must be excluded. One "worker" attempting the E-UFA test told me he could not be bothered with cleaning glassware and was going to use plastic. Indeed he added detergent to his reactants! His negative results were published within a few months in a reputable English journal. Another decided to incubate RBA with LA for 30 minutes at 38°C and gave me (the not very surprising) news that the cells underwent lysis! Seaman et al. (1979a) in their confirmation of the E-UFA test gave reasons why others have failed. A very full account of the practical aspects of E-UFA testing has been recorded (Field, 1983).

### Theoretical Basis

Since the fundamental work of Burr and Burr (1929; 1930), Swank (1950; 1970) and Sinclair (1956) (see review by Holman, 1978), it has been recognized that certain polyunsaturated fatty acids (PUFA) are essential for proper development and function. The acids are of two groups, the ω-6 and ω-3, and simple explanations are given in the FAO

Table 1. Laboratory Tests for MS.

| | |
|---|---|
| 1. MEM-LAD | Field et al. 1974 |
|     confirmed double blind by | Jenssen et al. 1976 |
| 2. E-UFA | Field et al. 1977 |
|     confirmed double blind by | Bisaccia et al. 1977 |
|     "    "    "    " | Seaman et al. 1979a |
|     "    "    "    " | Tamblyn et al. 1980 |
|     "    "    "    " | Jones et al. 1981 |
| 3. PGE$_2$ | Field and Joyce 1977 |
| 4. PL-EUFA* (Plasma-EUFA) | Field and Joyce 1982a |

* Developed from Seaman et al.'s (1979b) demonstration that electro-phoretic properties of RBC were conditioned by ambient plasma. Test admits of diagnosis within 30 minutes.

publication of the United Nations on "Dietary Fats and Oils in Human Nutrition" (Rome, 1977). Suffice it here to say that there are two ω-3 acids of considerable importance present in fish oils especially herring, mackerel and cod liver. They are:-

(i)  C22: 6 ω-3 (22 C atoms unbranched in a chain with 6 double bonds, the first beginning 3 C atoms from the $CH_3$ end of the molecule). Its "trivial" name is clupadonic acid.

(ii) C20: 5 ω-3 (20 C atoms in line with 5 double bonds, the first being 3 C atoms away from the terminal $CH_3$ group). Its "trivial" name is timnodonic acid.

The important acids for laboratory testing purposes are linoleic acid (LA) C18: 2 ω-6 (9.12 Octodecadienoic acid) and arachidonic acid (AA) C20: 4 ω-6 (5.8.11.14 Icosatetraenoic acid - usually mis-spelled "eicosatetraenoic" - Sinclair, 1982).

These acids, as components of phospholipids, are important constituents of cell membranes and if their mishandling were basic to the pathogenesis of MS, as suggested by Thompson (1966; 1973), they would make the membrane of all cells (both surface and internal) different from those in a non-MS subject. Bolton et al. (1968) showed that thrombocytes from an MS subject suspended in plasma showed different electrophoretic mobility from non-MS platelets. They have also been shown to have decreased linolenate content (Gul et al., 1970) as do MS-RBC (Homa et al., 1980).

Following publication by Millar et al. (1973) that supplementation of the diet with 60 ml per day sunflower seed oil (active principle LA) produced (in a double blind trial) a lesser number of exacerbations, of lesser degree and duration than occurred in a control group treated with placebo, further interest in LA was aroused. Although they do not explicitly say so, it appears that Millar et al. were working on the hypothesis of a simple replacement of linoleic acid in the blood in MS where they believed it to be low. In fact these original data have been challenged by Love et al. (1974) who found that reduced LA content of serum lipids was not specific to MS and occurred in all ill patients with acute non-neurologic disease.

Unsaturated fatty acids (UFA) might regulate the immuno-suppressive effect of corticosteroids (Turnell et al., 1973), increased linoleate enhancing immunosuppression. Early experiments with the MEM-LAD test (Mertin et al., 1973) showed that whilst oleic acid had no inhibitory effect upon lymphocyte-antigen reaction, both LA and AA and very marked effects. And so, since LA was easier to work with, the MEM-LAD test was born using the well marked difference in suppressive effect of LA on MS lymphocyte-antigen reaction as compared with non-MS lymphocytes. The test was later greatly

extended in scope (Field et al., 1974). In vitro, LA, AA and γ-linolenate all have well marked immunosuppressive effect on sensi-tized lymphocytes (Field and Shenton, 1975).

The claim by Ring et al. (1974) that LA suppresses in vivo immunological reaction (graft rejection in rats) was vitiated by the fact that LA was found to be irritative when injected intraperito-neally; their control group had been untreated (i.e. free from any treatment stress). Mertin (1974) found prolongation of skin graft in mice after subcutaneous injection of LA (though his controls received saline). Brock and Field (1975), however, found the subcutaneous injection of LA was highly toxic in mice; indeed, controls given oleic acid also died. "Graft survival was prolonged in the single mouse which survived LA treatment (16 days as compared with 11.2 ± 0.21 for control group)". Thus toxicity again could be the operative factor through ACTH release. Further experiments with gamma-linolenate (Naudicelle: Bio Oil Research Limited, England) showed that whilst this material was not toxic there was no prolongation of great survival time as compared with controls. This material did not produce a local granuloma as did LA and oleic acid. Brock and Field concluded that these grafting experiments provided no support for the hypothesis that PUFA are significantly involved in immunoregulatory mechanisms.

This point is being dealt with at some length because it is important in the possible mechanism of LA or γ-linolenate action in MS. Further evidence in the human is to hand.

(a)  Direct testing of the degree of reactivity of T lymphocytes to PPD by the MEM test was not reduced in subjects who had been taking γ-linolenate for 2 years (Field and Shenton, unpub-lished).

(b)  LA appeared to have a beneficial effect on suppression of a renal graft for up to six months; thereafter rejection did not differ significantly from the control group (McHugh et al., 1977).

We may conclude that whatever be the case in experimental guinea pigs or rats, PUFA have at most a short term cellular immunosuppress-ive activity in the human. This is an important conclusion respect-ing its mode of action, if any, in the management of MS. To antici-pate, the action of PUFA is believed to be on the structure of cell membranes themselves, via the plasma or tissue fluid in which the cells are living, and not through immunosuppression.

Results of the E-UFA Test (Field et al., 1977)

It must be emphasized that the E-UFA (and other) test was devel-oped for the immediate diagnosis of the young person who comes to his

medical attendant with a suspicious symptom or sign of MS and whose
condition has not yet been distorted by therapies such as ACTH,
prednisolone etc., etc.  A whole gamut of drugs (some of them
commonly regarded as innocuous, e.g. $FeSO_4$) have marked tendency to
adsorb to the surface of RBC, alter the charge, and so interfere with
the result of the E-UFA test.  Apart from those mentioned anti-
biotics, folic acid, very heavy smoking, furadantin, β-blockers (more
likely to be met with in elderly relatives of a proband), hygroton
(Geigy), prolonged amytal and no doubt many others will interfere, as
will sunflower seed oil (LA) and Naudicelle or Efamol (γ-linolenate).
Even the taking of soluble asprin within an hour or two of testing
can lead to a false result, especially if the more recent plasma
method is used.  The most careful enquiry must be made about
materials bought at Health Stores.  Patients, especially from outside
England, may come with whole lists of "drugs" they are taking (Field
and Joyce, 1981; Field, 1983).

When uncomplicated by drug therapy the E-UFA test shows:-

0.08 mg/ml LA or AA (Sigma London Chemical Company Limited, Poole,
England) reduces the electrophoretic mobility of well washed MS-RBC
(p < .001).  RBC from normal subjects or from OND (other destructive
neurological diseases) travel more rapidly in the presence of these
acids at this concentration.

    If 0.02 mg/ml LA or AA is used then these results are reversed:
MS-RBC travel more rapidly in the presence of 0.02 mg/ml acids,
whilst non-MS RBC travel slowly (p < .001).

    During the testing of the family of an MS proband "anomalous"
subjects will be encountered.  All MS mothers and the majority of
first born daughters are of this anomalous type.  They have not got
MS; on repetition of the test several years later the result has been
identical; none has developed any suggestion of MS over 6-7 years
observation period, but they may give rise to MS offspring (see
below).

## Results of the PGE$_2$ Test (Field and Joyce, 1977; 1979)

    These have been set out by Field (1980; 1983) and in greater
detail by Field and Joyce (1980).  The presence of 31.25 pg/ml $PGE_2$
(made up in medium 199, Hanks based; Gibco Europe, Scotland) causes
MS-RBC to travel more slowly than control (p < .001); non-MS cells
(including those of anomalous subjects) travel more rapidly.  At the
time of this discovery RBC-receptors for $PGE_2$ were not known but
recently they have been uncovered (Stengel and Hanoune, 1981).

    The $PGE_2$ test is simple and valuable in that if patients have
already taken γ-linolenate for about a year they will not give a

normal E-UFA test (Field and Joyce, 1978), but the PGE test remains positive until some 20-21 months later (Field and Joyce, 1983).

## Family Studies

Whilst as long ago as 1896 Eichorst had recorded MS in a 38 year old mother and 8 year old son, it was Mackay's series of papers and the excellent review of Moya (1962) leading up to the masterly presentation by Myrianthopoulos (1970) which really established the familial element in the disease (though there is a considerable number of papers in the intervening German literature).

Recent work with HLA haplotypes confirms the genetic element in MS though it does little more than tell us there is a diathesis. It may, however, lead to advances in mapping of human chromosomes.

Some 473 English families have been studied in as complete a manner as possible. No obvious Mendelian principles operate in the familial distribution of the disease. Reactivity to LA and AA when followed out in families shows that all mothers of the probands are "anomalous". All of 243 ("English" up to September 1983) mothers of MS probands exhibit such anomaly. (Four additional mothers were themselves suffering from clinical MS and gave a positive E-UFA test.) Here 0.08 mg/ml LA causes RBC to travel more slowly (p < .001) – though less so than in MS – whilst 0.08 mg/ml AA causes RBC to travel more rapidly (p < .001). Moreover, when such "anomalous" RBC are tested with 0.02 mg/ml LA and AA there is no simple reversal. With 0.02 mg/ml LA the RBC travel more slowly than control (absolute alcohol along added) (p < .001); 0.02 mg/ml LA gives the same effect as 0.08 mg/ml LA; but with 0.02 mg/ml AA there is reversal and the cells travel slowly (as with normals) (p < .001) (Field, 1980). More than 40% of near relatives, chiefly female, give "anomalous" results. The female descent of the anomally is mindful of mitochondrial inheritance (Fine, 1977; 1978). Both mitochondrial and cytoplasmic plasmic inheritance have not received consideration in MS.

Details of the families have been set out elsewhere (Joyce and Field, 1980; Field, 1980). The E-UFA positivity is found in about 1 in 50 of first degree relatives (parents, brothers, sisters) either as overt or as a "subclinical" or totally "silent" case. The "subclinical" are those with clear symptoms – occasional pins and needles in arms or legs; occasional heaviness in a leg; occasional vision blurring; great tiredness etc., none of which has taken them to their doctor. On casual enquiry, worded so as not to arouse suspicion or anxiety, about half admit to some of the minor symptoms or signs which they attribute to "sleeping on the wrong side in bed", etc. Truly "silent" cases make up the other half. These are people at risk but who may well go through life without any real trouble.

A full analysis of risk amongst relatives is currently under way

on clinical grounds, clinical + E-UFA test and relatives "said to have had" MS (based on corroboration).

To take some examples from "English" families only:-

Altogether 275 mothers (of English families) were examined of which 243 were "anomalous" (see above), 4 were clinical open MS, and 17 were "silent" or "subclinical" MS giving minor or suspicious symptoms or signs on oblique questioning; 11 more were "told" to us on good grounds making a total of 4 + 17 + 11 = 32.

Therefore, 32/275 = 11.64% of mothers of an MS proband were themselves MS.  If we assume 60 per 100,000 as the prevalence rate in England, then:-

$$\frac{100,000}{276} \times 32 \times 1/60 = \underline{194 \text{ x as common as in the general population.}}$$

There were in addition 4 cases where a silent MS sibling had a silent MS mother.

Turning to fathers of an MS proband:  altogether 114 fathers were available for study.  Of these 2 were clinical MS and 10 were subclinical.

Therefore, 2/114 = 10.53% of fathers of MS probands were themselves MS.  Taking into account those brought out by the E-UFA test we had 12 MS fathers.  Again assuming an English prevalence rate of 60 per 100,000 we have $\frac{100,000}{114} \times 12 \times 1/60 = \underline{175.4 \text{ x as common as in}}$ the general population.

Adding parents together, we get:-

32 + 12 = 44 MS out of 275 + 114 = 389 and 44/389 = 11.31% of parents of an MS proband are themselves MS.

Therefore, $\frac{100,000}{389} \times 44 \times 1/60 = \underline{188.5 \text{ x as common amongst}}$ parents of probands as in the general population (taken uncorrected) at 60 per 100,000.

Turning now to English siblings we have 99 brothers and 173 sisters available for study who were normal and 47 probands.

|                 | Brothers | Sisters |
|-----------------|:--------:|:-------:|
| Clinically MS   | 2        | 2       |
| "Silent" (E-UFA)| 18       | 21      |
| "Told"          | 2        | 2       |
|                 | 22       | 25      |

i.e. 47 MS out of 272 + 47 = 319

therefore 47/319 = 14.73% of siblings of an MS proband are themselves MS, so that:-

$\frac{100,000}{319}$ x 47 x 1/60 = 245.6 x as common as in the general population

(reckoned - uncorrected - as 60 per 100,000)

and $\frac{SIBLINGS}{PARENTS} = \frac{14.73}{11.31} = 1.3$

i.e. there are 1.3 times as many MS to be found amongst siblings of a proband as amongst the parents.

These figures illustrate how greatly the occurrence of MS is magnified when the E-UFA test is employed.

Percentage of families with more than one case in the family, i.e. a proband + at least one more case; we have on clinical ground alone 8/473 = 1.69%.

If we take those who are E-UFA positive then we have:-

Clinical + "silents" = 104/46 = 21.98%, i.e. percentage goes up by a factor of 13 if we use E-UFA.

If we include another 24 of which we were "told", then:- 128/473 = 27.06%.

Other workers who have not used E-UFA testing of all available family members will have used clinicals + "tolds", i.e. 8 + 24 = 32, i.e. 32/473 = 6.76% of MS families they will consider to have at least two in the family.

If Falconer's (1965) Figure 4 is used as a rough guide to hereditability then our figures suggest an 80-100% heritability in MS. However limited the usefulness of this estimate (which is much above that calculated by Berry (1969) from the figures of other workers) it does allow us to say with confidence that a high genetic element operates in the development of MS.

Several points emerge:-

(a) All mothers of MS patients are "red circles" (in so far as can be proved by "induction"), i.e. their RBC travel more slowly with 0.08 mg/ml LA and fast with 0.08 mg/ml AA.

(b) A high proportion of first daughters of an MS parent are also "red circles" and these, whilst never getting MS themselves, can actually produce MS offspring.

(c)  A normal female child can neither herself develop MS, nor become
     the mother of an MS offspring even if she has the misfortune to
     marry an MS husband.  Family testing does much to relieve
     scarcely concealed anxieties within MS families.

     Much of this work, with some elementary genetic counselling, has
been set out elsewhere (Field and Joyce, 1980).  Our limited experi-
ence with Italian families strongly suggests that Italy and Sicily
are high risk areas comparable with England; and about 40% of near
relatives are anomalous as in England (see below).


## Recurrent Acute Disseminated Encephalomyelitis

     This is relatively rare and great attention should be paid to
initial clinical history – drowsiness, fever, antecedent respiratory
or other infections and so on.  Such cases, when they are of the
(rare) recurrent type, can readily pass under the heading of MS.
However, they give a negative E-UFA test and their mothers are not
"red circles".

     Neurosarcoidosis, likewise, gives a negative result and the
mother, once again, is not a "red circle".  It must be remembered
that cases of Boeck's sarcoid may be confined to the CNS and give
general signs (reviewed by Matthews, 1979).  The way in which neuro-
sarcoidosis may imitate MS clinically is set out in great detail by
Zollinger (1941).


## DISCUSSION

## How widespread is MS?

     The E-UFA test not only enables diagnosis of MS to be made at
the first presenting symptom but uncovers an "MS diathesis" which in
the great majority of cases will eventuate in clinical manifes-
tations, though these may be very mild.  This diathesis is much more
frequent than clinical signs recognized.  The same phenomenon appears
to occur in all areas looked at, including Italy and Sicily.  There
are far more cases of MS brought to light than are supposed to occur
in this "low risk area".  In England the number of cases generally
set at about 50,000 is probably nearer 125,000.  The Vale of Trent
has been especially explored and is rife with MS.  Recently, indeed,
there have been epidemiological studies in Italy which put up the
prevalence and incidence rates on clinical grounds along.  Thus, Dean
et al. (1981) found "at least 32 per 100,000 in Agrigento city on the
south west coast of Sicily" but thought "this is likely to be a
considerable underestimate of the true prevalence".  Agrigento is
given as an example of the difficulty of studying MS in a rural city
with no specific interest in neurological problems and far from a

center.  We would agree that 32/100,000 is an underestimate and E-UFA
testing brought to bear upon the problem would assuredly at least
double the prevalence when relatives of probands (themselves to be
verified by testing!) are studied.  We disagree, however, that
absence of good neurological facilities make the recognition of MS so
much more difficult.  Epidemiologists must rid themselves of "poss-
ible" cases; there is MS, non-MS and an MS-like syndrome.  The appli-
cation of the E-UFA test to eliminate "possible" MS has recently been
outlined (Field and Joyce, 1982b).  The major practical difficulty is
transport of blood specimens or the setting up of a local testing
station.  If MS is of the nature of an abiotrophy then we would
expect many cases of MS to occur with small and unnoticed lesions
during life where disintegration had not gone beyond a few plaques
possibly located in non-critical areas.

All these "silent" and subclinical cases will be brought to
light by application of the E-UFA test under the proper conditions
(outlined above) and make a very considerable difference to the final
figure especially in the parent-proband and proband-sibling ratios,
and hence on the general frequency of the condition in the population
at large.


On the Nature of MS

If the basis of our tests be correct and there is indeed an
inborn mishandling of unsaturated fatty acids reflected in the
unusual phospholipid component of cell membrane, then it is highly
probable that this charge extends to that most membranous organ of
the body - the brain.  If the surface of the oligodendrocyte be
affected in the same way as the RBC, then myelin as a product in
large measure of the oligodendrocyte surface might also be expected
to be unusual (Field and Joyce, 1982b).  Here we enter into the
realms of (testable) hypothesis.  It may be supposed that the MS
modified oligodendrocyte membrane has different surface groupings
which do not exert the full and powerful electrostatic binding
between adjacent lamellae.  Consequently there is a tendency for
myelin lamellae to spring apart - like watch-spring leaves.  It may
be further supported that certain HLA groupings (known to be present
on glial cells, but apparently not on neurones) may also contribute
to lamellar-binding:  certain others may be weak, or entirely
deficient in this respect.  Those HLA haplotypes which are of the
latter nature may be just those found most frequently in MS.  The MS
inclined myelin would then be doubly at risk in that the lamellar-
binding forces would be deficient from two causes.  Thus we may
suggest that at root the cause of MS (i.e. the localized, isolated
regional breakdown or crumbling of myelin in situ which we call a
"plaque") is due to defective constitution and binding of myelin
lamellae.  MS myelin then is, in the great majority of cases, endowed
with inadequate "vitality" to hold out for a natural life span, and

we come to the notion of abiotrophy, introduced by Sir William Gowers
in 1902.  This postulates (in modern molecular biological language) a
tissue inadequately programmed - programmed, indeed, for early death.
Gowers - whose paper should be read by all interested in medicine and
not only neurology uses more homely (and equally enlightening) terms
- "lack of vitality" in the tissues.  MS according to this view comes
into the same category as osteoarthritis of the knee joint and is
probably just as common.  Certainly the range of affliction in the
latter varies from "normal wear and tear" at over 60 years through to
severe degeneration changes as early as 30 years.  Many people with
minor creaks and twinges in the knee never bother their doctor.  The
case is similar with MS.

All degrees of degeneration are to be found with little or no
correlation between plaque distribution and symptoms.  Many MS
sufferers never go to the doctor and those who do may be symptomati-
cally treated without the true nature of the complaint being recog-
nized.

There is, however, one respect in which osteoarthritis and MS
differ markedly.  It is possible to pick up the tell-tale marker of
MS soon after birth by the E-UFA test whilst the future osteo-
arthritic cannot be so diagnosed.  The analogy is closer to phenyl-
ketonuria, again a programmed disease but one in which the precise
biochemical defect is known, can readily be ascertained, and can be
rectified at once.

If the argument regarding MS has been followed, then it will
appear that once abnormal cell surface has been diagnosed, measures
should be taken to rectify it and return it, at least so far as
electrophoretic properties (and indeed biochemical constitution)
(Homa et al., 1981) are concerned, towards normal.  This can easily
be done by administering LA, or better, $\gamma$-linolenate, in the young
child as in the adult.  Clearly it is important that this rectifi-
cation be carried out as early as possible and certainly whilst
myelination is active, i.e. from mid-fetal life until the age of
about 16 years, although there is evidence (Jungawala and Dawson,
1971; Horrocks, 1973; Norton and Podulso, 1973; Dawson and Gould,
1976; Gould, 1977) that in rats some myelin constituents undergo
metabolism till relatively late in life.  Myelin cannot be considered
a discrete metabolic entity, but has many components, with different
turnover rates so that it may be possible to influence its compo-
sition well into adult life.

There appears to be a continual increase in the amount of myelin
in the brains of mice and rats throughout the animal's lifetime
(Horrocks, 1973).  Norton and Podulso (1973) have commented upon the
extraordinarily large amount of myelin produced by each oligodendro-
cyte.  For the rat they make the rough calculation that each
oligodendrocyte is making $175 \times 10^{-12}$ gm of myelin each day or about
threefold its own weight.

Although the older morphological studies of Yakovlev and Lecours (1967) suggested that active myelination in the human was going on until the age of 5 years and thereafter with diminishing intensity until about 16, other studies by Brante (1949), Tingey (1956) and others suggest that by 2 years of age 90% of the total lipid has been deposited and that by about 10 years adult values have been attained. However, the question of turnover or replacement of individual components remains an unsettled question and leaves open the possibility of replacement therapy.

## The EAE Mechanism in MS

Research should be directed to picking out individuals in whom an EAE mechanism has been superadded to the primary breakdown of myelin. If this can be demonstrated, e.g. by ratios of lymphocyte subspecies, then immunosuppression would be a rational treatment. However, it is clearly not initiated by brain trauma. Recurrent ADEM, too, is rationally treated with immunosuppressants.

We would appear to be at a turning point in our whole view of MS and an optimistic road lies ahead. But, as Sir Harold Himsworth has pointed out (1949), "The history of modern knowledge is concerned in no small degree with man's attempt to escape from his previous concepts". The view of MS to which we have been forced is, of course, by no means universally accepted; it is, however, to be hoped that for some thoughtful workers it has, at least, passed from the late Professor Haldane's stage (a) into stage (b).

APPENDIX

Amongst English families the "anomalous" E-UFA result is found in some 42% of near relatives of an MS index case. There is 100% occurrence amongst mothers, very low amongst fathers and other male relatives, but it is 66% amongst sisters, 63.8% amongst daughters and 33% amongst nieces.

Our Italian figures are much smaller, for geographic reasons, as soon from Table 2. This reinforces strongly our impression that MS is every bit as common in Italy (both north and south, including Sicily) as it is in England and makes systematic study of well defined areas more urgent. Clearly a rectification of prevalent ideas on geographic distribution must be important in solving MS, and we would hazard a suggestion that it has more to do with the wanderings of the Northmen and the distribution of their genes than with latitude.

Table 2. "Anomalous" Relatives (i.e. slow with LA, fast with AA)

|           | Number | "Anomalous" | %     |
|-----------|--------|-------------|-------|
| mothers   | 15     | 15          | 100   |
| fathers   | 7      | 0           | 0     |
| sons      | 17     | 1           | 5.8   |
| daughters | 12     | 8           | 66.7  |
| sisters   | 12     | 8           | 66.7  |
| brothers  | 8      | 3           | 37.5  |
| others    | 17     | 5           | 29.4  |
|           | 88     | 40          | 45.0% |

## Acknowledgements

## ADDENDUM

Myalgic encephalitis (ME) may on occasion have to be considered in the differential diagnosis of early MS. Since the above work was completed we have had the opportunity (through the courtesy of Dr. D.G. Smith, Medical Advisor to the ME Association) to study several classical examples of the disease. This has been made with a new (as yet unpublished laser method) which separates MS, OND and normal subjects. Those with ME fall into the OND category and can easily be set aside from MS.

## REFERENCES

Allen, I.V., 1983, Hydrolytic Enzymes in MS, in: "Progress in Neuro-
     pathology", Vol. 5, H.M. Zimmerman, ed., Raven Press, New
     York, 1-17.
Allen, I.V., Glover, G., and Anderson, R., 1981, Abnormalities in the
     microscopically normal white matter in cases of mild or spinal
     Multiple Sclerosis (MS)., Acta Neuropath.(Biol)., Suppl.
     IV:176-178.
Anton, G., and Wohwill, F., 1912, Multiple nicht eitrige Encephalo-
     myelitis und Multiple Sklerose., Z.ges.Neurol.Psychiat.,
     12:31-98.
Arstila, A. U., Riekkinen, P., Rinne, U. K., and Laitinen, L., 1973,
     Studies on the pathogenesis of multiple sclerosis: Partici-

pation of lysosomes on demyelination in the central nervous
system while matter outside plaques., Eur.Neurol., 9:1-20.

Berry, R. J., 1969, Genetical factors in the aetiology of Multiple
Sclerosis, Acta Neurol.Scand., 45:459-483.

Bisaccia, G., Caputo, D., and Zibetti, A., 1977, E-UFA test in
Multiple Sclerosis, Boll.Ist.sieroter Milan., 56:583-588.

Bolton, C. H., Hampton, J. R., and Phillipson, O. T., 1968, Platelet
behaviour and plasma phospholipids in MS., Lancet., 1:99-104.

Bornstein, M. B., and Appel, S. H., 1965, Tissue culture studies of
demyelination, Ann.NY Acad.Sci., 122:280-286.

Brante, G., 1949, Studies on lipids in the nervous system with
special reference to qualitative chemical determination and
topical distribution, Acta Physiol.Scand., (Suppl. 63),
Stockholm, 18:1-20.

Brock, J., and Field, E. J., 1975, Unsaturated Fatty Acids and
Transplantation, Lancet., 1:1382-1383.

Burr, G. O., and Burr, M. M., 1929, A new deficiency disease produced
by the rigid exclusion of fat from diet, J.Biol.Chem., 82:
345-367.

Burr, G. O., and Burr, M. M., 1930, On the nature and role of the
fatty acids essential in nutrition, J.Biol.Chem., 86:587-621.

Castaigne, P., Lhermitte, F., Escourolle, R., Huan, J. J., Gray, F.,
and Lyon-Caen, O., 1981, Les scléroses en plaques asympto-
matiques, Rev.Neurol.(Paris), 1937:729-739.

Charcot, J-M., 1872-1873, Lecons sur les maladies du système nerveux.
Bournville, ed., Delahaye, Paris, 187-190.

Charcot, J-M., 1886, Lecons sur les maladies du système nerveux, in:
"Oeuvres complètes de J-M Charcot", Tome 1, Bournville, ed.,
Paris, 158-163.

Charcot, J-M., 1892, Lecons du mardi à la Salpêtrière, Vol. 1,
Bataille, Paris, 392.

Curtius, F. C., 1933, "Multiple Sklerose und Erbanlage", Georh Thieme
Verlag, Leipzig, 215.

Dawson, J. W., 1916, The jistology of disseminated sclerosis,
Trans.Roy.Soc.Edin., 50:517-740.

Dawson, R. M. C., and Gould, R. M., 1976, Renewal of phospholipids in
the myelin sheath, Adv.Exp.Med.Biol., 72:95-113.

Dean, G., Savettieri, G., Tairi, G., Morreale, S., and Karhausen, L.,
1981, The prevalence of multiple sclerosis in Sicily, II
Agrigento City, J.Epidemiol.Commun.Health., 35:118-122.

DeJong, R. N., 1970, Multiple Sclerosis, History definition and
general consideration, in: "Handbook of Clinical Neurology",
P.J. Vinken and G.W. Bruyn, eds., North Holland Publishing
Co., Amsterdam and New York, 9:45-63.

Eichorst, H., 1896, Ueber infantile und hereditäre multiple Sklerose,
Archiv f.Pathol.Anat., 146:173-192.

Falconer, D. S., 1965, The inheritance of liability to certain
diseases, estimated from the incidence among relatives,
Ann.Hum.Genet.Lond., 29;51-76.

Ferraro, A., and Davidoff, L. M., 1928, The reaction of the
    oligodendroglia to injury of the human brain, Arch.Path.,
    6:1030-1063.
Field, E. J., 1957, Histogenesis of compound granular corpuscles in
    the mouse brain after trauma and a note on the influence of
    cortisone, J.Neuropath.Exp.Neurol., 16:48-56.
Field, E. J., 1967, The significance of astroglial hypertrophy in
    scrapie, kuru, multiple sclerosis and old age, together with a
    note on the possible nature of the scrapie agent, Dtsch Z.
    Nervenheilk., 192:265-274.
Field, E. J., 1980, "Multiple Sclerosis in Childhood: Treatment and
    Prophylaxis", C.C. Thomas, Springfield, Illinois.
Field, E. J., 1983, E-UFA Test, in: "Cell Separation Methods and
    Selected Application", Vol. 2, T.G. Pretlow II and T.P.
    Pretlow, eds., Academic Press, New York and London, 251-271.
Field, E. J., and Joyce, G., 1977, Prostaglandin (PGE$_2$) and human
    erythrocyte mobility: a specific test for multiple sclerosis?
    IRCS Med.Sci., 5:158-159.
Field, E. J., and Joyce, G., 1978, Effect of prolonged ingestion of
    gamma-linolenate by MS patients, Eur.Neurol., 17:67-76.
Field, E. J., and Joyce, G., 1979, Multiple Sclerosis: what can and
    cannot be done, Brit.Med.J., 2:1571-1572.
Field, E. J., and Joyce, G., 1980, Recent Methods of in vitro
    Diagnosis of Multiple Sclerosis, in: "Progress in Multiple
    Sclerosis Research", H.J. Bauer, S. Poser and G. Ritter, eds.,
    Springer-Verlag, Berlin and Heidelberg, 310-322.
Field, E.J., and Joyce, G., 1981, Simplified E-UFA test for Multiple
    Sclerosis (MS): Some sources of "false" results, J.Neurol.,
    226:149-155.
Field, E. J., and Joyce, G., 1982a, An Office Test for Multiple
    Sclerosis, IRCS Med.Sci., 10:560-561.
Field, E. J., and Joyce, G., 1982b, A laboratory test to achieve
    assured ascertainment of MS; frequency in epidemiological
    studies and clinical trials, in: "Multiple Sclerosis East and
    West", Y. Kuroiura and L.T. Kurland, eds., Kyushu University
    Press, Fukuoka, Japan, 265-272.
Field, E. J., and Joyce, G., 1983, Multiple Sclerosis: Effect of
    gamma-linolenate administration upon membranes and the need
    for extended clinical trials of unsaturated fatty acids,
    Eur.Neurol., 22:78-83.
Field, E. J., and Raine, C. S., 1966, Experimental allergic
    encephalomyelitis: an electron microscope study, Amer.J.Path.,
    49:537-553.
Field, E. J., and Shenton, B. K., 1975, Inhibitory effect of
    unsaturated fatty acids on lymphocyte-antigen interaction with
    special reference to Multiple Sclerosis, Acta Neurol.Scand.,
    52:299-309.
Field, E. J., Miller, H., and Russell, D. S., 1962, Observations on
    glial inclusion bodies in a case of acute disseminated
    sclerosis, Brit.J.Clin.Path., 15:278-284.

Field, E. J., Shenton, B. K., and Joyce, G., 1974, Specific
        laboratory testing for diagnosis of Multiple Sclerosis,
        Brit.Med.J., 1:412-414.
Field, E. J., Joyce, G., and Smith, B. M., 1977, Erythrocyte-UFA
        (E-UFA) mobility test for Multiple Sclerosis: Implications for
        pathogenesis and handling of the disease, J.Neurol., 214:
        113-127.
Fine, P. E. M., 1977, Analysis of family history data for evidence of
        non-Mendelian inheritance resulting from vertical trans-
        mission, J.Med.Genet., 14:399-407.
Fine, P. E. M., 1978, Mitochondrial inheritance and disease, Lancet,
        2:659-662.
Fog, T., 1977, Clues from clinical features, in: "Multiple Sclerosis:
        A Critical Conspectus", E.J. Field, ed., MTP Press Ltd,
        Lancaster, England, 13-33.
Georgi, W., 1961, Multiple Sklerose, Pathologischanatomische Befunde
        multipler Sklerose bei klinisch nicht diagnostizierten
        Krankheiten, Schweiz.Med.Wochenschr., 91:606-607.
Ghatak, N. R., Hirano, A., Litjmaer, H., and Zimmerman, H. M., 1974,
        Asymptomatic demyelinated plaque, Arch.Neur.(Chic)., 30:484-
        486.
Gould, R. M., 1977, Incorporation of glycoproteins into peripheral
        nerve myelin, J.Cell Biol., 75:306-338.
Gowers, W., 1902, A lecture on abiotrophy, Lancer, 1:1003-1007.
Gul, S., Smith, A. D., Thompson, R. H. S., Payling Wright, H., and
        Zilkha, K. J., 1970, The fatty acid composition of phospho-
        lipids from platelets and erythrocytes in Multiple Sclerosis,
        J.Neurol.Neurosurg. Psychiat., 33:506-510.
Hassin, G. B., 1922, Studies in the pathogenesis of Multiple
        Sclerosis, Arch.Neurol.Psychiat., 7:589-607.
Hassin, G. B., 1937, Pathologic features of multiple sclerosis and
        allied conditions, Arch.Neurol.Psychiat., 38:713-724.
Himsworth, H. P., 1949, The syndrome of diabetes mellitus and its
        causes, Lancet, 1:465-473.
Holman, R. T., 1978, How essential are essential fatty acids?
        J.Amer.Oil Chemists Soc., 55:774A-781A.
Homa, S. T., Belin, J., Smith, A. D., Monro, J. A., and Zilkha, K.
        J., 1980, Levels of linolenate and arachidonate in red blood
        cells of healthy individuals and patients with multiple
        sclerosis, J.Neurol.Neurosurg.Psychiat., 43:106-110.
Homa, S. T., Conroy, D. M., Belin, J., Smith, A. D., and Zilkha, K.
        J., 1981, Fatty acid patterns of red blood cell phospholipids
        in patients with multiple sclerosis, Lancet, 2:474.
Horrocks, L. A., 1973, Compounds and metabolism of myelin
        phosphoglycerides during maturation and ageing, Prog.Brain
        Res., 40:383-387.
Hughes, D., and Field, E. J., 1967, Myelotoxicity of serum and spinal
        fluid in MS: a critical assessment, Clin.Exp.Immunol., 2:295-
        309.

Jacob, H., 1969, Tissue process in MS and parainfections and post-
    vaccinal encephalomyelitis, Int.Arch.Allergy, 36:suppl. 22-36.
James, P. B., 1982, Evidence for subacute fat embolism as the cause
    of Multiple Sclerosis, Lancet, 1:380-386.
James, P. B., 1983, Oxygen for Multiple Sclerosis, Lancet, 2:632.
Jenssen, H. L., Meyer-Rienecker, H. J., Köhler, H., and Günther, J.
    K., 1976, The Linoleic Acid Depresseion (LAD) Test for
    Multiple Sclerosis using the Macrophage Electrophoretic
    Mobility (MEM) Test, Acta Neurol.Scand., 53:51-60.
Joyce, G., and Field, E. J., 1980, Further observations with the
    Erythrocyte-Unsaturated Fatty Acid Test. A contribution to
    the Genetics of Multiple Sclerosis, Eur.Neurol., 19:266-272.
Jones, R., Capildeo, R., Rose, C. F., Forrester, J. A., Luckman, N.
    P., and Preece, A. W., 1981, A diagnostic test for multiple
    sclerosis using glutaraldehyde fixed erythrocytes and laser
    cytopherometry, in: "Cell Electrophoresis in Cancer and Other
    Clinical Research", A.W. Preece and P. Ann Light, eds.,
    Elsevier/North Holland Biomedical Press, Amsterdam, New York,
    Oxford, 189-195.
Jungawala, F. B., and Dawson, R. M. C., 1971, The turnover of myelin
    phospholipids in the adult developing rat brain, Biochem.J.,
    123:683-693.
Lampert, P., and Carpenter, S., 1965, Electron microscope studies on
    the vascular permeability and the mechanism of demyelination
    in experimental allergic encephalomyelitis, J.Neuropath.Exp.
    Neurol., 24:11-24.
Love, W. C., Cashell, A., Reynolds, M., and Callaghan, N., 1974,
    Linoleate and fatty acid patterns of serum lipids in multiple
    sclerosis and other disease, Brit.Med.J., 2:18-21.
Lumsden, C. E., 1970, The neuropathology of MS, in: "Handbook of
    Clinical Neurology", P.J. Vinken and G.W. Bruyn, eds., North
    Holland Publishing Co., Amsterdam and New York, 9:217-309.
Mackay, R. P., and Hirano, A., 1967, Forms of benign Multiple
    Sclerosis, Arch.Neurol.(Chic.), 17:588-600.
Mackenzie, A., and Wilson, A. M., 1966, Accumulation of fat in the
    brains of mice affected with scrapie, Res.Vet.Sci., 7:45-54.
Matthews, W. B., 1979, Neurosarcoidosis, in: "Handbook of Clinical
    Neurology", P.J. Vinken, G.W. Bruyn and H. Klawans, eds.,
    North Holland Publishing Co., Amsterdam and New York, 38, part
    1:521-542.
McHugh, M. I., Wilkinson, R., Elliott, R. W., Field, E. F., Dewar,
    P., Hall, P. R., Taylor, R. M. R., and Uldall, P. R., 1977,
    Immunosuppression with polyunsaturated fatty acids in renal
    transplantation, Transplantation, 24:263-267.
Mertin, J., 1974, Unsaturated fatty acids and renal transplantation,
    Lancet, 2:717.
Mertin, J., Field, E. J., and Caspary, E. A., 1973, Unsaturated fatty
    acids in multiple sclerosis, Brit.Med.J., 2:777.
Millar, J. H. D., Zilkja, K. J., Langman, M. J. S., Payling Wright,
    H., SMith, A. D., Belin, J., and Thompson, R. H. S., 1973,

Double blind trial of linoleate supplementation of the diet in multiple sclerosis, Brit.Med.J., 1:756-768.

Morariu, M., and Klutzow, W. F., 1976, Subclinical Multiple Sclerosis, J.Neurol., 213:71-76.

Moya, G., 1962, Sclérose en plaques familiales. Etude critique de leur signification, Acta Neurol.et Psychiat.Belg., 62:40-94.

Müller, E., 1904, Die multiple Sklerose des Gehirns und Rückenmarks, Verlag von G. Fisher, Jena.

Myrianthopoulos, N. C., 1970, Genetic aspects of multiple sclerosis, in: "Handbook of Neurology", P.J. Vinken and G.W. Bruyn, eds., North Holland Publishing Co., Amsterdam and New York, 9:85-106.

Norton, W. T., and Podulso, S. E., 1973, Myelination in rat brain. Changes in Myelin composition during brain maturation, J.Neurochem., 21:759-762.

Peters, G., 1958, Multiple Sklerose, in: "Handbuch der Spezellen Pathologischen Anatomie und Histologie", P. Lubarsh, F. Henke, and R. Rössle, eds., Springer-Verlag, Berlin, Göttingen and Heidelberg, 13 part II:525-602.

Rindfleisch, E. E., 1863, Histologisches Detail zu der graven Degeneration von Gehirn und Rückenmark, Virchows Arch., 26:474-483.

Ring, J., Seifert, J., Mertin, J., and Brendel, W., 1974, Prolongation of skin allografts in rats by treatment with linoleic acid, Lancet, 2:1331.

Rossolimo, G., 1897, Zur Frage über die multiple Sklerose und Gliose, Deutsch.Zeitschr.f.Nervenheilk., 11:89-121.

Scherer, H. J., 1938, La "Glioblastomatose en plaques" sur les limites anatomiques de la gliomatose et des processus sclérotique progressifs (sclérose en plaques, sclérose diffuse de Schilder, sclérose concentrique), J.Belge Neurol., 38:1-17.

Seaman, G. V. F., Swank, R. L., Tamblyn, C. H., and Zukoski, C. F.iv, 1979A, Simplified red cell electrophoretic mobility test for multiple sclerosis, Lancet, 1:1138-1139.

Seaman, G. V. F., Swank, R. L., and Zukoski, C. F.iv, 1979b, Red-Cell membrane differences in multiple sclerosis are acquired from plasma, Lancet, 1:1139.

Siemerling, E., and Raecke, J., 1911, Zur pathologischen Anatomie und Pathogenese der multiplen Sklerose, Arch.f.Psychiat.u. Nervenkr., 43:824-840.

Siemerling, E., and Raecke, J., 1914, Beitrag zur Klinik und Pathologie der multiplen Sklerose mit besonderer Berücksichtigung ihrer Pathogenese, Arch.f.Psychiat.u. Nervenkr., 53:385-564.

Sinclair, H. M., 1956, Deficiency of essential fatty acids and atherosclerosis, etcetera, Lancet, 1:381-383.

Sinclair, H. M., 1982, Icosapentaenoic acid and ischaemic heart disease, Lancet, 2:383.

Stengel, D., and Hanoune, J., 1981, The catalytic unit of ram sperm adenylate cyclase can be activated through the guanine nucleo-

tide regulatory component and prostaglandin receptors of human
erythrocyte, J.Biol.Chem., 256:5394-5398.
Strähuber, A., 1903, Ueber Degenerations - und proliferationsvorgänge
bei multipler Sklerose des Nervensystems, Zieglers Beitr.zu
Path.Anat., 33:409-480.
Swank, R. L., 1950, Multiple Sclerosis: A correlation of its
incidence with dietary fat, Am.J.Med.Sci., 220:421-430.
Swank, R. L., 1970, Multiple Sclerosis: Twenty years on low fat diet,
Arch.Neurol., 23:460-474.
Tamblyn, C. H., Swank, R. L., Seaman, G. V. F., and Zukoski, C. F.iv,
1980, Red cell electrophoretic mobility test for early
diagnosis of multiple sclerosis, Neurol.Res., 2:69-83.
Thompson, R. H. S., 1966, A biochemical approach to the problem of
multiple sclerosis, Proc.Roy.Soc.Med., 59:269-276.
Thompson, R. H. S., 1973, Fatty acid metabolism in multiple
sclerosis, Biochem.Soc.Symp., 35: 103-111.
Tingley, A. H., 1956, Human brain lipids at various ages in relation
to myelination, J.Ment.Sci.London, 102:851-855.
Turnell, R. W., Clark, L. H., and Burton, A. F., 1973, Studies on the
mechanism of corticosteroid-induced lymphocytolysis, Cancer
Res., 33:203-212.
Vost, A., Wolochow, A., and Howell, D. A., 1964, Incidence of
infarcts of the brain in heart disease, J.Path.Bact., 88:463-
470.
Waksman, B. H., 1959, Experimental Allergic Encephalomyelitis and the
"Auto Allergic" diseases, Int.Arch.Allerg. and Appl.Immunol.,
Suppl. 14:1-87.
Yakovlev, P. I., and Lecours, A. R., 1967, The myelogenetic cycles of
regional maturation in the brain, in: "Regional Development of
the Brain", A. Minkowski, ed., Blackwell Scientific Pub-
lishers, Oxford and Edinburgh, 3-70.
Zollinger, H. U., 1941, Groszellig-granulomatöse Lymphangitis cerebri
(Morbus Boeck) unter dem Bilde einer multiplen Sklerose
verlaufend, Virchows Arch., 307:597-615.

PLASTICITY OF CONDUCTION PROCESSES IN DEMYELINATED NERVE

FIBERS: IMPLICATIONS FOR THERAPY IN MULTIPLE SCLEROSIS

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INTRODUCTION

Until fairly recently it had always been assumed that the complete loss of myelin over one or more internodes must necessarily produce an irreversible block of impulse conduction in myelinated nerve fibers. This view is a consequence of experimental observations showing that the essential ingredients for generation of nervous activity – voltage dependent channels selective for $Na^+$ ions – are normally only present at the nodes of Ranvier in mammalian and non-mammalian myelinated nerve fibers. Thus, the exposure of the internodal axon membrane which follows demyelination reveals a structure incapable of producing action potentials. A second obstacle to conduction following demyelination arises from the fact that, even if the internode were in principle excitable, the physiological function of the myelin sheath is to reduce the capacitance, and thus the amount of current necessary to charge the internodal membrane. Removal of this material, in the absence of any other changes, simply represents too great an electrical "load" on the excitation process to allow the continuation of conduction from the last normal node to the distal demyelinated segment(s).

In contrast to myelinated nerve fibers in which the impulse effectively "jumps" from one node to the next, in many mammalian fibers or nerve trunks (e.g. the vagus), as well as in all invertebrate species, impulse conduction occurs without an insulating myelin sheath. In this case, the machinery of excitation is distributed more or less uniformly along the fiber's length. At each point along its surface, the local nerve impulse provides the large current flow necessary to excite the immediately adjacent region of the fiber. The penalty such unmyelinated fiber systems pay for this mode

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of operation is greatly reduced conduction velocity, arising from the fact that each small area of membrane must be sequentially excited.

On the clinical side, everyone working with significant numbers of multiple sclerosis (MS) patients is struck by the immense variability of neurological signs and symptoms in this disease, both from one patient to another, and at different periods in the course of a particular individual's illness. It is not unusual for an MS patient to develop new findings, or undergo a dramatic exacerbation of existing symptoms, over just a few days; and then to spontaneously recover within an equally brief period. Occasionally, one even encounters the so-called "benign" patient who has symptoms so mild as to appear normal; and in many of these cases incidental autopsies have revealed widespread demyelination in functionally significant areas of the central nervous system (CNS), such as the optic nerve or dorsal column.

Currently, remyelination within the mammalian CNS, if it occurs at all, is considered to be a slow, very limited process (Prineas and Connell, 1979). Thus, it is necessary to reconcile a morphological situation which should not sustain impulse conduction, with the physiological fact that many nerve fibers may indeed be functional in the MS patient! The resolution of this apparent paradox lies in the recent observation that, at least in experimentally demyelinated nerve fibers, the machinery of impulse generation is sufficiently plastic to allow demyelinated regions of a nerve to behave as if they had never been myelinated in the first place - that is, to conduct impulses continuously over their entire length. If such a process also occurs in man, then it represents not only a key to unraveling the enormous variability that is seen clinically in MS, but also may point out a new direction for therapeutic investigations.

It is the aim of this Chapter to describe this axonal recovery process in some detail, and to discuss the pathophysiology of impulse conduction under such modified conditions. However, before embarking on these topics, it is necessary to briefly review the biophysical basis for impulse generation and conduction in nerve, and show how this becomes interrupted following demyelination.

## 101 CHANNELS IN MEMBRANES: THE BASIS OF EXCITABILITY

Perhaps the most fundamental biophysical advance of the last decade was the conclusive demonstration that there exist in nerve membranes quasi-permanent macromolecular pathways through which ions can move at rates exceeding $10^7$ ions/second. These pathways have been termed channels because, at least during the time that they exist in a conducting ("open") configuration, the permeant ions move relative to the channel. This contrasts with carrier systems (such as ion "pumps") in which only a few ions are translocated each time

the transport molecule moves across the membrane.  Transmembrane ion
channels come in all shapes and sizes, exhibit a wide range of physi-
cal behavior in different tissues, and in general may be regarded as
responsible for all aspects of transmission and information process-
ing in the nervous system.

In nerve fibers (as opposed to the cell bodies from which they
arise) there exist only two sets of ion-selective channels, each
operating quite independently of the other.  One pathway permits only
$Na^+$ ions to pass through, while the other is selective for $K^+$ ions.
For the purposes of understanding the basic properties of the nerve
impulse it is acceptable to consider the membrane to be impermeable
to other cations, or to anions such as $Cl^-$.  Each channel ($Na^+$ or $K^+$)
behaves as if the entry of ions into it were controlled by a kind of
"gate" whose state (open or closed) varies with changes in the volt-
age across the membrane.  By using the term "gate" we do not mean
that there must be a distinct structure controlling access to the
interior.  Most likely, the channel itself is only present in a
conducting configuration some small fraction of the time, and it is
this lifetime which depends on voltage.  At any particular time each
channel is either fully conducting or nonconducting, so that the
membrane permeability is proportional to the fraction of total
channels that are conducting.  In the resting stage of a nerve fiber
a small fraction of the $K^+$ gates are open, while essentially all the
$Na^+$ gates are closed.  Since the internal $K^+$ concentration is high,
more $K^+$ ions tend to leave the cell than enter, and a (resting)
potential develops such that the interior of the cell is negatively
charged relative to the exterior (approximately - 70mv in most
cells).

Suppose the membrane potential is suddenly displaced from its
resting potential to a more depolarized value, and is then held there
by appropriate electronic circuitry (this experimental procedure is
termed a "voltage clamp").  By recording the transmembrane current,
one can examine the way in which the $Na^+$ and $K^+$ gates respond to such
changes (Figure 1).  Here the number of conducting $Na^+$ and $K^+$ chan-
nels is shown as a function of time.  When the membrane is slightly
depolarized a few of the $Na^+$ channels open very quickly, and then
spontaneously close despite the fact that the membrane is still
depolarized (a process which is termed $Na^+$ inactivation).  For larger
depolarizations, the number of channels which open and subsequently
inactivate, as well as the speed with which both processes occur, is
increased.  Finally, with sufficiently large depolarizations all the
available $Na^+$ channels become conducting, and any further depolar-
ization has little additional effect.  Thus, the $Na^+$ permeability is
a sigmoid function of membrane potential.

The potassium gates behave differently.  Again progressively
more open with increasing depolarization (Figure 1); however, not
only is the time required for them to open much longer, but the $K^+$
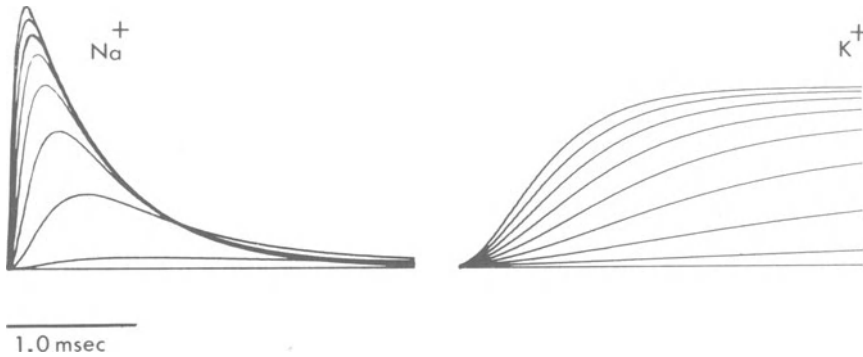
Fig. 1.  Sodium and potassium permeability as a function of time
         after a sudden shift in membrane potential.  The curves
         shown were calculated for depolarizations of 20-160 mV (in
         20 mV increments).  The na$^+$ and K$^+$ permeabilities can be
         assumed to equal the number of open channels (which has been
         plotted in arbitrary units).

gates remain open as long as the membrane is depolarized, shutting
only when the membrane potential is restored to its initial state.
They do not in general inactivate.  The time course of K$^+$ activation
is of the same order as the time course of Na$^+$ inactivation, and both
are about 10 times slower than the process of Na$^+$ activation.  This
timing turns out to be critical in the production of a nerve impulse.

    Figure 1 showed how the gates behave when we artifically de-
polarize the membrane and hold it there.  Suppose we do not control
the membrane potential, but permit it to react spontaneously to
changes in the relative permeability of the membrane to Na$^+$ and K$^+$?

    If we apply a pair of electrodes to a nerve fiber, and then pass
an appropriate current between them, there will be a slight depolar-
ization of the membrane in the vicinity of the cathodal electrode.
This will cause a small fraction of the Na$^+$ gates to open, while
little or no change occurs in the state of the K$^+$ gates simply be-
cause they react more slowly.  A net inward movement of positive
charge occur through the conducting Na$^+$ channels, while a net outward
movement of positive charge takes place through those K$^+$ channels
conducting at rest.  If sufficiently few Na$^+$ channels open, the net
rate of Na$^+$ entry will be less than the net rate of K$^+$ loss from the
cell, and upon termination of the external current the cell's voltage
will return to its resting level.

    As the external current is increased, more Na$^+$ gates open and
the net entry of Na$^+$ thus becomes progressively larger.  For depolar-
izations of 15-20 mV the net inward movement of Na$^+$ begins to exceed

the net outward movement of $K^+$. Then, even if the stimulating cur-
rent is removed, the net flow of positive charge is into the cell,
and this results in a further depolarization. This depolarization
opens more $Na^+$ gates; the imbalance between $Na^+$ entry and $K^+$ exit is
increased, leading to a larger depolarization; and another increment
in the number of open $Na^+$ gates is produced. The cycle is regener-
ative and, in less than a millisecond, all the available $Na^+$ gates
snap open. There are sufficient $Na^+$ channels so that the axon mem-
brane is now more permeable to $Na^+$ than $K^+$, and thus the voltage must
approach the equilibrium potential for $Na^+$ (reversed in polarity from
the resting state since $Na^+$ is at high concentration externally). We
have just described the phenomena of threshold. Either too few $Na^+$
channels open to overcome the tendency of the $K^+$ channels to repolar-
ize the membrane, in which case the nerve repolarizes when the exter-
nal stimulus is removed; or enough $Na^+$ channels become conducting to
trigger the regenerative opening of the remainder, and a transition
of the membrane potential to near the sodium equilibrium potential.

If activation of $Na^+$ channels were all that occurred, then a
nerve fiber would remain depolarized indefinitely. However, once
nearly all the $Na^+$ gates have opened, two additional factors come
into play. In the first place, as mentioned earlier, $Na^+$ channels do
not stay conducting. Once they have opened, they immediately begin
to close again (inactivation). Simply by closing down $Na^+$ channels,
the background leak of $K^+$ ions out of the nerve through those $K^+$
channels conducting at rest would eventually be able to repolarize
the membrane. However, in addition, more potassium channels begin to
activate at about the same time the $Na^+$ channels start to become
inactivated, so that at any given instant during the falling phase of
the action potential the membrane is relatively more $K^+$ permeable.
Repolarization is thus accelerated, shortening the action potential.
The potassium channels which are opened in response to a membrane
depolarization are not inactivated, rather they simply shut down as
the membrane repolarizes. The process of closing on repolarization
takes some finite period of time, however, so that the potassium
permeability remains slightly elevated even after the membrane has
completely repolarized. The permeability changes during a typical
action potential are illustrated in Figure 2.

It is a well-known classical phenomena that if one action poten-
tial is produced, a second cannot be elicited immediately following.
The presence of such a refractory period is a consequence of $Na^+$
inactivation because the conformational state of the $Na^+$ channel
following its opening and spontaneous inactivation is not identical
with its resting state. There are at least three different configur-
ations in which the $Na^+$ channel can exist - closed but activatable,
conducting, and inactivated. Following activation into the conduct-
ing state, and subsequent passage into an inactive configuration,
some period of time must pass before the channel finds itself in the
closed but activatable state. Clearly if the $Na^+$ gates are inacti-
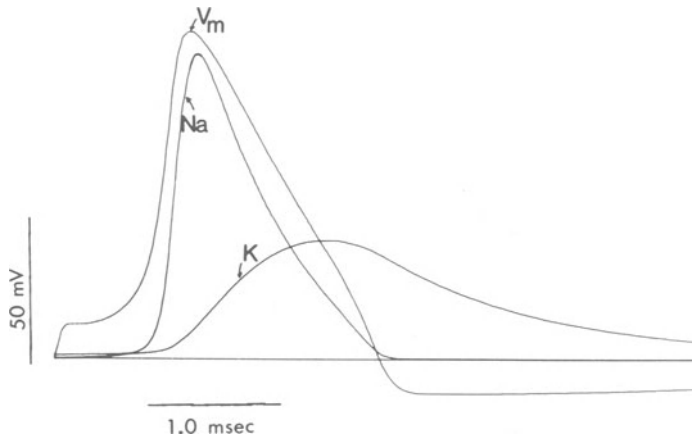
Fig. 2.   Time course of membrane voltage ($V_m$),sodium permeability,
          and potassium permeability during a nerve impulse.  Note
          that the nerve impulse is associated with a rapid increase
          in $Na^+$ permeability which reaches its maximum value near the
          peak of the impulse, and that the $K^+$ permeability remains
          elevated even after the membrane voltage has returned to
          near its resting level.


vated, and cannot open in response to a depolarization, there can be
no action potential.


CONDUCTION OF THE NERVE IMPULSE

     Up to this point we have been describing the production of a
nerve impulse at a single point on a nerve fiber, while deliberately
ignoring the problem of propagation.  In order to understand the
latter it is useful to initially consider the model myelinated nerve
fiber shown in Figure 3 (top).  Here areas at which the nerve fiber
membrane is exposed (nodes of Ranvier - approximately 1 micron wide)
are separated by long (millimeter or 1000 micron) stretches covered
by multiple turns of Schwann or oligodendrocyte cell myelin.  The
details of this structure is discussed in standard texts (e.g. Gray's
Anatomy) and reviews (Landon, 1982).  It is sufficient to note that
the multiple turns of membrane surrounding the internode act electri-
cally to increase the transmembrane resistance (axon to external
solution); and concomitantly to decrease its effective capacitance.

     Suppose node A is producing an action potential at some particu-
lar instant of time.  During the rising phase of the action potential
at node A, $Na^+$ ions are entering the fiber - that is, there is an
inward current.  This current flows mainly down the axis cylinder
(generally in both directions), with only a small amount leaking out
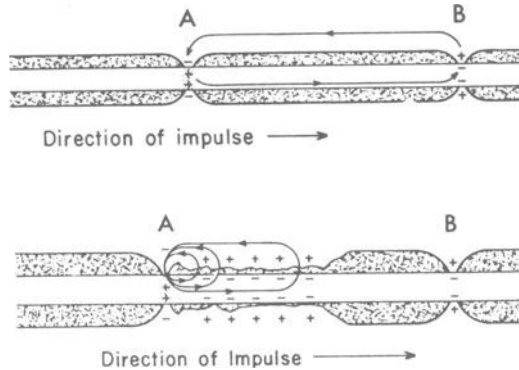
Fig. 3.  An illustration of the conduction process in a normal
         myelinated nerve fiber, and in a "model" demyelinated nerve
         fiber.  Note that the scale is greatly exaggerated.  A
         typical node is about 1 micron wide, with an internodal
         distance perhaps 500-1000 times as great.  The current flow
         is shown from an active node (node A) to the next most
         distal node (node B) in each case.

across the thick insulating layer of myelin.  Upon reaching the next
node, the positive charge neutralizes some of the excess negative
charge on the inside of the membrane at node B.  The now unbalanced
positive charges on the outside of node B flow back to node A, com-
pleting the electrical circuit.  The result of this process is a
depolarization of node B to threshold, and the initiation of an
action potential at B.  The excitation sequence is then repeated.
The action potential at node B again involves an inward movement of
$Na^+$ (inward current), which spreads in both directions along the
fiber, stimulating the next node; and so on for each successively
more distal node of Ranvier.  (The portion of current flowing back-
ward from node B to node A can be neglected simply because the proxi-
mal node, having just produced an action potential, is refractory.)
The action potential thus appears to "jump" from node to node down
the length of the fiber, a process termed "saltatory" conduction.

     What determines how fast such a nerve fiber conducts impulses?
The key to this issue is that any membrane subjected to a step change
in current does not change its potential instantaneously.  Rather,
the response to an applied current resembles that shown in Figure 4A.
On increasing the current from one level to another, the voltage in-
creases with a definite time lag.  This delay arises because the
membrane has a kind of electrical inertia (called "capacitance")
which severely limits the rate at which voltages can change.  Suppose
we consider a nerve fiber subjected to a series of increasing current
steps.  The steady state value of the membrane potential will always
be proportional to the applied current (Ohm's law), but in each case

the voltage will increase much more slowly than the current.  If the
line in Figure 4A represents the threshold for action potential
initiation then we can observe the following: (a) there are some
currents too small to attain threshold, and (b) for two currents both
giving steady-state voltages above threshold, the larger the current
the briefer the period of time it must flow to drive the nerve to
threshold.  This forms the basis for what was termed the strength-
duration relationship in the classical neurophysiology literature.

     The considerations in the preceeding paragraph can be applied to
the process of conduction in a myelinated nerve fiber.  In flowing
distally from one node to the next, some current leaks across the
myelin sheath.  The amount leaking out, compared to the amount flow-
ing longitudinally, depends on the ratio of the membrane resistance
to the internal resistance of the fiber (the larger the diameter the
smaller its longitudinal resistance).  The current loss can be quan-
titatively described by noting how far distally it is necessary to go
to lose about 2/3 of the initial longitudinal current - this is
termed the space constant.  The larger the space constant, the less
the attenuation of current from one node to the next, and the larger
the remaining current.  A larger exciting current means that a
shorter time is required to reach threshold, permitting a higher
overall conduction velocity.  The space constant, and consequently
the conduction velocity, is increased by increasing the membrane
resistance of a nerve and/or by increasing the diameter of the fiber.
Application of myelin is an extremely efficient way of increasing the
membrane resistance, while simultaneously maintaining a small dia-
meter.  Conduction in a non-myelinated nerve fiber is similar in
principle, except that the processes of excitation and current spread
occur simultaneously in each small area of membrane.  In a sense a
non-myelinated nerve fiber may be visualized as a myelinated fiber
with the internodal distance made infinitely short.  Propagation
occurs more slowly since there are in effect many more patches of
membrane to excite (a myelinated fiber of 20 μ diameter has a con-
duction velocity approximately 10 times a as large as an unmyelinated
fiber of the same diameter).

     Under normal physiological conditions, because of the high
resistance of the myelin sheath, the process of conduction in a
myelinated nerve fiber involves the net delivery of current from an
exciting proximal node to the next inactive node which is 5 to 7
times as great as necessary to drive the inactive node to threshold,
and thus sustain conduction.  This excess current is called the
"safety factor".  However, suppose a nerve fiber has one or more
internodes in which a portion of the myelin sheath is absent or
disrupted (Figure 3), such as occurs in multiple sclerosis.  In this
case relatively more current will be short-circuited between success-
ive nodes.  If we plot the potential of the inactive node as a func-
tion of time, we obtain the result illustrated in Figure 4B.  The
lower current means that more time is needed to excite the next node,
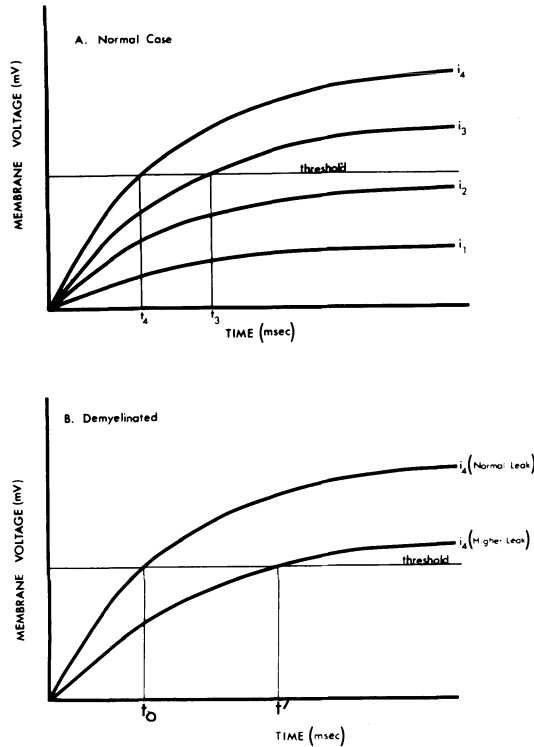
Fig. 4.  Determinants of conduction velocity.  In part A increasing
currents ($i_1$, $i_2$, $i_3$, and $i_4$) are applied to a nerve.  For
the lowest currents, the final voltage is subthreshold.  For
the larger currents, the final voltage is above threshold,
but for $i_4$ the time needed to reach threshold is less than
that for the smaller current.  For each curve, the time
course is similar, and is determined by the effective
capacitance.  Since the conduction velocity is inversely
proportional to the time needed to excite successive nodes,
the larger the effective stimulating current (the safety
factor), the greater the conduction velocity.  The consider-
ations of part A have been applied to the case of a demye-
linated nerve fiber in part B.  Here, the increased leakage
of current in the demyelinated internode results in a
decrease in the effective current reaching the distal node,
increasing the time needed to reach threshold, and thus
decreasing conduction velocity.

reducing the conduction velocity.  If sufficient myelin is lost there
may be so much leakage that not enough current will be available to
depolarize the inactive node to threshold, and conduction will fail.
What will be of particular interest to us in the remainder of this

discussion are situations in which conduction is still possible; but
where the total current leakage is sufficiently large so that the
safety factor is reduced to a value near unity.  Under such con-
ditions the conduction process becomes very sensitive to minor
changes in the local environment.


SPATIAL DISTRIBUTION OF EXCITABILITY

     In considering conduction, a valid question that could be posed
is why a demyelinated nerve fiber does not automatically revert to
the same mode of conduction as is seen in normally nonmyelinated
fibers – that is, a continuous propagation of the impulse along the
demyelinated segments.  As noted earlier, the answer is twofold.  In
the first place, even if the internodal membrane exposed as a result
of demyelination contained excitable $Na^+$ channels, there would be a
severe problem in initiating conduction from the last normal inter-
node to the subsequent, demyelinated segments.  The normal node of
Ranvier is only 1-2 microns long.  Thus, compared to the internode,
the membrane area is quite small.  Normally, the high resistance and
low capacitance of the myelin sheath combine to dwarf the contri-
bution of the internodal axon membrane to the overall properties of
the nerve fiber.  With demyelination, however, this situation is
completely changed, and the properties of even a single exposed
internode will dominate the behavior of the whole axon.  The last
normal node simply cannot deliver enough current to cause distal
regions of the fiber to reach threshold, unless some other electrical
characteristic of the fiber is also changed.

     However, ignoring for a moment the problem of impulse initia-
tion, a more serious difficulty is that myelinated nerve fibers only
possess the machinery for excitation at the nodes of Ranvier.  There
are simply no $Na^+$ channels present under the myelin sheath.  Support
for this statement comes from two different kinds of experimental
studies.  In the first, neurotoxins that are known to specifically
bind to $Na^+$ channels are radioactively labeled, and their uptake into
whole nerve is measured on normally myelinated fibers; on fibers
completely disrupted by vigorous homogenization; and on fibers im-
mediately following demyelination caused by application of toxic
substances such as lysolecithin.  The result is that the exposure of
an internodal membrane area perhaps 1000 times that of the node,
results in no increase in toxin binding, implying a very sparse
distribution of $Na^+$ channels along the normal internode (Ritchie and
Rogart, 1977).  Recently, we have been successful in examining the
distribution of $Na^+$ channels more directly in individual nerve fibers
using a radioactively labelled scorpion venom (Figure 5).  When
applied to longitudinal sections of normal myelinated nerve this $Na^+$
channel-specific toxin can be seen to be localized exclusively to the
nodes of Ranvier.  In contrast, label appears to be evenly distrib-
uted along nonmyelinated nerve fibers.  Quantitatively, for rabbit

myelinated nerve, the nodal $Na^+$ channel density is approximately
$10,000/\mu^2$ (equivalent to a distance between channels of perhaps 100 A
for a protein with a molecular weight of 250,000 daltons – very close
indeed), while the upper limit on internodal channel density is
perhaps $10/\mu^2$.  By way of comparison, nonmyelinated fibers in the
rabbit vagus nerve have 100 $Na^+$ channels/$\mu^2$ distributed uniformly
along their entire length (Ritchie and Rogart, 1977).

    A second demonstration of the same point comes from electrical
measurements.  Here, single myelinated fibers are isolated and placed
in an apparatus in which the currents flowing across a small length
(50–100 $\mu$) of the fiber can be accurately measured.  If the area
under study contains a node, large inward $Na^+$ currents are seen
(Figure 6A), while in the internode the myelin sheath serves to block
all current flow (not illustrated).  Next, the paranodal myelin is
acutely disrupted by the application of detergents such as lyso-
lecithin; osmotic shock; or specific antisera.  The magnitude of the
$Na^+$ currents are unchanged (Figure 6B) even though one can demon-
strate (by measuring total membrane capacitance) that there has been
exposure of a significant amount of paranodal axon membrane.  If the
same experiment were to be performed at some point along the inter-
node, no indication of an inward $Na^+$ current would be seen.  If there
were $Na^+$ channels beyond the node, they should have been observed
following these procedures (Chiu and Ritchie, 1980; Smith and Schauf,
1981b).
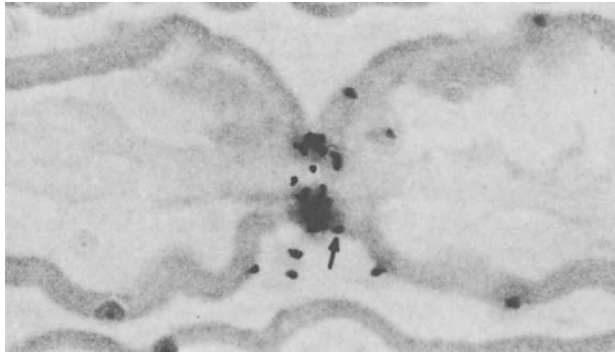


Fig. 5.  Localization of $Na^+$ channels along a normal myelinated
         nerve fiber.  Scorpion venom, a toxin which specifically
         binds to the $Na^+$ channel was labelled with I-125, and
         applied to a rat sciatic nerve.  After incubation, the nerve
         was prepared for autoradiography.  The dark grains represent
         venom molecules, and thus presumably $Na^+$ channels.  Note the
         selective localization on the node of Ranvier and low
         background nonspecific binding.
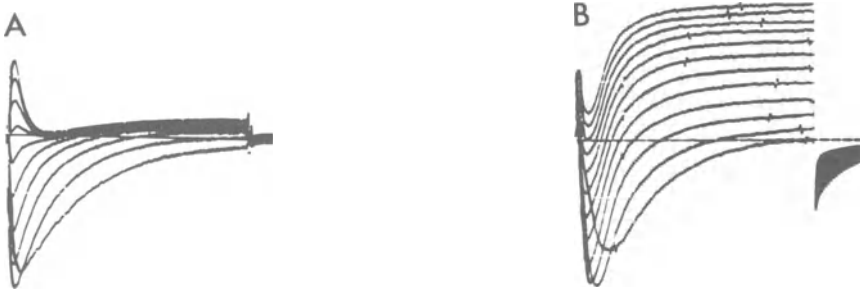
Fig. 6.   Membrane currents at the node of Ranvier in a mammalian
          myelinated fiber before (left) and after (right) disruption
          of the paranodal myelin.   In the normal situation only
          inward (at voltages below the $Na^+$ equilibrium potential) and
          outward (at voltages more positive than the $Na^+$ equilibrium
          potential) sodium currents are seen.   There is a conspicuous
          absence of $K^+$ channels.   Exposure of paranodal membrane does
          not increase the $Na^+$ current, but reveals the presence of $K^+$
          channels which were previously beneath the myelin sheath,
          and thus electrically silent.   (Data reproduced with
          permission from Smith and Schauf, 1981b).

        Along with the selective localization of $Na^+$ channels at nodes
of Ranvier, there seems to be a deliberate exclusion of $K^+$ channels
from the same region.   This is also evident from the records in
Figure 6, where exposure of the paranodal membrane can be seen to
cause a significant increase in the $K^+$ current.   (In the internode, a
similar disruption also reveals the presence of $K^+$ channels).   The
reason for such an exclusion is not known.   Perhaps the high nodal
$Na^+$ channel density, which must be deliberately controlled, simply
precludes the presence of significant numbers of $K^+$ channels (whereas
in nonmyelinated fibers with a much lower $Na^+$ channel density these
two macromolecules have room to coexist).   But why then have them in
the internode which normally is electrically silent?   Perhaps it is
unnecessary to prevent their synthesis, given that other areas of the
neuron clearly need such channels.   In any case, the fact that such
channels are present, and are exposed as a result of demyelination,
has important implications for the therapeutic approaches to be
discussed later.


RECOVERY OF EXCITABILITY FOLLOWING DEMYELINATION

        Some 35 years ago Huxley and Stampfli (1949) first demonstrated
the existence of saltatory conduction in myelinated nerve by record-
ing (extracellularly) the currents flowing longitudinally along
single nerve fibers.   Recently a more refined, but essentially simi-

lar technique has been used to reveal the changes in conduction that
follow demyelination in animal model systems (Rasminsky and Sears,
1972; Bostock and Sears, 1978, Smith, Bostock, and Hall, 1982).  In
this procedure, single rat motor fibers are selectively stimulated in
the tail, while the longitudinal currents flowing in an undissected
spinal root are recorded using very closely spaced electrodes.  As
the recording electrodes are moved along the roots, the differences
between the longitudinal currents at successive electrode positions
can be used to determine the sites of inward membrane current, corre-
sponding to the location of voltage dependent $Na^+$ channels.  In
normal fibers, as expected, such sites of inward current are restric-
ted to the usual internodal spacing (Figure 7).  The experimental
results are best presented visually as contour maps (see Bostock and
Sears, 1978) in which the sites of inward current (solid concentric
circles) and outward current (broken lines) are indicated as a dual
function of distance and time.  In this way both the distances bet-
ween successive nodes (abcissa), as well as the time taken for the
impulse to pass from node to the next (ordinate), can be readily
identified.  In the particular fiber shown in Figure 7 the conduction
velocity was about 40 m/sec and the internodal distance approximately
1 mm.

    Nerves can be experimentally demyelinated by a variety of tech-
niques, but the use of the detergent lysophosphatidyl choline (lyso-
lecithin - LPC) to disrupt the central and/or peripheral myelin
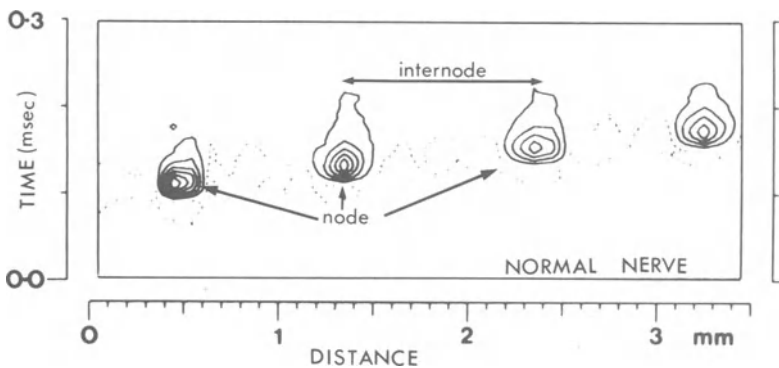


Fig. 7.  The distribution of inward and outward currents in normal
         rat myelinated nerve.  Inward ($Na^+$) currents are indicated
         by solid lines, and outward ($K^+$) currents by broken lines.
         The number of concentric circles is proportional to the
         density of current at a given point.  The distance along the
         fiber is plotted on the x-axis, and the time on the y-axis.
         (Data reporoduced with permission from Smith, Bostock and
         Hall 1982.  The details of the experimental procedures used
         to generate such contour maps are described in Bostock and
         Sears, 1978).
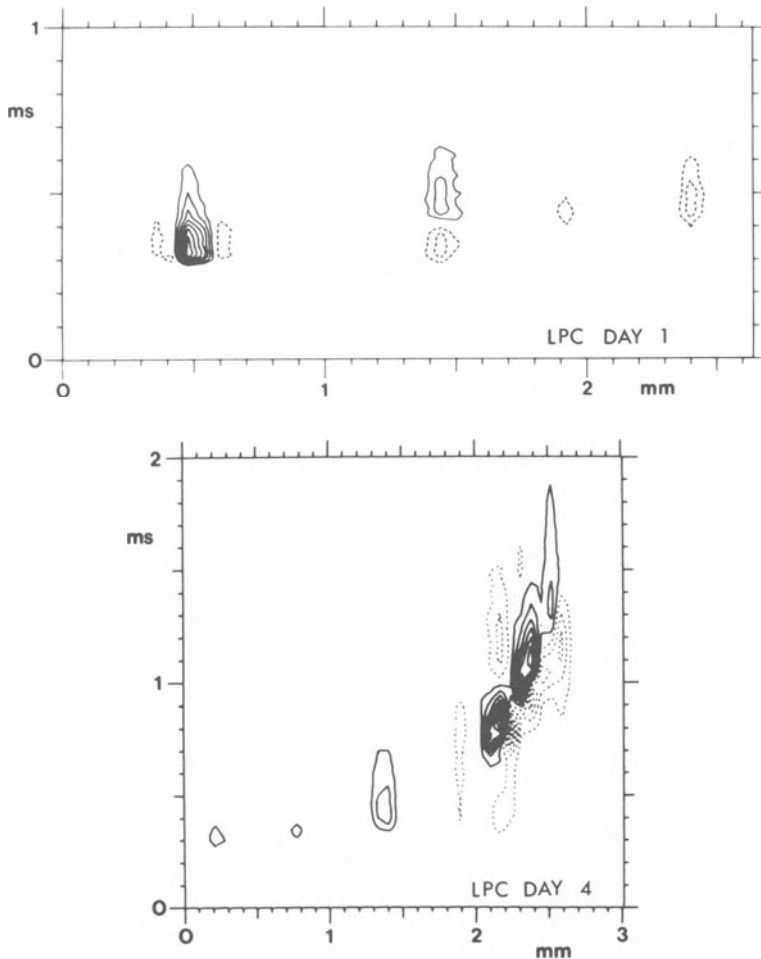
Fig. 8.  Maps of current distribution similar to those shown in
         Figure 7 following the demyelination produced by lyso-
         phosphatidylcholine (LPC).  The data in A were obtained at
         day 1 and illustrate the acute block of conduction, while
         those in B were from a lesion 4 days post LPC injection and
         show the initial appearance of sites of inward current in
         the newly exposed internode.  Data reproduced with
         permission from Smith, Bostock, and Hall (1982).

sheath has proved to be particularly useful in examining the recovery
processes which occur following demyelination because the events
occurring in all the affected fibers in this lesion are well
synchronized (Smith, Bostock, and Hall, 1982).  As with other agents,
rat nerves immediately following LPC treatment show segmental demye-
lination with a concommitant failure of impulse conduction (Figure
8A).  There is no sign of any inward $Na^+$ current in the internode.
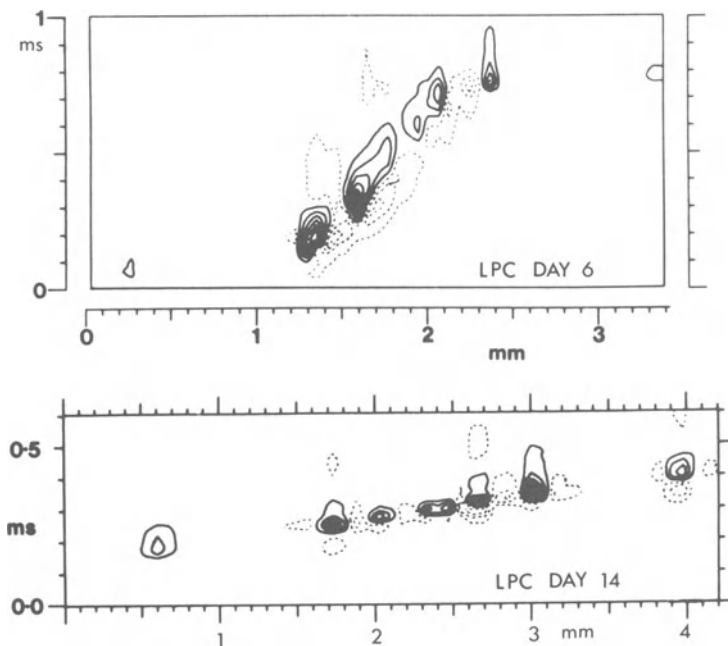
Fig. 8.   (Cont'd).   At 6 days, the contour map in part C shows that
          propagation occurs across the entire internode at low
          velocity, with normal conduction resuming beyond.   However,
          within the internode, there is an uneven distribution of
          inward current.   At 14 days following LPC injection, the
          countour map suggests that remyelination has occurred.


Remyelination begins only after 7-8 days following LPC treatment.
However, as soon as 4 days post-demyelination, strong inward cur-
rents, not previously detectible, can be seen arising from axon
membrane previously covered with myelin (Figure 8B).   At this time
the currents are usually not sufficiently large to sustain propa-
gation across the entire internode.   By 5-6 days, however, the re-
covery process has progressed to the point that conduction can occur
over the length of the entire demyelinated internode, with normal
conduction continuing more distally (Figure 8C).   However, in the
case of LPC treatment, internodal conduction does not become truly
continuous, but remains "microsaltatory" with new, strong foci of
inward current located some 100μ apart throughout the length of the
internode.   SInce no morphological evidence of remyelination is seen
as this time, it must be the case that internodal Na$^+$ channels are
nonuniformly distributed in the LPC lesion, perhaps due to a cellular
control of the insertion sites.   The distance between what have been
termed "φ-nodes" (Smith, Bostock, and Hall, 1982) increases over the
next few days, and eventually corresponds to the length of the new,
shorter internodes in the remyelinated fibers (Figure 8D).   It has

thus been suggested that the axon, by localizing its Na$^+$ channels, may in fact be "instructing" the remyelinating glial cells where it is to place new myelin.

A somewhat different recovery process is seen following dip-theria toxin induced demyelination (Bostock and Sears, 1978). As with lysolecithin, within a few hours following demyelination induced by the injection of diphtheria toxin into the cauda equina, the internodal conduction time is first increased (corresponding to a decrease in local conduction velocity to about 5-6 m/sec), and slightly later conduction is blocked, with no evidence of any inward Na$^+$ current beyond the last active node. Again, if one waits for a period of 4-6 days following the initial treatment with diphtheria toxin, recovery of conduction occurs.

In the earliest stages of this recovery, it is possible to resolve net inward Na$^+$ currents which extend beyond the last normal node into the previously inexcitable internodal region, but the impulse fails to propagate completely across the demyelinated inter-node. A day or two later, net inward currents can be detected throughout the internode, with local internodal conduction velocities that are comparable to those seen in normally nonmyelinated fibers of similar diameter (e.g. 102 n/sec in a fiber initially conducting at 40 m/sec). What is significant about the response to diphtheria toxin is that the countour maps show no sign of "hot spots", even at high spatial resolution. Thus, there is no evidence for an uneven distribution of inward currents along the internode (Figure 9) such
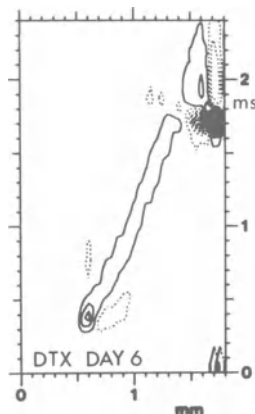


Fig. 9.   Contour map in a rat myelinated nerve fiber 6 days after
          injection of diphtheria toxin.  Again conduction throughout
          the internode is occuring,  but now there is a relatively
          uniform inward current throughout the internode, suggesting
          a continuous distribution of Na$^+$ channels, such as might be
          seen in a normal, nonmyelinated nerve fiber.  Data repro-
          duced with permission from Smith, Bostock, and Hall (1982).

as seen with LPC.  Beyond the area of segmental demyelination, conduction again proceeds normally in a saltatory fashion at high velocity.

Given the absence of internodal $Na^+$ channels in normal myelinated fibers, it is clear that the recovery of conduction following LPC and diphtheria toxin induced demyelination can only be explained by postulating the insertion of $Na^+$ channels (either at specialized sites or more or less uniformly) into regions previously devoid of them.  Such an observation is perhaps not surprising when one realizes that remyelinated axons are clearly capable of conducting impulses, and characteristically have much shorter internodes than do the parent fibers.  Obviously the sites of excitation have changed.  Nevertheless, interesting questions are raised by these experiments.  Do the internodal $Na^+$ channels represent newly synthesized and inserted macromolecules, or is there a spread of channels from the adjacent nodal regions?  The fact that with diphtheria toxin one can often find demyelinated stretches extending over more than one internode, while the position of the "old" node is not marked by a corresponding peak in inward current, suggests that some decrease in nodal density occurs following demyelination.  However, other experiments on a variety of excitable membranes argue that $Na^+$ channels may be relatively firmly anchored to the cytoskeleton and are not free of diffuse in the place of the membrane (Stuhmer and Almers, 1982).  Probably what is occurring in both lesions is that the internodal $Na^+$ channels are being newly inserted, while those at the old nodes gradually turn over, and are not replaced at anything like their original high density.


HOW IS THE INTERNODE EXCITED?

It was noted earlier that a consideration of the passive electrical properties of a myelinated nerve fiber permit one to conclude that, even if the internodal region contains $Na^+$ channels, and thus is intrinsically excitable, the nerve impulse must still fail to invade a demyelinated internode because of the large electrical load it represents.  Nevertheless, conduction can occur in experimental lesions.  What is the mechanism responsible?  Probably the changes in the $Na^+$ channel distribution along a demyelinated internode which allow conduction to occur are accompanied by alterations in passive fiber properties that facilitate the spread of an impulse into the demyelinated region (Waxman, 1978; Waxman and Brill, 1978).  The adaptations that are needed to permit passage of the impulse across this transition region are well understood theoretically, but there is little or no experimental evidence in favor of any particular mechanism.

In general, the electrical problem is that, because of the increased capacitance, the last normal node usually delivers insufficient current to depolarize the internodal membrane to threshold.

Two kinds of adaptations are theoretically possible. On the one
hand, there may be an increase in the current delivered by the prox-
imal, exciting node. This may involve an actual change in its prop-
erties or, alternatively, the internodal length between the last few
proximal nodes prior to the demyelinated stretch of axon may be
decreased, thus decreasing the amount of current attenuation and
increasing the effective safety factor for conduction. There is some
morphological evidence of such changes in experimental lesions.
Another possibility is that the new internodal $Na^+$ channels may have
slightly different properties which effectively lower their threshold
for excitation. Further studies are needed.


## CAN HUMAN FIBERS RECOVER THE ABILITY TO CONDUCT IMPULSES?

Experimentally demyelinated fibers clearly can recover the
ability to conduct electrical impulses in the absence of any signifi-
cant degree of remyelination. Obviously it is impossible to perform
in man the kind of electrical recordings that are used to demonstrate
such internodal excitability in the rat. Moreover, multiple
sclerosis lacks a completely comparable animal equivalent and, given
the different responses that can be seen experimentally with LPC
versus diphtheria toxin, one cannot a priori say very much about the
expected sequelae to demyelination in man. Nevertheless, there is
strong circumstantial evidence that some sort of recovery is poss-
ible, at least in a subset of the MS patient population.

Perhaps the strongest evidence for this kind of long-term plas-
ticity in multiple sclerosis arises from the disparity between clini-
cal signs and symptoms and pathological findings in significant fiber
tracts referred to previously, and from the persistence of slowed
conduction (as manifest in evoked responses) in the face of appar-
ently normal neurological findings. More generally, provided one
believes that remyelination within the human CNS is indeed rare, the
fact that many patients recover a major portion of lost sensory and
motor function itself suggests that internodal conduction of the type
described does occur.

It is also significant that the clinical signs and symptoms in
MS are completely understandable in terms of the conduction defects
observed in experimental demyelination. Complete segmental demye-
lination in the absence of internodal excitability must result in
conduction block. However, in experimental lesions, both paranodal
demyelination, and segmental demyelination combined with the in-
itiation of continuous (in the case of DTX) or microsaltatory (with
LPC) conduction, yield fibers with reduced safety factors. Both
conditions manifest themselves in terms of a marked slowing of con-
duction. It is quite common in multiple sclerosis to observe in-

creases in somatosensory or visual evoked response latencies of 10-40 msec, even in individuals who have apparently recovered from acute exacerbations (McDonald, 1976; Halliday et al., 1977).  Clearly slowing of impulse conduction in fiber tracts will most impair those functions (e.g. vibration sensitivity; ability to track moving objects; etc.) which depend on the delivery of precisely timed impulses in separate neural pathways.

Another characteristic of experimentally demyelinated fibers is their inability to faithfully transmit trains of impulses at high frequencies.  Although able to conduct widely spaced impulses, the presence of a reduced safety factor causes such fibers to have an increased sensitivity to the small elevations in external $K^+$ concentration that are invariably associated with repetitive firing, leading to block at higher frequencies.  Clinically, MS patients exhibiting sensory loss often show a decrease in critical flicker fusion frequency and loss of vibration sensitivity at frequencies as low as 40 Hz (Sclabassi et al., 1974).

In general, both paranodally demyelinated and segmentally demyelinated, continuously conducting fibers show decreased safety factors for conduction - the site in the latter case being at the junction between the last normal node and the demyelinated, conducting internode.  It has been shown quite unequivocally that conduction in low safety factor fibers must have an increased sensitivity to small changes in temperature with heating producing block in conducting fibers and, conversely, cooling restoring conduction to blocked fibers (Schauf and Davis, 1974 - see following section).  Clinically, such behavior is the basis for the hot bath test in multiple sclerosis, and has also been seen in investigations of the pattern visual evoked response after exercise (Persson and Sachs, 1978).  Finally, experimentally demyelinated nerve fibers often show an increase in mechanical irritability (Howe et al., 1977), and such behavior mimics the clinical presence of Lhermitte's sign (Lhermette et al., 1924), visual phosphenes (Davis, et al., 1976), and paresthesias.

This close correspondence between the clinical pathophysiology of MS and the behavior seen in experimental lesions is not likely to be fortuitous.  While paranodal demyelination can produce all of the foregoing phenomena, it is probable that in MS, as well as in the majority of toxic and allergic lesions, a complete segmental demyelination is the rule.  If this is true, then conduction lability is most likely to arise at the low safety factor site between the last normal node and a demyelinated, excitable internode since, once it becomes excited, the internode itself would be expected to conduct quite securely (as would remyelinated fibers - see Smith and Hall, 1980; Smith, Blakemore, and McDonald, 1980).

LABILITY OF CONDUCTION IN MULTIPLE SCLEROSIS

If one accepts the argument that multiple sclerosis, like the toxic demyelinating lesions that have been more throughly studied physiologically, involves a conduction process which is proceeding with substantially reduced safety factors, then much can be learned by considering the pathophysiology of this situation in more detail. The precise events that reduce the safety factor become largely irrelevant, because the ways in which conduction can be blocked or restored under such conditions turn out to be quite indpendent of the exact details of the process (Schauf and Davis, 1974).

As noted above, minor changes in body temperature (less than 1°C) can produce dramatic, reversible alterations in neurological signs and symptoms in MS, with heat causing a worsening (Nelson and McDowell, 1959; Namerow, 1968) and cooling an improvement (Watson, 1959). It has been shown both theoretically and experimentally that this clinical phenomenon is due to a direct effect of temperature on the action potential duration in low safety factor demyelinated nerve fibers (Schauf and Davis, 1974; Davis et al., 1976; Rasminsky, 1978). Borderline conducting axons block with small temperature increases, whereas just barely blocked fibers are restored to conduction by a temperature decrease. The main effect of a temperature increase is to shorten the action potential duration. The decreased action potential duration leads to a reduction in inward $Na^+$ current. In a nerve fiber where the safety factor is already severely decreased to a value near unity, further lowering of this inward current by temperature elevation produces conduction block. Conversely, a lowering of temperature increases the action potential duration, and can restore conduction in borderline blocked demyelinated nerve fibers, no matter where the low safety factor site is. These phenomena are illustrated in Figure 10, which also shows that the conduction velocity itself depends on temperature in a way opposite to the degree of conduction block.

The conduction velocity of an unblocked nerve increases with increasing temperature, reaches a maximum, and then decreases slightly just prior to block. As the safety factor is lowered, conduction fails at much lower temperatures. Given a population of fibers with varying, but low safety factors, a small increase in temperature would cause a decrease in the number of conducting fibers. A demyelinated axon thus exists in a delicate balance between conducting and blocked states. Factors other than temperature which also act to slightly alter the safety factor can have dramatic effects, while causing little or no change in normal fibers. In fact, Schauf and Davis (1974) suggested that a practical symptomatic therapy for MS could be attained by using agents that could either lower threshold, and/or prolong the action potential by mimicking the effects of a temperature decrease – i.e. slowing $Na^+$ inactivation and/or inhibiting $K^+$ activation (Figure 11). An early example of
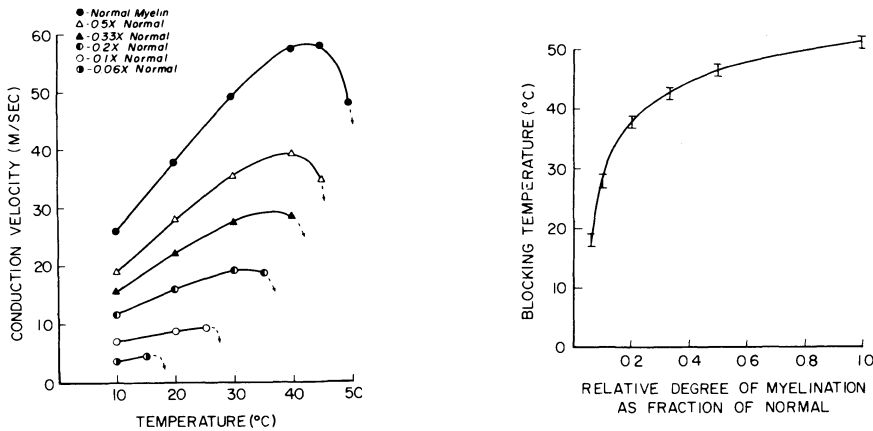
Fig. 10.  The origin of the temperature sensitivity of conduction
          block.  For a normal fiber the conduction velocity in-
          creases with temperature until conduction is blocked.  In
          demyelinated fibers conduction velocity still increases
          with temperature, but the actual temperature at which
          conduction is blocked falls dramatically.  This is illus-
          trated more directly in B, where blocking temperature is
          plotted as a function of myelination.  Data reproduced with
          permission from Schauf and Davis (1974).


this approach was the acute improvement in neurologic function in MS
patients obtained by reducing plasma calcium levels (Davis et al.,
1970).  In later studies, Bostock et al., (1978) confirmed another
prediction using scorpion venom, an agent known to slow $Na^+$ inacti-
vation.  In a rat spinal root preparation in which demyelination was
produced by local injection of diptheria toxin, they were able to
raise the blocking temperature of single demyelinated fibers by
4-12°C.

    Reduction of the potassium permeability is also expected to
improve conduction by increasing the blocking temperature, and Schauf
and Davis (1974) first suggested that such drugs might prove useful.
Although in normal fibers the potassium conductance plays little or
no significant role in controlling action potential duration, inter-
nodal $K^+$ channels are exposed on demyelination.  Both Pencek et al.,
(1978) and Sherratt et al., (1980) have demonstrated that 4-
aminopyridine, a compound that inhibits the delayed potassium con-
ductance at low concentration, facilitates conduction in blocked
demyelinated nerve.  Smith and Schauf (1981) recently reported a
similar restoration of conduction by gallamine.

    These ideas offer hope for the development of a symptomatic
therapy.  More work is needed on the pharmacological modification of
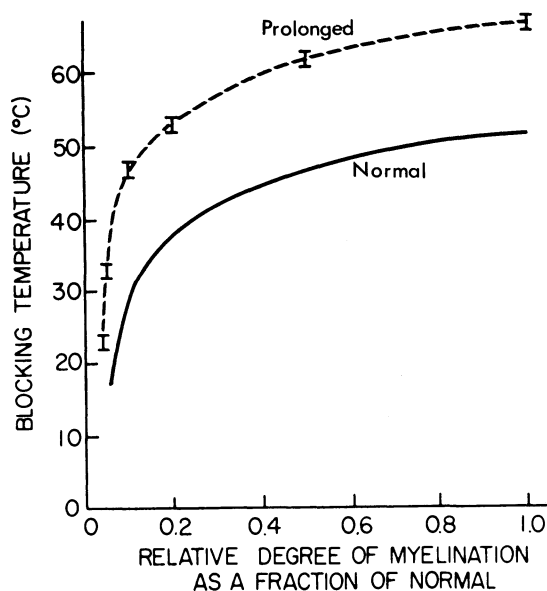
Fig. 11. The effects of prolonging the action potential (dashed
curve) on the blocking temperature of a demyelinated nerve
fiber. For any particular degree of myelin loss, slowing
$Na^+$ inactivation or, alternatively, blocking $K^+$ channels,
results in an increased blocking temperature. Data re-
produced with permission from Schauf and Davis, 1974.

ion channels, and in careful drug studies in patients. Many of the
drugs capable of restoring conduction in experimentally demyelinated
fibers may have side effects that render them impractical for thera-
peutic use in man. In particular, agents that prolong action poten-
tials in nerve fibers may have similar effects at nerve terminals,
leading to increases in transmitter release. Such actions might have
to be selectively blocked to eliminate side effects, unless some new
level of specificity is discovered.


DO INTRINSIC INHIBITORS OF CONDUCTION EXIST IN MAN?

     Given that many fibers in MS patients appear to be conducting
with low safety factors, and are thus thermolabile, it is reasonable
to inquire whether some intrinsic modulation of conduction other than
temperature plays any role in the generation of transient clinical
signs and symptoms in MS, without there being any substantial change
in the degree of myelination itself.

     Some years ago, we provided evidence that one or more factors
capable of inhibiting the ventral root response in isolated spinal

cord segments subjected to supramaximal dorsal root stimulation was
present in the serum of MS patients.  The activity of such "neuro-
electric blocking factors" appeared closely correlated with vari-
ations in neurological signs and symptoms, both between different
individuals and longitudinally within particular patients.  Such
blocking activity was not present in normal serum, and was eliminated
or preserved by experimental manipulations in a manner consistent
with the presence in MS sera of neurally directed antibodies (Schauf
et al., 1981; Schauf and Davis, 1981).

Unfortunately, although such blocking activity is quite repro-
ducible, the interpretation in terms of the role such antibodies have
in the production of signs and symptoms in MS remains less certain.
Multiple sclerosis is not thought to be a disease in which there
occur significant changes in synaptic efficacy, and therefore for
extrinsic agents to alter neurological signs and symptoms seems to
require that they directly affect axonal conduction.  We have shown
that sera with potent blocking activity on spinal cord transmission
do not have any detectable effect on conduction in either normal or
demyelinated nerve.  Rather, such sera inhibit both spontaneous and
evoked release of neurotransmitters from nerve terminals.  In add-
ition, we have demonstrated that plasmapheresis consistently de-
creases blocking activity of patient sera, but does not in general
alter clinical signs and symptoms.  We were also unable to observe
significant blocking activity in the cerebrospinal fluid itself.

Why, then, are anti-synaptic antibodies observed peripherally?
It may be that the production of such blocking factors is the result
of a generalized defect in immune regulation, leading to the pro-
duction of antibodies to a variety of "nonsense" antigens unrelated
to the primary event(s) of the disease process.  This would be con-
sistent with the presence of a variety of antiviral antibodies in
oligoclonal CSF of MS patients, and would be expected to result in a
situation in which levels of neuroelectric blocking antibodies were
closely correlated with disease activity.  However, their occurrence
would then be a peripheral manifestation of the disease, rather than
a cause of clinical exacerbations.  Peripheral deficits have been
observed in MS in studies of curare sensitivity and single fiber
electromyographic jitter, although these particular experiments could
not distinguish an effect on conduction in distal nerve terminals
from a transmission defect.  The anti-synaptic activity we have
characterized would provide a sufficient explanation of these
phenomena.

CONCLUSIONS

In summary, studies of experimentally demyelinated lesions over
the past several years have provided an attractive explanation for
the extreme lability of neurological deficits in multiple sclerosis

patients, and for the degree of variability seen between different
individuals affected with this disease.  According to this hypo-
thesis, the critical variable is the extent to which human demye-
linated nerve fibers can develop the ability to conduct impulses in
the same fashion as normal nonmyelinated fibers - that is, continu-
ously over the entire length of the lesion.  Studies of the basic
cellular processes which control the synthesis and insertion into the
membrane of excitable ion channels, as well as their detailed pro-
perties, then become most relevant to the search for an ultimate
therapeutic intervention in this disease.


## Acknowledgements

## REFERENCES

Bostock, H., and Sears, T. A., 1978, The internodal axon membrane:
      electrical excitability and continuous conduction in segmental
      demyelination, J.Physiol.(London) 280:273-301.
Bostock, H., Sherratt, R. M., and Sears, T. A., 1978, Overcoming
      conduction failure in demyelinated nerve by prolonging action
      potentials, Nature 274:385-387.
Chiu, S. Y., and Ritchie, J. M., 1980, Potassium channels in nodal
      and internodal axonal membrane of mammalian myelinated fibers,
      Nature (London), 284:170-171.
Davis, F. A., Schauf, C. L., Reed, B. J., and Kesler, R. L., 1976,
      Experimental studies of the dependence of conduction in normal
      and demyelinated nerve on extrinsic factors, I. Temperature.
      J.Neurol.Neurosurg.Psychiatry, 39:441.
Davis, F. A., Becker, F. O., Michael, J. A., and Sorensen, E., 1970,
      Effect of intravenous sodium bicarbonate, disodium edetate
      (Na$_2$EDTA), and hyperventilation on visual and oculomotor signs
      in multiple sclerosis, J.Neurol.Neurosurg.and Psychiatry, 33:
      723-732.
Davis, F., Bergen, D., Schauf, C. L., McDonald, I., and Deutsch, W.,
      1976, Visual phosphenes in multiple sclerosis, Neurology, 26:
      1100-1102.
Halliday, A. M., McDonald, W. I., and Mushin, J., 1977, Visual evoked
      potentials in patients with demyelinating disease, in: "Visual
      Evoked Potentials in Man," J. E. Desmedt, ed., Claredon Press,
      Oxford.
Howe, J. F., Loeser, J. D., Calvin, W. H., 1977, Mechnosensitivity of
      dorsal root ganglia and chronically injured axons: a physio-
      logical basis for the radicular pain of nerve root compression,
      Pain 3.

Huxley, A. F., and Stampfli, R., 1949, Evidence for saltatory
     conduction in peripheral myelinated nerve fibers, J.Physiol
     (London), 108:315-339.
Landon, D. N., 1982, The structure of the nerve fiber, in: "Abnormal
     Nerves and Muscles as Impulse Generators," W. Culp and J. Ochoa,
     eds, Oxford University Press, pp. 27-53.
Lhermitte, J., Bollack, J., Nicholas, M., 1924, Les douleurs a type
     de decharge electrique a la flexion cephalique dans le sclerose
     en plaque, Rev.Neurol., 2:56-62.
McDonald, W. I., 1976, Pathophysiology of conduction in central nerve
     fibers, in: "New Developments in Visual Evoked Potentials in the
     Human Brain," J. E. Desmedt, eds., Oxford University Press.
Namerow, N. S., 1968, Circadian temperature rhythm and vision in
     multiple sclerosis, Neurology (Minneap) 18:417-422.
Nelson, D. A., and McDowell, F., 1959, The effects of induced
     hyperthermia on patients with multiple sclerosis, J.Neurol.-
     Neurosurg. and Psychiatry, 22:113-116.
Pencek, T. L., Schauf, C. L., Low, P. A., et al., 1980, Disruption of
     the perineurium in amphibian peripheral nerve:  Morphology and
     physiology, Neurology, 30:593-599.
Persson, H. E., and Sachs, C., 1978, Provoked visual impairment in
     multiple sclerosis studied by visual evoked responses, Electro-
     encephalographic J., 44:664.
Prineas, J. S., and Connell, F., 1978, The fine structure of
     chronically active multiple sclerosis plaques, Neurology, 28:68.
Rasminsky, M., 1978, Physiology of conduction in demyelinated axons,
     in: "Physiology and Pathobiology of Axons," S. G. Waxman, ed.,
     pp.361-367, Raven Press, New York.
Rasminsky, M., and Sears, T. A., 1972, Internodal conduction in
     undissected demyelinated nerve fibers, J.Physiol.(London),
     227:323-250.
Ritchie, J. M., and Rogart, R. B., 1977, The density of sodium
     channels in mammalian myelinated nerve fibers and the nature of
     the axonal membrane under the myelin sheath, Proc.Nat.Acad.Sci.,
     (USA), 74:211-215.
Sclabassi, R. J., Namerow, N. S., and Enns, N. F., 1974,
     Somatosensory response to stimulus trains in patients with
     multiple sclerosis, Electroencephalography and Clinical Neuro
     physiology, 37:23.
Schauf, C. L., and Davis, F. A., 1974, Impulse conduction in multiple
     sclerosis: A theoretical basis for modification by temperature
     and pharmacological agents, J. Neurol.Neurosurg.Pyshciatry,
     37:152-161.
Schauf, C. L., and Davis, F. A., 1981, Neuroelectric blocking factors
     in multiple sclerosis: a perspective, in: "Demyelinating
     disease: Basic and Clinical Electrophysiology," S. G. Waxman and
     J. M. Ritchie, eds., Raven Press, New York, p.267.
Schauf, C. L., Pencek, T. L., Davis, F. A., and Rooney, M. W., 1981,
     Physiological basis for neurelectric blocking activity in mul-
     tiple sclerosis, Neurology, 31:1337.

Sherratt, R. M., Bostock, H., and Sears, T. A., 1980, Effects of
    4-aminopyridine on normal and demyelinated mammalian nerve
    fibers, Nature (London), 283:570-572.
Smith, K. J., and Hall, S. M., 1980, Nerve conduction during
    peripheral demyelination and remyelination, J.Neurol.Sci.,
    48:201-219.
Smith, K. J., and Schauf, C. L., 1981a, Effects of gallamine
    triethiodide on membrane currents in amphibian and mammalian
    peripheral nerve, J.Pharmacol.Exp.Ther., 217:719-726.
Smith, K. J., and Schauf, C. L., 1981b, Size-dependent variation of
    nodal properties in myelinated nerve fibers, Nature, 293:297.
Smith, K. J., Blakemore, W. F., and McDonald, W. I., 1980, Central
    remyelination restores secure conduction, Nature (London),
    280:395-396.
Smith, K. J., Bostock, H., and Hall, S. M., 1982, Saltatory
    conduction precedes remyelination in axons demyelinated with
    lysophosphatidyl choline, J.Neurol.Sci., 54:13-31.
Stuhmer, W., and Almers, W., 1982, Photobleaching through glass
    micropipettes: sodium channels without lateral mobility in the
    sarcolemma of frog skeletal muscle, Proc.Nat.Acad.Sci., 79:
    946-950.
Watson, C. W., 1959, Effect of lowering of body temperature on the
    symptoms and signs of multiple sclerosis, New England J.Med.,
    261:1253-1259.
Waxman, S. G., 1978, Prerequisites for conduction in demyelinated
    fibers, Neurology, 28:27-33.
Waxman, S. G., and Brill, M. H., 1978, Conduction through
    demyelinated plaques in multiple sclerosis, Computer simulations
    of facilitation by short internodes, J.Neurol Neurosurg.
    Psychiatry, 41:408-416.

RECENT STATUS OF EPIDEMIOLOGICAL STUDIES

ON MULTIPLE SCLEROSIS IN ITALY

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INTRODUCTION

Multiple sclerosis (MS) is difficult to study by epidemiological
methods, since it shows unfavorable characteristics for the epidemi-
ologic approach.

In fact, its low frequency, long latency and long duration
reduce the possibilities of the epidemiological approach, and, for
these reasons, almost exclusively descriptive studies are used, above
all prevalence surveys, which are, however, more suitable for quanti-
fying the condition of the general public health than for answering
etiological questions.

In contrast analytic studies, namely retrospective or case-
control studies, and prospective or cohort studies, electively advis-
able to test the hypothesis resulting from descriptive surveys,
clinical observations and laboratory studies, are either difficult or
impossible to carry out.  In fact, a MS case-control study ought to
be devised for investigation into childhood and adolescence events,
but the difficulties involved are obvious.  Moreover, prospective
studies are practically impossible to organize because it would be
necessary to keep thousands of people under observation for many
years.

Since low frequency prevents the conduction of door-to-door
inquiries, MS cases can only be discovered from various medical
sources.  So, qualitative level, organization and accessibility to
these sources are decisive factors in determining reliability and
comparison of the estimates.

Given these characteristics of the disease, when the sources of study material have not undergone essential changes in time, the use of incidence is not very reliable as compared to prevalence. For these reasons, most authors have always used prevalence. However, it must be emphasized that incidence expresses the proportion of individuals belonging to a given population at risk who develop a disease in a definite time interval: as for MS, the incidence measures the frequency of additional new cases of the disease per 100,000 per year; thus, the incidence rates are important indicators of the temporal trend of MS and, therefore, besides being necessary for preventive measures, they can provide etiological clues.

In contrast, prevalence measures the proportion of individuals belonging to a specific population at risk who have the disease at a given point in time: for MS, at prevalence day. The prevalence rate, by assessing the frequency of all current cases of disease (existing cases and new cases) within a specific population, quantifies the current need and is particularly useful as an indicator for medical and social care to cope with the existing cases. Contrary to incidence, prevalence is likely to be biased by other geographic variables such as migratory flux within the population, accessibility to the neurological centers, level of assistance and consequent survival of the sick. These problems, more evident in a disease with particular characteristics such as MS, tend to make the prevalence unsuitable for comparing prevalence rates found in different areas and years.

Long latency and wide variability of the interval between onset and diagnosis cause an under estimation of the true frequency.

Finally, in MS a really specific and easily feasible diagnostic test does not exist. The diagnostic criteria of Allison and Millar (1954), McAlpine et al., (1965), Schumacher committee (1965), Rose et al., (1976) and McDonald and Halliday (1977) still used in epidemiology are all descriptive and classify the MS cases according to the probability of the diagnosis. In the processing of the data, the MS epidemiological studies consider only the cases in which the two more important clinical criteria are satisfied simultaneously: the temporal dissemination of the symptoms and the spatial dissemination of the signs. The above mentioned clinical diagnostic classification (probable cases of Allison and Millar, definite cases of Schumacher, clinically definite cases of McAlpine) satisfy all such criteria, and correspond to one another. However, the use of descriptive clinical criteria alone, without support of paraclinical tests and laboratory procedures, gives rise to subjective bias and so can introduce unquantifiable variables when accepting MS cases.

These characteristics of MS pose serious methodological problems. We feel that they were worth mentioning but they should not be a matter of scepticism. In fact, in spite of all these

limits, the use of the epidemiological approach has brought to light
many pathogenetic connections that might otherwise never have been
revealed by other investigative methods.  We should like to bring
your attention to an essential point: To carry out epidemiological
studies means to work with constant care in order to pick out each
possible source of bias.  A survey can be considered valid if it has
been able to minimize the biases, since it is impossible to remove
them all in the observational studies (Rosati, 1981).

    Despite these limits, the epidemiological approach is still one
of the eventual hopes for a better knowledge of the enigmatic nature
of MS.  About 250 descriptive studies on MS prevalence in different
areas of the world have been performed in the last 30 years, which
allows us to elaborate a pattern of the natural history of MS casting
some light on etiological research.

    At first, the current opinion was that MS was differently dis-
tributed throughout the world, and its frequency was a function of
geographic latitude: the higher the latitude, the higher the MS
prevalence rate (Alter, 1968; Kurland, 1970).

    Soon afterwards, according to data from many other prevalence
surveys, a division of the world into three parts for the distribu-
tion of MS was proposed by Kurtzke in 1975.  Such distribution was
confirmed by the same author in 1980, on the basis of 234 prevalence
surveys.  High frequency rate of 30 cases, or more, per 100,000
population is limited to northern and central Europe (especially in
the United Kingdom where highest figures were estimated), southern
Canada, and the United States north of 37° north latitude, Australia
and New Zealand, and South-Africa.  In other words, MS seems to
involve Europe and those extra-European countries in which, through-
out various historical periods, there were strong settlements of
Europeans, and in particular the British colonies.  According to some
opinions, this pattern of distribution could indicate that MS origin-
ated in Europe, and was spread through the rest of the world by means
of the strong migrations of northern Europeans.  Such an inference
points to the epidemic nature of the disease.  Recently, several
authors, prompted by two epidemic foci in Faroë Islands and in
Iceland, are trying to test this hypothesis through the search of
temporal and spatial clusters (Poskanzer et al., 1981).

    Medium frequency regions with rates between 5 and 25 per 100,000
population comprise northern Scandinavia, the Mediterranean basin,
the southern United States, most of Australia, and perhaps the middle
part of South America.

    Asia seems to be globally, or nearly, a low risk band, with the
exception of western Siberia and Transcaucasia.

    Apart from one white group of South Africa, all known parts of
the African continent are low risk areas, even if it must be empha-

sized the the MS prevalence of 10, estimated in the African littoral, in Tunisia, raises the possibilities that Afro-Asiatic Mediterranean countries might share the MS prevalence of the northern regions of the Mediterranean.

That Mediterranean Europe is a medium frequency zone, is not at all certain.  On one hand, when considering the Iberian peninsula, we have no descriptive study to refer to; but, if we set a value on the Spanish mortality rate of 1.0 per 100,000 established 20 years ago by Dominguez Carmona (1961), Spain ought to be included within the high risk zones.  From Greece and the Balkans, data are still lacking: the only prevalence study carried out using only in-patients seen at the Neurological Clinic of Tessaloniki, offers, therefore, an under-estimate rate.  But a good study from Rijeka city, on the high Adriatic littoral, puts Yugoslavia into the high frequency zone.

However, the current opinion that Mediterranean countries are medium frequency areas for MS is to a great extent based on the results of about 30 Italian prevalence studies between 1962 and 1982. These surveys, reporting MS probable prevalence rates from 7.2 to 27.1 per 100,000, were undertaken on large populations, generally exceeding 300,000 inhabitants, that is to say, a methodological approach was used which in Italy involves some problems of organi-zation and thus increasing the risk of bias.Surveys on large popula-tions can be exposed to errors in estimates, especially when one works in a health organization like the Italian one, which is not very suitable for epidemiologic research.  The figures of such studies in Italy cannot be compared to those found in northern and central European countries, which have a historically greater experi-ence in the epidemiological field.  Current medical organization in Italy shows several defects including poor accessibility to the sources of study material, low level of organization in many hospital archives, differences in systems of medical records, shortage of rehabilitation facilities for the handicapped, tendency of many patients to attend medical centers outside their health districts, and lack of up-to-date population data.  These problems tend to underestimate the true prevalence, especially when large populations are studied.

Likewise, the reported crude prevalence rates, found in 19 Italian provinces, would indicate a decreasing frequency from north of Italy to southern regions (Table 1).  However, this finding, too is open to criticism.  Foremost, lower figures in southern Italy probably reflect major incompleteness in case-collection practices in that part of the Peninsula owing to the lower level of public health services.  Secondly, it must be underlined that no prevalence rate was standardized from the study area's population for both age and sex.  In Italy the composition of southern populations is quite different from that of northern populations, in which the proportion of individuals in the middle age groups is much higher.  This diff-

Table 1. MS Prevalence Studies Carried Out in Italy Using Large Populations

| AUTHORS | | STUDY AREA | LATITUDE | POPULATION | PROBABLE MS PREVALENCE RATES PER 100,000 | 95% C.I. | STANDARDIZED PREVALENCE RATES PER 100,000 |
|---|---|---|---|---|---|---|---|
| Marforio | (1980) | Sondrio | 46° | 174,327 | 24.1 | 17-33 | - |
| Rossi | (1980) | Trento | 46° | 442,873 | 27.1 | 22-32 | - |
| Marforio | (1980) | Varese | 45° | 790,046 | 24.2 | 21-28 | - |
| Mamoli | (1980) | Bergamo | 45° | 891,153 | 15.8 | 13-19 | - |
| Bortolon | (1982) | Vicenza | 45° | 727,296 | 32.2* | 28-37 | - |
| Caputo | (1979) | Novara | 45° | 507,394 | 19.5 | 16-24 | - |
| Borri | (1976) | Torino | 45° | 2,287,016 | 17.1 | 15-19 | - |
| Nardi | (1979) | Venezia | 45° | 831,657 | 18.6 | 16-22 | - |
| Tavolato | (1974) | Padova | 45° | 760,649 | 16.0 | 13-19 | 17.8 |
| Rosati | (1981) | Ferrara | 44° | 386,896 | 26.9 | 22-33 | 24.8 |
| Macchi | (1962) | Parma | 44° | 389,279 | 11.6 | 8-15 | - |
| Borri | (1976) | Firenze | 43° | 1,146,367 | 10.6 | 9-13 | - |
| Borri | (1976) | Perugia | 43° | 552,136 | 17.0 | 14-21 | - |
| Paci | (1980) | Terni | 43° | 229,034 | 17.9 | 13-24 | - |
| Megna | (1980) | Bari | 41° | 1,351,288 | 15.5 | 13-18 | - |
| Palma | (1981) | Avellino | 40° | 433,864 | 7.6** | 5-11 | - |
| Palma | (1981) | Napoli | 40° | 2,812,996 | 9.1** | 8-10 | - |
| Rosati | (1977) | Sassari | 40° | 397,891 | 16.6 | 13-21 | 17.6 |
| Rosati | (1977) | Nuoro | 40° | 273,021 | 15.8 | 12-21 | 17.9 |
| Rosati | (1977) | Cagliari | 39° | 802,888 | 9.5 | 8-12 | 10.2 |
| Bramanti | (1978) | Messina | 38° | 673,791 | 12.6 | 10-15 | - |
| Savettieri | (1978) | Palermo | 38° | 666,175 | 12.3 | 10-15 | 13.1 |
| Reggio | (1979) | Catania | 37° | 938,273 | 7.2 | 6- 9 | - |

\* Includes suspected and possible MS.

\*\* Includes possible MS.

erence of the age composition of the Italian population clearly
appears in Figure 1, which compares the Emilia Romagna population,
northern Italy, with the Sardinian population, insular-southern
Italy.  The Emilian population structure is columnar, with a high
proportion of individuals in the mid-age classes; in contrast the
Sardinian population is pyramidal, with a lower proportion of indivi-
duals in middle age.  The crude rates estimated in these two popula-
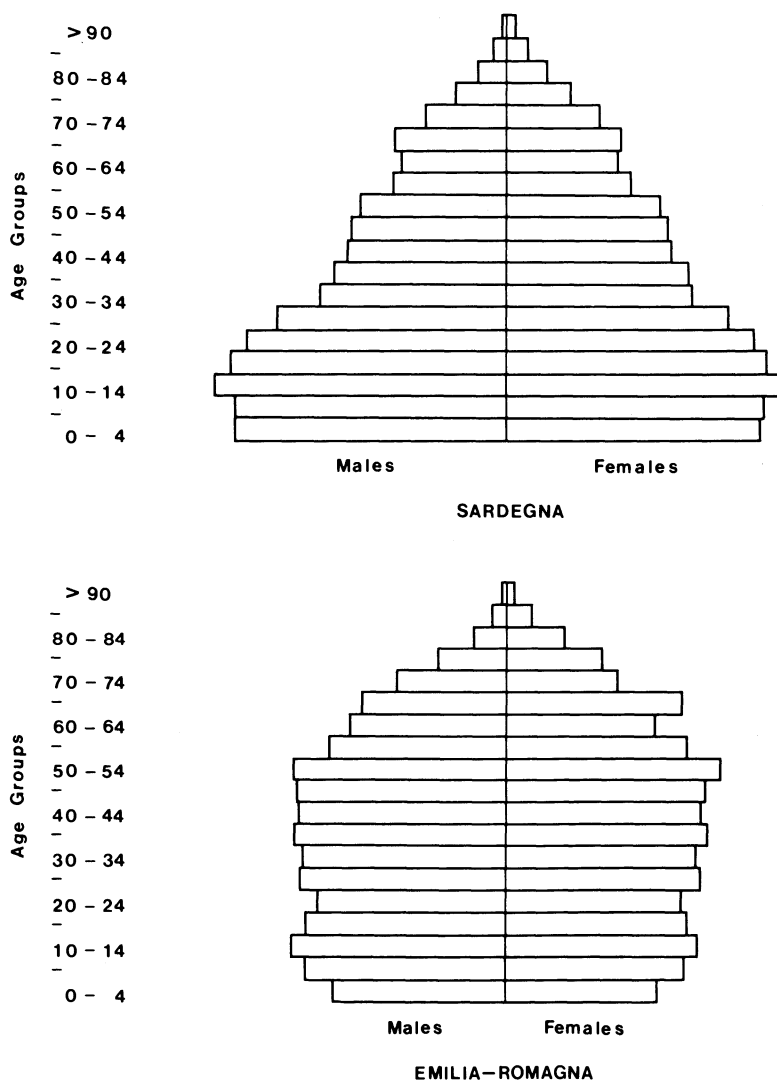


Fig. 1.  Population structures according to age- and sex-composition
         in Sardinia and in Emilia Romagna in 1971 (Rosati and
         Granieri, 1982).

tions are not comparable.  As we adjust rates versus the Italian
population, the rates found in Emilia Romagna decrease, and those
found in Sardinia rise considerably.

An indirect confirmation of the limits in comparing inter-
national data carried out using large populations is suggested by the
results of a study of Dean et al., (1976) on the frequency of first
admissions for MS to London hospitals among Italian immigrants.  The
survey seems to indicate that Italian immigrants have an analogous
risk to that observed among the English.  In fact, the number of
first admissions found among the Italian community was like that
expected for the British population.  So, it is not very credible
that Italians who have migrated to London increase their risk for MS:
moreover, it would contradict most studies on migrants.  This obser-
vation, on the contrary, stresses the possibility that the true MS
frequency in Italy is much higher than reported by Italian studies on
large populations.

Attempts, have, therefore, been made to verify the MS prevalence
in Italy by using more appropriate methods to minimize the risk of
biased data.  Since it is not possible either to change rapidly the
current health situation, or to increase the efficiency of the
studies (and thus the precision of the rates), some authors have
decided to concentrate all their efforts, including increase in staff
and expenditure, in relatively small homogeneous communities (30,000
- 50,000 inhabitants).  On this basis, Dean et al., (1979) first
contested the inclusion of Italy within the medium-risk zone with his
survey of Enna in Sicily, where a rate of 53 per 100,000 was
recorded.  Soon after, between 1979 and 1982, the results of other 7
intensive surveys were published, offering considerable support to
Dean's study.  All these studies estimated prevalence rates higher
than 30 per 100,000 (Table 2), and are all concordant in implying
that Italy is also a high-frequency zone, and in suggesting that the
frequency does not decrease from North to South according to a grad-
ient of latitude.  Moreover, with regard to the importance of adjust-
ing the rates versus the Italian population for both age and sex, we
must take into consideration the rates estimated both in northern
Italy (Copparo in Emilia Romagna) and in southern Italy (Barbagia and
Ozieri in Sardinia).  The standardized rates from Copparo decrease,
and those from Barbagia and Ozieri increase considerably (Rosati et
al., 1980a; Rosati et al., 1980b; Granieri and Rosati, 1982).

In conclusion, it is likely that accurate estimates of MS pre-
valence in Italy can be obtained only by intensive surveys of limited
population groups.  Surveys of this kind, although too few and ex-
clusively based on prevalence estimation, do not permit definitive
conclusions.  However, they are comparable in indicating that the
risk for MS is similar to that of most communities in central and
northern Europe.  In other words, all these findings, supporting the
possibility that Italy is a high-risk area, seem to refute the "zonal

Table 2.  MS Prevalence Studies Carried Out in Italy Using Limited Population Groups

| AUTHORS | STUDY AREA | LATITUDE | POPULATION | PROBABLE MS PREVALENCE RATES PER 100,000 | 95% C.I. | STANDARDIZED PREVALENCE RATES PER 100,000 |
|---------|-----------|----------|-----------|------------------------------------------|----------|-------------------------------------------|
| Rossi et al. (1980) | Bassa Valsugana | 46° | 24,570 | 44.8 | 22-80 | - |
| Rosati et al. (1980a) | Copparo | 44° | 45,153 | 31.1 | 17-52 | 29.1 |
| Dean (1981) | San Marino | 43° | 25,000 | 32.0 | 14-63 | - |
| Rosati et al. (1980b) | Ozieri | 40° | 56,294 | 30.2 | 18-48 | 35.0 |
| Granieri et al. (1982) | Barbagia | 40° | 51,611 | 40.7 | 25-62 | 48.5 |
| Savettieri et al. (1981a) | Monreale | 38° | 25,403 | 43.0 | 22-77 | - |
| Dean et al. (1981) | Agrigento | 37° | 49,979 | 32.0 | 18-52 | - |
| Savettieri et al. (1981b) | Caltanissetta | 37° | 60,022 | 38.0 | 24-57 | - |
| Dean et al. (1979) | Enna | 37° | 28,189 | 52.9 | 30-88 | - |

concept" for MS distribution in Europe and, therefore, the theory of the risk gradient with geographic latitude.

To confirm this hypothesis, many other similar investigations on homogeneous communities are urgently needed in Italy.  In fact for two years now the Section of Neuroepidemiology of the Italian Society of Neurology has been concentrating most of its efforts to this end, having planned a series of descriptive and analytic studies in some Italian regions, which have different climatic situations and socio-economic conditions.  These multicentric studies aim at ascertaining, once and for all, the true frequency of MS in Italy, and, at the same time, hope to discover factors, both genetic and environmental, that may play a role in the natural history of the disease in our country.

## REFERENCES

Allison, R. S., and Millar, J. H. D., 1954,  Prevalence and familial incidence of disseminated sclerosis: a report to the Northern Ireland Hospitals authority on the results of a three year survey, Ulster Med.J., 23:1.

Alter, M., 1968, Etiologic considerations based on the epidemiology of multiple sclerosis, Am.J.Epidem., 88:318.

Dean, G., McLoughlin, H., Brady, R., Adelstein, A. M., and Tallet-Williams, J., 1976, Multiple sclerosis among immigrants in Greater London, Br.Med.J., 2:861.

Dean, G., Grimaldi, G., Kelly, R., and Karhausen, L., 1979, Multiple sclerosis in southern Europe. I: Prevalence in Sicily in 1975, J.Epidem.,Commun.Health, 33:107.

Dean, G., Savettieri, G., Taibi, G., Morreale, S., and Karhausen, L., 1981, The prevalence of multiple sclerosis in Sicily. II: Agrigento city, J.Epidem.Commun.Health, 35:118.

Dominguez Carmona, M., 1961, Esclerosis en placas.  Epidemiologia en Espana, Rev.Sanid,Hig.Pùbl., 35:113.

Kurland, L. T., 1970, The epidemiologic characteristics of multiple sclerosis, in: "Multiple Sclerosis and other Demyelinating Diseases,"  P. J. Vinken and G. W. Bruyn, eds., North Holland Publ.Co., Amsterdam.

Kurtzke J. F., 1975, A reassessment of the distribution of multiple sclerosis, Part one, Part two, Acta Neurol.Scand., 51:110.

Kurtzke J. F., 1980, Geographic distribution of multiple sclerosis: an update with special reference to Europe and the Mediterranean region, Acta Neurol.Scand., 62:65.

Granieri, E., and Rosati, G, 1982, Italy: A medium- or high-risk area for multiple sclerosis?  An epidemiologic study in Barbagia, Sardinia, southern Italy, Neurology, 32:466.

McAlpine, D., Lumsden, C. E., and Acheson, E. D., 1972, "Multiple Sclerosis.  A reappraisal,"  Churchill Livingstone, Edinburgh.

McDonald, W. I., and Halliday, A. M., 1977, Diagnosis and classifications of multiple sclerosis, Br.Med.Bull., 33:4.

Poskanzer, D. C., Walker, A. M., Prenney, L. B., and Sheridan, J. L., 1981, The etiology of multiple sclerosis: temporal-spatial clustering indicating two environmental exposures before onset, Neurology, 31:708.

Rosati, G., Granieri, E., Carreras, M., and Tola, R., 1980a, Multiple sclerosis in southern Europe: a prevalence study in the socio-sanitary district of Copparo, northern Italy, Acta Neurol. Scand., 62:244.

Rosati, G., Granieri, E., Carreras, M., Pinna, L., Tola, R., and Paolino, E., 1980b, Epidemiologia della sclerosi multipla in piccole communità dell'Italia Settentrionale ed Insulare, in: "Atti del Secondo Convegno Nazionale di Neuroepidemiologia," R. Boeri, and G. Filippini, eds., Associazione Promozione Ricerche Neurologiche, Milan, December 12-13, 1980.

Rosati, G., 1981, Epidemiologia della sclerosi multipla, in: "Atti XXII Congresso Nazionale della Società Italiana di Neurologia, Scalea, November, 5-7, 1981," T. Caraceni, ed., Crippa & Berger s.p.a., Milan.

Rosati, G., and Granieri, E., 1982, Ruolo degli studi descrittivi nella comprensione della eziologia della sclerosi multipla: analisi critica e proposte, in: "Atti del Terzo Convegno Nazionale di Neuroepdiemiologia," R. Boeri, and G. Filippini, eds., Associazione Promozione Ricerche Neurologiche, Milan, February 26-27, 1982.

Rose, A. S., Ellison, C. W., Myers, L. W., and Tourtellotte, W. W., 1976, Criteria for the clinical diagnosis of multiple sclerosis, Neurology, 26 (Suppl.):20.

Rossi, G., Ferrari, G., and Dalri, E., 1980, Sclerosi multipla nella provincia di Trento: ricerca epidemiologica, in: "Atti del Secondo Convegno Nazionale di Neuroepidemiologia," R. Boeri, and G. Filippini, eds., Associazione Promozione Ricerche Neurologiche, Milan, December 12-13, 1980.

Savettieri, G., Karhausen, L., and Dean, G., 1981a, The prevalence of multiple sclerosis in Sicily: Monreale city. J.Epidem.Commun. Health, 35:114.

Savettieri, G., Grimaldi, G., Giordano, D., Ventura, A., Karhausen, L., and Dean, G., 1981b, La sclerosi multipla in Sicilia: altri dati epidemiologici, in: "Atti XXII Congresso Nazionale della Società Italiana di Neurologia, Scalea, November 5-7, 1981", T. Caraceni, ed., Crippa & Berger s.p.a., Milan.

Schumacher, G. A., Beebe, G., and Kibler, R. F., 1965, Problems of experimental trials of therapy in multiple sclerosis: report by panel on the evaluation of experimental trials of therapy in multiple sclerosis, Ann.N.Y.Acad.Sci., 122:552.

RECENT DESCRIPTIVE SURVEYS ON MULTIPLE SCLEROSIS

IN SARDINA AND IN THE PROVINCE OF FERRARA, ITALY

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The Italian findings from small populations, which seem to deny the zonal concept for multiple sclerosis (MS) distribution in Europe and the more widely held interpretation of the gradient of latitude, although appealing, must certainly be reviewed with caution (Kurtzke, 1983).  In fact, since MS is a disease with particular characteristics, studies based only on prevalence rather than incidence are likely to be biased by several geographic variables such as natural evolution of the disease, migratory flux, of assistance level, and accessibility to neurological centers.  These problems make it difficult to compare prevalence rates for different years and areas. Moreover, owing to the size of the populations investigated, the high prevalence rates in the intensive surveys show very wide confidence intervals, which could reduce the precision rate in measuring the true frequency of MS in Italy.  In particular, the large confidence intervals, due to the very few cases do not allow us to reject the idea that the true MS rate is below 30 per 100,000.

For these reasons and in the light of methodological considerations which had emerged in the previous investigations in Sardinia and in Emilia Romagna (Rosati et al., 1977; Rosati et al., 1980; Granieri and Rosati, 1982), the research groups of the Neurological Clinics of the Universities of Ferrara and Sassari, planned a further descriptive study in Barbagia, Sardinia to establish the MS incidence in the last 20 years and to estimate a new prevalence.

Barbagia covers a small area (1242 $km^2$, nearly 50,000 inh.) in the east-central part of Sardinia.  The living population is a self contained community that was isolated for centuries and excluded from any contact with the other ethnically distinct populations occupying the island originating from southern Europe and Afro-Asiatic Mediter-

ranean littorals.  Even from a genetic point of view, Barbagia's
population is different in that some of the more frequent HLA anti-
gens found in white people are almost absent, but there are high
frequencies of HLA antigens similar to those observed in Negroids and
in Arabs, Turks and Lebenese (Piazza et al., 1973; Bodmer, 1973).  In
many ways, Barbagia appears to be appropriate for epidemiological
investigations, and our groups, having used this area for years, have
previously verified the suitability of this region for epidemiologic
purposes.

Based on 31 MS incident cases selected according to the diagnos-
tic criteria of Allison and Millar (1954), the mean incidence per
year for the years 1961-1980 was 2.9 per 100,000 (95% confidence
intervals: 1.9 - 4.1).  The rate, adjusted for both age and sex
versus the Italian population, was 3.2.  No significant temporal
trend was noted during the period considered.  On October 24th, 1981
the crude prevalence rate for "probable" MS was 65.3 per 100,000 (95%
confidence intervals: 44 - 93).  The rate, standardized to the
Italian population, was 77.9.  Although incidence and prevalence
rates were higher among females, no significant differences were
noted between the sexes.

So, these findings, the highest figures that have yet been
estimated for any community in Italy, confirm that in Barbagia MS
occurs more frequently than expected in a Mediterranean area.  More-
over, the high confidence intervals on the rates, both lower and
upper, and the high incidence rate do in fact demonstrate that MS
frequency is high.  The rates give further support to the idea that
MS frequency in Italy is similar to that established for most of
central and northern Europe.

On the other hand, a second stage of our descriptive studies on
MS in Italy includes an incidence investigation in the province of
Ferrara (2632 km$^2$, nearly 380,000 inh.), in Emilia Romagna, northern
Italy.  The choice of such a large population might seem to be in
contrast with what has emerged from previous Italian surveys, es-
pecially if we consider some problems which make it too difficult to
obtain accurate estimates of MS frequency in our country when using
large populations.  However, it is necessary to bear in mind that
studies on small areas can be exposed to large casual fluctuations in
rates, and therefore do not permit definitive conclusions.  With
regard to Ferrara, a series of favorable circumstances (good level of
medical organization, subdivision of the area in six homogeneous
health districts, full cooperation from the doctors and the social
workers engaged in the territorial services) provide us with a great
amount of workable data for planning a descriptive study, in particu-
lar an incidence study, in a big population.  During the years 1965-
1979, 121 subjects residing in the province of Ferrara presented the
onset of the disease which later developed into "probable" MS.  Thus,
the average annual incidence rate was 2.08 per 100,000 (2.1 if age-

and sex-standardized to the Italian population).  The 95% confidence
intervals are 1.7 - 2.5.  During the period considered, the incidence
remained stable.  On October 24th, 1981 the prevalence per 100,000
for the whole province was 43.5 (95% confidence intervals: 37 - 50).
The standardized rate for Italy is 40.9 per 100,000.

So, these results carried out on a large area with the same
methodology used for small communities, further support the view that
the frequency of MS in Italy is higher than that indicated in the
literature and, on the contrary, they strengthen the idea that the
risk for the disease in the Mediterranean region is similar to that
found in most countries in central and northern Europe.  It is pos-
sible also that in Italy in recent years there has been a real in-
crease in the incidence of the disease, perhaps due to a progressive
westernization following the Second World War, which has made the
Italian life-style more similar to that in western Europe and U.S.A.

According to this latter view, a "pilot" study on the incidence
of MS in the Sardinian commune of Macomer during the period 1912-1981
(Rosati et al., 1983) seems to indicate that the frequency of MS in
Sardinia increased dramatically in the years following the last war.
The commune of Macomer covers a small area (123 km$^2$, nearly 11,000
inh.) in the western part of the province of Nuoro, Sardinia situated
between 39°54' and 40°13' N and 8°58' and 9°39' E.  The living popu-
lation is a self-contained community that remained isolated until the
late forties and early fifties.  In those years there was an immi-
gration of workers and technicians from norther Italy and the Nether-
lands since a number of industrial activities (brewery, wool indus-
try, cheese factory, etc.) were initiated.

The incident cases were drawn from all possible sources:  ar-
chives of discharge diagnosis for the years 1912-1983 from the
Sassari and Cagliari University Hospitals and from the hospitals of
Nuoro and Ozieri; death certificates for the years 1911-1980 from the
commune of Macomer, local section of the Italian Association for MS;
and directly from the old municipal doctor of Macomer who practised
in the area from 1933 to 1978.  During the years 1912 through 1981,
13 (9 women and 4 men) native-born subjects living in the study area
presented onset of disease which later developed into "definite" MS,
according to the criteria of the Schumacher Committee (1965).  All
these cases had clinical onset of MS between 1952 and 1981; in con-
trast the disease was completely absent before 1952.  During the
period 1952 - 1981 the mean annual incidence was 6.3 per 100,000 (95%
confidence intervals: 3.4 - 10.8).  By estimating the incidence rates
for 5-year intervals, the onset of MS in Macomer showed the following
temporal distribution: 2.8 cases per 100,000 population in the years
1952 - 1956; 10.2 in the years 1957 - 1961; 7 in the years 1962 -
1966; 4.2 in the years 1967 - 1971; 3.9 in the years 1972 - 1976; and
1.8 in the years 1977 - 1981 (Figure 1).  In 1961 the point preva-
lence rate was 61.5 per 100,000 population (95% confidence intervals:

Table 1.  MS Incidence among Native-born Residents in Macomer,
Sardinia, during the Years 1912 through 1981.
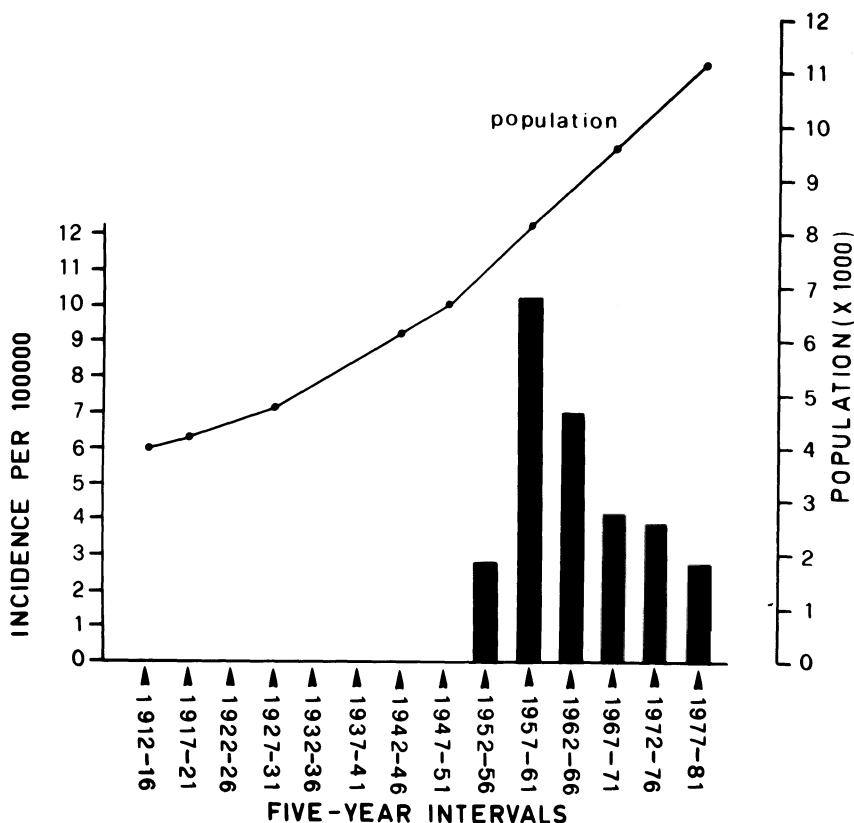


20 - 143); in 1971, 73.4 (95% confidence intervals: 30 - 151); and in
1981, 72 (95% confidence intervals: 31 - 141).  In other words, MS
appeared in Macomer in the early fifties, reached its highest inci-
dence in the years 1957 to 1967, and then decreased slowly until
1981.

These findings lead to the conclusion that MS was introduced in
Macomer, and probably in other parts of central Sardinia, in the
years following the Second World War, when the rise of industrializ-
ation put an end to the century-old isolation of the native popu-
lations.  These data, moreover, seem to confirm the hypothesis posed
in the famous study by Kurtzke and Hyllested (1979) on the Faroe
Islands that MS is an exogenous disease with epidemic trend.

Acknowledgement

REFERENCES

Allison, R. S., and Millar, J. H. D., 1954, Prevalence and familial incidence of disseminated sclerosis: a report to the Northern Ireland Hospitals authority on the results of a three year survey, Ulster Med.J., 23:1.
Bodmer, W. F., 1973, Population genetics of the HLA system: retrospect and prospect, in: "Histocompatibility testing 1972", J. Dauset and J. Colombani, eds., Munksgaard, Copenhagen.
Granieri, E., and Rosati, G., 1982, Italy: A medium- or high-risk area for multiple sclerosis? An epidemiologic study in Barbagia, Sardinia, southern Italy, Neurology, 32:466.
Kurtzke, J. F., and Hyllested, K., 1979, Multiple sclerosis in the Faroe Islands: I. Clinical and epidemiological features, Ann.Neurol., 5:6.
Kurtzke, J. F., 1983, Some epidemiological trends in multiple sclerosis, Trend Neuro.Sci., 6:75.
Piazza, A., Belvedere, M. C., and Bernoco, D., 1973, HLA variation in four Sardinian villages under differential selective pressure by malaria, in: "Histocompatibility Testing 1972", J. Dausset and J. Colombani, eds., Munksgaard, Copenhagen.
Rosati, G., Pinna, L., Granierei, E., Aiello, I., De Bastiani, P., Tola, R., Agnetti, V., and Pirisi, A., 1977, The distribution of multiple sclerosis in Sardinia, Riv.Pat.Nerv.Ment., 98:46.
Rosati, G., Granieri, E., Carreras, M., and Tola, R., 1980, Multiple sclerosis in southern Europe: A prevalence study in the socio-sanitary district of Copparo, northern Italy, Acta Neurol. Scand., 62:244.
Rosati, G., Aiello, I., Pirastru, M. I., Becciu, S., Zoccheddu, A., De Montis, G., and Mannu, L., Studio sull'andamento temporale della sclerosi multipla a Macomer dal 1912 al 1981: conferma epidemiologica della natura esogena della malattia, in: "Atti XXIII Congresso Nazionale della Societa` Italiana di Neurologia, Parma, October 20-22, 1983", in press.
Schumacher, G. A., Beebe, G., and Kibler, R. F., 1965, Problems of experimental trials of therapy in multiple sclerosis: report by panel on the evaluation of experimental trials of therapy in multiple sclerosis, Ann.N.Y.Acad.Sci., 122:552.

EVOKED POTENTIALS IN THE INVESTIGATION OF

MULTIPLE SCLEROSIS

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The use of evoked potential (EP) techniques in the investigation
of a number of aspects of multiple sclerosis (MS) has been a major
growth industry.  The technique has been used in the diagnosis of MS,
in attempts at monitoring the progress or activity of the disease,
including the effects of treatment, and in exploring its pathophysio-
logy.  The time is ripe for a critical appraisal of how far claims
for the value of EP in the investigation of MS are justified.


DIAGNOSIS

In the evaluation of any diagnostic technique, it is first
essential to demonstrate that the test under investigation is posi-
tive or abnormal in a high proportion of known cases of the disease.
In the whole of medicine very few diagnostic tests have been shown to
be 100% positive and much lower figures are valuable if they do not
include an undue proportion of false positives.  In MS there is the
further complication that the duration of the disease is nearly
always extremely prolonged and even if it were possible to await a
fatal outcome, there may be no opportunity to confirm the diagnosis
as the autopsy rate is notoriously low.  Diagnosis must therefore be
on clinical grounds.  Every few years a new attempt is made to estab-
lish criteria on which a diagnosis of 'clinically definite' MS can be
made.  The latest criteria include abnormal results of laboratory
tests, among these notably the results of EP recording, but this is
clearly inappropriate when the objective is to assess the diagnostic
value of these same techniques.

Most authors who have studied EP in the diagnosis of MS have
used McAlpine's[1] criteria.  These are reasonable when applied to

the clinically definite category but careful perusal at once reveals
that the definition of 'probable' and 'possible' cases contains
important ambiguities.  However, most other criteria have been
devised for a different purpose, in particular for the recognition of
cases suitable for trial of therapeutic agents, when it would be
clearly inadmissible to include patients in whom the diagnosis was
doubtful.

    The first diagnostic application of EP in MS was in the use of
the averaged visual potential (VEP) evoked by stimulation of each eye
separately by means of an alternating chequerboard pattern[2].
Earlier attempts using the simpler stimulus of a flash of light had
not produced consistent results although this technique may still be
occasionally useful[3].  The actual stimulus in the shifting pattern
appears to be the edges of the squares.

    Halliday et al.[2] applied this technique to patients known to
have had a recent episode of optic neuritis (ON).  The finding of a
delayed response was of great interest but was, of course, of no
diagnostic value, as demyelinating disease of the optic nerve was not
in doubt.  The latency was measured from stimulus, that is to say the
movement of the pattern, to the peak of the most prominent component
of the evoked response, the so-called P100.  There are obvious
sources of error.  The pattern shift is not instantaneous and indeed
in the television screen devices now in common use the pattern
develops on the screen in sequence, usually from the upper left
corner, although this is not detectable to the eye.  The P100 is
chosen because it is the most consistent response, although certainly
not the earliest component.  Using this technique Halliday et al.[2]
found a notable asymmetry of latency between the responses evoked
from the affected eye and that from the presumed normal eye.  Even at
this stage, however, it should be noted that this abnormality was not
present in all affected eyes, a normal response being obtained on one
occasion.  This may be regarded as an illustration of the failure to
find 100% positive results with any diagnostic test.

    These findings were confirmed in other series.  Prolonged
latency was found on stimulation of 22 of 28 eyes known to have been
affected by optic neuritis within the preceding three months, the
response from the other six eyes being normal.  Asymmetry of latency
was less striking, and indeed in one patient latency was more pro-
longed on stimulation of the eye not known to have been involved[4].
Shahrokhi et al.[5] examined 51 patients without evidence of multiple
lesions but who have a history of optic neuritis at some time in the
past.  A response normal in all respects was obtained from three of
59 affected eyes, latency being increased in the majority of the
remainder.  Such results indicate that optic neuritis in the past was
often associated with prolonged latency of VEP.

The next stage was to record VEP in patients with clinically
definite MS not suffering from recent ON.  In the original series
Halliday et al.[6] found abnormal VEP in 97% of such patients.  This
figure has been approached or even surpassed in a number of later
studies[7-9], but somewhat lower proportions are more usual[4,10-13]
but still above 75%.  These findings, while an essential step in the
exploration of the technique, were again of no diagnostic value as
the patients were already accepted as having MS.  Of great importance
to the extension of the technique to the examination of suspected
cases is the proportion of abnormal VEP in definite cases with no
clinical evidence of involvement of the visual system; that is to
say, no history of ON, no abnormal pallor of the optic discs, normal
acuity and color vision, the usual routine evaluation.  The visual
field changes in such patients, when present at all, are subtle and
difficult to record.

Not unexpectedly the percentage of abnormal VEP is considerably
higher in patients with a past history of ON than in those without.
Even the notoriously fallible clinical sign of pallor of the temporal
half of the optic disc is associated with a higher incidence of
abnormal VEP[4].  Nevertheless, even in the absence of any evidence
of visual involvement, present or past, VEP are abnormal in clini-
cally definite MS in from 57 to 94% of cases[6,7].  The significance
of this is that the method appears to be capable of detecting abnor-
mality where none was otherwise suspected – the silent lesion.  The
importance of this finding to the diagnosis is obvious enough.
Multiple lesions may be demonstrated by means other than clinical
history and examination.

The technique was therefore legitimately applied to the exam-
ination of patients in whom the clinical features did not satisfy the
criteria of definite MS.  In the original series[6] such abnormali-
ties were indeed found in 100% of 'probable' cases of MS and in 92%
of 'possible' cases.  McAlpine's criteria for the probable category
are difficult to apply at all strictly and this may account for the
much lower figures for abnormal VEP in such cases reported in most
series, 33%[14,15], 50%[10] and 58%[4,9].  The remarkable figure of
92% of abnormalities in patients in the possible category has never
been approached.  By definition very few such cases will have con-
vincing clinical evidence of optic nerve affection, so that any EP
abnormalities detected are likely to be of greater diagnostic value.

If decisions are to be made affecting the management of the
patient it is clearly of the utmost importance that strictly defined
standards of normality are established.  The technique has shown
itself to be remarkably robust in that consistent results can be
obtained despite variation in such factors as the size of the squares
in the stimulus pattern and the luminance of the black and white
areas[16].  Nevertheless it is not safe to base conclusions on normal
results published by others, and each laboratory must establish

normal values. Latency measurements beyond 2.5 or 3 SD above the mean are usually taken as abnormal but clearly more weight will be attached to a figure 50% greater than the mean than a latency a few milliseconds beyond the upper limit chosen. Asymmetry of latency beyond limits observed in normal series is also often accepted as indicating pathology even if latency is not unduly prolonged. Gross asymmetry of amplitude may also be abnormal but low amplitude symmetrical responses of normal latency are not uncommon in healthy subjects. Amplitude is often much greater in women and mean latency is also slightly shorter. There are unresolved difficulties in interpreting certain wave forms of unusual shape. There is little effect of age on VEP latency within the age range of patients with MS. Visual acuity below 6/12, from whatever cause, may result in an abnormal response[17].

Taking all these factors into account, a large number of reliable surveys have now been published and all demonstrate that the less certain the clinical diagnostic category the lower the rate of abnormal VEP found. This must mean either that the investigation is relatively seldom abnormal in early cases of MS or that a high proportion of patients in the lower categories of diagnostic certainty do not have the disease. A further difficulty arises, that will be discussed later in detail. What evidence exists to justify 'transferring' patients from one diagnostic category to another, implying a greater degree of certainty, on the evidence of an abnormal VEP recording? Many authors appear to have sensed this difficulty and announce the intention to follow up such patients to determine the outcome, but very few have succeeded in doing so. Others have accepted without question that such results indicate 'proven dissemination of the demyelinating process'[13]. It is, perhaps, unwise to speak of 'proof' of a biological phenomenon from indirect evidence.

The collection of data on percentage of abnormalities and the theoretical 'transferring' of blocks of patients as a result is, perhaps, rather far removed from the practical problems of the clinic. The possible diagnostic value of VEP may be considered in two common clinical problems. The first concerns the young adult who presents with a single monosymptomatic episode compatible with the onset of MS - diplopia or sensory symptoms and signs in the limbs. The immediate diagnosis of MS in such circumstances carried no immediate benefit for the patient except that other causes of, for example, a VIth cranial nerve palsy, need not be sought. A single episode of this kind does not qualify a patient for any of McAlpine's diagnostic categories but would be regarded as 'suspect' by McDonald and Halliday[18]. The frequency with which recording VEP provides evidence of an unsuspected lesion of the visual system in such patients has seldom been examined but is certainly small: three out of 39[4] and two out of 22[19] in two series.

The other clinical problem to which VEP recording might be
expected to contribute is that of the patient with progressive
spastic weakness of the legs and no clinical evidence of multiple
lesions.  It is well known that a high proportion of such patients do
indeed have MS but there are unpleasant possibilities of diagnostic
error, including overlooking a benign cause of spinal cord com-
pression.  Abnormal VEP in such a patient should indicate multiple
lesions and therefore obviate the necessity for myelography,
Blumhardt et al.[20] attempted to find the answer in 64 patients with
chronic progressive paraparesis, out of a total of 131 cases of
apparently isolated spinal cord disease.  In the chronic progressive
category VEP were abnormal in 36%, the percentage being much higher
in those with symptoms for more than three years.  Unfortunately for
the purely scientific value of the study the clinicians involved were
undoubtedly influenced by the results of VEP recording and myelo-
graphy was not done in a high proportion of those in whom VEP were
abnormal.  This was the precise result the investigation was designed
to test and it is not known how often the decision not to proceed to
invasive radiology was justified.

The question of the specificity of abnormal VEP is of obvious
importance.  Prolonged latency may be found in hereditary
ataxias[21], hereditary spastic paraplegia[21], vitamin B12 de-
ficiency[22], the Chiari malformation[23], neurosyphilis[24] and
sarcoidosis[25], all conditions that may be confused with MS.
Abnormal VEP in patients with spinal cord disease and myelographi-
cally demonstrated narrowing of the cervical spinal canal may be
explained by the well recognized coexistence of MS and spondylotic
myelopathy[26].  Conditions that do not cause spinal cord disease but
which may result in prolonged VEP latency and cause diagnostic con-
fusion include glaucoma without visual loss[27] and diabetes without
retinopathy[28].  Abnormal VEP are certainly of diagnostic use in MS
if used judiciously but cannot be regarded as 'a valuable test in the
early diagnosis' as was at first hoped[6].


SOMATOSENSORY EVOKED POTENTIALS (SEP)

Namerow[29] reported that the latency of the scalp recorded SEP
evoked by stimulation of the median nerve was prolonged in patients
with MS with sensory loss in the limb tested.  In diagnostic use
attention has been mainly directed to short latency potentials
recorded over the neck and scalp from stimulation of main nerve
trunks in the limbs[30].  By the latter method a clear potential
can be recorded from the scalp but there are great difficulties in
obtaining consistent records over the spinal cord above the lower
dorsal region.  In relaxed normal subjects and by averaging a very
large number of responses an extremely small amplitude potential can
sometimes be recorded from surface electrodes over the cervical
spinal cord from stimulation of the tibial nerve[31].  This naturally

gave rise to hopes of measurement of spinal cord sensory conduction
velocity in disease but this has not proved possible.  In spinal
disease such potentials cannot be recorded at all but it is not
possible to accept this as evidence of disease in cases of doubt as
the response is often unrecordable in normal subjects.

In routine clinical practice the median nerve is stimulated and
the averaged response recorded over the neck, referred to the vertex,
and the short latency scalp component is also recorded.  The complex
series of potentials in the neck recording include an initial wave
arising from the brachial plexus and if latency of this component is
normal this provides good evidence of normal peripheral conduction.
Naturally latency will vary according to length of arm.  The site of
origin of the main component of the normal cervical response is
probably the dorsal column nuclei rather than the cervical spinal
cord.

In MS both the cervical and early scalp potentials are often
abnormal.  This is not surprising as the posterior columns in the
cervical spinal cord are sites of predilection for MS plaques.  Small
et al.[32] reported abnormal cervical SEP in 69% of clinically defi-
nite cases and Mastaglia et al.[15] in 15 of 17 such cases.  The
abnormality observed was usually absence of the cervical potential
rather than prolonged latency.  It is tempting to regard wave forms
of unfamiliar shape as indicating pathology but this is almost
certainly unjustified.  The early scalp SEP, N20, is seldom abnormal
if the cervical response is normal.  Prolonged latency of this com-
ponent may be observed.

In patients in whom the diagnosis of MS is uncertain, SEP are
also frequently abnormal.  Small et al.[32] reported abnormal SEP in
42% of probable and 52% of possible cases, the latter category
including, by definition, cases of progressive spastic paraparesis.
In many of these patients the abnormal SEP could not be accepted as
revealing leasions in regions of the CNS not known to be involved as
spinal cord disease was clinically evident.  However, in this series,
21% of patients with no sensory loss in the upper limbs and no
evidence of spinal cord disease had abnormal SEP.  These stricter
criteria are obviously sound and were adopted by Green et al.[33] and
by Purves et al.[34].

The use of SEP in the early diagnosis of MS has encountered
difficulties similar to those with VEP.  In patients with a single
symptomatic episode SEP are seldom abnormal.  It is probable that
many of these patients do not have MS and will never develop the
disease, but there is one group in whom the probability of developing
MS is high:  patients with isolated optic neuritis.  In a series of
40 such cases SEP from the median nerve were abnormal in only two, a
far smaller proportion that the expected incidence of MS in these
patients.  The conclusion must be either that plaques in the cervical

spinal cord are seldom present at this stage of the disease or that they are not detectable by this method.

## AUDITORY EVOKED POTENTIALS

Brainstem auditory EP (BAEP) at first appeared to provide much greater precision of interpretation as each of the seven components usually recordable could be duplicated in animal experiments and attributed to activity in a closely localized site in the auditory pathway.  This is in contrast to VEP and SEP where there is still much controversy concerning the origin of the potentials.  Experience has shown that localization of lesions by means of BAEP is less precise than was hoped.  This is scarcely surprising as pathological processes are seldom as sharply defined as those produced in the laboratory and spreading effects from pressure, oedema and other factors commonly occur.  A further disadvantage of BAEP is their remarkable sensitivity to variations in stimulus, method of recording and status of the subject examined[36].

In the diagnosis of MS it has been found that BAEP are less often abnormal in clinically definite MS than VEP or SEP, although figures have varied widely between different series.  Chiappa et al.[37] found only 57% of abnormalities in definite cases with clinical evidence of brainstem disease, whereas Stockard et al.[38] reported 77% of abnormalities in probable cases who had never had brainstem symptoms.  In a further report[36] they described results in a large series of patients with a single lesion suggestive of MS, either ON or myelopathy.  When examined in the first attach only 10% of patients had abnormal BAEP.  Most exceptionally an adequate follow up study was mounted and it was found that all those with abnormal BAEP in the first attack developed MS within three years.

## MULTIPLE EP RECORDINGS

The availability of several different methods of recording EP naturally led to the application of such multiple examinations in the hope of increasing the proportion of abnormalities detected.  The combined use of VEP, SEP by various techniques, and BAEP has been described by a number of authors[19,33,34,39,40,41,42,43].  There is no doubt that if all three modalities are used abnormalities may be found in individual patients by one method alone.  Many authors have claimed that SEP is the most useful in this respect but the criteria employed have been defective.  Abnormal SEP cannot be claimed as demonstrating a silent lesion in a patient with spastic weakness of both legs, even if no sensory loss is found, as disease of the spinal cord is already evident.

It may be doubted whether the submission of a patient with suspected MS to two hours' intensive and sometimes uncomfortable and often frightening investigation in an EP laboratory – the so-called routine MS workup – can be justified by results. Papers claiming success have mainly been written by clinical neurophysiologists who are not in charge of the patients.

## CAN ABNORMAL EP INDICATE SILENT LESIONS IN MS?

The diagnostic use of EP has been largely built on assumptions; first that abnormalities can result from asymptomatic plaques, and secondly that the discovery of such abnormalities is diagnostic of MS. Anatomical verification of the first assumption is almost entirely lacking. I recorded SEP from a woman with very mild MS[44]. She had experienced sensory symptoms in the right limbs in the past but at the time I saw her there was no sensory loss, although the right leg was weak and spastic. Shortly afterwards she died suddenly from a subarachnoid hemorrhage. At autopsy a plaque was found encroaching on the dorsal root entry zone and on the posterior columns in the cervical spinal cord. This could confidently be accepted as the cause of the abnormal SEP in the absence of sensory loss.

That the second assumption is correct requires prolonged follow up of patients in whom EP abnormalities have appeared to indicate the presence of silent lesions allowing their 'transfer' to some more definite diagnostic category. Do such patients actually have MS? Follow up of such patients is extremely difficult. They are young adults who have mostly had transient symptoms and who may not know that MS has even been suspected. They are not inclined to waste time attending hospital. Few such studies have been reported. Deltenre et al.[45] examined 133 patients suspected of having MS by all three techniques and followed them up for up to four years. In 27 some diagnosis other than MS was established and in five of these abnormal EP indicating a silent lesion had been found – false positive for MS. Disturbingly, BAEP were abnormal in two patients with spondylotic myelopathy. Of the 44 patients who developed clinically definite MS, 36 had abnormal EP when first examined. Of the 62 in whom no diagnosis had been reached on follow up 12 had abnormal EP of uncertain significance.

In a personal study[19] containing a higher proportion of patients with a single episode of neurological symptoms, 17 of 56 patients developed definite MS within three years and of these nine had abnormal EP when first seen. Absence of such abnormalities was of no prognostic significance. The lack of follow up studies should be remedied. For example, abnormal BAEP have been reported in patients with ON[46] but it is not known whether MS developed.

## PRACTICAL DIAGNOSTIC VALUE

A serious contribution to the management of patients would be methods of diagnosis that would obviate the use of invasive radiology, particularly myelography. Mastaglia et al.[47] specifically examined this aspect. Of 129 patients with probable or suspected MS such procedures were avoided in 23 after taking into account neurophysiological findings and perhaps should have been avoided in a further 24. No follow up was reported so it is not known whether the omission of radiology was always justified by the event.

The most useful application of EP in MS is still that of recording VEP in patients with disease of the spinal cord. Prolonged latency is strong evidence of more widespread disease, and the probability of MS is much increased if the abnormalities are markedly asymmetrical.

## MONITORING

There is a serious need for an objective method of monitoring the activity of the disease process in MS and of measuring the change in clinical status. This is particularly relevant to the assessment of methods of treatment. The concept that these objectives might be attained by serial recordings of EP is at first sight attractive but contains a fundamental flaw. The value of EP in diagnosis lies in their ability to demonstrate persistent abnormality in the absence of relevant clinical signs, and in apparently quiescent disease. This property is almost the reverse of those required for an efficient monitor. What is needed is a measurement that will reliably indicate relapse and remission. The well known lack of relationship between lesions and symptoms in MS naturally renders the interpretation of any such indications extremely difficult. To demonstrate the persistence of a plaque or even an increase in its neurophysiological effect, in the absence of relevant symptoms, cannot readily be translated into persistent 'activity' of disease or deterioration of the clinical state. Nevertheless it was essential to explore these possibilities.

Detailed serial recordings of VEP and SEP were performed in a small number of patients by Matthews and Small[48]. Records were obtained at monthly intervals and correlated with the clinical state. In one particular monitoring appeared to be successful as sudden and persistent increase in latency of VEP accompanied brief slight changes in visual acuity. SEP, in contract, either remained constant if recordings were normal or, if abnormal, fluctuated widely without relationship to any change in clinical features. Similar fluctuations in abnormal BAEP in MS were observed by Robinson and Rudge[46].

Less detailed serial records repeated once or twice after a prolonged period in patients with definite MS have shown that with the passage of time the mean latency of a number of components increases in relation to the total disability rather than to clinical abnormalities in the systems tested[49]. In contrast, Confavreux et al.[50] found that the latency of VEP, measured at an interval of a year, was not related to the degree of disability, but increased in relapse, whether the visual system was clinically involved or not. Visual function does not, however, seem to have been examined in any detail.

Likosky and Elmore[51], among others, have claimed that fluctuations in EP values in the absence of any clinical change indicate subclinical relapses. This may be so, but is impossible to confirm without correlation with some other indication of disease activity such as myelin basic protein in the CSF or new plaques detected on NMR scan. Detailed monitoring of individual patients for disease activity and clinical status does not seem practicable, partly because we do not know the significance of the changes observed.


PATHOPHYSIOLOGY

Halliday and McDonald[52] interpreted the prolonged latency of VEP in ON as due to persistent demyelination and the reduction in amplitude to conduction block resulting from reversible swelling and oedema. The amplitude was shown to return rapidly to normal with restoration of visual acuity, while latency remained prolonged indefinitely. The role of oedema in the production of symptoms in MS is somewhat speculative and certainly amplitude of VEP can remain depressed long after an acute attack of ON.

It is well known that an increase in environmental temperature will cause reduction of visual acuity in MS patients with optic nerve lesions. This is in conformity with the observed effect of heat in causing conduction block in demyelinated fibers at physiological temperatures. In such patients raising body temperature results in a reduction in amplitude of VEP and cervical SEP with usually no effect on latency[53]. It therefore appears probable that persistent reduction in amplitude is due to conduction block of a proportion of fibers due to demyelination in contrast to any rapid changes noted in acute ON.

It is customary to speak of delay of the P100 in the visual system or of wave V in the BAEP but it is of course by no means certain that these waves are indeed the 'same' waves as those present in the normal, simply occurring later because of slowed conduction in the white matter. This may indeed to be but it has never been easy to explain, for example, a 40 msec delay in latency of the P100 in terms of slowed conduction through the optic nerve. McDonald[54],

however, calculated that a 1 cm long plaque could introduce a 25 msec delay.  The nature of the recorded response must surely be suscep- tible to such factors as conduction block in faster fibers and dis- persion of the potential due to unequal slowing in different fibers.

The mechanism of remission in MS remains mysterious but is clearly central to any hope of restoration of function by therapeutic means.  There is really no certain evidence of remyelination in MS and this is in accord with the persistence of abnormal EP, even when function has somehow returned to normal.  Restitution of normal latency of VEP has occasionally been observed[48] but usually in relation to significant improvement in acuity following acute ON. We[55] were recently able to demonstrate marked prolongation of latency, with normal vision, persisting for 3½ years at least, but returning to normal five years after the episode of ON.  This has proved to be a rare, but not isolated, occurrence on similar extended follow up, but if prolonged latency is due to demyelination, this finding suggests that delayed remyelination may occur.


CONCLUSION

The commercial availability of excellent equipment for recording EP has naturally led to excessive use of these techniques in the diagnosis of MS.  Early cases can seldom be detected and in more advanced disease such diagnostic aids are less often needed.  There are many technical and clinical pitfalls for the inexperienced.  In contrast, the potentialities of EP for investigating the pathophysi- ology of MS have not been fully exploited.


REFERENCES

1.  D. McAlpine, "Multiple Sclerosis: A Re-Appraisal," D. McAlpine, C.E. Lumsden and E.D. Acheson, eds., Churchill Livingstone, Edinburgh (1972).
2.  A. M. Halliday, W. I. McDonald and J. Mushin, Delayed visual evoked response in optic neuritis, Lancet, 1:982 (1972).
3.  A. Neetens, Y. Hendrata and J. van Rompaey, Pattern and flash visual evoked responses in multiple sclerosis, J.Neurol., 220:113 (1979).
4.  W. B. Matthews, D. G. Small, M. Small and E. Pountney, Pattern reversal evoked visual potential in the diagnosis of multiple sclerosis, J.Neurol.Neurosurg.Psychiat., 40:1009 (1977).
5.  F. Shahrokji, K. H. Chiappa and R. R. Young, Pattern shift visual evoked responses:  two hundred patients with optic neuritis and/or multiple sclerosis, Arch.Neurol., 35:65 (1979).
6.  A. M. Halliday, W. I. McDonald and J. Mushin, Visual evoked response in diagnosis of multiple sclerosis, Brit.Med.J., 4:661 (1973).

7.  M. Hennerici, D. Wenzel and H. J. Freund, The comparison of
    small-size rectangle and checkerboard stimulation for the
    evaluation of delayed visual evoked responses in patients
    suspected of multiple sclerosis, Brain, 100:119 (1977).
8.  F. Chain, J. Mallecourt, M. Leblanc and F. Lhermitte, Apport de
    l'enregistrement des potentials évoqués visuels au diagnostic
    de la sclérose en plaques, Rev.Neurol., 133:81 (1977).
9.  W. Trojaborg and E. Petersen, Visual and somatosensory evoked
    cortical potentials in multiple sclerosis, J.Neurol.
    Neurosurg.Psychiat., 42:323 (1979).
10. D. W. K. Collins, J. L. Black and F. L. Mastaglia, Pattern-
    reversal visual evoked potentials: method of analysis and
    results in multiple sclerosis, J.Neurol.Sci., 36:83 (1978).
11. P. Asselman, D. W. Chadwick and C. D. Marsden, Visual evoked
    responses in the diagnosis and management of patients
    suspected of multiple sclerosis, Brain, 98:261 (1975).
12. F. Shahrokji, K. H. Chiappa and R. R. Young, Pattern shift
    visual evoked responses, Arch.Neurol., 35:65 (1978).
13. K. Lowitzsch, Pattern-evoked visual potentials in 251 multiple
    sclerosis patients in relation to ophthalmological findings
    and diagnostic classification, in: "Progress in Multiple
    Sclerosis Research," H.J. Bauer, S. Poser and G. Ritter,
    eds., Springer, Berlin (1980).
14. G. G. Celesia and R. F. Daly, VECA - a new electrophysiological
    test for the diagnosis of optic nerve lesions, Neurology,
    27:637 (1977).
15. F. L. Mastaglia, J. L. Black and D. W. K. Collins, Visual and
    spinal evoked potentials in diagnosis of multiple sclerosis,
    Brit.Med.J., 2:732 (1976).
16. A. M. Halliday, Event-related potentials and their diagnostic
    usefulness, in: Motivation, Motor and Sensory Processes of
    the Brain, H.H. Kornhuber and L. Deecke, eds., Elsevier,
    Amsterdam (1980).
17. D. W. K. Collins, W. M. Carroll, J. L. Black and M. Walsh,
    Effect of refractive error on the visual evoked response,
    Brit.Med.J., 1:231 (1979).
18. W. I. McDonald and A. M. Halliday, Diagnosis and classification
    of multiple sclerosis, Brit.Med.Bull., 33:4 (1977).
19. W. B. Matthews, J. R. B. Wattam-Bell and E. Pountney, Evoked
    potentials in the diagnosis of multiple sclerosis:  a follow-
    up study, J.Neurol.Neurosurg.Psychiat., 45:303 (1982).
20. L. D. Blumhardt, G. Barrett and A. M. Halliday, The pattern
    visual evoked potential in the clinical assessment of undiag-
    nosed spinal cord disease, Adv.Neurol., 32:463 (1982).
21. L. Pedersen and W. Trojaborg, Visual auditory and somatosensory
    pathway involvement in hereditary verebellar ataxia,
    Friedreich's ataxia and familial spastic paraplegia, Electro
    enchep.Clin.Neurophysiol., 52:283 (1981).
22. E. J. Fine and M. Hallett, Neurophysiological study of subacute
    combined degeneration, J.Neurol.Sci., 45:331 (1980).

23. A. M. Halliday and W. I. McDonald, Pathophysiology of demyeli-
    nating disease, Brit.Med.Bull., 33:21 (1977).
24. B. Conrad, R. Benecke, H. Müsers, H. Prange and W. Behrens-
    Baumann, Visual evoked potentials in neurosyphilis, J.Neurol.
    Neurosurg.Psychiat., 46:23 (1983).
25. L. J. Streletz, R. A. Chambers, H. B. Sung and H. L. Israel,
    Visual evoked potentials in satcoidosis, Neurology, 31:1545
    (1981).
26. W. R. Brain and M. Wilkinson, The association of cervical
    spondylosis and disseminated sclerosis, Brain, 80:456 (1957).
27. C. Huber and T. Wagner, Electrophysiologic evidence for glauco-
    matous lesions in the optic nerve, Ophthalmol.Res., 10:22
    (1978).
28. K. Puvanendran, G. Devathasan and G. Wong, Visual evoked re-
    sponses in diabetes, J.Neurol.Neurosurg.Psychiat., 46:643
    (1983).
29. N. S. Namerow, Somatosensory evoked responses in multiple
    sclerosis patients with varying sensory loss, Neurology,
    18:1197 (1968).
30. W. B. Matthews, M. Beauchamp and D. G. Small, Cervical somato-
    sensory responses in man, Nature, 252:230 (1974).
31. S. J. Jones and D. G. Small, Spinal and sub-cortical evoked
    potentials following stimulation of the posterior tibial
    nerve in man, Electroenceph.Clin.Neurophysiol., 44:299
    (1978).
32. D. G. Small, W. B. Matthews and M. Small, The cervical somato-
    sensory evoked potentials in the diagnosis of multiple
    sclerosis, J.Neurol.Sci., 36:147 (1978).
33. J. B. Green, R. Price and S. G. Woodbury, Short-latency somato-
    sensory evoked potentials in multiple sclerosis: comparison
    with auditory and visual evoked potentials, Arch.Neurol.,
    37:630 (1980).
34. S. J. Purves, M. D. Low, J. Galloway and B. Reeves, A comparison
    of visual, brainstem auditory and somatosensory evoked poten-
    tials in multiple sclerosis, Acta Neurol.Scand., 62:220
    (1981).
35. W. B. Matthews, Somatosensory evoked potentials in retrobulbar
    neuritis, Lancet, 1:443 (1978).
36. J. J. Stockard, E. J. Stockard and F. W. Sharborough, Brainstem
    auditory evoked potentials in neurology: methodology, inter-
    pretation, clinical application, in: "Electrodiagnosis in
    Clinical Neurology," M.J. Aminoff, ed., Churchill
    Livingstone, New York (1980).
37. K. H. Chiappa, J. L. Harrison, E. B. Brooks and R. R. Young,
    Brainstem auditory evoked responses in 200 patients with
    multiple sclerosis, Ann.Neurol., 7:135 (1980).
38. J. J. Stockard, E. J. Stockard and F. W. Sharborough, Detection
    and localisation of occult lesions with brainstem auditory
    responses, Mayo Clin.Proc., 52:761 (1977).
39. P. Deltenre, A. Vercruysse, C. van Nechel, P. Ketelaer, A.
    Capon, F. Colin and J. Manil, Early diagnosis of multiple

sclerosis by combined multimodal evoked potentials, J.Biomed. Engineering, 1:17 (1979).

40. K. H. Chiappa, Pattern shift visual brainstem auditory, and short latency somatosensory evoked potentials in multiple sclerosis, Neurology, 30, 7 pt 2:110 (1980).

41. M. Kjaer, The value of brainstem auditory, visual and somatosensory evoked potentials and blink reflexes in the diagnosis of multiple sclerosis, Acta Neurol.Scand., 62:220 (1980).

42. S. Khoshbin and M. Hallett, Multiple evoked potentials and blink reflex in multiple sclerosis, Neurology, 31:138 (1981).

43. W. Tackmann, H. Strenge, R. Barth and A. Sojka-Raytscheff, Evaluation of various brain structures in multiple sclerosis with multimodality evoked potentials, blink reflex and nystagmography, J.Neurol., 224:33 (1980).

44. W. B. Matthews and M. Esiri, Multiple sclerosis plaque related to abnormal somatosensory evoked potentials, J.Neurol. Neurosurg.Psychiat., 42:940 (1979).

45. P. Deltenre, C. van Nechel, A. Vercruysse, S. Strul, A. Capon and P. Kietelaer, Results of a prospective study on the value of combined visual, somatosensory, brainstem auditory evoked potentials and blink reflex measurements for disclosing subclinical lesions in suspected multiple sclerosis, Adv.Neurol., 32:463 (1982).

46. K. Robinson and P. Rudge, The use of the auditory evoked potential in the diagnosis of multiple sclerosis, J.Neurol.Sci., 45:235 (1980).

47. F. L. Mastaglia, J. L. Black, L. A. Cala and D. W. K. Collins, Electrophysiology and avoidance of invasive neuroradiology in multiple sclerosis, Lancet, 1:144 (1980).

48. W. B. Matthews and D. G. Small, Serial recording of visual and somatosensory evoked potentials in multiple sclerosis, J.Neurol.Sci., 40:11 (1979).

49. J. C. Walsh, R. Garrick, J. Cameron and J. G. McLeod, Evoked potential changes in clinically definite multiple sclerosis, J.Neurol.Neurosurg.Psychiat., 45:494 (1982).

50. Ch. Confavreux, F. Mauguière, J. Courjon, G. Aimard and M. Devic, Evolution des potentiels évoqués visual dans la sclérose en plaques, Rev.Neurol., 137:121 (1981).

51. W. Likosky and R. S. Elmore, Exacerbation detection in multiple sclerosis by clinical and evoked potential techniques: a preliminary report, Adv.Neurol., 32:535 (1982).

52. A. M. Halliday and W. I. McDonald, Pathophysiology of multiple sclerosis, Brit.Med.Bull., 33:21 (1977).

53. W. B. Matthews, D. J. Read and E. Pountney, Effect of raising body temperature on visual and somatosensory evoked potentials in patients with multiple sclerosis, J.Neurol. Neurosurg.Psychiat., 42:250 (1979).

54. W. I. McDonald, Pathophysiology of conduction in central nerve fibres, in: "Visual Evoked Potentials in Man," J.E. Desmedt, ed., Clarendon Press, Oxford (1977).

55.  W. B. Matthews and M. Small, Prolonged follow up of abnormal
        visual evoked potentials in multiple sclerosis:  evidence for
        delayed recovery, J.Neurol.Neurosurg.Psychiat., 46:639
        (1983).


DISCUSSION

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     I am delighted to learn from Professor Matthews that evoked
potentials have more variability in MS than my reading of the litera-
ture had led me to believe.  As I mentioned in my address on the
opening day, we have been interested in using the evoked potential
technique in EAE partly to confirm objectively our relatively crude
neurological examinations of monkeys, partly to detect subclinical
changes that might better define the onset of EAE and partly to
follow the course during remissions and exacerbations to see if EAE
and MS resemble each other or not.

     The definition of the onset of a disease obviously depends on
the techniques used to evaluate the disease:  clinical, histological,
chemical, physiological, etc.  In our experiments evaluating various
treatments of experimental allergic encephalomyelitis (EAE), we have
generally used purely clinical observations to define the onset of
mild, moderate or severe EAE (Shaw et al., 1976; Alvord et al.,
1979b).  In attempts to make more objective observations not only to
corroborate our clinical observations but also to be compared with
similar observations in humans with multiple sclerosis (MS) and other
diseases, we have been studying changes in the cerebrospinal fluid
(CSF) and in evoked potentials (Alvord et al., 1979a; Hruby and
Alvord, 1980; Slimp and Alvord, 1982).  We have used measurements of
evoked cortical potentials to visual and somatosensory stimulation
and evoked brainstem potentials to auditory stimulation as objective
in vivo indicators of the probable sites of certain lesions; measure-
ments of albumin and immunoglobulin (IgG) in the CSF as indicators of
the state of the blood-CSF barrier; and measurements of myelin basic
protein (BP) and anti-BP antibodies in the CSF as indicators of
damage to myelin and immunologic response to this antigenic stimu-
lation.

     Male monkeys (macaca fascicularis), weighing 2-3 Kg, were
injected in the hind foot pads with 0.1 ml of a water-in-oil emulsion
containing 5 mg BP (pig or monkey) and 0.5 mg heat-killed myco-
bacterium tuberculosis.  Each animal was obtained from the University
of Washington Regional Primate Center, where the animals were also
quarantined and housed.  The Center conforms to the National
Institutes of Health Standards for the care and use of experimental
animals.

All animals were observed daily, one half especially carefully as we were looking for the first suggestive signs of EAE, such as abnormal behavior, anorexia or enlarged pupils, at which time evoked cortical potentials were recorded, CSF was obtained by lumbar puncture and blood was obtained from the femoral vein for the preparation of serum. At the appearance of definite signs for EAE, such as blindness, weakness or ataxia, most of the animals were treated with BP and/or dexamethazone by implanting subcutaneously one or more osmotic minipumps (Alza Corporation, 950 Page Mill Road, Pal Alto, California 94304). The animals remained under close clinical observation and evoked potentials and CSF samples were taken repeatedly throughout the periods of recovery and of any subsequent relapse.

Analyses of evoked potentials have been described in detail elsewhere (Kraft and Slimp, 1983). For the purpose of this presentation the latencies and amplitudes obtained in normal monkeys were averaged, the standard deviations calculated, and abnormalities considered definite if they exceeded 3x SD and questionable if between 2x and 3x SD.

In the 31 monkeys we observed 50 attacks of EAE, in 27 of which the observations of CSF and evoked potentials could be simultaneously correlated with the clinical observations at the onset of the attach. In 17 attacks the recordings were actually on the same day, and in the initial tabulations these were kept separate. An additional 4 attacks had recordings within the next day and another 6 within 2 to 3 days; since these were very similar to the first 17, they were combined in the tables which follow. The day of onset was defined as that day when any definite laboratory or clinical abnormality was recorded.

Analysis of these 27 attacks (Table 1) reveals that 20 were characterized by definite abnormalities in evoked potentials and CSF. Of these, 10 animals were definitely abnormal clinically but 9 were only questionably abnormal clinically and 1 actually appeared normal. Evoked potentials were definitely abnormal at the onset of EAE in 20 (74%), CSF abnormal in 25 (93%) and clinical abnormalities definitely present in only 10 (37%). If questionable clinical abnormalities are included, this last increases to 21 (78%).

The specific abnormalities of the evoked cortical potentials are analyzed in Table 2. The expected changes (i.e. an increase in latency and/or decrease in amplitude) were most commonly observed in the auditory and leg systems, whereas the reverse (facilitated or paradoxical) changes (i.e. a decrease in latency and/or increase in amplitude) were most often seen in the visual system. The visual system was least often normal, the auditory most. All 4 of the systems tested were simultaneously abnormal 5 times, 3 systems 10 times, 2 systems 3 times and 1 system 9 times.

Table 1.  Correlation of clinical, electrophysiological and CSF
           observations at the onset in each of 27 attacks of EAE.

| Evoked potentials | CSF | Clinical Observations | | | Total |
|---|---|---|---|---|---|
| | | 0 | ? | + | |
| N | N | | | | 0 |
| | Abn. | | 1 | | 1 |
| ? | N | | | | 0 |
| | Abn. | 4 | | | 4 |
| Abn. | N | 1 | 1 | | 2 |
| | Abn. | 1 | 9 | 10 | 20 |
| Total | | 6 | 11 | 10 | 27 |

Table 2.  Abnormalities in evoked potentials at onset of EAE.

| | Evoked cortical potentials* | | | |
|---|---|---|---|---|
| | visual | auditory | arm | leg |
| A.  No response | 2 | | | |
|     Increased latency | 2(3) | 10(2) | 7(2) | 10(1) |
|     Decreased amplitude | 2(2) | 1(1) | 2(2) | 2(3) |
| B.  Decreased latency | 7(3) | | 1(0) | 1(2) |
|     Increased amplitude | 1(0) | | 4(2) | 1(2) |
| C.  Normal | 10 | 14 | 11 | 10 |

*The first number represents definite, the number in parentheses
 questionable abnormalities.  Definite abnormalities exceeded 3x SD,
 and questionable abnormalities between 2x and 3x SD.

A rather remarkable coincidence appears from these studies,
namely that about 75% of attacks of EAE were first detectable by each
of 4 of the 5 major techniques employed:  clinical observations
(including questionable signs), evoked potentials, anti-BP antibodies
in the CSF and damage to the BBB.  However, if one eliminates the
subtle or questionable clinical signs, only 37% of attacks were
defined clinically.  The 5th measure, an increase in BP in the CSF,
occurred at the onset of EAE in only about 25%, but this may relate
to the early appearance of anti-BP antibodies precipitating most of
the BP from the CSF.

Such variability in definition of the onset of EAE can be under-
stood most easily when one recognizes that EAE is a multifocal
disease, not a diffuse one, in which different parts of the CSC-CSF
can be rather independently affected.  On the one hand, anyone who
has looked at the histologic preparations in EAE must admit this

variability; on the other hand, anyone who regards BP as occurring exclusively in myelin must admit to being surprised that the blood-CSF barrier may be involved without significant involvement of the white matter in which the tissue-specific antigen is located.

With regard to recovery from EAE, Dr. Jefferson Slimp tells me that of 23 occurrences of clinical EAE with clinical recovery thus far studied in 17 monkeys, over half of these showed concomitant improvement in evoked potentials. About one-fourth showed no change and a few actually worsened. Improvement occurred equally often in the four sensory systems tested; however, the brainstem auditory evoked potentials became worse during recovery three times as often as any other system. Six bouts of EAE were followed by complete recovery of all evoked potentials to normal. Quite often this involved three or four sensory systems. Of the cases with partial improvement, however, it was characteristic that only one system returned to normal.

Thus, while the clinical recovery from EAE may appear to be complete, evoked potentials may remain abnormal in as many as two-thirds of the animals. Such subclinical abnormalities should not be surprising. The variable nature of the improvement of the evoked potentials emphasizes again that EAE is a multifocal disease with significant similarities with MS.

REFERENCES

Alvord, E. C., Jr., Hruby, S., and Sires, L. R., 1979a, Degradation of myelin basic protein by cerebrospinal fluid: Preservation of antigenic determinants under physiological conditions, Ann.Neurol., 6:474-482.
Alvord, E. C., Jr., Shaw, C. M., and Hruby, S., 1979b, Myelin basic protein treatment of experimental allergic encephalomyelitis in monkeys, Ann.Neurol., 6:469-473.
Hruby, S., and Alvord, E. C., Jr., 1980, Studies on the proteolytic activity of the cerebro-spinal fluid in multiple sclerosis and other neurological diseases, Clin.Chem., 26:1013.
Kraft, G. H., and Slimp, J. C., 1984, Electrophysiological monitoring of experimental allergic neuritis and experimental allergic encephalomyelitis, in: "Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis," E.C. Alvord, Jr., M.W. Kies, and A.J. Suckling, eds., Alan R. Liss, Inc., NYC.
Shaw, C. M., Alvord, E. C., Jr., and Hruby, S., 1976, Treatment of experimental allergic encephalomyelitis in monkeys. I. Clinical studies, in: "The Aetiology and Pathogenesis of the Demyelinating Diseases," H. Shiraki, T. Yonezawa, and Y. Kuroiwa, eds., Japan Science Press, Tokyo, pp. 367-376.
Slimp, J. C., and Alvord, E. C., Jr., 1982, Somatosensory visual, and auditory evoked potentials in monkeys with experimental allergic encephalomyelitis (EAE), Neurosci.Abst., 8:351.

CLINICAL IMPLICATIONS OF STUDIES INVOLVING

CEREBROSPINAL FLUID T CELL SUBPOPULATIONS

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INTRODUCTION

Multiple sclerosis (MS) is a demyelinating central nervous system (CNS) affliction manifested by exacerbations and remissions of clinical symptoms.

It has been recognized that the progressive course of MS may be associated with changes in the proportion of peripheral blood (PB) and cerebrospinal fluid (CSF) T cell subpopulations and B cells.

In PB, an exacerbation of MS may be reflected by decreased percentages of T cells,[1,2] Fc$\gamma$ cells,[3] and T-suppressor-cytotoxic (OKT$_8$+) cells[4]. The T helper-inducer (OKT$_4$+)/OKT$_8$+ cell ratio may be increased[4,5,6] and there may be an increased percentage of B cells[7] and/or their clonal restriction[8].

In CSF, the relapse and/or progressive course of MS is manifested by an increased percentage of T cells[9,6] a decreased percentage of Fc$\gamma$ cells[10] and an increased percentage of OKT$_4$ cells [11,12]. The percentage of OKT$_8$+ cells may be decreased with an increased OKT$_4$+/OKT$_8$+ cells ratio[13]. Sandberg-Wollheim[6], in her series, found, in active MS, decreased percentage of CSF OKT$_8$+ cells, but the reduction was not satistically significant.

Weiner et al.,[14] on examining CSF in 21 MS patients, and Hauser et al.,[15] on CSF studies in 40 MS patients did not establish

characteristic changes in CSF phenotypes which could be related to
changes in circulating $OKT_{8+}$ cells or to disease activity.  In their
series, Hauser and his coworkers[15] did not find evidence that in
patients with low numbers of PB $OKT_8$+ cells, an accumulation of $OKT_8$+
cells would occur in CSF.  In four age and sex matched controls
followed with six MS patients over a four to six month period at
weekly intervals, Hauser et al.,[16] did not establish abnormalities
in PB $OKT_8$+ or $OKT_4$+ cells.  In two of the MS patients studied, an
elevated PB $OKT_4$+/$OKT_8$+ cell ratio was associated only with fatigue.
Huddlestone and Oldstone[17] noticed increased numbers of PB Fcγ
cells and/or $OKT_8$+ cells in MS patients in remission following an
acute episode of MS.

    In this presentation, we are comparing findings in our MS
patients with the above mentioned observations.


MATERIALS AND METHODS

    In 132 patients with definite MS (92 females, average age 38.7 ±
9.7 years and 40 males, average age 38.6 ± 9.3 years) the percentage
of PB T, Fcγ, $OKT_8$+, $OKT_4$+, and B cells was determined.  In 87 of
these patients, more than one examination was performed (4.15 ± 2.21
examinations per one patient) over a period of time extending over
13.5 ± 8.1 months.  In 44 MS patients with progressive neurological
symptomatology observed up to one month prior to the CSF examination,
the percentages of CSF $OKT_8$+, $OKT_4$+, T and/or Fcγ cells were also
established.

    The generally used techniques applied on examination for CSF T
cell subpopulations were modified to obtain the maximum number of
preserved mononuclear cells from the 6-8 ml of CSF submitted for a
routine CSF examination.

    As soon as possible following the spinal tap, the CSF specimen,
in a 15 ml plastic conical centrifuge tube, was centrifuged at 400 g
for 30 minutes at 5° C.  One ml of CSF was left at the bottom of the
tube and the remaining CSF was thereafter carefully removed.  The
cells collected at the bottom of the tube were then gently re-
suspended and divided into four labeled 12 x 75 mm tubes using a
serologic pipet.  0.15 ml of AET-treated sheep red blood cells were
added in the tube for examination of rosetting T cells.  0.15 ml of
ox IgG coated red blood cells were placed in the tube for examination
of the Fcγ cells.  Following spinning at 200 g at 5°C for five
minutes, the tubes were kept on ice for one hour.  Thereafter, the
supernatant was carefully removed, leaving 0.3 ml of the cell sus-
pension at the bottom of the tube.  The cells were subsequently
gently resuspended and rosetting cells were counted in a hemacyto-
meter.  For examination of the T suppressor-cytotoxic cells and the
helper cells, 5 microliters of reconstituted Ortho-mune $OKT_8$ and $OKT_4$

antibody solutions (Ortho Pharmaceutical Co., Raritan, NJ) were added
to the remaining two tubes.  Following incubation on ice for 30
minutes, one drop of a goat-anti-mouse IgG fluorescein conjugated
serum (Meloy Lab., Inc., Springfield, VA) was placed in each tube
using a Pasteur pipette.  After incubation on ice for an additional
30 minutes, 2 ml of PBS were added to each tube and the tubes were
centrifuged at 300 g for 10 minutes at 5°C.  The supernatant was
subsequently removed leaving approximately 20 microliters of the cell
suspension in the tube.  Following gentle resuspension of cells, 10
microliters of the cell suspension were released from the pipette
onto a coverslip.  The labeled slide was then carefully placed over
the coverslip which was sealed with vasoline and the slide was read
without delay.

In case a larger amount of CSF is submitted for examination, or
there is a special reason to establish subgroups of CSF B cells,
fluorescein conjugated antisera to immunoglobulins G, A, M and D
and/or to kappa and lambda light chains may be used similarly as the
above mentioned solutions of monoclonal antibodies.

The median number of CSF mononuclear cells evaluated on deter-
mination of the percentages of T and $Fc\gamma$ cells was 67 and of the
$OKT_4+$ and $OKT_8+$ cells was 50.  The CSF specimen examination also
included cell count and cytomorphology; cellulose polyacetate protein
electrophoresis, immunoelectrophoresis using antisera to human serum,
Fab fragments of IgG, kappa and lambda chains, and determination of
the IgG concentration and the IgG/Albumin ratio as previously des-
cribed[18].  Protein electrophoresis, immunoelectrophoresis and
examination of IgG, IgM and IgA levels in the corresponding serum
specimens were also performed.

The percentages of PB T, $Fc\gamma$, $OKT_8+$, $OKT_4+$ and B cells were
determined in 30 control subjects (average age 37.5 ± 9.9 years).  In
13 of them, more than one examination (2.8 ± 1.0) was performed over
15.6 ± 8.2 months.  In addition, the percentages of CSF T cells
(N=22, 86.59 ± 8.16), $Fc\gamma$ cells (N=20, 19.70 ± 10.64), $OKT_4+$ (N=12,
47.33 ± 16.04), and $OKT_8+$ cells (N=20, 30.97 ± 13.00) were determined
in a control group of individuals without neuropsychiatric symptoms
and with normal CSF cell count, total proteins, CSF cytomorphology
and protein electrophoresis.  For statistical evaluation, Spearman
and Kendall correlation coefficients, Wilcoxon, Kruskal-Wallis, Van
Der Waerden, Median and Savage tests were applied.


RESULTS

In the controls without neuropsychiatric symptoms, the percent-
ages of CSF T cells and $Fc\gamma$ cells were found to be higher as compared
to PB in control subjects (T cells 73.36 ± 8.33; $Fc\gamma$ cells 12.56 ±
6.76).

In our series of MS patients with progressive neurological
symptomatology (N=44) the average CSF cell count was 11.09 ± 9.24
white blood cells per cubic millimeter.  The average percentage of
CSF lymphocytes was 91.21 ± 5.92.  In the MS patients studied, the
percentage of CSF T cells (90.23 ± 8.14) was higher compared to the
corresponding PB T cells (p < 0.001) and only moderately elevated
compared to normal CSF specimens (p < 0.040).  In MS patients with
progressive neurological symptoms, the percentages of CSF Fcγ cells
and OKT$_8$+ cells were lower (6.40 ± 5.91, p < 0.001; 22.53 ± 10.87, p
< 0.012) compared to controls.  In contrast, the percentage of OKT$_4$+
cells (61.67 ± 12.87, p < 0.011) was higher.  In patients with pro-
gressive MS, the percentage of PB B cells was found to be higher
(23.98 ± 9.17) as compared to controls (18.33 ± 7.67, p < 0.008) and
to MS patients with stable neurological symptoms (19.28 ± 8.16, p <
0.036).  In these patients, the percentage of PB lymphocytes, carry-
ing IgG (9.65 ± 5.03), was higher when compared to individuals with
stable MS (6.65 ± 3.81, p < 0.007) and controls (6.00 ± 8.57, p <
0.002).

In MS patients with progressive neurological symptoms, a nega-
tive correlation was noticed between the percentage of CSF lympho-
cytes and the CSF OKT$_4$+/OKT$_8$+ cell ration (p < 0.018), and between
the percentage of CSF lymphocytes and the percentage of PB lympho-
cytes stained with fluorescein conjugated antiserum to kappa light
chains (p < 0.006).  There was an indication of a positive corre-
lation between the CSF IgG concentration and the PB OKT$_4$/OKT$_8$+ cell
ration (p < 0.044).

In MS patients presenting with progressive neurological symp-
toms, the most frequent abnormalities found were a decreased per-
centage of Fcγ cells (86%) and a decreased OKT$_8$+/OKT$_4$+ cell ratio
(75%) in the CSF.  There was no correlation between the percentage of
CSF Fcγ cells and the CSF OKT$_8$+/OKT$_4$+ cell ratio.  In 41% of MS
patients with progressive neurological symptoms, an increased per-
centage of CSF OKT$_4$+ cells was observed.  A decreased percentage of
CSF OKT$_8$+ cells was found in 32% of MS patients in this series.

In 14% of MS patients with progressive neurological symptoms, a
decreased percentage of CSF Fcγ cells was found in individuals with
a normal CSF OKT$_8$+/OKT$_4$+ cell ratio.  In 9% of the MS patients with
progressive symptoms, examination of CSF and PB for T cell subpopu-
lations revealed abnormal findings only in the CSF.  Considering MS
patients with progressive neurological symptoms in whom only one of
the PB immunologic parameters studied was abnormal (one patient with
a decreased percentage of T cells, an increased percentage of OKT$_4$+
cells, and an increased percentage of OKT$_3$+ cells, and two patients
with an increased percentage of B cells) and patients with progress-
ive MS with normal PB studies, negative or inconclusive findings in
PB were obtained in 20% of MS patients with progressive neurological
symptoms in whom simultaneous CSF examination revealed definite
abnormalities in the distribution of T cell subpopulations.

     In 87 MS patients with repeated PB studies, marked changes in
the percentages of PB, T, Fcγ, $OKT_4+$, $OKT_8+$, and B cells were
noticed, not infrequently with nonsignificant changes in their
objective neurological symptomatology.  In 13 controls subjects, with
a total of 37 PB examinations over a period of time extending up to
two years, a decreased percentage of Fcγ cells was established on one
examination (2.7%), a decreased $OKT_8+/OKT_4+$ cell ratio in two
instances (5.4%) increased percentage of B cells in three instances
(8.1%), and a decreased percentage of T cells on seven examinations
(18.9%).


DISCUSSION

     In MS patients with progressive objective or subjective neuro-
logical symptomatology, abnormal distribution of CSF T cell subsets
may be expected in about 20% of individuals with nonrevealing PB
studies.  MS patients with progressive neurological symptoms may have
a decreased percentage of Fcγ cells in CSF or PB in the absence of
abnormalities in the distribution of $OKT_4+$, and/or $OKT_8+$ cells.  In
age and sex matched controls of our MS patients, transient decrease
or increase in the percentages of PB T cells and their subpopulations
and/or B cells were found in the absence of abnormal clinical signs.
However, the incidence of abnormal findings in controls was lower as
compared to MS patients with a decreased percentage of PB Fcγ cells
being the least frequent and decreased percentage of rosetting T
cells, the most frequent abnormalities.  Abnormal percentages in PB
Fcγ, $OKT_4+$, $OKT_8+$, and B cells may be found in MS patients complain-
ing of progressive motor deficits, muscle spasticity or increasing
fatigue without objective signs of worsening in neurological symp-
toms.  Similarly, in two of four MS patients studied, who indicated
progressive subjective neurological symptoms, abnormalities in the
distribution of CSF T cell subpopulations were found.  In agreement
with Hauser et al.,[15] we were unable to observe an accumulation of
$OKT_8+$ cells in CSF in MS patients showing decreased percentages of PB
$OKT_8+$ cells.

     In view of the reported predominance of $OKT_4+$ cells in peri-
vascular infiltrates in active demyelinating brain lesions[19,20] the
negative correlation between the percentage of CSF lymphocytes and
the CSF $OKT_4+/OKT_8+$ cell ratio in individuals with progressive MS and
an elevated CSF cell count and the higher incidence of an increased
percentage of CSF $OKT_4+$ cells (41%) compared to PB (25%) in the MS
patients studied, the abnormal proportion of the T cell subsets
established is very similar to the $OKT_4+$, and $OKT_8+$ cell distribution
in lungs and PB of individuals with sarcoidosis and high intensity
alveolitis[21] and in skin lesions of the tuberculoid form of leprosy
[22].

Pathogenetic mechanisms in the active phase of MS appear to be mediated predominantly by $OKT_4+$ cells crossing the blood/brain barrier and by the subsequently released lymphokines. The lymphokines possibly induce alteration of myelin and activate a restricted number of locally available B cell clones which produce predominantly IgG with resulting oligoclonal gammopathy manifested most noticeably in the CSF.

Because there are no indications of significant, persistent correlations between PB T cells, T cell subsets and B cells in MS patients on repeated longitudinal studies, one can postulate that in MS patients, the immunopathologic process, controlled by the extra-neural immune system, and particularly the immunopathologic mechanisms inside the blood/brain barrier, present themselves in overlaping phases involving, in succession, immunoregulatory T cells, B cells and the secondary production of immunoglobulins.

## SUMMARY

The active phase of multiple sclerosis is associated with abnormal proportion of T cell subpopulations, more frequently demonstrated in the cerebrospinal fluid as compared to peripheral blood. No significant correlations were found between T, Fc$\gamma$, $OKT_4+$, and $OKT_8+$ cells in cerebrospinal fluid and peripheral blood of multiple sclerosis patients. In progressive multiple sclerosis, a decreased percentage of cerebrospinal fluid and circulating Fc$\gamma$ cells may be found in absence of abnormalities in the percentage of $OKT_4+$, and/or $OKT_8+$ cells. An understanding of the dynamic changes in the proportion of T cells, their subsets and B cells in cerebrospinal fluid and peripheral blood of multiple sclerosis patients is essential for optimal application of intensive immunoregulatory therapy in patients with active multiple sclerosis.

## Acknowledgements

## REFERENCES

1.  R. P. Lisak, A. I. Levinson, B. Zweiman, and N. I. Abdou, T and B lymphocytes in multiple sclerosis, Clin.Exp.Immunol., 22:30 (1975).
2.  H. J. Sagar and I. D. Allonby, Lymphocyte subpopulations in multiple sclerosis, J.neurol Sci., 43:133 (1979).

3.  J. R. Huddlestone and M. B. A. Oldstone, T suppressor ($T_G$)
    lymphocytes fluctuate in parallel with changes in the clinical
    course of patients with multiple sclerosis, J.Immunol 123:1615
    (1979).
4.  E. L. Reinherz, H. L. Weiner, S. L. Hauser, J. A. Cohen, J. A.
    Distaso, and S. F. Schlossman, Loss of suppressor T cells in
    active multiple sclerosis, N.Engl.J.Med., 303:125 (1980).
5.  M.-A. Bach, F. Phan-Dinh-Tuy, E. Tournier, L. Chatenoud, J.-F.
    Bach, C. Martin, and J.-D. Degos, Deficit of suppressor T
    cells in active multiple sclerosis, Lancet 2:1221 (1980).
6.  J. Sandberg-Wollheim, Lymphocyte populations in the
    cerebrospinal fluid and peripheral blood of patients with
    multiple sclerosis and optic neuritis, Scan.J.Immunol. 17:575
    (1983).
7.  J. F. Oger, B. G. W. Arnason, S. H. Wray, and J. P. Kistler, A
    study of B and T cells in multiple sclerosis, Neurology 25:444
    (1975).
8.  S. L. Hauser, H. L. Weiner, and K. A. Ault, Clonally restricted
    B cells in peripheral blood of multiple sclerosis patients:
    Kappa/Lambda staining patterns, Ann.Neurol 11:408 (1981).
9.  J. C. Allen, W. Sheremata, J. B. R. Cosgrove, K. Osterland, and
    M. Shea, Cerebrospinal fluid T and B lymphocyte kinetics
    related to exacerbations of multiple sclerosis, Neurology
    26:579 (1976).
10. P. K. Coyle, B. R. Brooks, R. L. Hirsch, S. R. Cohen, P. O'
    Donnell, R. T. Johnson, and J. S. Wolinsky,
    Cerebrospinal-Fluid lymphocyte populations and immune com-
    plexes in active multiple sclerosis, Lancet 2:229 (1980).
11. H. S. Panitch and G. S. Francis, T-lymphocyte subsets in
    cerebrospinal fluid in multiple sclerosis, N.Eng.J.Med.,
    397:560 (1982).
12. J. Oger, J. P. Antel, A. Noronha, and B. G. W. Arnason, Changes
    in T-cell subpopulations in the cerebrospinal fluid of multi-
    ple sclerosis patients, Neurology 32:A148 (1982).
13. N. Cashman, C. Martin, J.-F Eizenbaum, J.-D. Degos and M.-A.
    Bach, Monoclonal antibody-defined immunoregulatory cells in
    multiple sclerosis cerebrospinal fluid, J.Clin.Invest., 70:387
    (1982).
14. H. Weiner, S. L. Hauser, S. F. Schlossman, and E. L. Reinherz,
    Analysis of CSF mononuclear cells in multiple sclerosis using
    monoclonal antibodies:  Correlation with disease activity and
    peripheral blood T-cell subset changes, Neurology 33:A165
    (1982).
15. S. L. Hauser, E. L. Reinherz, C. J. Hoban, S. F. Schlossman and
    H. L. Weiner, CSF cells in multiple sclerosis, Monoclonal
    antibody analysis and relationship to peripheral blood T-cell
    subsets, Neurology 33:575 (1983).
16. S. L. Hauser, E. L. Reinherz, C. J. Hoban, S. F. Schlossman and
    H. L. Weiner, Immunoregulatory T-cells and lymphocytotoxic
    antibodies in active multiple sclerosis:  Weekly analysis over
    a six-month period, Ann Neurol., 13:418 (1983).

17. J. R. Huddlestone and M. B. A. Oldstone, Suppressor T cells are activated in vivo in patients with multiple sclerosis coinciding with remission from attack, J.Immunol., 129:915 (1982).
18. O. J. Kolar, P. H. Rice, F. H. Jones, R. J. DeFalque, and J. Kincaid, Cerebrospinal fluid immunoelectrophoresis in multiple sclerosis, J.Neurol Sci., 47:221 (1980).
19. H. Nyland, R. Matre, S. Mørk, J.-R. Bjerke, and A. Naess, T-lymphocyte subpopulations in multiple-sclerosis lesions, N.Engl.J.Med., 307:1643 (1982).
20. C. J. J. Brinkman, H. H. ter Laak, O. R. Hommes, S. Poppema, and P. Delmotte, T-lymphocyte subpopulations in multiple sclerosis lesions, N.Engl.J.Med., 307:1644 (1982).
21. G. W Hunninghake and R. G. Crystal, A disorder mediated by excess helper T-lymphocyte activity at sites of disease activity, N.Engl.J.Med., 305:429 (1981).
22. W. C. Van Voorhis, G. Kaplan, E. N. Sarno, M. A. Horwitz, R. M. Steinman, W. R. Levis, N. Nogueira, L. S. Hair, C. R. Gattass, B. A. Arrick, and Z. A. Cohn, The cutaneous infiltrates of leprosy: Cellular characteristics and the predominant T-cell phenotypes, N.Engl.J.Med., 307:1593 (1982).

DISCUSSION

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Dr. Kolar has contrasted his results with those of other groups and indicated the differences in results obtained in different series. These differences are clearly of concern if one expects the results to influence treatment. Why is this variability present? First, one can wonder whether the monoclonal antibodies presently used as markers for lymphocyte subpopulations are the best ones, or whether they are used merely because they are the first ones. Second, one can question whether the study of cerebrospinal fluid (CSF) cells adequately reflects the status and activity of inflammatory cells within the central nervous system (CNS). Third, flow cytometry, rather than fluorescent microscopic enumeration of the cells, may be a preferable and more accurate means of analyzing the small numbers of CSF lymphocytes; it is also possible of course that flow cytometry may be measuring somewhat different parameters. Last, some of the clinical correlations of studies may be flawed because of difficulties in assessing disease activity; the availability in certain centers of nuclear magnetic resonance and position emission tompgraphy may improve our ability to determine disease activity.

Despite these controversies there is a general agreement concerning the presence of an increased number of T cells in the CSF and a decline in suppressor cells in blood and the CNS. Suppressor T cells may not only differ in number in MS but also may be <u>functionally</u> different. The reason for the decline and changes in the suppressor T subpopulation is unclear. Several explanations have been suggested. (1) Suppressor T cells move from the blood into the CNS compartment. The decreased number of T cells in the CSF and their absence from the perimeter of the CNS parenchyma makes this hypothesis unlikely, although it is also possible that our autopsy CNS studies have not been idea. (2) Suppressor T cells are destroyed. (3) Suppressor T cells are immunologically modulated under the influence of anti-lymphocyte antibodies, which are known to occur in multiple sclerosis (MS). Antibody could strip the cell surface markers, reduce suppressor activity, but not destroy the cell. The absence of cytodestruction seen with lymphocyte antigenic modulation may make future prospects of monoclonal antibody immunotherapy difficult.

What is the importance of a decline in suppressor cells? The suppressor cell decline may merely reflect the immunodysregulatory state, i.e. the suppressor cells are nonsense T cells directed against antigens irrelevant to MS pathogenesis (or perhaps "missense" T cells is a better expression). Or perhaps the suppressor cell decline may permit the production of an important pathogenic antibody. Alternatively, the suppressor cell decrease could be the result of a pathologic antibody directed against white matter which happens to cross-react with suppressor cells. The availability of MS T cell clones may make studies of their antigenic target(s) possible.

IMMUNOLOGICAL TREATMENTS IN MULTIPLE SCLEROSIS:

RATIONALE, RESULTS AND NEW AVENUES

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INTRODUCTION

Cytotoxic drugs have been used in multiple sclerosis (MS) since 1966. At that time however, our understanding of immune mechanisms involved in the development of the disease was very limited.

At first, the rational of immunotherapy in MS was based on the existence of an increased antibody production in the central nervous system (CNS), revealed by the oligoclonal aspect of immunoglobulins G (IgG) in the cerebrospinal fluid (CSF). This humoral immune response appeared to be of importance in the pathogenesis of the disease, and the goal was therefore to abrogate this abnormal IgG's synthesis by killing the antibody producing B cells.

Since then, it appeared that most of the CSF antibodies and IgG production are non relevant phenomena that do not correlate either with the disease activity or with its pathogenesis, and that imbalance in cellular immunity is probably more important. From the other side, our knowledge of the effects of cytostatic or cytotoxic agents on the immune system is progressing and those substances are used more and more frequently as immunomodulators rather than as cytotoxic drugs. Moreover, non cytotoxic immunomodulators have been recently proposed even though their exact mechanisms of action remain to be defined.

Our concepts concerning the pathogenesis of MS are certainly less obscure than 10 years ago, and the first table summarizes what we know and what remains controversial concerning the putative causes and biological mechanisms involved in the disease progression.

Table 1.  Possible Pathogenic Mechanisms in Multiple Sclerosis

| What we know | What we do no know |
|---|---|
| Causes: | Causes: |
| External factor | Virus(es)? |
| Associated immune | |
| processes | Auto-immune disease? |
| Genetic background | Nature of the genetic predisposition? |
| Mechanisms: | Mechanisms: |
| Demyelination | Target: oligo and/or myelin sheath? |
| Immune process | Humoral and/or cellular? |
| Immune injury | Antibody + complement? |
| | Immune complexes? |
| | Cytotoxic T cells? |
| Immune imbalance | Antibody dependant cellular toxicity? |
| (H/S) associated to | |
| disease activity | Loss of S cells induces demyelination? |
| | Demyelination induces loss of S cells? |
| | Both induced by a third factor? |

There are still more questions than answers, but we have to accept that abnormal immune reactions are associated with the evolution of the disease, particularly during active phases.

In spite of all those uncertainties, concerning both the immune basis of MS pathogenesis and the exact mode of action of immunosuppressors, considerable efforts have been made since 1966 to reduce or correct immune abnormalities observed in MS.  The aim was of course to influence the inexorable evolution of the disease in the long run by reducing the relapse rate frequency and above all, the slowly progressing neurological and functional disability.

The first paper dealing with immunosuppression in MS: "Sclérose en Plaques et Processus d'Auto-Immunisation: Traitement par les Antimitotiques" was published in 1966 by Aimard et al., Cyclophosphamide was used but, because of its subjective intolerance, it was soon replaced by Azathioprine.  Since this first attempt, many other clinical studies have been published using various substances, and from 1966 to 1977, nearly 800 MS patients have been treated.

The results of those efforts were summarized during the First Workshop on Immunosuppressive Treatments (April 1,2 - 1977 Melsbroek, Belgium).  At that time, some beneficial effects were found in uncontrolled studies, and adverse reactions appeared acceptable.  It was therefore decided to continue, using a methodology as strict as possible and particularly:

- controlled groups and when feasible, blind studies
- appreciation of the progression of the disease determined by usual disability and/or neurological scales
- large number of patients and a follow-up for at least 3-4 years
- treatment begun within the first 10 years of the evolution when a progression of the disease is still to be expected.

The purpose of this paper is to review the clinical trials published since 1977, using different techniques (Table 2). Some of them have been discussed during the Second Workshop on Immunosuppressive Treatments in MS (June 10,11,12 - 1982, Nijmegen, The Netherlands). Since then however, new progress has been made in neuroimmunology and within the framework of this meeting, we thought it interesting to review those therapeutic efforts again and for each treatment to state the rationale and the possible effects on the immune system, as well as the therapeutical scheme and the clinical results. To make the text as clear as possible, these data will be summarized in tabular form.


RECENT IMMUNE TREATMENTS IN MULTIPLE SCLEROSIS

Azathioprine

Azathioprine is certainly the most widely used substance. Its immune effects in laboratory experimental models are summarized in Table 3.

Clinical immune effects of Azathioprine in MS patients have been studied by Oger et al. (Table 4).

According to those observations, T cell suppressor activity is lower in MS patients when compared to healthy persons, and Azathioprine reduces this suppressor activity still further. On the other hand, the intrathecal production of IgG is restored to normal values.


Table 2. Immune Treatments Discussed in this Review (1977-1983).

| | |
|---|---|
| Antilymphocyte Serum | Levamisole |
| Antithymocyte Globulin | Lymphocytapheresis |
| Azathioprine | Methotrexate |
| Cyclophosphamide | Myelin Basic Protein |
| Cyclosporine A | Plasmapheresis |
| Cytarabine | Polyunsaturated Fatty Acids |
| Hyperbaric Oxygen | Synthetic Polypeptide (COP I) |
| Immunoglobulin G | Transfer Factor |
| Interferon | Thymectomy |

Table 3.    Immune Effects of Azathioprine.

| Humoral Immunity | |
|---|---|
| B cells: | marked depression (?) |
| | lowered IgG secretion |
| **Cellular Immunity** | |
| T cells: | ? |
| H cells: | resistant |
| S cells: | marginally lowered (Oger 1982) |
| | markedly decreased (Trotter 1982) |

NB.   In conventional dosages corrects abnormal B
      cells without altering T cell regulation
      At high doses S cells are affected

Table 4.    Effect of Azathioprine on Suppressor Activity and IgG
            Production.   (2 mg/kg/day) (Oger et al., 1982).

| Group | Patients n = | IgG Production ng/ml | Patients n = | Con A induced suppressor activity (%) |
|---|---|---|---|---|
| Controls | 32 | 1.377 | 44 | 40 |
| MS stable | 18 | 2.372 | 22 | 30 |
| MS treated | 12 | 1.238 | 10 | 23 |

Table 5.   Azathioprine (Rosen et al., 1979).

| 127 patients | |
|---|---|
| Daily dose: | 2 mg/kg |
| Mean follow-up: | Pilot study: 9 years (n=85) |
| | Randomized group: 4.5 years (n=42) |
| Results: | |
| Pilot study: | "<10% of the patients progressed from ambulatory to immobile status" |

| Randomized group: | | Aza | Untreated |
|---|---|---|---|
| | Stable patients | 91% | 35% |

Azathioprine appears therefore mostly effective on humoral
immunity even though its depressive effect on B cells is not always
accepted.

The results of Azathioprine chronic treatment in MS have been
published by Rosen et al., in 1979 (Table 5).

According to this randomized study, Azathioprine seems to exert a favorable effect on the progression of the disease since stable patients are found more frequently in the treated than in the non-treated group.

The results of Aimard et al., have been recently published (1983) (Table 6).

The results of this controlled study with long-term administration of Azathioprine appear in favor of a beneficial effect of the drug on the progression as well as on the annual relapse rate (ARR).

The series of Sabouraud et al., has been published in 1982 (Table 7).

In this uncontrolled study, patients with a pure remittent form seem to benefit the most from Azathioprine since there is a striking

Table 6.  Azathioprine (Aimard et al., 1983).

175 patients, mean follow-up 9 years
Daily dose:           150 mg
Control group:        128 patients
Results:

|  | Treated | Control |
|---|---|---|
| Stable | 45% | 13% (P 0.001) |
| Stable + bouts | 36% | 35% N.S. |
| Worsened | 18% | 53% (P.0.001) |

Relapse rate (mean interval of time between bouts):

Before Aza:    2.5 years
After Aza:     3.8 years

Table 7.  Azathioprine (Sabouraud et al., 1982).

67 patients, follow-up ⩾ 5 years
No control group
Daily dose: 3 mg/kg
Results: - no effect in
            * remittent progressive forms (n = 17)
            * pure progressive forms (n = 10)

         - in 40 pure remittent forms

|  | Before treat. | After treat. |
|---|---|---|
| ARR | 0.77 | 0.16 |

         - global stabilization: 40/67 cases (60%)

Table 8.  Azathioprine (Zeeberg et al., 1982).

| | |
|---|---|
| 22 patients: | 12 Aza |
| | 10 placebo |
| Follow-up: | 18 months |
| Dose: | 2.5 mg/kg/day |
| Results: | no difference between both groups |

reduction of the ARR.  In addition, the progression of the disease appears lower in 60% of the patients.

The first Azathioprine blinded study has been published by Zeeberg et al., in 1982 (Table 8).

No difference was found between both groups, but the number of patients is small and the follow-up rather short.  An interesting observation in this study is that the progression of the disease was slower in both groups when compared to untreated patients.  This of course may result from a placebo effect, but a placebo effect lasting for 18 months is unlikely.  We rather think that this study demonstrates the necessity of a continuous follow-up of MS patients in order to prevent and/or to treat minor complications early (e.g. urinary bladder infections) and to avoid their influence on the progression of the disease.

The final report concerning a double blind trial of Azathioprine has been published by Mertin et al. (1982) (Table 9).

In fact, this study combines an acute immunosuppression (associating antilymphocyte globulin and prednisone) and a chronic administration of Azathioprine for 14 months.

No significant differences were found between the treated and the placebo groups, but the authors consider that there is a definite trend in the treated patients towards a slower evolution and a lower ARR.

The interval of time between the discontinuation of treatment and first relapse is longer in the treated group, and this is in favor of a potential efficacy of Azathioprine.  The better response in female patients observed in the preliminary publication (1980) was not confirmed, and no conclusion could be drawn in this study about the role of HLA antigens in determining the response to the treatment.

Another controlled study has been published by Patzold et al., in 1982 (Table 10).

Table 9.  Azathioprine (Mertin et al., 1982).

| 43 patients: | 21 treated |  |
|---|---|---|
|  | 22 placebo |  |
| **Acute treatment:** |  |  |
| - Antilymphocyte globulin: | 500-750 mg daily, for 15 days |  |
| - Prednisone: | 150 mg/day, subsequently tapered (4 weeks) |  |
| **Chronic treatment:** |  |  |
| Azathioprine: | 3 mg/kg/day, for 14 months |  |
| **Evolution:** | Aza | Placebo |
| Stable | 16 | 13 |
| Worsened | 5 | 9 |
| **Annual relapse rate:** |  |  |
| Before treatment | 1 | 1 |
| After treatment | 0.7 | 1 |

Table 10.  Azathioprine (Patzold et al., 1982).

| 115 patients: | 60 treated |  |
|---|---|---|
|  | 55 placebo |  |
| Daily dose: | 2 mg/kg |  |
| Follow-up: | 1 year  n = 17 |  |
|  | 2 years n = 98 |  |
| **Results (2 years follow-up):** |  |  |
| Mean progression ($\bar{x}$) | Aza | Controls |
| Intermittent progressive | 15.10 | 28.37 |
| Intermittent | 9.33 | 10.95 |
| Chronic progressive | 27.96 | 12.76 |

In this randomized controlled study, in opposition to the results of Sabouraud et al., there is no clear influence of Azathioprine on the course of patients with a pure relapsing form, as well as with a pure progressive evolution.

In opposition, in the group of patients experiencing intermittent progressive course, there is a favorable effect since the mean progression is significantly lower in treated patients (P=0.01019).  Of note is that the standard deviation is quite important in each group.

A recent clinical study has been published by Lauer et al. (1984) (Table 11).

This study emphasizes the difficulties inherent to randomized blinded studies since the course of the disease was more active in the treated group. When comparing similar periods of time before and after treatment however, a favorable incidence of Azathioprine on both the progression index and the ARR was observed.

Adverse effects of Azathioprine have been reviewed by Patzold et al. (1982) (Table 12).

In fact, no serious side-effects have been reported so far and the incidence of cancer in Azathioprine treated MS patients does not seem higher than in the general population.

## Cyclophosphamide

Cyclophosphamide (CY) has been less widely used in MS because of its subjective intolerance. The immune response to CY is summarized in Table 13.

Table 11.  Azathioprine (Lauer et al., 1984).

---

62 patients:              46 relapsing remittent
                          16 progressive
Control group:            59 matched patients
                          (sex, onset, type, course)
Dose:                     150 mg daily
Follow-up:                3-6 years
Results:

| Treated Patients | Entry | End | After Withdrawal |
|---|---|---|---|
| ARR | 1 | 0.48 | 1 |
| Progression index | 0.76 | 0.16 | 0.58 |

| Treated Patients vs controls | Aza | Untreated |
|---|---|---|
| ARR | 0.48 | 0.32 |
| Progression index | 0.16 | 0.25 |

Bias: treated patients had a more active course
Comparison of similar pre- and post-treatment periods:

| | Aza | Untreated |
|---|---|---|
| Reduction ARR | 0.60 | 0.23 (P<0.05) |
| Reduc.Progr.Index | 0.57 | 0.32 (N.S., P>0.1) |
| % Pat. not progress. | 67% | 50%  (N.S., tendency: P<0.10) |

---

Table 12.  Adverse Effects of Azathioprine (Patzold et al., 1982)

| Material: | 105 cases |
|---|---|
| Adverse effects: | |
|     - Haematological changes | 2 severe anaemia |
|     - Gastrointestinal | |
|       complications: | 3 major intolerances |
|     - Hepatotoxicity: | increased liver enzymes:24% |
| | 2 severe hepatitis |
|     - Infections: | seldom observed (acne) |
|     - Allergic reactions: | 2 cases |
|     - Malignancies: | 4 cases |
| | no proved causal relationship |


Table 13.   Immune Effects of Cyclophosphamide

| Humoral Immunity | |
|---|---|
|   B cells: | marked depression |
| | dose dependent |
| | long lasting |
| | superior to other alkal. agents |
| Cellular Immunity | |
|   T cells: | depression |
|   H cells: | highly vulnerable |
|   S cells: | depression (Turk 1979) |
| | increase (Brinkman 1982) |

NB:  Changes are not due to cell death
     Altered B and T cells functions are reversible
     Peak CSF concentrations vs serum: parent subst.
     50%  alkal. fraction  20%


On experimental basis, CY influences both humoral and cellular immunity and this could be an advantage if both immune responses are involved.  As a matter of fact, in a preliminary study of acute CY immunosuppression in 5 patients, we observed that T cells are more severly depressed than B cells, and H cells than S⋅cells.  According to Brinkman (1982), S cells would be even increased during chronic administration, with a simultaneous return of the H/S ratio normal values.

CY has been used by Hommes et al., in chronic progressive forms and his therapeutic scheme as well as his recent results are summarized in Table 14.

Progressive froms of MS are in theory the most reliable ones to appreciate the potential effect of treatments, since the evolution is slowly and inexorably progressing, without any stabilization nor improvement. Keeping that in mind, it appears that after a mean follow-up of 2.5 years, 43% of CY treated patients are stable. According to Hommes, this high percentage of stable patients does not correspond to the natural evolution of the progressive form of the disease. Of note is that Prednisone was systematically associated to CY in this series.

Since 1966, over 250 patients have been treated with CY at the Belgian National Center for MS in Melsbroek. The first protocol study (Gonsette et al., 1977) is summarized in Table 15.

Table 14.  Cyclophosphamide (Hommes et al., 1982)

---

CY (400 mg) + Prednisone (100 mg) daily for about
3 weeks
Pure progressive form:      n = 47
Follow-up:                  1 - 5 years (mean 2.5)
Results:

|  | n = |
|---|---|
| Stable | 10 (43%) |
| Worsened (DSS+1) | 12 (25%) |
| DSS + 2 | 15 (32%) |

Best results in patients with
        - a higher progression rate
        - an evolution shorter than 6 years
        - HLA typing: DR W 2

---

Table 15.  Cyclophosphamide (Gonsette et al., 1977).

---

Phase I: Methodology

    Acute i.v. CY immunosuppression (mean dose 4.6 g)
    Duration of treatment: 3 weeks
    Leucopenia ≤2000 - Lymphopenia ≤1000
    No cortisone or ACTH during or after CY
    Relapsing remittent or relapsing progressive forms
    GOAL: possible effect on the ARR
    Evaulation:
      - ARR calculated over a 2 years period before and after CY in
        the same patients
      - ARR in treated patients vs retrospectively matched control
        group

---

The aim of this phase I study was to determine if a transient, acute, intensive immunosuppression was able to influence the ARR in patients with frequent exacerbations. Of note is that patients with a recent relapse (less than 8 weeks) were not included and that no corticoid therapy was associated. It was therefore to be expected that, if observed, clinical changes would be the results of CY therapy.

The results observed in 119 patients with a follow-up for 2 years and over are summarized in Table 16.

When patients are taken as their own controls, and when the ARR is calculated over a short period (2 years before and after treatment), a definite reduction of the ARR was observed after CY immunosuppression. This reduction was more pronounced in patients with a shorter evolution.

To overcome the objection of patients serving as their own controls, the ARR of treated patients was compared to that of untreated patients, retrospectively matched, according to the duration of the disease, the clinical form, the evolution, the sex and age (Table 17).

The mean spontaneous ARR decrease in a group of 91 non-treated patients, calculated over the 2 preceeding years is of 35% at the end of the 4th year, 18% at the end of the 6th year and 13% at the end of the 8th year. In the CY treated patients, the reduction of the ARR is definitely higher, even in patients with a pre-treatment evolution of 8 years.

However, in spite of its short term favorable influence on the ARR, CY acute immunosuppression was unable to change the progression rate of the disease and a second trial protocol associating acute and chronic CY immunosuppression was designed (Table 18).

Table 16.  Cyclophosphamide (Gonsette et al., 1977).

Phase I:  Results (Patients as their own controls)

| Duration of disease before CY | Patients n = | Annual Relapse Rate | |
|---|---|---|---|
| | | Before CY | After CY |
| 1 - 2 | 24 | 2.6 | 0.3 |
| 3 - 4 | 35 | 1 | 0.27 |
| 5 - 6 | 30 | 0.90 | 0.31 |
| 7 - 8 | 21 | 0.77 | 0.25 |
| 9 -10 | 19 | 0.46 | 0.46 |
| Total | 119 | | |

Table 17.  Cyclophosphamide (Gonsette et al., 1977).

Phase I: Results (Treated patients vs control group)
         Percentage decrease of ARR vs 2 precedent years

| Duration years+ | Untreated patients n = 91 (spontaneous ARR decrease) | Treated patients n = 91 |
|---|---|---|
| 1-2 | – | 81% |
| 3-4 | 35% | 73% |
| 5-6 | 18% | 65% |
| 7-8 | 13% | 67% |

+ Treated patients   :   duration of disease before CY
  Untreated patients :   duration of disease


Table 18.  Cyclophosphamide (Gonsette et al., 1984)

Phase II: Methodology
      Acute CY immunosuppression followed by a chronic
      treatment with cyclophosphamide 50 mg/day
                   or isophosphamide 150 mg/day

      Patients
      –  experiencing frequent relapses (mean ARR 1.62)
      –  with a short evolution (mean duration 4 years)
      –  low disability status (Kurtzke $\leqslant$ 6)

      Evaulation:
      DSS after $\geqslant$ 2 years (mean follow-up 4 years) in
      treated patients and matched control group


     The aim of phase II study was to evaluate the longterm influence
of the treatment on the disability score.  Chronically treated
patients were compared to patients treated by a single acute CY
immunosuppression and matched according to their age, sex, length of
evolution, handicap at treatment, form of the disease, etc.. (Table
19).

     After a mean follow-up for 4 years, 29/35 patients were stable
in the chronically CY treated group compared to 12/35 in the control
group.  The difference between both groups is highly significant
($P<0.005$).

     A study of Hauser et al. (1983) has been widely discussed in the
recent literature.  The results of this three arms clinical trial are
summarized in Table 20.

Table 19.  Cyclophosphamide (Gonsette et al., 1984)

Phase II: Results
    Mean follow-up: 4 years (2-8 years)

| Treatment | Patients n = | Stable n = | Worsened n = |
|---|---|---|---|
| Acute CY only | 35 | 12 | 23 |
| Acute + chronic CY | 35 | 29 | 6 |

Table 20.  Hauser et al., 1983

58 patients with severe relapsing and/or progressive forms

| Treatment (2-3 weeks) | Patients N = | Stabilization DSS | Clinical | Aggravation Ambulant at entry Wheelchair 1 year later |
|---|---|---|---|---|
| ACTH | 20 | 20% | 10% | 11 |
| CY + ACTH | 20 | 80% | 70%+ | 2 |
| Plasm. Exch. | 18 | 50% | 44% | 6 |

As a matter of fact, there is a strong tendency in this study in favor of CY treated patients when compared to ACTH or plasma exchange groups.

Unharmful side-effects of CY are well known: nausea, vomiting, hairloss, and discoloration of the nails.  Non-specific adverse reactions are exceptionally observed: water intolerance, hyponatremia, drop in blood sugar in diabetic patients, prolonged apnoea after anesthesia and blurring of vision.  Specific adverse reactions have been described, but their frequency is still controversial: carcinogenicity (reticulum cell sarcoma, squamous cell carcinoma, acute myeloid leukemia), organ toxicity (acute myopericarditis, hemorrhagic cystitis, acute hemorrhagic pancreatitis) and teratogenicity (azoospermia, amenorrhea, chromosome abnormalities, foetal death, extremity defects).

In our experience, side-effects of CY chronic administration mostly concern gastro-intestinal intolerance and their frequency is usually reduced by slowly increasing the dose (mean daily dose 50 mg).  The cancer incidence in over 250 patients treated with CY since more than 15 years, is not higher than in a normal population.


Mechloretamine

Mechloretamine (Mustagen) is an analogue of CY.  It has been

used in MS patients by Alexandrowicz et al. (1982) (Table 21).

The results of this preliminary study, associating an immuno-
suppressor (nitrogen mustard) and an immunomodulator (levamisole),
encouraged the authors to design a multicenter longterm evaluation
(10 years) with this treatment.

The results of an open clinical trial associating CY and Aza-
thioprine have been published by Dachsel et al. (1982) (Table 22).

Table 21.  Mechloretamine (Alexandrowicz et al., 1982).

50 patients with relapsing and/or progressive forms
"Mustagen" i.v. inj. for 5 days, monthly Levamisole 150 mg,
twice a week

Duration of treatment: 2 years
Results:

|                      | n =        |
|----------------------|------------|
| Stable, no bouts     | 15 (30%)   |
| Stable, less bouts   | 27 (50%)   |
| Worsened             | 8 (16%)    |

Table 22.  CY + Azathioprine (Dachsel et al., 1982).

82 patients:
        – remittent progressive:      31
        – secondary progressive:     39
        – primary progressive:       12

Follow-up: 18 months

1.  Intensive immunosuppression:
        – CY: 200–300 mg/day i.v. for 4–5 days
              100–150 mg/day (tablets) for 5 weeks
        – Simultaneously, Prednisone: 25 mg/day

2.  Subsequent chronic immunosuppression:
        – Imuran: 100–150 mg/day for 18 months
        – Week-end drug holdiay

Results:
        – Remittent progressive: * no influence on ARR
                          * definite beneficial
                            effect on progressive
                            deterioration
        – Secondary progressive:  no influence
        – Primary progressive:   markedly reduced
                            progression

According to this open study, combined CY + Aza immunosuppression seems mostly effective during the active stages of the disease.


## Methotrexate

Association of intramuscular and intrathecal injections of Methotrexate has been used by Dominguez et al. (1979).

There is no evaluation of the clinical results in this study but adverse effects are severe enough to preclude this particular treatment. Meningeal reactions are possibly due to the preservative substances added to Methotrexate solutions.


## Cytarabine

The same holds for intravenous and intrathecal injections of Cytarabine as published by Tourtellotte et al. (1980) (Table 24).


## Antilymphocyte Globulin or Serum

Longterm results of antithymocyte globulin (ATG) in MS have been published by Kastrukoff et al. (1978) (Table 25).

This open study failed to demonstrate a favorable influence of ATG therapy in the long run. Those results confirm therefore previous observations indicating that the beneficial effect observed in some patients immediately after antilymphocyte globulin therapy does not last very long.


Table 23.  Methotrexate (Dominguez et al., 1979).

---

7 patients

Dosage:
  - 10-12 mg i.m. per square meter body surface weekly for 6 weeks
  - 1 intrathecal injection monthly for ?

Adverse effects:
    visual alteration
    delirium
    aseptic meningitis

Results:
    Clinical?
    Immunological:      CSF IgG synthesis decrease
                        Oligoclonal aspect disappeared in 1 case

---

Table 24.  Cytarabine (Tourtellotte et al., 1980).

---

10 stable patients: 3 i.v
                    7 intrathecal

Dosage:
    - i.v.: 67 mg per square meter body surface 3 times daily for
          4-5 days
    - intrathecal : 50 mg-1000 mg, 2-10 times

Follow-up:
    2.5 years

Side-effects:
    hemianopia
    meningeal syndrome

Results:
    Clinical: no improvement or worsening
    Immunological: no change of CSF IgG synthesis oligoclonal aspect

---

Table 25.  Antithymocyte globulin (Kastrukoff et al.,
           1978).

---

21 patients in relapse

Treated group (n = 10):
    -ACTH:  40 IU/day i.v.
    -ATG:   10 mg/kg/day i.v. for 28 days
            or 20 mg/kg/day i.v. for 14 days

Control group (n = 11):
    ACTH: 40 IU/day i.v.

Follow-up: 5 years

Results: no difference between both groups (P > 0.5)

---

Antilymphocyte serum (ALS) in association with Aza and Pred-
nisone has been used in France by de Saxce et al. (1984) (Table 26).

de Saxce et al., conclude that Group I possibly yields better
response than Group II, and that the difference is mostly marked
during the first year.  After discontinuation of ALS therapy, no
significant difference could be found between both groups.  This
combined ALS and Azathioprine therapy does not seem therefore to
influence the disease in the long run.  Moreover, side-effects of
antilymphocyte serum are not to be underestimated.

Table 26.   Antilmyphocyte Serum (de Saxce et al., 1984).

Group I (n = 45)

      ALS i.v. 125 mg active proteins/day for 2 weeks
                        3 times a week for 2 weeks
                        once a week for 1 year

      Azathioprine 2 mg/Kg/day for 3 years
      Prednisone 20 mg/day for 1 year

Group II   (n = 22)

      Same regime except no ALS

| Results: | | Group I | Group II |
|---|---|---|---|
| Follow-up | | ALS + AZA + Pred. | Aza + Pred. |
| 1st year | stable | 76% | 59% |
|  | worsened | 24% | 41% |
| 2nd year | stable | 55% | 33% |
| (stop ALS) | worsened | 45% | 67% |
| 3rd year | stable | 50% | 24% |
|  | worsened | 50% | 76% |

Table 27.   Thymectomy (Ferguson et al., 1983).

Group   I (n = 16) : thymectomy + Azathioprine (1 year)
        II (n = 16) : thymectomy alone
       III (n = 32) : untreated control matched group

Follow-up:          2 years

Results (Groups I and II compared to Group III):
Group I:            significant improvement (Kurtzke scale) more
                    pronounced for relapsing remittent forms

Group II:           no difference when compared to untreated patients

N.B.:   The lack of side-effects of thymectomy in MS patients
        contrasts to usual experience in patients with myasthenia
        gravis

## Thymectomy

      The clinical results in thymectomy in MS have been recently
published by Ferguson et al. (1983) (Table 27).

      Apparently, association of thymectomy and Azathioprine yields
favorable effects in relapsing remitting forms.  However, even though
thymectomy appears to be safer in MS than in myasthenia gravis, one
may be reluctant to expose MS patients to a surgical procedure.

## Lymphocytapheresis and Plasmapheresis

Removal of presumed cytotoxic or immune secreting lymphocytes is possibly obtained by lymphocytapheresis, and the results of Giordano et al. (1982) in MS are listed in Table 28.

Patients with relapsing or progressive forms seem to benefit the most from this treatment.

In their report, Giordano et al. (1982) briefly review the results obtained at other centers (Table 29).

Nearly 45% of the patients are reported to have shown improvement in these combined data. Of note is that in a blind study, lymphocytes were returned in 5 patients at the end of the procedure, and that 3 of them rapidly deteriorated afterwards. The length of the follow-up is not known and it is not possible to comment on the duration of the improvement.

Another short-term study of lymphocytapheresis has been published by McFarland et al. (1982) (Table 30).

Table 28. Lymphocytapheresis (Giordano et al., 1982).

---

70 out-patients

Lymphocytes yield per procedure $1 \times 10^9 - 1 \times 10^{10}$
10 procedures over a 12 days period
When improved 1 session every 2 weeks for 3 months

Follow-up: 1 year

Results (Kurtzke DSS):

|                  | n = | Before | After            |
|------------------|-----|--------|------------------|
| Remitt. Relaps.  | 11  | 4.44   | 2.22 (P < 0.005) |
| Remitt. Progr.   | 45  | 6.02   | 5.48 (P < 0.005) |
| Chronic Progr.   | 14  | 7.92   | 7.64 (P < 0.05)  |

Table 29. Lymphocytapheresis: Data from a Multicenter Study (Giordano et al., 1982).

---

| Authors           | n = | Results (Kurtzke Scale) | |
|-------------------|-----|----------|--------|
|                   |     | Improved | Stable |
| Hamburger et al.  | 6   | 4        | 2      |
| De Green et al.   | 14  | 5        | 9      |
| Kasprisin et al.  | 21  | 13       | 8      |
| Pitts et al.      | 9   | 3        | 6      |

Table 30.  Lymphocytapheresis (McFarland et al., 1982).

---

7 patients
Lymphocytes removed per procedure: 6 to 6 x $10^9$
3 procedures a week for 3-4 weeks then weekly procedure
  for 3-4 weeks
Follow-up: 1-3 months
Results: patients improved:      3
                  unchanged:     2
               deteriorated:     2

---

Table 31.  Lymphocytapheresis (Kennes et al., 1984)

---

Protocol I:
    22 out-patients (chronic progressive)
    1 x $10^9$ lymphocytes yield
    once a week for 3 weeks
        once every 3 weeks for 6 months

Protocol II:
    7 out-patients (chronic progressive)
    once a week for 3 months

Follow-up: 9-12 months

Clinical results: modest but significant improvement in
                  29/29 patients

Immunological studies:
    - Definite IgG serum level decrease (50%)
    - T or B cells distribution unaffected
    - H/S ratio normal before and after treatment

---

Cell-mediated immunity was studied in those patients by measuring _in vitro_ response of lymphocytes to mitogen, and fluorescent activated cell scatter analysis was performed.  No change of immunological functions could be observed.

A recent clinical and immunological study with lymphocytapheresis has been published by Kennes et al. (1984) (Table 31).

Up till now, available results do not suggest that lymphocytapheresis, an expensive procedure, carrying some risks has any utility in MS patients.

Rationale for plasmapheresis is summarized in Table 32.

Results of plasmapheresis in MS are summarized in Table 33.

Table 32.   Rationale for Plasmapheresis.

---

Existence (?) in serum of MS patients of
   - circulating immune complexes
   - brain extracts complement fixing anti-
     bodies
   - antimyelin antibodies
   - antiligodendroglia antibodies
   - demyelinating factor
   - neuroelectric blocking activity factor

In 1980, 20 clinical groups have undertaken
plasma exchange therapy in MS

Drawbacks: expensive and carries some risk

---

N.B.: concomitant Azathioprine and Prednisone
        administration


Table 33.   Combined Data of Plasmapheresis in MS.

| Year | Authors | Patients n = | Exchange n = | Results |
|------|---------|--------------|--------------|---------|
| 1980 | Dau et al. | 11 | 10-20 | 10/11 modest improvement |
| 1980 | Weiner et al. | 8 | 3-5 | 6/8 modest improvement for 6 months |
| 1981 | Valbonesi et al. | 11 | 5 | 4/6 moderate improvement |
| 1982 | Stefoski et al. | 7 | 5 | 2/7 improvement |

Immunological studies

| 1984 | Caparelli et al: | marked decrease of IgG (serum + CSF) CSF oligoclonal aspect unchanged |
|------|------------------|----------------------------------------------------------------------|
| 1984 | Guarneri et al: | significant decrease of polymorphonuclear leucocytes proteinase activity in active cases |

Neurophysiological studies

| 1981 | Rosen et al: | reduction in visual evoked potential latencies |
|------|--------------|-------------------------------------------------|
| 1982 | Stefoski et al: | decrease of serum neuroelectric blocking activity |

It has been suggested that plasmapheresis, in association with immunosuppression drugs could improve our ability to modulate the immune system but no clinical studies supporting or denying its clinical utility in MS are available so far.

## Levamisole

Levamisole is considered as an "immunomodulator" and its effects on the immune system are dose dependent (Table 34).

Usual therapeutical levels of Levamisole are considered as producing a decrease in activity of B cells and an increase of suppressor activity.

Levamisole has been used in MS since 1976 and the clinical results are summarized in Table 35.

In most studies, no definite effect of Levamisole on MS could be demonstrated and, moreover, it has been suggested by Dau et al., that this substance could be harmful.  In a double blind study concerning 54 patients followed for 2 years, Gonsette et al., did not observe adverse reactions and it appeared that there was a tendency for treated patients to experience a slower progression of the disease and a lower ARR.  The difference between the placebo and treated groups however is not significant.  Most of the patients had a long evolution and a severe handicap at entry in the trial and it could be that Levamisole is more effective when applied earlier during the first years of the disease.  Levamisole has been used with possible

Table 34.   Immune Effects of Levamisole.

---

1.   Low doses (potentiation)
     Humoral Immunity:
           B cells:          activated

     Cellular Immunity:
           T cells:          activated
           H cells:          marked increased
           S cells:          mild increased

   N.B.:      increases heldper to a much important degree
              than suppressor activity
              net effect: immunopotentiation

2.   High doses (suppression)
     Humoral Immunity:
           B cells:          not directly suppressed
                             antibody synthesis reduced as
                             a consequence of increased
                             suppressor activity

     Cellular Immunity:
           T cells:          activated
           H cells:          ?
           S cells:          probably increased

   N.B.:      marked increase of suppressor activity

Table 35.  Clinical Trials with Levamisole.

| Year | Authors | Patients n = | Follow-up | Results |
|------|---------|--------------|-----------|---------|
| 1976 | Dau et al. | 7 | 3 months | 5 patients deteriorate |
| 1977 | Myers et al. | 4 | 1 year | no effect |
| 1977 | Cendrowski et al. | 17 | 30 days | no effect |
| 1977 | Camenga et al.+ | 33 | 6 months | no difference treat./plac. |
| 1979 | Patzold et al. | 23 | 10 months | no effect |
| 1981 | Massaro et al. | 26 | 1 year | no conclusion |
| 1982 | Fonsette et al.+ | 54 | 2 years | Levamisole group more stable |

+ Blind control study

benefit as a chronic immunomodulator after acute nitrogen mustard treatment (Alexandrowicz et al., 1982).

Hyperbaric Oxygen

Hyperbaric oxygen is able to reduce CNS lesions in EAR (Warren et al., 1978) and has been used therefore since as early as 1970 (Table 36).

From those preliminary open trials, it seems that HBO is able to provoke a mild and transient improvement in MS.

Longterm results of HBO have been published by Fisher et al. (1983) in a blinded randomized study (Table 37).

In fact, it is still difficult to comment on longterm benefit of this treatment, even though there seems to be a tendency for treated

Table 36.  Combined data concerning HBO clinical trials.

| Year | Authors | n = | Improvement |
|------|---------|-----|-------------|
| 1970 | Boschetty et al. | 26 | transient in 16 |
| 1978 | Baixe et al. | 11 | moderate in 11 |
| 1980 | Neubauer et al. | 600 | dramatic (39%) moderate (52%) |
| 1980 | Formai | ? | positive results |
| 1980 | Pallotta | 6 | substantial in 6 |

N.B.  no control group

Table 37.   Longterm Results of HBO Therapy (Fisher et al., 1983).

---

40 patients:       20 MS (3 drop out)
                   20 controls

2 athmosphere absolute for 90 minutes daily for 20 days

Longterm results (1 year):

|  | Treated n = 17 | Placebo n = 20 |
|---|---|---|
| Patients improved | 5 | – |
| stable | 10 | 9 |
| worse | 2 (12%) | 11 (55%) |

---

patients towards a slower progression.  For this reason, a 2 years multicenter double blind control study has been designed in order to more definitely establish the value of HBO therapy in MS (Kindwall et al., 1983).


Transfer Factor

    The exact nature and mechanisms of action of transfer factor (TF) are not known yet, but this substance is supposed to restore deficient cell-mediated immune functions.   Several blinded studies have been published with conflicting results (Tables 38 to 41).

    Behan et al. (1976) observed no evident difference between treated patients and the control group, and the same holds true for Fog et al. (1978) as well as for Collins et al. (1978).


Table 38.   Transfer Factor (Behan et al. 1976).

---

| 29 patients: | 14 transfer factor |
|---|---|
|  | 15 placebo |
| TF: | isolated from one pint of venous blood |
|  | Random donors |
| Dosage: | once a month for 3 months |
|  | same procedure 3 months later |
| Follow-up: | 1 year |

Results:

|  | TF | Placebo |
|---|---|---|
| Improved | 3 | 2 |
| Stable | 8 | 9 |
| Worsening | 3 | 4 |

---

Table 39.   Transfer Factor (Fog et al., 1978).

| 32 patients: | 16 transfer factor | | |
|---|---|---|---|
| | 16 placebo | | |
| TF: | 1 unit = 1 - 2 x $10^7$ leucocytes | | |
| | Random donors | | |
| Dose: | 3 units monthly for 13 months | | |
| Follow-up: | 1 year | | |
| Results: | | Neurological Examination | |
| | | TF | Placebo |
| | Stable | 23 | 25 |
| | Worse | 9 | 7 |

Table 40.   Transfer Factor (Collins et al., 1978).

| 48 patients: | 28 transfer factor | | |
|---|---|---|---|
| | 20 placebo | | |
| TF: | 2 x $10^7$ lymphocytes/ml | | |
| | Random donors | | |
| Dose: | 10 ml every 2 months | | |
| Follow-up: | 1 year | | |
| Results: | | Neurological TF | Evaluation Placebo |
| | Improved | 3 | 1 |
| | Stable | 13 | 11 |
| | Worse | 12 | 8 |
| Conclusions: | Results disappointing No effect in a number of serological and immunological studies Is the preparation biologically active? | | |

Another blind study has been recently published by Lamoureux et al. (1983).

During a follow-up period of 1 year, there is no clear influence of this short-term (3 months) treatment with TF.

A longterm study has been published by Basten et al. (1980) with more encouraging results (Table 42).

Table 41.  Transfer Factor (Lamoureux et al., 1983).

| | |
|---|---|
| 27 MS patients: | 14 transfer factor<br>13 placebo |
| TF: | $2 \times 10^7$ lymphocytes/ml<br>Unrelated donors |
| Dose: | 2 ml subcutaneously at weekly interval<br>Treatment period: 3 months |
| Immunological Studies: | TF do no stimulate the humoral response<br>Significant decrease of total CSF protein IgG levels |

In this prospective double-blind trial, the benefit of treatment with TF was not apparent until 18 months to 2 years.  This could explain the failure of previous short-term studies to demonstrate any favorable effect.  Another explanation may be that in other studies, random donors were used as the source of leucocytes for TF production.  Even though statistical analyses are in favor of F therapy, it must be stressed that standard deviations in disability scores evaluation are relatively high.  A carefully designed longterm study is underway at the Belgian Centre for MS (Van Haver et al., 1984) in order to demonstrate a possible differential effect of TF of MS relatives or random donors, and to establish the possible influence of a prolonged administration of this substance.

Table 42.  Transfer Factor (Basten et al., 1980).

| | |
|---|---|
| 51 patients: | 26 transfer factor<br>25 placebo |
| TF: | 1 unit = $4 \times 10^8$ cells equivalents (mononuclear)<br>Relatives as donors |
| Dose: | every 2 weeks for 1 month<br>every 4 weeks for 6 months<br>every 8 weeks thereafter<br>Total: 25 doses |
| Immunological studies: | no clear cut effect |
| Results: | Percentage of Patients without Clinical Signs of Progression |

| | 6 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|
| Treated | 58 | 46 | 37 | 48 |
| Placebo | 56 | 36 | 16 | 24 |

Desensitization Techniques

Desensitization against a hypothetical encephalitogenic deter-
minant has been tried in MS using myelin basic protein (MBP)
(Table 43).

According to Campbell's and our own experience, no influence of
MBP treatment in MS could be demonstrated. More recently, Romine et
al. (1983) have used definitely higher doses without any therapeutic
effect as well. Of note is that the development of a primary take by
some patients in this study suggests that they had not been sensit-
ized to MBP previously. Moreover, high doses of MBP may be associ-
ated with neurological adverse reactions. In neurophysiological
studies, Seil et al. (1983) have shown that there is no correlation
between serum demyelinating activity and the presence or absence of
MBP therapy in MS patients.

Desensitization with a non-encephalitogenic synthetic polypep-
tide (COP-I) has been used by Bornstein et al. (1982) (Table 44).


Table 43.   Combined Data of MBP Treatments in MS.

| | | | |
|---|---|---|---|
| 1973 Campbell et al., | 30 | 5 mg/week | some amelioration |
| 1977 Gonsette et al., | 35 | 5 mg/week | no therapeutic effect |
| 1983 Romine et al., | 19 | 75 mg/daily | no therapeutic effect |

> NB:  In Romine's study, hypersensitivity to MBP was observed
>       in 17/19 patients
>         * 15: delayed type (injection site)
>               + neurological changes
>                    6 reversible
>                    2 residual
>         * 2: immediate type


Table 44.   Synthetic Polypeptide -COP-I (Bornstein et
            al., 1982).

| | |
|---|---|
| Open study: | 16 patients (mostly chronic progressive) |
| Daily dose: | 20 mg i.m. |
| Follow-up: | 18 months |
| Results: | no effect  11/16 |
| | improvement 6/16 |

The results of this open study being in favor of a possible therapeutic effect of COP-I, a preliminary blinded trial was developed in exacerbating-remitting patients. According to a first analysis of the results, still blinded for the investigator, an external advisory committee has recommanded to extend the program to a full clinical trial in 1984.

## Polyunsaturated Fatty Acids

The longterm results of a trial with polyunsaturated fatty acids (PUFA) have been recently published by Dworkin et al. (1984). Fifty-eight patients were included in a total series of 172 patients from other centers in UK and Canada (Table 45).

Treatment with PUFA significantly reduced the severity and duration of relapses and there was a tendency to a slower progression rate. Patients with remitting forms and with the shorter duration of illness appear to benefit more.

Table 45.  Polyunsaturated Fatty Acids (Dworkin et al., 1984).

| | | | |
|---|---|---|---|
| Blind Study: | 58 patients: | 29 linoleic acid | |
| | | 29 oleic acid | |
| Daily dose: | 23 g | | |
| Follow-up: | 2 years | | |
| Results: | | Treated | Control |
| Kurtzke scale | | | |
| Improved | | 8 }21 | 7 }18 |
| Stable | | 13 | 11 |
| Worsened | | 8 | 10 |
| Millar scale | | | |
| Score per relapse | | 8.2 | 18.2 |
| Combined study: | 172 patients: | 87 treated patients | |
| | | 85 controls | |
| Follow-up: | 28 months | | |
| Results: | | Treated | Control |
| Kurtzke DSS Before | | 3.33 | 3.15 |
| After | | 3.79 | 3.93 |
| Mean relapse score | | 15.29 | 26.47 |
| Annual relapse rate | | 0.66 | 0.61 |

Interferons

     Besides its well-known effect on the K-NK system, other influences of Interferons (IFN) on immune system are not clear yet (Table 46).

     IFN production by peripheral blood mononuclear cells in MS patients has recently attracted attention (Table 47).

     According to the most recent studies, IFN responders are less frequent among MS patients, but in those responders, IFN production appears normal.

Table 46.   Immune Effects of Interferons

| | |
|---|---|
| **Humoral Immunity** | |
| B cells: | antibody synthesis decreased |
| **Cellular Immunity** | |
| T cells: | ? |
| H cells: | ? |
| S cells: | increased |
| N.B.: | – NK system |
| | – cytotoxic cells } activated |
| | – macrophages |

Table 47.  Production of IFN in MS patients (Peripheral Blood. Mononuclear cells)

| Year | Authors | Type of IFN | IN yield |
|---|---|---|---|
| 1979 | Neighbour | $\alpha$ | significant decrease |
| 1980 | Benczur | $\alpha,\beta$ | id. |
| 1981 | Neighbour | $\alpha,\beta,\gamma$ | id. |
| 1981 | Santoli | $\alpha$ | no difference |
| 1982 | Salonen | $\alpha$ | decr.: mumps-meales no diff. : Herpes simplex |
| 1982 | Zander+ | $\alpha,\beta,\gamma$ | no difference |
| 1983 | Kaudewitz+ | $\gamma$ | id. |
| 1983 | Vervliet++ | $\alpha,\gamma$ | id. |
| 1983 | Tovel | $\alpha$ | id. |

+   Design study excluding pre-existing genetic bias
++  IFN $\gamma$ Responders: MS 37%, controls 85%

Table 48.   Interferons (Clinical Trials).

---

1979 Ververken et al. (3 patients):
- i.m. 0.05 x $10^6$ units IFN β /Kg body weight
  every other day for 12 days
- Follow-up: 9–18 months
- Results:   no effect

1980 Fog et al. (6 patients):[2]
- i.m. HUIFN α 5 x $10^6$ units daily for 2 weeks
                 2.5 x $10^6$ units daily for 5–13 months
- Follow-up: 1 year
- Results:   no effect

1982 Cook et al. (9 patients):
- i.m. HUIFN β 1  $10^6$ daily for 2 months
- Follow-up: "over years"
- Results:   "distinct improvement in some patients but not all"
             no firm conclusion
  N.B.:      general decrease in measles viral protein in the
             jejunum

---

Table 49.   Intrathecal Interferon in MS (Jacobs et al., 1982).

---

- 20 patients:          10 MS
                        10 controls

- intrathecal HUIFN  β 1 x $10^6$ IRU body square meter
  semiweekly for 4 weeks
  once a month for the next 5 months

- Mean follow-up: 1.9 years

- Results:

|           | Improved | Stable | Worse |
|-----------|----------|--------|-------|
| IFN       | 5        | 3      | 2     |
| Untreated | 2        | 4      | 4     |

ARR (2 y):

|           | Before | After |
|-----------|--------|-------|
| IFN       | 1.8    | 0.2   |
| Untreated | 0.8    | 0.7   |

---

Clinical trials with IFN have been published by Ververken et al. (1979), Fog et al. (1980) and Cook et al. (1982), with disappointing results (Table 48).

The clinical study of Jacobs et al. (1982) using intrathecal injections of IFN seems more encouraging but has raised many reactions in the literature (Table 49).

The most impressive results concern the reduction of the ARR, but it has been objected that both groups of patients are small and not homogeneous concerning relapse rate, clinical form and progression of the disease.

Ruutiainen et al. (1983) have reported their experience with intrathecal injection of interferon α (Table 50).

No conclusion could be drawn on the therapeutic results owing to the limited number of patients as well as the restricted observation period.

In order to assess more definitely the therapeutic value of IFN in MS, several double-blind, randomized, cross-over studies are presently underway, but results will not be published until 2 or 3 years. A preliminary evaluation of the Californian study seems to indicate that IFN treated patients experience less attacks, but that the number of patients going into the progressive stage is the same in both groups. This apparent lack of influence on the progression of the disease is possibly due to IFN ability to activate macrophages (Johnson et al., 1983).


Immunoglobulin G

Clinical studies with immunoglobulin G (IgG) have been recently published by Schuller et al. (1983) and Guenther et al. (1984). Administration of exogenous IgG would produce an immunosuppression by a subsitution and a reduction of endogenous IgG synthesis. Moreover, IgG would induce a marked activation of T suppressor cells (Table 51).

After a 4 years follow-up, a slowing of the progression was observed by Schuller et al., in 2/3 of the patients and an objective improvement in 1/3. The study of Guenther et al., is more limited in


Table 50.   Intrathecal IFN in MS (Ruutiainen et al., 1983).

- 5 severely disabled patients
  chronic progressive forms

- 4 weekly intrathecal injections

- Increasing doses:   0.1, 0.3, 1, 3 x $10^6$ HUIFN α

- Follow-up:          3 months

- Side-effects:       Dose dependent (> 0.3 IU)
                      Fever in all patients
                      Extreme fatigue

time but again, a significant decrease of the disability score was
noted after 1 year therapy.  On the other hand, the modest reduction
of the ARR must be viewed with caution.


## THE PRESENT AND THE FUTURE

Attempts to influence the immune system in order to reduce the
progression of MS disease fall into various categories: immunosup-
pression with cytostatic drugs, selective action on lymphocytes,
possible immunomodulation, desensitization, plasma "toxic factors"
extraction, and finally somewhat empirical techniques.

Among immunosuppressive substances, Aza and CY are mostly used.
Usually, an acute intensive immunosuppression is achieved with high
doses of CY, whereas Aza is chronically administered for several
years.  It must be reminded that high doses of cytostatic drugs alter
lymphocytes viability, whereas chronic low doses exert a reversible
influence on their functions.

During the last four years, 8 clinical studies using Aza in 524
patients have been reported.  With the exception of 2 open studies,
all clinical trials were blinded, including matched control groups.
No significant differences were found between treated and untreated
patients when the follow-up period did not exceed 2 years.  In
patients with a longer follow-up period did not exceed 2 years.  In
patients with a longer follow-up (3-9 years), a significant reduction
of the progression index as well as the ARR was observed.  Patients
experiencing active forms of the disease seemed to benefit the most.
However, due to the limited number of patients in each trial and to
important standard deviations in some studies, those results must be
interpreted rather as a significant tendency than as a definite
evidence.

According to the recent literature, about 350 patients have been
treated with CY.  The subjective side-effects of this drug make
blinded studies quite impossible and CY immunosuppressed patients are
usually compared with matched untreated patients or with patients
receiving other treatments.  As previously mentioned, CY is generally
used as an acute (pulse) intensive immunosuppression for 3 to 4
weeks, associated or not with Prednisone therapy.

It appears that after short term CY immunosuppression, a sig-
nificant tendency towards a lower ARR and a slower evolution is
observed in about 70% of the patients.  This beneficial effect is
mostly marked in patients with a definite progression or with a high
ARR.  However, this influence on the disease is transient, lasting
for 1 to 2 years.  For this reason, in recent studies chronic immuno-
suppression with CY, Levamisole or Aza was used after the acute
treatment.  Compared the transient effects of a single acute CY

immunosuppression, it appears from those preliminary studies that, after a mean follow-up for 4 years, a combined acute and chronic CY therapy produced a significant reduction of the progression rate of the disease.

Immune treatments more specifically directed against lymphocyte cells (ALS, ALG, thymectomy, lymphocytapheresis) seem to yield only moderate and transient improvement, if any.

Plasmapheresis appears of marginal benefit in spite of its favorable effects on serum and CSF IgG production, visual evoked potential conduction, and serum neuroelectric blocking activity.

No clinical studies are available to date, definitely supporting or denying the clinical utility either of immunomodulators (Levamisole, Transfer factor, Interferons) or desensitization techniques (Basic Protein, Copolymer I).

The mechanisms by which hyperbaric oxygen, linoleic acid and Immunoglobulin G possibly influence the immune system remain unclear. Longterm linoleic acid supplementation reportedly reduces the severity and length of the relapses, without any evident influence on the progression rate. The utility of HBO is strongly debated and will only be established by the results of a multicenter clinical study presently underway. In the same way the potential improvement observed after infusion of high doses of IgG must be confirmed in controlled studies.

An analysis of the preceeding paragraphs provides definite arguments in favor of a real, even though moderate, effect of some immune treatments on the natural history of MS disease.

Prolonged immunodepression with cytostatic agents appears more effective than other techniques. CY, an agent acting on both humoral and cellular immunity, seems more potent than Aza, a drug mainly depressing the humoral effector arm.

Unfortunately, alkylating agents (CY, chlorambucil) are more carcinogenetic than antimetabolites (Aza). It is admitted that prolonged use of chlorambucil for non malignant diseases must be discouraged, and so far, 8 cases of acute leukemia have been reported in MS patients chronically treated with this alkylating agent.

Acute leukemia has been documented in some patients after prolonged administration of CY for non-neoplastic diseases. However, as far as we know, no malignancies related to CY immunosuppression in MS patients have been described.

For those two alkylating agents, the risk seems dose related and no acute leukemia has been reported after administration of a total

dose smaller than 1 g for chlorambucil and 50 g for CY and urinary bladder cancer induced by CY has been observed after prolonged treatment with high doses (over 170 g for 4 years).

Acute myelogenous leukemia in patients with non-malignant diseases maintained on chronic immunosuppression with Aza, has been less frequently observed, and to date, no cases have been reported in Aza immunosuppressed MS patients.

In our experience, the prevalence of cancer for MS is the same as for the whole general population. In a series of 3000 patients, 21 certain cases of malignant disease were found (0,7%). Of note is that the prevalence of acute leukemia in this group (3 cases = 0,1%) is 10 times that of the general population (Seidenfeld et al., 1976). The same holds for rheumatoid arthritis, another immune disease (Isomaki et al., 1975).

Even though longterm complications of bladder cancer, leukemia or other teratogenic effects have not been reported after maintained immunosuppression in MS (with the exception of chlorambucil), they remain potential severe side-effects that must not be underestimated. Intermittent, repeated chemotherapy, or limiting treatment to as short duration as possible according to the evolution of the disease, may reduce the incidence of severe adverse reactions. However, we still need reliable parameters for an objective assessment of the biopathological status of MS patients. There is a reasonable hope that longitudinal monitoring of new immune parameters will help to delineate the real effects of immunosuppression as well as the fluctuations of the disease. Preliminary observations presented during this meeting by Kolar seem very promising to this effect.

The principle of immune treatments in MS is a valid one but we have to investigate new avenues in immunological manipulations to make them more effective at lower risks. It has been suggested that some actual treatments could be improved. Where EAE prevention by MBP is concerned, it has been shown that the efficacy is enhanced when administered together with Galactocerebroside. According to Raine (1983), MBP should act through a T cell response and Galactocerebroside through an antibody dependant response. In the same way desensitization in MS should be improved by addition of a lipid hapten to MBP.

Immunological effect of immunosuppressive agents have been recently studied by Shih et al. (1983) with a view to their possible therapeutical application in MS (Table 52).

According to the authors, CCNU presents several advantages including reduction of IgG synthesis, enhancement of suppressor activity, andy easy access to the CNS. As far as we know, no clinical studies have been performed with this agent in MS.

Table 51.  Immunoglobulin G in MS.

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Schuller et al. (1983)
- 31 patients
- i.m. injection of 5 g gammaglobulin, 3 times a week or i.v.
  injection, once a week
- Results:

|           |    |    |
|-----------|----|----|
| Improved  | 11 |    |
| Stable    | 9  | 20 |
| Worsened  | 11 |    |

Guenther et al. (1984)
- 20 patients
- 5 g i.v. at 2 months interval for 12 months
- Results:

|                | Fog scale | Annual R.R. |
|----------------|-----------|-------------|
| Before treat.  | 28.8      | 1.13        |
| After 6 months | 21.3      | 1.02        |
| After 1 year   | 19        | 0.99        |

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Table 52.  Immunological Effects of 3 Immunosuppressive Agents
           (modified from Shih et al., : Cl.Exp.Imm. 1983).

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| Parameters    | Cyclophosphamide | CCNU      | 5-FU      |
|---------------|------------------|-----------|-----------|
| T cells       | augmented        | no effect | decreased |
| B cells       | decreased        | no effect | no effect |
| S cells (FcR) | no effect        | augmented | no effect |
| K activity    | no effect        | augmented | no effect |
| NK activity   | decreased        | no effect | decreased |
| IgG synthesis | no effect        | decreased | augmented |

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Cyclosporine A (CS-A) belongs to a new generation of immuno-competent agents and it has some potential advantages on previous substances.  Its possible effects on the immune system are listed in Table 53.  (Klaus, 1981).

An important advantage of CS-A should be its lack of antimitotic and cytostatic effects, but more experimental studies are needed.

The suppressive effect of CS-A on EAE has been studied as early as 1977 (Levine and Sowinski) and CY A is as effective as CY.  Since there are strong arguments in favor of an influence of CY in MS patients, CS-A could possibly be beneficial too at lower risk. However prolonged administration of CS-A seems limited by its toxic effects on kidney and liver.  Double blind studies are presently

Table 53.  Immune Effects of Cyclosporine A.

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Humoral Immunity
|       B cells: | not directly suppressed antibody synthesis reduced as a consequence of H cells depression |

Cellular Immunity
|       T cells: | High affinity for proliferating T cells |
|       H cells: | markedly impaired |
|       S cells: | depressed (Borel 1980) mild induction (Routhier 1980) |

| N.B.: | Interferes with the inductive phase Specifically inhibits production of interleukines Acts selectively on immunocompetent cells Lacks antimitotic and cytostatic effects |

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under way in two neurological centers, but no results will be available before 1986.

Monoclonal antibodies (Mab) are wonderful biological tools not only because of their ability to define single antigenic determinants but also because of their potential capacity to modulate antigens specifically.  Since our knowledge of immunoregulation is progressing faster and faster, the answer for the treatment of MS might be found in the manipulation of the immune system by idiotypic or anti-idiotypic antibodies directed at the clones of immunoreactive cells implicated in the pathogenesis of the disease.  Of note is that Brinkman et al. (1984) have already been able to suppress EAE in Lewis rats by Mab to all peripheral T lymphocytes (W3/13).

CONCLUSION

The pressure to find relief for MS has never been so strong and we are indeed at a critical point in the search for a treatment.

In the beginning, immunosuppressive treatments were based more on hope and challenge than on clear scientific basis.  Since many years most scientists are convinced that immune reactions are associated with the pathogenesis of Ms disease, but until recently, we were ignorant of the involved mechanisms.

During the last few years, our understanding of immune effector arms is advancing fast.  Where MS is concerned, several observations suggest that the immune mechanisms involved in the progression of the disease are not necessarily restricted to the CNS but are possibly shared with other systems such as the lympoid tissues.  It is strik-

ing to observe that MS lesions are preferentially localized around capillaries and ventricular walls, where peripheral immunocompetent cells come into contact with the CNS.

The exact nature of a putative interaction between CNS and extracerebral tissue remains unclear. One possibility is that MS is a "lymphoid-CNS" disease, which means that the immunizing event initiating the production of demyelinating factors occurs in extra-neural lymphoid system years earlier, and that CNS demyelination represents a delayed reaction secondary to an autoregulation defect in lymphoid tissues. Another possibility is that specific antibodies produced in the brain enter the peripheral blood and recruit peri-pheral lymphocytes to migrate in CNS and bring about an antibody-dependant cellular cytotoxicity reaction against glial cells.

There are more and more arguments in favor of a peripheral origin of the lymphoid cells associated in the CNS with the develop-ment of MS lesions. Those observations circumvent the presence of the blood brain barrier and encourage attempts to influence the evolution of MS disease by manipulations of the immune system.

The major problem in evaluating the results of tenative treat-ments in MS is the impredictable evolution of the disease. There is little doubt however that this difficulty will be partly solved by a longitudinal monitoring of biopathological events with the recently discovered immunological tests and by appreciating the evolution of brain lesions by the nuclear magnetic resonance technique.

It is therefore to be expected that an objective appreciation of the efficacy of immune treatments in MS will be possible within the next five years. In the meantime it would be most unfortunate if untimely conclusions or inflated publicity about the results brought discredit on those clinical trials.

REFERENCES

Aimard, G., Girard, P. F., and Raveau, J., 1966, Sclérose en plaques et processus d' autoimmunisation. Traitement par les anti-mitotiques, lyon Med., 215:345.

Aimard, G., Confavreux, C., Ventre, J. J., Guillot, M., and Devic, M., 1983, Etude de 213 cas de sclérose en plaques traités par l'azathiorpine de 1967 á 1982, Rev.Neurol., 139:509.

Aleksandrowicz, J., Skotnicki, A. B., and Zduńczyk, A., 1982, The combined immunomodulatory treatment in patients with multiple sclerosis - A long-term study, Personal Communication.

Baixe, J. H., 1978, Bilan de onze années d'activité en médecine hyperbare, Med.Aer.Spatiale Med., Subaquatique Hyperbare, 17:90.

Basten, A., Pollard, J. D., Stewart, G. J., Frith, J. A., McLeod, J. G., Walsh, J. C., Garrick, R., and Van Der Brink, C. M., 1980, Transfer factor in treatment of multiple sclerosis, The Lancet, II:931.

Behan, P. O., Durward, W. F., Melville, I. D., McGeorge, A. P., and Behan, W. M. H., 1976, Transfer factor therapy in multiple sclerosis, The Lancet, I:988

Benczur, M., Petranyi, G. Gy., Palffy, Gy., Varga, M., Talas, M., Kotsy, B., Földes, I., and Hollan, S. R., 1980, Dysfunction of natural killer cells in multiple sclerosis: a possible pathogenetic factor, Clin.Exp.Immunol., 39:657.

Bolton, C., Borel, J. F., Cuzner, M. L., Davison, A. N., and Turner, A. M., 1982, Immunosuppression by cyclosporin A of experimental allergic encephalomyelitis, J.of the Neurol.Soc., 56:147.

Borel, J. F., 1980, Essentials of cyclosporin A. A novel type of antilymphocyte agent, Trends Pharmacol.Sci., 1:146.

Bornstein, M. B., Miller, A. I., Teitelbaum, D., Arnon, R., and Sela, M., 1982, Multiple Sclerosis: Trial of a synthetic polypeptide, Ann.Neurol., 11:317.

Boschetty, V., and Cernoch, J., 1970, Aplikace kysliku za pretlaku u nekterych Neurologiekych onemochemy, Bratisl.Lek.Listy, 52:298.

Brinkman, C. J. J., and Homes, O. R., 1982, The effect of cyclophosphamide on T lymphocyte and T lymphocyte subsets in patients with chronic progressive multiple sclerosis, in: "Immunosuppression in Multiple Sclerosis, Proceedings of the Nijmegen Workshop June 10-12, 1982. Clinical trials Journal 1984". O. Hommes, J. Mertin, W. W. Tourtellotte, eds., (to be published).

Camenga, D. L., 1977, Safety and efficacy of Levamisole in multiple sclerosis, in: "Excerpta Medica Proceedings of the 11th World Congress of Neurology, Amsterdam".

Campbell, B., Vogel, P. J., Fisher, E., and Lorenz, R., 1973, Myelin basic protein administration in multiple sclerosis, Arch.of Neurol., 29:10.

Capparelli, R., Inzitari, D., Sita, D., Guarnieri, B. M., Fratiglioni, L., Amaducci, L., Avanzi, G., Franco, C., and Lombardo, R., 1984, Plasmapheresis in multiple sclerosis: cerebrospinal fluid changes after treatment, in: "Immunological and Clinical Aspects of Multiple Sclerosis," R. E. Gonsette, P. Delmotte, eds, MTP Press Limited, Lancaster.

Cendrowski, W., and Czlonkowska, A., 1978, Levamisole in multiple sclerosis, with special reference to immunological parameters, Acta.Neurol.Scand., 57:354.

Cook, A. W., Carter, W. B., and Nidzgorski, F., 1982, Interferon responses of leukocytes in MS, Neurol., 32:104 .

Collins, R. C., Espinoza, L. R., Plank, C. R., Ebers, G. C., Rosenberg, R. A., and Zabriskie, J. B., 1978, A double-blind trial of transfer factor vs placebo in multiple sclerosis patients, Clin.Exp.Immunol., 33:1.

Dachsel, R., Wieczorek, V., and Voigt, W., 1982, Klinische
    Längschnittuntersuchungen unter immunsuppressiver therapie bei
    multipler sklerose, Dt.Gesundh.-Wesen, 37:2087.
Dau, P. C., Johnson, K. P., and Spitler, L. E., 1976, The effect of
    levamisole on cellular immunity in multiple sclerosis, Clin.
    Exp.Immunol., 26:302.
Dau, P. C., Petajan, J. H., Johnson, K. P., Panitch, H. S., and
    Bornstein, M. B., 1980, Plasmapheresis in multiple sclerosis:
    preliminary findings, Neurol., 30:1023.
De Saxce, H., Marteau, R., and Lhermitte, F., 1984, Treatment of
    progressive multiple sclerosis by the combined application of
    antilymphocyte serum, azathioprine and prednisone, in: "Immuno-
    logical and Clinical Aspects of Multiple Sclerosis," R. E.
    Gonsette, P. Delmotte, eds., MTP Press Limited, Lancaster.
Dominguez, R., Chamoles, N., and Somoza, M., 1978, Intrathecal
    methotrexate in multiple sclerosis, in: "Humoral Immunity in
    Neurological Diseases," D. Karcher, A. Lowenthal, eds, Plenum
    Press, N. Y., London.
Dworkin, R. H., Bates, D., Millar, J. H. D., Paty, D. W., and Shaw,
    D. A., 1984, Dietary supplementation with polyunsaturated fatty
    acids in acute remitting multiple sclerosis, in: "Immunological
    and Clinical Aspects of Multiple Sclerosis," R. E. Gonsette, P.
    Delmotte, eds., MTP Press Limited, Lancaster.
Ferguson, T. B., Clifford, D. B., Montgomery, E. B., Bruns, K. A.,
    McGregor, P. J., and Trotter, 1983, Thymectomy in Multiple
    Sclerosis.  Two preliminary trials, J.Thorac.Cardiovasc.Surg.,
    85:88.
Fischer, B. H., Marks, M., and Reich, T., 1983, Hyperbaric-oxygen
    treatment of multiple sclerosis. A randomized, placebo-
    controlled, double-blind study, The New England J. of Med.,
    308:181.
Fog, T., Raun, N. E., Pedersen, L., Kam-Hansen, S., Mellerup, E.,
    Platz, P., Ryder, L. P., Jakobsen, B. K., and Grob, P., 1978,
    Long-term transfer-factor treatment for multiple sclerosis, The
    Lancet, I:851.
Fog, T., 1980, Interferon treatment of multiple sclerosis patients. A
    pilot study, in: "Search for the cause of multiple sclerosis
    and other chronic diseases of the central nervous system," A.
    Boese, eds., Verlag Chemie, Weinheim.
Formai, C., Sereni, G., and Zannini, D., 1980, L'ossigenoterapia
    iperbarica nel trattamento della sclerosi multipla, in: "4th
    Congresso Nationale di Medicina subacquea ed Iperbarica,"
    Napoli.
Giordano, G. F., Masland, W., Ketchel, S. J., Holland, K, Tilmann,
    K, Wallace, B. A., and Jones, R. M., 1982, An investigation of
    lymphocytapheresis in multiple sclerosis, Plasma Ther.Transfus.
    Technol., 3:417.
Gonsette, R. E., Demonty, L., and Delmotte, P., 1977, Intensive
    immunosuppression with cyclophosphamide in Multiple Sclerosis,
    Follow-up of 110 patients for 206 years, J.Neurol., 214:173.

Gonsette, R. E., Delmotte, P., and Demonty, L., 1977, Failure of
    basic protein therapy for Multiple Sclerosis, J.Neurol.,
    216:27.
Gonsette, R. E., Demonty, L., Delmotte, P., Decree, J., de Cock, W.,
    Verhaeghen, H., and Symoens, J., 1982, Modulation of immunity
    in multiple sclerosis: a double-blind levamisole-placebo con-
    trolled study in 85 patients, J.Neurol., 228:65.
Gonsette, R. E., Demonty, L., De Smet, Y., and Delmotte, P., 1984,
    Immunosuppression with cyclophosphamide in multiple sclerosis,
    in: "Immunological and Clinical Aspects of Multiple Sclerosis,"
    R. E. Gonsette, P. Delmotte, eds., MTP Press Limited,
    Lancaster.
Guarnieri, B. M., Amaducci, L., Cambi, F., Capparelli, R., Arfaioli,
    C., Avanzi, G., and Franco, C., 1984, Changes of polymorpho-
    nuclear neutral proteinase activity in multiple sclerosis
    patients before and after plasma exchanges, in: "Immunological
    and clinical aspects of Multiple Sclerosis," R. E. Gonsette, P.
    Delmotte, eds., MTP Press Limited, Lancaster.
Guenther, W., Neu, I. S., Koenig, N., and Rothfelder, U., 1984,
    Experiences with immunoglobulin-G infusions in the treatment of
    acute multiple sclerosis, in: "Immunological and Clinical
    Aspects of Multiple Sclerosis," R. E. Gonsette, P. Delmotte,
    eds., MTP Press Limited, Lancaster.
Hauser, S. L., Dawson, D. M., Lehrich, J. R., Beal, M. F., Kevy, S.
    V., Propper, R. D., Mills, J. A., and Weiner, H. L., 1983,
    Intensive immunosuppression in progressive multiple sclerosis.
    A randomized, three-arm study of high-dose intravenous cyclo-
    phosphamide, plasma exchange, and ACTH, The New Engl.J.of Med.,
    308:173.
Hauser, S. L., Dawson, D. M., and Weiner, H. L., 1983, Immunosup-
    pression for Multiple Sclerosis, letter, New Engl.J.of Med.,
    309:241.
Hommes, O. R., Lamers, K. J. B., and Reekers, P., 1982, Long-term
    follow-up after intensive immunosuppression of 47 patients with
    chronic progressive multiple sclerosis, in: "Immunosuppression
    in Multiple Sclerosis, Proceedings of the Nijmegen Workshop
    June 10-12, 1982. Clinical Trials Journal 1984," O. Hommes, J.
    Mertin, W. W. Tourtellotte, eds, (to be published).
Isomaki, H. A., Mutru, O., and Koota, K., 1975, Death rate and cause
    of death in patients with rhumatoid arthritis, Scand.J.Rhum.,
    4:205.
Jacobs, L., O'Malley, J., Freeman, A., Murawski, J., and Ekes, R.,
    1982, Intrathecal interferon in multiple sclerosis, Arch.
    Neurol., 39:609.
Johnson, K., 1983, Viruses as initiator of central nervous system
    autoimmune disorders, in: "Symposium on immune regulation and
    its application to Multiple Sclerosis, Vancouver, Canada".
Kastrukoff, L. F., McLean, D. R., and McPherson, T. A., 1978,
    multiple sclerosis treated with antihymocyte globulin - A five
    year follow-up, J.Canc.Sc.Neurol., 5:175.

Kaudewitz, P., Zander, H., Abb, J., Ziegler-Heitbrock, H. W., and
     Riethmüller G., 1983, Genetic influence on natural cytotoxicity
     and interferon production in multiple sclerosis studies in
     monozygotic discordant twins, Hum.Immunol., 7:51.
Kennes, B., Leroy, C-P., Dumont, J-P., Brohée, D., Jacquy, J., Noël,
     G., and Nève, P., 1984, Lympho-plasmapheresis in multiple
     sclerosis: effect on clinical and immunological parameters, in:
     "Immunological and Clinical Aspects of Multiple Sclerosis," R.
     E. Gonsette, P. Delmotte, eds., MTP Press Limited, Lancaster.
Kindwall, E. P., 1983, National multiple sclerosis registry, HBO
     Review, 4:38.
Klaus, G. G. B., 1981, The effects of cyclosporin A on the immune
     system, Immunology today, 83.
Kolar, O. J., 1983, Clinical application of examination of CSF
     subpopulation of T cells, in: "International Workshop on multi-
     ple sclerosis, Erice."
Lamoureux, G., Cosgrove, J., Duquette, P., Lapierre, Y., Jolicoeur,
     R., and Vanderland, F., 1981, A clinical and immunological
     study of the effects of transfer factor on multiple sclerosis
     patients, Clin.Exp.Immunol., 43:557.
Lauer, K., Firnhaber, W., and John, D., 1984, Longterm treatment with
     azathioprine in multiple sclerosis patients, in: "Immunological
     and clinical aspects of Multiple Sclerosis," R. E. Gonsette, P.
     Delmotte, eds., MTP Press Limited, Lancaster.
Levine, S., and Swoinski, R., 1977, Suppression of the hyperacute
     form of experimental allergic encephalomyelitis by drugs,
     Arch.int.Pharmacodyn., 230:309.
McFarland, H. F., and Rose, J. W., 1982, Lymphocytapheresis in the
     treatment of multiple sclerosis, Plasma Ther Transfus Techn.,
     3:411.
Massaro, A. R., and Burrai, I., 1981, Levamisole treatment in
     multiple sclerosis, in: "Excerpta Medica. Proceedings of the
     12th World Congress of Neurology, Kyoto".
Mertin, J., Kremer, M., Knight, S. C., Batchelor, J. R., Halliday, A.
     M., Rudge, P., healey, M. J. R., Compston, A., Thompson, E. J.,
     and Denman, M., 1982, Double-blind controlled trial of immuno-
     suppression in the treatment of multiple sclerosis: final
     report, The Lancet II:351.
Myers, L. W., Ellison, G. W., Levy, J., Holevoet, M., and
     Tourtellotte, W. W., 1977, Experience with Levamisole in multi-
     ple sclerosis, in: "Excerpta Medica.Proceedings of the 11th
     World Congress of Neurology, Amsterdam".
Neighbour, P. A., and Bloom, B. R., 1979, Absence of virus-induced
     lymphocyte suppression and interferon production in multiple
     sclerosis, Proc.Natl.Acad.Sci.USA, 76:476.
Neighbour, P. A., Miller, A. E., and Bloom, B. R., 1981, Interferon
     responses of leucocytes in multiple sclerosis, Neurol., 31:561.
Neubauer, R. A., 1980, Exposure of multiple sclerosis patients to
     hyperbaric oxygen at 1,5-2 ATA: a preliminary report, J.Fla.
     Med.Assoc., 67:498.

Oger, J. J-F., Antel, J. P., Kuo, H. H., and Arnason, B. G. W., 1982, Influence of azathioprine (Imuran) on in vitro immune function in multiple sclerosis, Ann.Neurol., 11:177.

Pallotta, R., Anceschi, S., and Costagliola, N., 1980, Prospettive di terapia iperbarica nella Sclerosi a Placche, Ann.Med.Navale, 85:57.

Patzold, U., Haller, P., Haas, J., Pocklington, P., and Dreicher, H., 1979, Therapie der Multiplen Sklerose mit Levamisole und Aza-thioprin, Nervenarzt, 49:285.

Patzold, U., Hecker, H., and Pocklington, P., 1982, Azathioprine in treatment of multiple sclerosis.  Final results of a 4½ year controlled study of its effectiveness covering 115 patients, J.Neurol.Sci., 54:377.

Patzold, U., and Haas, J., 1982, Adverse effects of longterm treatment with azathioprine, in: "Immunosuppression in Multiple Sclerosis, Proceedings of the Nijmegen Workshop, June 10-12, 1982.  Clinical trials Journal 1984", O. Hommes, J. Mertin, W. W. Tourtellotte, eds., (to be published).

Raine, C. S., and Traugott, U., 1983, Chronic relapsing experimental autoimmune encephalomyelitis.  Ultrastructure of the central nervous system of animals treated with combinations of myelin components, Labor. Invest., 48:275.

Romine, J. S., and Salk, J., 1983, A study of myelin basic protein as a therapeutic probe in patients with multiple sclerosis, in: "Multiple Sclerosis," J. F. Hallpike, C. W. Adams, W. W. Tourtellotte, eds., Chapman and Hall, London.

Rosen, J. A., 1979, Prolonged azathioprine treatment of non-remitting multiple sclerosis, J.Neurol.Neurosurg. & Psych., 42:338.

Rosen, A. D., and Hamburger, M. I., 1981, Plasmapheresis in multiple sclerosis: effect on the visual evoked potential, Plasma Ther. Transfus.Techn., 2:239.

Routhier, G., Janossy, G., Epstein, O., Thomas, H. C., Sherlock, S., Kung, P. C., and Goldstein, G., 1980, Effects of cyclosporin A on suppressor and inducer T lymphocytes in primary biliary cirrhosis, The Lancet, II:1223.

Ruutiainen, J., Panelius, M., and Cantell, K., 1983, Toxic effects of interferon administered intrathecally, British Med.J., 286:940.

Sabouraud, O., Madigand, M., and Merienne, M., 1982, Continuous immuno-suppressive therapy for multiple sclerosis: appraisal of 67 cases started before 1972, in: "Immunosuppression in Multi-ple Sclerosis, Proceedings of the Nijmegen Workshop.  June 10-12, 1982.  Clinical trials Journal 1984" O. Hommes, J. Mertin, W. W. Tourtellotte, eds., (to be published).

Salonen, R., Ilonen, J., Reunanen, M., and Salmi, A., 1982, Defective production of interferon-alpha associated with HLA-DW2 antigen in stable multiple sclerosis, J.Neurol.Sc., 55:197.

Santoli, D., Hall, W., Kastrukoff, L., Lisak, R. P., Perussia, B., Trinchieri, G., and Koprowski, H., 1981, Cytotoxic activity and interferon production by lymphocytes from patients with multi-ple sclerosis, J.of Immunol., 126:1274.

Schuller, E., and Govaerts, A., 1983, First results of immunotherapy
    with immunoglobulin G in multiple sclerosis patients, Eur.
    Neurol., 22:205.
Seidenfeld, A. M., Smythe, H. A., Ogryzlo, M. A., Urowitz, M. B., and
    Dotten, D. A., 1976, Acute leukemia in rheumatoid arthritis
    treated with cytotoxic agents, J.Rheum., 3:295.
Seil, F. J., Westall, F. C., Romine, J. S., and Salk, J., 1983, Serum
    demyelinating factors in multiple sclerosis, Ann.Neurol.,
    13:664.
Shih, W. W. H., Baumhefner, R. W., Tourtellotte, W. W., Haskell, C.
    M., Korn, E. L., and Fahey, J. L., 1983, Difference in effect
    of single immunosuppressive agents (cyclophosphamide, CCNU,
    5-FU) on peripheral blood immune cell parameters and central
    nervous system immunoglobulin synthesis rate in patients with
    multiple sclerosis, Clin.Exp.Immunol., 53:122.
Stefoski, D., Schauf, C. L., McLeod, B. C., Haywood, C. P., and
    Davis, F. A., 1982, Plasmapheresis decreases neuroelectric
    blocking activity in multiple sclerosis, Neurol., 32:904.
Tourtellotte, W. W., Potvin, A. R., Mendez, M., Baumhefner, R. W.,
    Potvin, J. H., Ma, B.I., and Syndulko, K., 1980, Failure of
    intravenous and intrathecal cytarabine to modify central ner-
    vous system IgG synthesis in multiple sclerosis, Ann.Neurol.,
    8:402.
Tovell, D. R., McRobbie, I. A., Warren, K. G., and Tyrrell, D. L. J.,
    1983, Interferon production by lymphocytes from multiple
    sclerosis and non-MS patients, Neurol., 33:640.
Trotter, J. L., Rodey, G. E., and Gebel, H. M., 1982, Azathioprine
    decreases suppressor T cells in patients with multiple
    sclerosis, The New Engl.J. of Med., 306:365.
Turk, J. L., and Parker, D., 1979, The effect of cyclophosphamide on
    the immune response, J. of Immunopharmacol., 1:127.
Valbonesi, M., Garelli, S., Mosconi, L., Zerbi, D., and Forlani, G.,
    1981, Plasma exchange in the management of patients with multi-
    ple sclerosis: preliminary observations, Vox Sang., 41:68.
Van Haver, H., Lissoir, F., Theys, P., Droissart, Chr., Van Hees, J.,
    Ketelaer, P., Carton, H., Gautama, K., Vandeputte, I., and
    Vermylen, C., 1984, Transfer factor treatment in MS: a 3 year
    prospective double-blind study (1982-1985), in: "Immunological
    and Clinical Aspects of Multiple Sclerosis," R. E. Gonsette, P.
    Delmotte, eds., MTP Press Limited, Lancaster.
Ververken, D., Carton, H., and Billiau, A., 1979, Intrathecal
    administration of interferon in MS patients, in: "Humoral
    Immunity in Neurological Diseases," D. Karcher, A. Lowenthal,
    eds., Plenum Press, N. Y. and London.
Vervliet, G., Claeys, H., Van Haver, H., Carton, H., Vermylen, C.,
    Meulepas, E., and Billiau, A., 1983, Interferon production and
    natural killer (NK) activity in leukocyte cultures from multi-
    ple sclerosis patients, J.Neurol.Sci., 60:137.
Warren, J., Sacksteder, M. R., and Thuning, C. A., 1978, Oxygen
    immunosuppression: modification of experimental allergic enc-

ephalomyelitis in rodents, J. of Immunol., 121:315.Weiner, H. L., Dawson, D. M., 1980, Plasmapheresis in multiple sclerosis: preliminary study, Neurol., 30:1029.

Zander, H., Abb, J., Kaudewitz, P., and Riethmüller, G., 1982, Natural killing activity and interferon production in multiple sclerosis, The Lancet, I:280.

Zeeberg, I., Heltberg, A., Kristensen, J. K., Raun, N. E., and Fog, T., 1982, A longterm double-blind, controlled trial - of aza-thioprine versus placebo, - in treatment of progressive multi-ple sclerosis, in: "Immunosuppression in multiple sclerosis, Proceedings of the Nigmegen Workshop June 10-12, 1982. Clini-cal Trials Journal," O. Hommes, J. Mertin, W. W. Tourtellotte, eds., (to be published).

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