Summary and Concluding Remarks

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Experimental Models in Demyelinating Disease

At the Locarno symposium on the pathogenesis and etiology of demyelinating diseases in 1967, Seitelberger stressed that on neuropathologic grounds human MS is a process and has features of its own which simply cannot be compared to the changes found in experimental models. This statement is still valid today. Meanwhile, however, at least one of the animal models of experimental demyelination, chronic relapsing EAE, has come closer to MS in the human. The undeniable value of experimental models, even though they clearly represent a pathologic process different from MS, is the possibility of focusing on phenomena that are, or may be, key reactions in the chain of pathogenetic events leading to demyelination and its recurrence in the human disease.

Raine pointed out the similarities of Stone and Lerner's model of chronic relapsing EAE in strain 13 guinea pigs, produced by a single injection of isogenic spinal cord in complete Freund's adjuvant. They reported on the permanent suppression of the experimental disease by injections of myelin basic protein (MBP) and incomplete Freund's adjuvant. Wisniewski and Glassman were able to improve the model and to produce a chronic disease with predictable remissions and relapses in 80% of strain 13 guinea pigs. They were not able to induce chronic EAE with plaquelike demyelination by sensitization with MBP, however, and they found a sensitization to MBP only in the acute episode of the experimental disease. They concluded that antigens other than MBP play an important role in recurrence of EAE and the formation of large demyelinated plaques similar to those in MS. These observations may be relevant to current therapeutic trials with MBP and copolymers in MS. Considering the fact that no adjuvants are being used in these trials, and furthermore that MBP is subject to rapid proteolysis in the blood stream (Alvord and co-workers), Wisniewski's findings could question the chances for success in the treatment of recurrent chronic MS with these substances. The report of Nagai and co-workers on the prevention of EAE by complete Freund's adjuvant alone should also be mentioned in connection with this question.

Lipton and Dalcanto consider Theiler's virus infection of the central nervous system of mice an excellent model for MS because there is selective demyelination in the chronic phase of the infection, with simultaneously occurring active and inactive lesions, and because a chronic progressive disease, with immune-mediated myelin breakdown, can be produced using tissue-culture adapted virus. In their report they also point out, however, that in the Theiler virus infection there is often severe leptomeningitis, and that immunosuppression produces an overall potentiation of the

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infection, with increased microglial proliferation and neuronal necrosis and an increase in mortality.

Acute and recurrent demyelination due to virus-induced destruction of oligodendrocytes caused by mutants of mouse hepatitis virus was described by Lampert and co-workers. Nagashima et al. reported on subacute demyelinating encephalomyelitis in rats due to infection with murine corona virus JHM. Corona virus JHM was also used in an in vitro carrier culture system of persistently infected mouse neuroblastoma cells by Stohlman, Sakguchi, and Weiner to study the question of defective viral infection, in which viral antigens are undetectable by conventional techniques even in the presence of complete viral genome. One of the intriguing aspects of this work is the possibility that latent virus genome, not interfering with cell function under ordinary conditions, could produce cell damage and even death under stress. Referring to MS, a mechanism of this type could serve to explain how an oligodendrocyte, subject to infection by an unidentified defective virus, could suffer damage and how demyelination could be triggered by unspecific and transient stress. Of interest in relation to this experimental approach is the possible isolation of corona virus from autopsy material in two cases of MS by Burks and co-workers. A laboratory contamination appeared unlikely in view of the positive serologic findings in body fluids of the cases from which the virus was isolated. The authors were cautious with respect to a causal relationship, and further confirmation appears necessary.

Chronic demyelination due to visna-virus remains an interesting experimental approach: since the visna-virus possesses a reverse transcriptase and is capable of producing a provirus not recognized by the immune defense mechanism of the body, latent infection can persist in a small proportion of brain cells. The possible significance of similar mechanisms in MS requires further study.

A possible biochemical approach to the question of virus infection in MS may be the development of techniques to detect viral genomic information in organ material. An example of this was presented by Dörries and ter Meulen with the examination of MS brain material for genomic information of herpes simplex type 1 and adenovirus type 2 by DNA-DNA reassociation kinetics. This technique, which proved useful in herpes encephalitis, did not detect any signs of herpes or adenovirus in the brain material of ten cases of MS.

Chronic relapsing EAE appears to be the most noteworthy recent advance in the study of models of experimental demyelination. However, the use of different virus models in research on the cause of MS testifies to the fact that, in addition to the pathogenetic mechanisms operative in EAE, other possibilities demand consideration. The impression could arise that increasing knowledge in the field of experimental demyelination is not narrowing the possibilities of developing one valid concept, but leading to further diversification. Naturally occurring viruses and mutants provide us with a variety of models for demyelination, each of which may have a lesson to offer in the study of the cause or causes of demyelination in MS, but none providing the precise experimental counterpart to the human disease.

In line with this is the situation with respect to the epidemiologic and virologic investigations on common viruses and MS: while myxoviruses, especially measles, appear as prime candidates, increased antibody titers have also been found in some MS cases against herpes simplex, varicella, rubella, and cytomegalovirus. Of the va-

rious isolates that have been reported, none have been sufficiently confirmed to date. With this background, the tenuous situation of laboratory investigations in relation to the etiology and pathogenesis of MS is pinpointed. The relevance of laboratory findings to MS thus far is predominantly based on their casuistic or statistical correlation to the clinicopathologic condition, insofar as this is identifiable on the basis of clinical findings and, in a comparatively minute proportion of cases, on histopathologic confirmation.

Laboratory Investigations in Relation to Etiology and Pathogenesis of MS

The broad spectrum of laboratory methods used in the examination of body fluids and tissue material from MS cases encompasses three objectives:

1. Improvement of reliability of the diagnosis of MS;
2. Assay of process activity;
3. Investigations relevant to the etiology and pathogenesis of MS.

The last of the three objectives was the topic of the second section of our symposium.

In his survey on IgG, Tourtellotte points out that *de novo* IgG synthesis in the nervous system can be measured by the use of an empiric formula (his own, Ganrod-Laurell's, or modification of these), an IgG isotopic test, or the demonstration of oligoclonal bands. He presents available evidence and accepted arguments for the hypothesis that CNS IgG synthesis is related to the production of MS lesions, and he also proposes that its quantification may serve as a useful parameter in assessing the value of putative therapies in MS. Numerous studies have shown that local IgG-synthesis, though of considerable diagnostic value, is not a practicable parameter for assessing process activity. However, in their studies on immunosuppressive therapy with cytostatic drugs, Hommes et al. found that the IgG concentration in CSF responds to immunosuppression.

Oligoclonal bands are found in 80%–90% of CSF specimens from MS patients, with the figures in the literature ranging from 45% (Clausen) to 90%–95% (R. M. Schmidt et al.). In a comparison of the agar gel technique, the most widely used method, with isoelectric focusing (IEF), Delmotte found the latter to be the more sensitive method. IEF also showed a 100% correlation with capillary isotachopheresis in the detection of local IgG synthesis; both methods present a more accurate methodological basis for the differentiation of locally synthesized IgG and a disturbance of blood-CSF barrier function than other procedures do.

An approach different from the analysis of oligoclonal bands is the quantitative determination of IgG subclasses in CSF and serum of MS patients by a radioimmunoassay described by Morell and Skvaril. By this technique, Kaschka and associates found Ig1 significantly higher in MS patients, with correspondingly lower values for the three other IgG subclasses.

One of the important results emerging from recent work on the analysis of IgG components of CSF is the possibility of differentiating an increase of IgG in CSF

due to local synthesis within the CNS from an increase that results from damage to the blood-CSF barrier. The demonstration of oligoclonal bands is a valuable technique supporting the diagnosis of MS. In addition to the possibility of correlating antigens to oligoclonal bands, the identification of antibodies composing these sub-fractions promises to become an important area of laboratory research on pathogenetic problems of MS.

This of course poses the problem of antigens relevant to the demyelinating process. Wajgt et al. found increased titers of measles and parainfluenza type 3 specific antibodies in serum and CSF, and a correlation to the IgG response. Sindic and co-workers found an association of oligoclonal bands with elevated IgM concentration in CSF, and they considered this as a useful index of immunologic disorders affecting the CNS. Analyzing IgG oligoclonal bands in CSF, a predominance of light chains type kappa was found, with kappa and lambda components presented in the same band in 27 of 39 MS patients. Studying CSF lipids in demyelinating disease, Seidel, Heipertz, and Buck proposed that the cerebral origin of sphingomyelin and linoleic acid is unlikely. This work is in line with the fact that many previous attempts to correlate the concentration of lipid constituents in CSF to a demyelinating process have not been useful in diagnosis. With respect to MBP the situation appears to be different: the results of several investigators in different parts of the world (Cohen and McKhann, Whitaker, Hempel, Kohlschütter and others) all indicate that the concentration of MBP in CSF is a useful criterion of process activity. Panitch and K. Johnson demonstrated antibodies against MBP more frequently in active stages of MS, but they found no correlation to IgG or oligoclonal bands.

Clausen et al. found two "MS-specific" antigens in brain tissue, one of which caused antibody formation in experimental animals, the nature of which (microbial, brain cell protein or complexes) is still unknown. Measles antigen was found in three of seven brains, but not considered as specific for MS.

The reports mentioned exemplify only in a fragmentary manner various approaches endeavoring to elucidate the nature of oligoclonal bands found in a high percentage of CSF specimens from MS patients. This is a field of work still in its beginnings. A critical prerequisite in all of these studies is the reproducibility of bands. Since it is accepted that oligoclonal bands consist of antibodies with restricted heterogeneity, reliable quantitation is important for the identification of antibodies involved. But even with these problems solved, there still remains the question of whether we are dealing with etiologic and/or pathogenetic determinants, or only with epiphenomena.

A most instructive contribution to this question was the report by Vandvik and Norrby on viral antibody responses in the CNS of patients with MS. Whereas in a number of diseases (SSPE, chronic-progressive rubella encephalitis, mumps, meningitis, and neurosyphilis) the bulk of oligoclonal bands consists of antibodies relevant to the causal agents, this is not the case in MS. With the "imprint electroimmunofixation" technique, they found that local synthesis was restricted to antibodies against measles, mumps, rubella, and herpes simplex in nine of ten MS and in five of ten optic neuritis patients. Later varicella zoster antibody was found (10 of 12 cases) to be even more frequent than measles (6 of 12 cases). However, no relationship was established between the locally synthesized antibodies identified thus far and the major fractions of oligoclonal IgG demonstrable by agarose electrophoresis of

the CSF. As yet the question remains unanswered, whether nonspecific activation could explain all of the locally synthesized oligoclonal IgG, or whether the bulk of oligoclonal IgG in the CSF of MS patients is due to antibody against a still unknown agent. The study of antibodies that result from nonspecific coactivation of cells in diseases of known etiology is in progress. In this connection it should be recalled that there are rapidly progressing MS cases with special symptomatology, but devoid of characteristic CSF alterations. A careful study of these cases with respect to antibody patterns in their oligoclonal fractions might provide new leads.

By the use of skin tests, delayed hypersensitivity to measles antigen – an anergy indicating a selective defect in cell-mediated immunity – was shown to be less in active MS than in patients with stable disease or in healthy adults (Valdimarsson and Agnarsdottir). These authors also observed an impaired release of infectious measles virus by MS lymphocytes. They point out that these results suggest the importance of investigations on the cellular immune response to other enveloped RNA viruses as well. During the past years, the relationship of measles antibody titers to MS has been the subject of numerous studies; in most cases, however, without precise relationship to the clinical stage, i.e., process activity of the disease. Höher and associates found peak titers against subunits of measles virus, hemolysin and nucleoprotein within 1–3 weeks during the acute phase. No titer increases were seen with parainfluenza and poliomyelitis. By radioimmunoassay of antibodies against measles, rubella, and respiratory syncytial virus antigens, Salmi and associates demonstrated significant changes in intrathecal synthesis of antibodies against one or more of the viruses during the course of the disease in 8 of 20 patients, but no correlation to the clinical stage was observed. Salmi and his group are in the process of studying further viruses, and their results indicate that the intracerebral production of antibodies varies from one individual to another, suggesting no single virus as the responsible agent for MS.

An experimental approach to the problems is discussed in the study by Sandberg-Wollheim, Koprowski and associates on hybridomas of mouse myeloma cells versus human lymphocytes. Since antibodies against viruses account at best for only a very small fraction of the CSF-IgG in MS, it is hoped that the hybrids produced by CSF cells of MS patients may help to characterize some of the antibodies involved.

With the rapid advances in knowledge concerning lymphocytes and cell-related immune phenomena, studies on serum and CSF cells – one of the oldest laboratory methods in the diagnosis of MS – have become one of the “hot areas” in research on the immunology of MS.

The findings of Antel and Arnason, that suppressor cell function and mitogenic reactivity vary with disease activity, have pointed the way to an informative laboratory parameter for assessing process activity. The reduction in suppressor cell activity in the increment stages of MS bouts and the sharp rise in the period of recovery is an impressive finding, with further development perhaps a practicable tool for assessing process activity and monitoring of therapeutic studies.

An interesting aspect of this work is the relationship between suppressor cell activity and immune complex levels. Jans et al. detected immune complexes in 25% of sera and 47% of CSF specimens from MS patients. They found a positive correlation to CSF pleocytosis, but none to IgG, duration, severity, and disease activity.

Cuzners study on proteinase inhibitors touches on a problem long recognized, but still in dire need of more systematic study: the proteinase activity of CSF in relation to clinical activity. Cuzner points out that proteolytic activity and increased fragments of MBP appear in periods of exacerbation of MS, with a corresponding decrease of proteinase inhibitors.

Several symposium reports were devoted to studies on the distribution of B and T cells in various stages of MS and in the course of immunosuppressive therapy, as well as to basic investigations such as receptor functions of T cells (Eder and co-workers). The response of T lymphocytes from MS patients to antigens in MS brain material in a high percentage of cases and the pathognomonic nature of the reaction seen by Offner et al. may be of practical diagnostic value and should be analyzed for its potential use as a criterion of process activity. The fact that a drop in E rosette-forming lymphocytes under ACTH therapy was transitory in the investigations of Kaschka and co-workers could reflect limitations in the immunosuppressive effect of the therapy used. The question whether persisting T cell depression is obligatory for positive results of ACTH or steroid therapy merits further study.

Logically, stress on cellular immune reactions in MS has placed chief emphasis on investigations of T cells and their functions. Sayk's reminder, that in the initial stage of an MS bout plasma cells and monocytes are important components of the CSF-cytogram, and his reference to the "blast-like" cells described by Lumsden, should not be forgotten in the overall analysis of cellular interactions in the course of MS and during therapeutic programs. A survey of electrophoretic mobility techniques and the assay of lymphokine activity was given by Meyer-Rienecker et al. They propose a diagnostic program that includes CSF cell count and CSF-cytogram, protein analysis with electrophoretic subfraction, lymphokine assay, cytophoretic blood cell studies, cytotoxicity test, and genetic typing. All-inclusive diagnostic regimes of this type would be desirable and could be proposed in many variants. In practice, there always remains the inevitable compromise reconciling available technical facilities, know-how, costs involved, and the limit of what a patient will concede in the way of diagnostic manipulations.

Weiner and Stohlman's remark that immunopathologic and immunosuppressive states are two sides of the same coin should be borne in mind. Although recognized many decades ago, it still is difficult for some investigators to accept the idea that immune incompetence (or defect) is not irreconcilable with immunosuppression as a principle of therapy. Experimental evidence is presented that viruses interfere with cellular immune responses, producing an autoaggressive response to host protein, a host antigen cross-reacting with a virus antigen, or an altered host antigen derived from a previous CNS viral infection. Such an impairment of immune regulatory mechanisms may result in an autoimmune disease. Considerations of this type are important as basic concepts for the broad spectrum of laboratory approaches, probing for key mechanisms in the pathogenicity of MS. That evidence for many assumptions still remains incomplete, despite extensive and noteworthy experimental work and laboratory studies, is reflected by the investigations of Silberberg and associates on the role of antibodies in MS. Their findings show that not only protein components like MBP, but also lipid haptens, can induce central and peripheral nervous system demyelination, and that it remains unclear whether one or

more, or varying antigen-antibody mechanisms are operative in producing demyelination in MS.

In dealing with laboratory methods of diagnosis, it has become a cliché to set morphology apart from the biochemical, serologic, and virologic studies. With the exception of some CSF and T cell cytology this is somehow also reflected by the program of this symposium. It is all the more noteworthy that the morphological contributions deal with questions of basic importance in diagnosis.

Adams' characterization of the active lesion drives home a simple, but extremely important, lesson to the clinician and laboratory expert: since modern methodology provides practicable *in vivo* approaches, we must endeavor to recognize signs of myelin breakdown, neuroglial hyperplasia, edema and special features of meningeal inflammation. It would be advantageous to interpret laboratory results on the basis of what the morphologist teaches us concerning the features of the active lesion.

Ingrid Allen gives a positive answer to the old question whether macroscopically normal white matter in MS is different from normal tissue, pointing out that 72% of her histologic samples were abnormal. Since this is an expression of microlesions in only 13% of them, there is obviously more to look for in the morphogenesis of the demyelinating process in MS than the perivascular lesion. The demonstration by Prineas of lymphatic capillaries and lymphoid tissue in the brain and spinal cord pinpoints what is still almost a *terra incognita* of CNS morphology. Confirmation and more detailed study of the barrier surfaces between the lymphatic system and CNS tissue is urgently necessary because it may well provide key information on the morphogenesis of the demyelinating focus. Electron microscopy is producing a wealth of morphological data where the prime challenge, as indicated by Argyrakis, is that of correct interpretation.

Recent Trends in the Therapy of MS

In the discussions on recent trends in ("cause-related") therapy of MS, consideration was given to three topics: cytostatic drugs, MBP and copolymers, and polyunsaturated fatty acids. All three approaches are based on hypotheses concerning the pathogenesis of MS. The variability in the natural history of this disease precludes any deductions not based on careful documentation and statistical analysis. Thus far, only the treatment of MS with cytostatic drugs has progressed to the point where statistical evaluations are beginning to emerge.

The two alternatives of cytostatic therapy of MS are long-term therapy with azathioprine and courses of intensive immunosuppression with cyclophosphamide with or without corticosteroids.

Aimard and associates reported on 77 MS patients treated with azathioprine for longer periods of time (mean duration, 5 years). In 77 patients treated during the "progressive phase" there was no improvement, and deterioration continued. Of 38 cases treated in the "remittent phase" 23.5% became worse, as compared with 52.5% in 78 controls. In 12 cases, treatment had to be discontinued because of hematologic complications (4), gastrointestinal disturbances (4), thrombophlebitis (1), cause unknown (3).

Long-term treatment was carried out by Frick et al. in 88 patients. In 64 patients treated for at least 1 year, the relapse rate dropped from 0.42 to 0.16 cases per year during treatment. After discontinuation the relapse rate in 47 patients was 0.33. In the total group of 73, 8 patients improved, 15 deteriorated, and 50 remained unaltered. Of 24 cases with chronic-progressive course, 13 deteriorated and 10 remained unchanged. The authors recommend azathioprine treatment as early as possible and administration over a period of at least 2 years. In spite of their relatively favorable figures, they summarize their experience in the statement: "immunosuppressive therapy in its present form is problematic and unsatisfactory; it should be understood as a precursor of a specific intervention in the immunopathogenesis of MS."

Dommasch and co-workers also reported on fewer relapses during azathioprine treatment of relapsing-remitting cases ($n = 8$). Secondary chronic progression was apparently not influenced at all ($n = 8$), and there was also no change in the course of 7 patients with primary chronic progression. Effectiveness of azathioprine therapy did not last beyond the period of treatment. These authors also considered azathioprine therapy as unsatisfactory in a large number of cases, and further trials with intensive immunosuppression necessary.

Patzold et al. reported on 52 patients treated with a dose of 2 mg azathioprine/kg body wt. and 45 controls. The two groups were of comparable age (mean age, 29.6 and 32.8 years) and mean duration of disease (5.4 and 5.0 years). The mean duration of treatment was 433 days. The relapse rate in the azathioprine group was 1.08 and 1.2 per year for the control group, with a mean degree of progression of 0.016 and 0.026 respectively. These preliminary results were not considered statistically significant.

Gonsette and co-workers of the Belgian National Center for MS have by far the greatest experience with immunosuppressive therapy using cyclophosphamide. Reporting on 201 patients treated with a dose of cyclophosphamide sufficient to maintain a lymphopenia of <1000 cells/mm³ during 2–3 weeks, a marked reduction of annual relapse rate was observed in 70% and an improvement of neurologic signs in 60%. A stabilization period of 2–3 years was achieved, and relapses occurring after treatment appeared less severe and easier to control with corticosteroids. However there was no influence in acute cases and in advanced chronic forms.

Eighty-six patients with chronic-progressive MS were treated with 400 mg cyclophosphamide + 100 mg prednisone per day for a period of 3 weeks by Hommes et al. They saw "strong and long lasting improvement" and a decrease of CSF-IgG% persisting for 3 months after treatment. DR_{w2} homozygous MS patients with high progression rate responded best to treatment. This raises the question whether identifiable genetic determinants can prognosticate the result of this type of immunosuppressive therapy.

Summarizing results thus far on the cytostatic therapy of MS, it can be said that there appears to be a reduction of relapse rates especially in the early cases, that there is no benefit in chronic-progressive forms, and that the long-term benefit, while definitely outlasting the effect of corticosteroids and corticotrophins, is transient. In severe relapses, combination therapy with corticosteroids is warranted. On the basis of present knowledge and experience it may be said that there exists a rationale for treatment with cytostatics insofar as this type of therapy may limit the amount of ir-

reversible damage produced by the acute demyelinating episodes, which seem to be less frequent and less severe at least for a certain period in the course of the disease. The question of complications requires further study and a longer period of experience, especially with respect to oncogenicity and the influence on gestation processes.

Double-blind studies have repeatedly been brought into the discussion. The great difficulties in any such attempt are quite apparent, and the consensus appears to be that in view of grave technical problems, but also on ethical grounds, double-blind studies with cytostatics presently available are impracticable.

Therapeutic trials with myelin basic protein must be considered logical if the analogy of EAE and MS, i.e., of similar basic mechanisms with respect to the demyelinating process, is accepted. On the other hand, it may be said that therapeutic trials in MS with MBP can help to clarify the question whether this analogy is valid or not. Obviously, the first prerequisite in a speculative trial of this type must be that the administration of MBP is innocuous. This question was meticulously considered in the study by Salk and associates, and the absence of adverse effects provided the basis for a dose of 0.5–3.0 mg/kg body wt. per day for protracted periods of time. Atypical patterns of anti-MBP IgG, and IgG were found in MS patients, indicating defective immunoregulation in patients with MS. The preliminary results by Romine do not permit any conclusions concerning the question whether MBP therapy is beneficial in MS or not. Both presentations were reports on work in progress, and considerable time will still be required before definite results can be presented. Regardless of whether they are positive or negative, they should be an important contribution, aiding our comprehension of pathogenetic mechanisms in MS.

It is generally known that minute doses (1–10 μ g) of MBP with Freund's adjuvants induce EAE, whereas large doses (antigen excess) suppress EAE. This effect can also be produced by peptide fragments of the MBP molecule. In these fragments encephalitogenicity depends on the presence of tryptophan, but suppression is possible with MBP peptide residues lacking any tryptophan. Following these findings, Arnon, Sela, and Teitelbaum synthesized nonencephalitogenic, randomized basic polymers, which proved effective in suppressing EAE. One of these a copolymer with molecular weight of 23,000 designated COP1, consisting of L-alanine, L-glutamic acid, L-lysine and L-tyrosine, is being used in clinical trials analogous to those carried out by Salk and co-workers with MBP. Arnon reported on three patients with acute disseminated encephalitis who recovered completely under COP1 treatment, but in four patients with advanced chronic MS results were inconclusive. This was also the case in short term trials in 16 patients of the Göttingen University Neurologic Department. In both trials a small daily dose of 2–3 mg COP1 was injected IM. This dose may have been too small; further trials and informative tests for monitoring the response are necessary.

That a number of polyunsaturated fatty acids or essential fatty acids (PUFA = EFA) essential to body growth and function cannot be synthesized by the body and that recent studies have indicated an altered composition of myelin and/or a deficiency of these substances in persons with MS has led to the use of diets rich in PUFAs and to dietary supplements with substances like sunflower oil and preparations such as *naudicelle* (an extract of evening primrose containing a high percentage of linoleic acid and γ -linoleate). Publications on these problems have come up with di-

vergent results, and the question is still open whether and to what extent they are pathogenetically relevant factors in MS.

In an experimental study Mertin found that PUFAs suppressed EAE in Lewis rats and discussed the concept that, in addition to physicochemical effects on cell membranes, the immunosuppressive effect of EFAs may be determined by the action of prostaglandins.

Field pointed out that in the human the immunosuppressive effect of PUFAs is transient, being lost after some months. His own hypothesis is based on the existence of an inborn metabolic defect in the handling of PUFAs. He attributes a prophylactic value to the administration of γ -linoleate early in life. Results of cytopherometric tests (MEM-LAD, E-UFA, PGE₂-test) are presented and the hypothesis of a metabolic defect in handling PUFAs in the production and maintenance of myelin is stressed on the evidence of results in family studies (Field and Joyce).

In studies on patients receiving sunflower oil and naudicelle capsules as dietary supplements, Seidel and Heipertz found no differences in the serum linoleic acid levels in comparison with controls. The loss of long-chained fatty acids of sphingolipids, with a corresponding increase of short-chained fatty acids was identified as a pathogenetic factor responsible for myelin instability in an experimental study by Heipertz and Seidel.

Both possibilities, the role of PUFAs in cell-mediated immunity and the metabolic defect of myelin, require further basic study. This also applies to the therapeutic or prophylactic value of PUFAs in MS and the diagnostic role that can be attributed to Field's cytopherometric tests, since results in the literature are still controversial.

A double-blind study of amantadine treatment of MS by Ellison et al. revealed no differences between this substance and placebo. Brage reported on 166 patients treated with thymectomy, in 33,7% of which he saw a favorable effect.

In all of the procedures discussed in this section, the intention was "cause-related" therapy. Without exception they are characterized by a more or less tenuous hypothetical basis. To a certain extent results seen thus far appear encouraging and justify further therapeutic trials, but without exception more ground work and critical controls are required.

Epidemiology of MS

Studies of histocompatibility antigens in MS patients in various parts of the world have established an increased frequency of the tissue types A3, B7, and especially Dw2 in Eurocaucasians, but a predominance for other tissue types in Japanese, Israeli, and Arab MS patients. A survey of these differences and of possible correlations to immune reactions is given by Oger and Arnason. Raun, Fog et al. pointed out that the relative MS risk for Eurocaucasians with type Dw2 is 4 : 1. They also found a statistically higher rate of progression of the disease in Dw2-positive MS patients. This was also found by Hommes to be the case in the Netherlands. The particular importance of the HLA-D system in MS was borne out in a study of 300 MS patients by Grosse-Wilde et al.

An indication that the predominance of certain histocompatibility types in populations may have a bearing on MS prevalence rates is seen by Dean in that B7 is found in an unusually high percentage of the inhabitants of northeastern Scotland (34.4%), where MS prevalence is also high.

According to Zander, who analyzed the correlation between HLA-A, B-haplotypes, and MS in 34 pairs of siblings, a dominant type of inheritance with low penetrance may be operative with respect to MS susceptibility. He also considers the possibility, however, that additional genetic factors are codeterminant. Oger and Arnason, reviewing the literature on family studies, could not find adequate evidence of a single gene in the HLA complex as the susceptibility-determinant for MS. Alter asserts that efforts to establish the existence of an MS susceptibility gene by tracing inheritance of a shared HLA haplotype in multiplex MS families have failed.

Though representing only a brief section of the agenda, the reports and discussions on population genetics and MS clearly show the present state of our knowledge in this field, as well as the necessity to correlate investigations on histocompatibility types in MS not only with geographic and racial data, but also with specific exogenic conditions and the clinical situation. It must also be considered that, even with respect to methodology, the science of histocompatibility antigens is still in an early stage of development, and that new techniques may necessitate the revision of some of our present data.

Regarding geographic distribution, Alter pointed out discrepancies in the relationship of latitude and MS frequency, the local variations on examination of the "fine structure" of MS epidemiology, the gaps in our knowledge concerning the MS frequency at the extremes of latitude, in large areas of South America, the oriental countries, central and northern Africa. Referring to the multiplicity of environmental factors, the information on MS in migrant populations and the genetic aspects, Alter considers it necessary to supersede the phase of descriptive epidemiology, which has supplied a large body of information but no final answers regarding the cause and pathogenesis of MS, with an "experimental epidemiology" of combined field and laboratory studies.

Reporting on prevalence rates in Japan, Kuroiwa stressed that, in spite of great environmental changes, there has not been an increase in the last 20 years. He also pointed out that, compared to MS in the western countries, there were differences in clinical features, with more severe spinal lesions and visual loss, more intensive inflammatory reactions in the CSF, as well as no comparable HLA patterns and no increase in measles antibodies. Iwashita described the CSF alterations in 66 consecutive cases. Several patients with acute exacerbations had pleocytosis with polymorphonuclear cells predominating. The percentage of IgG increase (44%–51%) and the presence of oligoclonal bands (45%) was lower than that observed in most western case series. There were no differences in measles antibody titers in serum and CSF by hemagglutination inhibition and radioimmunoassay in MS and non-MS patients.

Dean examined the frequency of "hospitalized prevalence" of MS among immigrants to the United Kingdom. He found MS to be as common or nearly as common among immigrants from Europe and the old Commonwealth countries (Australia, Canada, New Zealand) as in people born in the United Kingdom. Immigrants from Italy had almost the same risk, from Ireland and Cyprus $\frac{2}{3}$ of the risk, and

from Spain about $\frac{1}{2}$ of the risk of the British. As for the new Commonwealth countries (West Indies, Africa, Malta, India, Pakistan) the risk of being hospitalized with MS was very small. A prevalence study in Malta and parts of Sicily revealed remarkable differences: in the city of Enna on the island of Sicily a prevalence of 53 per 100,000 was found. Malta had a prevalence of only 4 per 100,000. These remarkable differences are being examined more carefully to analyze ethnic, genetic, and exogenous factors involved.

Cazullo and co-workers presented prevalence rates from various areas in Italy, ranging from 5–7 per 100,000 in Messina and Naples to 20 in Aosta. A more detailed study in Novara and Varese provinces produced prevalence rates of 19.5 and 24, respectively.

Epidemiologists have constantly been on the search for MS clusters and the epidemic occurrence of this disease; results thus far have been meager and controversial. Within the high frequency areas of Europe Kurtzke identified spatial clustering in Norway, Sweden, Denmark, Switzerland, Finland, northern Scotland, and possibly the Netherlands. In a careful survey of all MS patients on the Faroe Islands, Kurtzke and Hyllested found 24 patients who met all criteria for a point source epidemic. They consider this to be the result of an infectious agent brought in by British occupation forces during World War II.

Palfy reviewed the clinical features in 219 MS cases observed in southwest Hungary (Pecs district) during the past 30 years. A remarkable observation was the complete nonexistence of MS in the gypsy settlements of the area.

These studies reflect two aspects that appear noteworthy: the global picture that is gradually emerging follows the accepted latitudinal pattern, and the observation that in far eastern countries of comparable latitude MS is rare, but that there are conspicuously abrupt differences in rather narrowly defined areas that do not fit into the large scale pattern. One of these is the abrupt drop of prevalence within the area of the Swiss canton of Wallis described by Bärtschi-Rochaix. Such differences may provide essential pathogenetic clues. Unquestionably, the validity of such figures is not only a statistical problem, but also one of the meticulous exploration of the areas in question, and of comparable methodology. The potential information involved underlines the necessity of what Alter calls experimental epidemiology. One of the approaches is the thorough and long-term epidemiologic study of pilot areas.

Two studies of this type were briefly described: a unique long-term study by Broman and associates has been under way for almost 30 years in the Göteborg area in Sweden. Some of the data on 312 patients, to be published in a detailed monograph, were presented at the symposium. An incidence rate of 5.3 per 100,000 was given, and in discussion Broman mentioned a prevalence rate of 130 per 100,000. A long-term epidemiologic study is also under way in the Göttingen area, where a prevalence rate of 76 per 100,000 was found. The 195 MS patients of the area have been carefully documented, and follow-up now covers a period of 12 years.

In studies of this type, the inclusion of optic neuritis is desirable. Haller et al., studying the epidemiology of optic neuritis in Hannover, found the same association of HLA types A3 and B7 as in MS; they estimated an annual incidence rate of 5 per 100,000.

One of the chief problems in bringing the relation of optic neuritis and MS into proper perspective is the great difference in data concerning the percentage of cases of optic neuritis as an initial symptom of MS. Figures range from 10% to 87%. The figures calculated by Poser et al. from our own data pool are 16% optic neuritis as the sole initial symptom and 39% as an initial manifestation along with other neurologic symptoms. It would therefore be a major error to equate optic neuritis with MS. On the other hand, Kuroiwa's observation is noteworthy that optic neuritis is an especially severe disturbance in the Japanese cases.

The Clinical Definition and Assessment of the Course of MS

The validity of epidemiologic studies depends on the thoroughness of survey of a given area and on the quality of the clinical data registered. Standardized diagnostic criteria are prerequisites for the comparability of results. Progress in clinical research, especially the development of laboratory techniques, which, though nonspecific, are of great value in supporting the diagnosis of MS, have motivated the International Medical Advisory Board of the International Federation of MS Societies (IFMSS) to carry out an investigation on the question of updating the diagnostic criteria of the Schumacher Committee of the US National MS Society. The results of the investigation were presented at the Göttingen symposium and provided the basis for a subsequent decision at the 1978 meeting of the IFMSS to establish a working group that should make recommendations. The most crucial questions, which led to considerable diversity of opinion in the discussion, were (1) whether the results of laboratory methods should be considered as criteria for establishing a definite, probable, or possible diagnosis of MS and (2) whether first bouts should be included as probable or possible MS. In the discussions it became apparent that the chief difficulties are encountered in defining the purpose that a set of diagnostic criteria is to serve: either to establish precise diagnostic standards for therapeutic studies (as originally intended by the Schumacher Committee) and for sophisticated clinicopathologic studies in relation to etiology and pathogenesis, or to provide, in a more limited way, general directives for diagnostic classification on the practical level and in epidemiologic field work where the facilities for laboratory studies are not available. The proceedings of the symposium could do no more than to provide a stimulus by raising questions. It may appear curious that after more than 100 years of clinicopathologic experience and research, the diagnosis of MS is still a problem; on the other hand, the influx of virologic, immunologic, biochemical, morphological, and neurophysiologic data obtained with modern research technology confronts us with baffling questions: for example, do we really know what MS is and is it a specific disease entity? To remain on solid ground in the face of such questions we need a well-defined clinical basis of description and classification, reconsidered from time to time to meet the challenge of changing concepts and new knowledge.

In addition to the refinement of CSF cytology, the measurement of IgG synthesis in the CNS and the demonstration of oligoclonal bands, a number of neurophysiologic methods and computer axial tomography are valuable new additions to the diagnostic armamentarium in MS.

McDonald reviewed the essential features of visual, auditory, and somatosensory evoked reactions and their clinical value in diagnosis. Although not specific for a demyelinating lesion, they can be determinants in establishing the polytopic nature of lesions and thereby the probability of MS, even when clinical features and laboratory tests are not typical. Ketelaer presented a multimodal evoked potentials program for the detection of clinically silent lesions, consisting of visual, brain-stem auditory, somatosensory evoked reactions and the blink reflex. He found subclinical evoked potentials in at least one of the tests in 62.5% of cases with clinically definite MS. In a study of 251 MS patients Lowitzsch found that diagnosis could be improved by 23% if visual evoked reactions were taken into account. Neetens et al. found the change in wave-shape even more reliable than latency in establishing a lesion in the optic system. They also stressed the generally accepted fact that visual evoked reactions are not specific for MS, and that sectional ischemic neuropathy and open-angle glaucoma give the same findings. The value of static perimetry for the precise quantitative evaluation and follow-up of visual disturbances in optic neuritis was described by Haller and co-workers.

By its very nature computer axial tomography cannot convey specific information concerning the demyelinating character of a lesion, but it has taught us two important things concerning MS: (1) that extensive transient focal edematous lesions (hypodense zones) can appear in the hemispheres in an early stage of acute MS, and (2) that a moderate degree of brain atrophy is a frequent finding even in cases not presenting conspicuous signs of functional impairment. Wüthrich presented data on 150 consecutive cases in which he found focal hypodense areas in about 20% and atrophic changes in 50% of the cases. This method can be a most useful tool in following the course of acute exacerbations of MS and the effect of therapeutic procedures.

In a discourse on the effort to update the diagnostic criteria of MS, Ellison and Myers recommended a systematic approach according to the rules of taxonomy, presenting brief examples for their concept.

Basic rules for the quantitation and assessment of the course of MS were summarized by Fog, and a number of systems for diagnostic evaluation were briefly described. He pointed out particularly that the assessment of the course of MS, with its polytopic, variable, and transient symptomatology presents intricate problems. A standardized documentation system was described by Poser et al. and was used by the MS Research Program of the Deutsche Forschungsgemeinschaft, registering on optical mark reader sheets and providing computer analysis with printouts.

Diagnostic evaluation comprises (1) the establishment of diagnosis, (2) the assessment of the course of the disease, and (3) the evaluation of functional impairment. Careful case histories with meticulous registration of symptoms often convey little or no information on what the patient can still do. Evaluation of function is difficult because correct assessment requires numerous parameters and their correlation; factors like motivation and fatigue – one of the important, early, and often neglected symptoms in MS – are nonmeasurable variables.

Potvin et al., who have spent almost 20 years developing their neurofunction laboratory as an instrumentarium for the evaluation of neurologic function, gave a description of methods employed, the analysis of reproducibility, the role of learning, and other influences that must be considered in evaluation and clinical trials. Benecke and Conrad described the evaluation of motor deficits by the neurophysio-

logic analysis of stereotyped motions, using the model of bicycling for the study of spasticity and paresis of the lower extremities.

The reports and discussions of the Göttingen Symposium covered a wide range of topics, undertook a realistic, honest inspection of many unsolved problems and accepted no euphemistic explanations for them. It is gratifying that the sum total of this endeavor is on the positive side: the assurance that we are forging ahead, recognizing lines of advance that offer reasonable promise of success, and identifying approaches that may be mere blind alleys. One of the noteworthy features of this meeting was its organizational basis: it was a joint effort of many scientists, research funding organizations, National MS Societies, and the Medical Advisory Board of the International Federation of MS Societies. Our sincere thanks to all is the most pertinent concluding remark that can be made. May the cooperation that became visible in making the Göttingen meeting possible be a continuum, serving to intensify the international effort in the search for the cause and cure of MS.

The following addendum to the “scientific” program may seem to some almost irrelevant in view of the highly specialized reports that fill this monograph – all too far away from the interests of the scientists from many countries whose contributions sum up to an informative survey of our present knowledge concerning MS. However, there is one central reality in the complicated topography of MS research bringing us all together: the MS patient.

The panel discussion that concluded the symposium program was devoted to a brief survey of organizational concepts for the long-term care of MS patients. In reports from various countries, the experience with existing institutions was summarized and discussed in relation to the prerequisites for clinical research – careful assessment of the patient’s condition and continuity in observations on the course of MS – but also beyond that and independent of the research aspect in relation to the everyday practical needs, the medical, psychological, and socioeconomic problems of the individual patient. The discussion was no more than a fragmentary entrée, but it strengthened the motivation and provided the groundwork for a forthcoming conference of the International Federation of MS Societies on this important topic.