MULTIPLE SCLEROSIS
Immunology, Virology, and Ultrastructure

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Fig. 3.6. A macrophage has penetrated the basement membrane (long arrows), lifted the Schwann cell (S) away from the sheath, and disrupted and undermined several superficial myelin lamellae (short arrows). x 10,000
Fig. 3.12. Denuded axon surrounded by a macrophage containing myelin debris. Note the 2 islands of Schwann cell cytoplasm associated with few intact myelin lamellae (arrows). x 10,000
the rate of MS in the northern hemisphere. While several good surveys have been conducted in the southern hemisphere, I would hesitate to say that a similar gradient by distance from the equator has been established. Incidence (average number of new cases per year) in low rate areas are about one per 100,000 while prevalence (number of existing cases at a given time) is about 10 per 100,000. In high risk areas the incidence is perhaps 3 or more per 100,000 with a prevalence of 40 to 60 per 100,000 or more. Between these two extremes there are intermediate areas. At any given latitude there is considerable regional variation in rates indicating that latitude is not the sole factor influencing risk.

Micro-foci or clusters of cases have been observed occasionally. The occurrence of these foci have not been striking and the probability for clustering given the prevalence of the disease in a given area must be weighed in assessing the significance of a given cluster. The Shetland and Orkney Islands off the north of Scotland present a fascinating epidemiologic challenge. On these islands the prevalence is not less than 200 per 100,000 or about 3 times higher than the next highest area in the world. Hopefully through continued exploration on these islands some explanation for this phenomenon will evolve.

The observation of geographic differences in rate of MS is a strong argument for an infectious etiology for this disease. Disappointingly little, however, has been contributed from these data to the understanding of the pathogenesis of multiple sclerosis. I am personally optimistic, however, that given this geographic distribution, recent and sophisticated studies of migrant populations may lead to more helpful concepts.

The initial migrant studies were confined to people who moved from high risk areas to low risk areas. These studies, although based on small numbers, indi-
latitude and risk correlation exists in Japan and apparently in other Oriental populations. Better understanding of this phenomenon could be achieved through more extensive studies in the Orient and among Oriental migrants to high risk areas. Further study is also necessary to determine if a latitude gradient occurs in the southern hemisphere and also to take advantage of large populations who have migrated from low risk areas (the Mediterranean basin) and high risk areas (England and Ireland) to a similar area in the southern hemisphere (Australia).

Seasonal Variations

There is no conclusive evidence of seasonal variation in the onset of multiple sclerosis. This may be a reflection of the fact that the onset of the disease is frequently subtle and even unnoticed. There are suggestions that retrobulbar neuritis as the initial symptom of MS may occur more frequently in north temperate areas in the winter, and there is even more fragmentary evidence that exacerbation may be more frequent in the winter. Detailed knowledge of precipitating events of exacerbation of specific symptoms such as retrobulbar neuritis may provide a clue as to the precipitating factors which trigger appearance or extention of disease. Certainly this pursuit is of great value if not in detecting the etiology of the disease, at least in developing a rationale to avoid potentially precipitating events. Should even a seasonal pattern for exacerbations emerge, it would perhaps be of some value to those favoring a virus hypothesis, inasmuch as during winter months in temperate climates the circulation of respiratory and myxoviruses is increased while during the warmer months enteroviruses become more prevalent.

Events of Childhood and Adolescence

As a result of studies among migrants there is accumulating interest in the period around adolescence
plaques. The yields of myelin from the MS cases varied greatly with the most myelin being obtained from the normal appearing white matter and the least from the plaques; however, the amino acid composition of all the samples of myelin was entirely normal.

Riekkinen and co-workers (13) have reported a large or total loss of basic protein from the myelin of MS patients. In Figure 2 of their paper the cases that are designated 1 and 2 show a large loss of basic protein while those designated 4 and 8 show a total loss. In Figure 3 of their paper the densiometer tracing shows a total loss of basic protein in MS myelin. Their results are inexplicable and they are certainly incorrect. The basic protein comprises approximately 30% of the total protein of CNS myelin. If even half of it were lost, this would be easily detected in the amino acid analyses. Yet the amino acid composition of MS myelin differs in no way from that of normal myelin. Furthermore, as will be dealt with later, basic protein has been demonstrated histochemically to be present in MS myelin.

**Enzymatic Degradation of the Proteins of Myelin**

The proteolipid which was discovered by Folch and Lees (6) was the first protein of myelin to be isolated. It is remarkably resistant to digestion with proteolytic enzymes such as trypsin, pepsin and pronase. Yet in Wallerian degeneration or in the demyelination in MS, this protein, which comprises 50% of the total protein of CNS myelin, must be enzymatically degraded. It is possible that there exists in the lysosomes of some cells of the CNS endopeptidases which will digest this proteolipid protein. It is also possible that it is simply sequentially digested by aminopeptidase or carboxypeptidase.

The basic protein of myelin and the acidic protein(s) (17) which together comprise the remaining
50% of the myelin proteins are readily degraded by most proteolytic enzymes. Nothing is known about what happens to the acidic protein in demyelination; however, a considerable amount of work has recently been done on the degradation of basic protein at the margins of MS plaques.

Both a peptidase (10) and a neutral proteinase (11) have been reported to be in purified myelin. It is not known what role, if any, these have in the degradation of the myelin proteins in Wallerian degeneration or MS. An acid proteinase is present in nervous tissue which rapidly degrades the basic protein and the products of this digestion include encephalitogenic peptides (2). Einstein, Dalal and Csejtey (5) have shown that the activity of this enzyme is greatly increased at the edges of MS plaques. Riekkinen and co-workers (12) have reported a similar but lesser increase in the activity of the enzyme. Einstein and co-workers (5) have also shown a partial loss of basic proteins from the white matter at plaque edges and from plaques. It has been speculated (12) that this degradation of basic protein by acid proteinase may give rise to encephalitogenic peptides which might escape from the central nervous system and stimulate the formation of immunocompetent clones of lymphocytes. These cells, in turn, would presumably invade the CNS and cause more demyelination.

Histochemistry of the Plaque Edge

The group at Guys' Hospital Medical School under the direction of Dr. C. W. M. Adams has done some admirable histochemical studies of MS plaques and plaque edges (1). They were, in fact, the first to demonstrate the increased proteolytic activity at the edges of plaques. Recently they have studied an acute plaque from the brain of a woman who had suffered an accidental death (1). This woman had "an acute depressive illness" for two years before her death but
apparently no diagnosis of MS was made. Three plaques were found in the left cerebral hemisphere and one of them showed extensive perivenous infiltration with lymphocytes. They regarded this plaque as a particularly early lesion and it showed, as was to be expected, increased proteolytic activity. Histochemical stains for cholesterol and glycerophosphatides showed an abrupt loss of these lipids at the plaque edge. On the other hand, there was "an irregular patchy loss" of cerebroside extending up to 0.4 mm beyond the edge of the plaque. Of most interest was the loss of basic protein up to 1.5 mm beyond the plaque edge. The other two plaques, without lymphocytic infiltration, did not show this loss of cerebroside and basic protein beyond the edge of the plaque. Adams theorized that the increased acid proteinase and the consequent loss of basic protein is one of the initial events in the demyelination in MS. This is followed by the loss of cerebroside and then the degradation of the other lipids such as cholesterol and the glycerophosphatides.

The cellular origin of the proteolytic activity around MS plaques has been studied by Hallpike, Adams and Bayliss (8). Active plaques were characterized by increased cell populations and increased NADH-dehydrogenase at their edges and 5 of 9 such plaques showed increased proteolytic activity. Inactive plaques showed little or no proteolytic activity. They concluded that the gitter cells (reactive microglia) at the edges of the plaques were probably not the source of the proteolytic activity. They advanced the tentative hypothesis that the proteinase may come from the oligodendroglia although some proteinase is known to be present in the myelin sheath.

Conclusions

It seems to this reviewer that the myelin from normal appearing white matter of MS brains is chemically normal. Such differences in lipid content as
have been reported are probably due to analytical methods rather than to the myelin. Certainly there is no significant shortening of the fatty acid chains, nor an increase in unsaturation, nor is there a generalized loss of basic protein from MS myelin.

Strangely enough, the myelin that is isolated from plaque edges also appears to be normal. While degenerating myelin is certainly present at plaque edges, we have been unable to locate it in the sucrose gradients. If this degenerating myelin could be isolated, it would be a most interesting material to study.

It is not particularly surprising that less basic protein can be isolated from plaques and plaque edges than from normal appearing white matter. This simply shows that there is less myelin in those areas. However, the finding of Adams and co-workers, if confirmed, that there is a selective loss of basic protein in otherwise normal appearing myelin for a considerable distance beyond the plaque edge is a very important contribution to our knowledge of the initial events in demyelination.

REFERENCES

can react with the altered determinants. Effector cells then arise from the B cell population which produce antibody to the native antigen (thyroglobulin) and thyroiditis ensues. Tolerance in this case can be reestablished by giving thyroglobulin along with cyclophosphamide (41).

Does the thyroiditis model apply to EAE? The main difference in these two autoimmune diseases is in their immunologic etiology, i.e. one is antibody mediated and the other probably cell mediated. The nature of the antigens is also relevant. Thyroglobulin is a large glycoprotein, easily denatured, probably existing at low levels in body fluids. By contrast, the Al protein, a small unraveled protein, is localized in a membrane from which it can only be extracted with difficulty (15). Its presence in body fluids is thus improbable, particularly in view of the blood brain barrier. If it were to escape, it would no doubt be rapidly degraded by proteolytic enzymes. Thus, the rabbit thyroiditis model does not appear applicable to the EAE problem. The immune response to the Al protein, therefore, may be likened to that of a foreign antigen having a predilection for thymus dependent cells. It is possible, however, that peptide fragments from the Al protein are normally released in low concentration which may induce a T cell tolerance. The question that arises in the induction of EAE, therefore, is whether we are breaking tolerance or simply initiating a response to a "foreign" antigen.

The Immune Response in MS. How might MS be elicited by an immune response to basic protein? Based on the possibilities presented above, it is necessary to postulate an insult occurring at the target tissue to initiate the immunopathologic events. From epidemiological arguments (23) concerning the geographic and age distribution of MS, a likely event is viral infection. The possible role of measles virus in MS has long intrigued investigators (39). Myxoviruses or pseudomyxoviruses (RNA-containing) are of special