8

The Neuropathology of Myelin Diseases

Cedric S. Raine and Herbert H. Schaumburg

I. Introduction

The following terminology and classification form the scaffold of this neuropathological chapter and the closely integrated chapter (Chapter 9) on the clinical aspects of the myelin diseases. Appropriate naturally occurring and experimental conditions of animals will be briefly covered. Naturally occurring disorders of myelin in animals are covered in greater detail in Chapter 14. Experiments dealing with metabolism of myelin in experimentally induced animal disorders are discussed in Chapter 13.

II. Terminology

Before embarking upon a detailed classification of the primary myelin diseases, it is pertinent to point out that loss of myelin is a common sequela of a multitude of conditions, many of which initially affect other components of white matter, in particular blood vessels, glia, and axons. Myelin, therefore, although the major element, is not the only component of white matter, and is secondarily damaged by neoplasia, trauma, infarct necrosis, abscess, edema, anoxia, and hemorrhage, and may also be altered after degeneration of the overlying cortex, for example, in the

Cedric S. Raine and Herbert H. Schaumburg • Departments of Pathology (Neuropathology) and Neuroscience, the Saul R. Korey Department of Neurology, and the Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, New York 10461.

P. Morell (ed.), *Myelin*

case of the diffuse atrophy of white matter seen subsequent to neuronal loss in Alzheimer's disease—see Blackwood *et al.* (1971), Adams and Sidman (1968), Brain and Walton (1969), and Baker and Baker (1974). It is possible, however, to segregate a significant number of central and peripheral nervous system (CNS and PNS) diseases in which myelin *per se* appears to be primarily and selectively affected. The present chapter deals with the varied neuropathology of these conditions in which the myelin loss is related to a host of different factors.

The reader should further realize that, in the past, some authors have referred to *all* diseases affecting myelin as "demyelinating." While this seems reasonable on a strictly semantic basis, the majority of neuropathologists and neurologists now reserve the term *demyelinating* to include only the acquired inflammatory demyelinating diseases such as multiple sclerosis (see Adams and Sidman, 1968) in which there is loss of myelin with striking sparing of axons.

In this chapter, the various diseases in which myelin is considered the primary target will be discussed according to etiology and neuropathology. In some cases, evidence is accumulating which suggests that a nosology based on biochemical data might soon become feasible. Where available, such evidence will be briefly mentioned in the present schema.

III. Classification

It is probably impossible to classify the myelin diseases to the satisfaction of all neuropathologists and neurologists. A major problem is the subgrouping of diseases in which some of the diseases may not completely fulfill all the criteria of a particular subgroup. For instance, progressive multifocal leukoencephalopathy (PML), a noninflammatory demyelinating disease, is usually included among the acquired inflammatory conditions. A classification similar to that outlined in Table I has recently been presented elsewhere (Morell *et al.*, 1976).

IV. Class I: Acquired Allergic (Inflammatory) and Infectious Diseases of Myelin

Diagnostic Criteria. With only two exceptions, PML and diphtheritic neuropathy, the cardinal features of lesions typifying the acquired allergic (inflammatory) and infectious diseases of myelin are the perivascular

Table I. Classification of Myelin Diseases

Class I: Acquired allergic (inflammatory) and infectious diseases of myelin (the demyelinating diseases)

A. Human
1. Multiple sclerosis
2. Possible variants of multiple sclerosis
3. Acute disseminated encephalomyelitis
4. Acute hemorrhagic leukoencephalopathy
5. Progressive multifocal leukoencephalopathy
6. Idiopathic polyneuritis
7. Diphtheritic neuropathy

B. Animal
1. Canine distemper encephalomyelitis
2. Visna
3. Coonhound paralysis
4. Marek's disease
5. Mouse hepatitis virus encephalomyelitis
6. Experimental allergic encephalomyelitis
7. Experimental allergic neuritis

Class II: Hereditary metabolic diseases of myelin

A. Human
1. Metachromatic leukodystrophy
2. Krabbe's disease
3. Adrenoleukodystrophy
4. Refsum's disease
5. Pelizaeus–Merzbacher disease
6. Spongy degeneration of white matter
7. Alexander's disease
8. Phenylketonuria

B. Animal
1. Canine Krabbe's disease
2. Jimpy mice
3. Quaking mice
4. Border disease
5. Murine muscular dystrophy

Class III: Acquired toxic–metabolic disorders of myelin

A. Human
1. Hexachlorophene neuropathy
2. Hypoxic encephalopathy—anocix anoxia and anemic anoxia (carbon monoxide poisoning)

B. Animal
1. Diphtheritic neuropathy
2. Hexachlorophene intoxication
3. Triethyl tin intoxication
4. AY 9944 intoxication

Class IV: Nutritional diseases of myelin

A. Human
1. Vitamin B_{12} deficiency

(continued)

Table I. (continued)

2. Central pontine myelinolysis
3. Marchiafava–Bignami disease
B. Animal
1. Malnutrition
Class V: Traumatic diseases of myelin
A. Human and animal
1. Edema
2. Compression
3. Barbotage following repeated lumbar puncture
4. Pressure release

demyelination and cuffs of inflammatory cells. A potentially more significant unifying feature, emanating from recent work from a number of disciplines, is the possibility that most of these conditions may be related to a viral infection.

In the various CNS conditions in this group, brains upon gross examination invariably show distinct white matter lesions which microscopically are devoid of myelin. The chronically demyelinated, gray-colored, gelatinous, sclerotic plaques in cases of multiple sclerosis (MS), the most common example of this family, are widely believed to be the end product of the fusion of myriads of small perivascular cuffs around each of which local demyelination had occurred. In the beginning, therefore, demyelination appears to be perivascular. In most cases, older CNS lesions in this group contain fewer inflammatory cells. Oligodendroglia appear to be lost relatively early in the disease process. In the case of the relapsing demyelinating diseases, it is commonly believed that the fluctuating clinical picture may be related to a reactivation of the inflammatory components within and around plaques. Chronically demyelinated plaques in all cases show a marked reduction in the number of intact, naked axons, and it is not uncommon in chronic MS to find lesions almost completely devoid of axons. Macrophage activity, as judged by oil-red-O or PAS-positive staining material, is common in acute or subacute lesions. Meningeal inflammation and subpial demyelination occur in the more acute members of the group, particularly those linked to a viral infection. In all cases, an intense, fibrous, astroglial response is a sequela of the demyelinative process.

It is interesting to note that inflammatory demyelinating diseases are not restricted to the CNS. They also occur in the PNS. This occurrence has been held to be strong evidence that these diseases result from an autoimmune process related to the different basic proteins of CNS and PNS myelin.

A. Human Examples

1. Multiple Sclerosis

Pathology. Despite its early recognition as a distinct disease entity by Charcot toward the end of the nineteenth century, the extensive neuropathological investigations of Dawson (1916), and the numerous analyses by contemporary neuropathologists (see Adams and Kubik, 1952), the underlying disease process in multiple sclerosis remains an enigma. The varied topography of plaques and the chronicity of the changes are consistent with the protracted clinical course of the disease, discussed in detail in the following chapter. In general, two variants of MS can be recognized on the basis of both neuropathology and clinical course—chronic MS, by far the most common, with a clinical course often extending more than 20 years, and the rare acute MS, ranging from a few weeks to a few months from onset to death. While chronic and acute MS are classified here as variants of the same disease, it is possible that they represent distinct disease entities, and indeed many investigators consider them as such.

Externally, the brain from a patient who has died of chronic MS, is covered with a cortical gray mantle and appears relatively unremarkable. The spinal cord often has grossly visible plaques superficially since it has myelinated fibers on its surface. Coronal section of the brain, however, reveals multiple, disseminated, grossly visible plaques ranging in size from about 1.0 mm to several centimeters (Fig. 1). The lesions can be differentiated from the surrounding normal CNS tissue on the basis of color and texture, which can also be used an index of lesion age. Recent (acute) lesions have a pinkish hue, subacute lesions (containing, by light microscopy, an abundance of fat-filled macrophages) appear whitish, while chronic, "burnt-out" plaques are gray due to the proliferation of glial scar tissue. Serial reconstruction of plaques demonstrates that some are interconnected and anastomose throughout the CNS much like the branches of a tree. There is a strong tendency for plaques to be associated with venules and paraventricular regions, the latter being one of the most common features in MS. Plaques are also found on gross examination in the optic nerves (which often appear atrophied due to the loss of myelin and some axons), and spinal cord. The lesions are not restricted to the white matter and may encroach upon myelinated areas of gray matter. In such regions there is remarkable sparing of nerve cell bodies. The PNS is usually spared but a few reports exist which describe changes in the spinal nerve roots. Whether such PNS changes are primary or secondary is not known.

Light microscopy of a typical chronic MS plaque reveals a total lack of

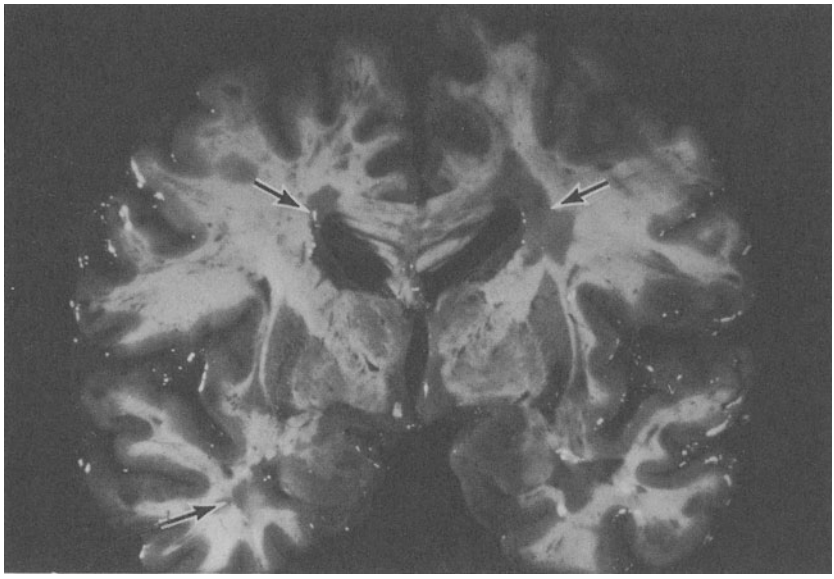

Fig. 1. Chronic MS—coronal slice. The demyelinated plaques are clearly visible in this gross specimen (arrows). Note their predilection for white matter and their greatest development in the paraventricular areas.

myelin (Figs. 2 and 3) with preservation of many demyelinated axons. In older lesions, however, axons may be lost. An intense astroglial response is common and the parenchyma is usually replaced by fibrous astroglial processes emanating from large cell bodies, frequently demonstrating multilobate nuclei. Oligodendroglia are lost early in the disease and are absent from the chronic plaques. At the peripheries of lesions it is not unusual to find a zone of subacute activity containing macrophages filled with lipid material which stains positively with Sudan black, oil-red-O, and PAS. More active regions of lesions or younger plaques contain varying numbers of inflammatory cuffs around blood vessels and large numbers of macrophages. The inflammatory cells are comprised of small lymphocytes, large mononuclear cells, plasma cells, and macrophages.

Not infrequently, a chronic plaque will display an almost acellular and gliotic center and a peripheral area which appears hypercellular but not completely devoid of myelin. Such an area is termed a "shadow plaque" and is believed to be indicative of remyelination or incomplete demyelination.

The fine structure of chronic MS plaques adds relatively little to what can be seen at the level of the light microscope (Périer and Grégoire, 1965). Naked axons lie in a matrix of fibrous astroglial processes (Fig. 4)

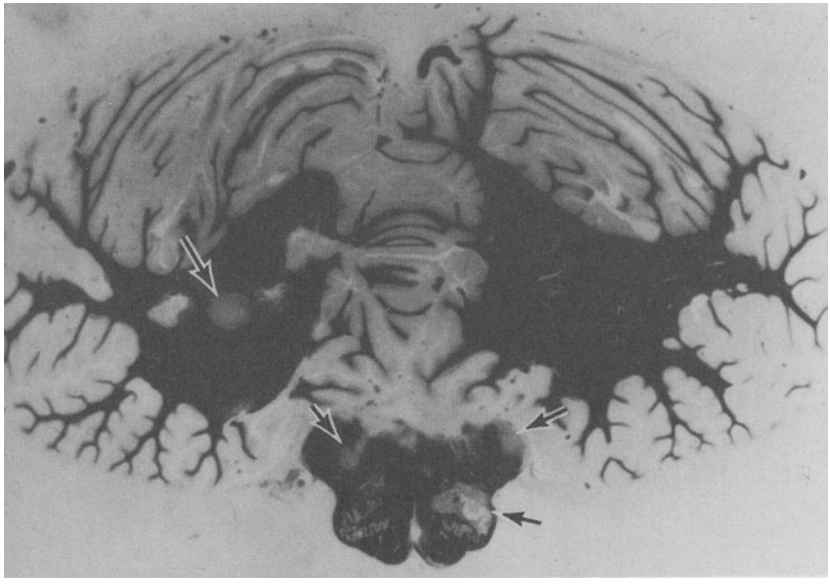

Fig. 2. Chronic MS—myelin stain, whole mount. In this section taken vertically through the cerebellum and medulla, demyelinated plaques stand out as unstained areas of white matter (arrows).

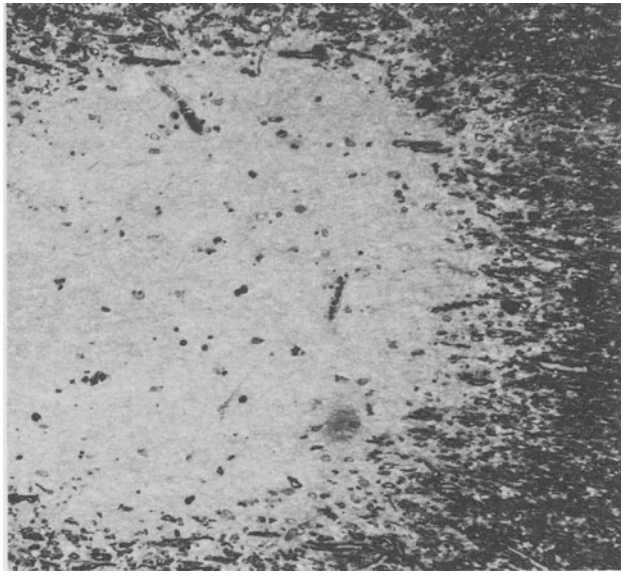

Fig. 3. Chronic MS—toluidine blue stained 1-μm epon section. A demyelinated plaque is seen, its edges clearly delineated where incoming fibers lose their myelin sheaths. ×120.

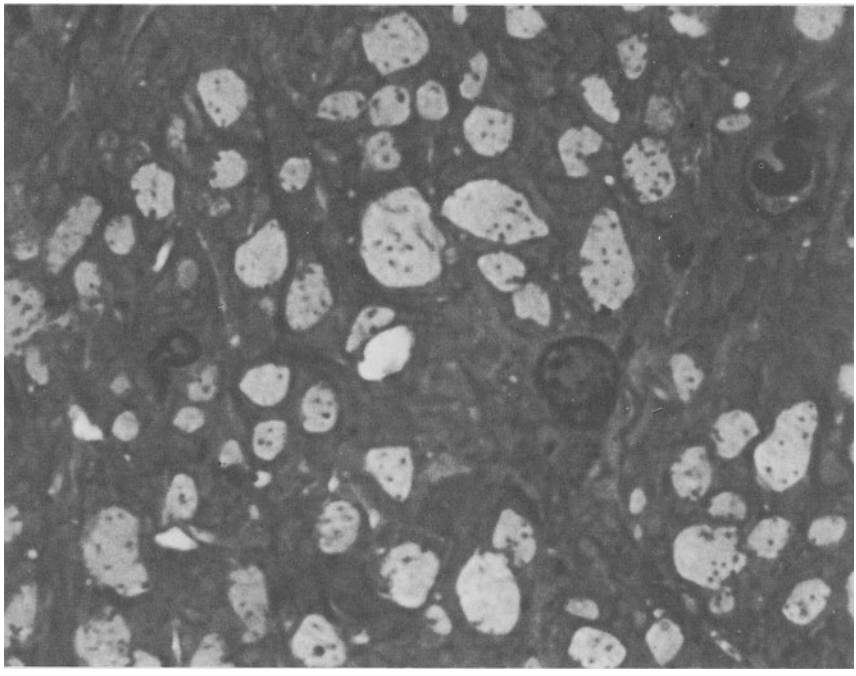

Fig. 4. Chronic MS—toluidine blue stained 1-μm epon section. A chronically demyelinated plaque from the spinal cord displays naked axons (note mitochondria in the axoplasm) and an intense astroglial fibrosis. ×1800.

and there is an increase in the amount of extracellular space. Many ultrastructural reports on unusual, possibly viral, material have been reported; however, most have been found not to be specific for MS and some of them have been found to be normal constituents of CNS tissue.

In many cases of chronic MS and in all cases of acute MS, lesions contain ongoing myelin destruction in the presence of inflammatory foci. In the brain of a patient having acute MS with a relatively short clinical course, for example, 7–12 months, while it may be possible on gross examination to find some chronic lesions, the majority of plaques will be pink to the naked eye, and microscopically will consist of confluent inflammatory zones. Elsewhere, regions of white matter apparently normal on gross examination might contain diffusely scattered perivascular cuffs, each with a narrow rim of macrophages and local demyelination (Fig. 5).

Traditionally, inflammation has served as the hallmark for recent activity and, on the basis of comparison with human and animal models of autoimmune demyelination, is often regarded as the first change. While perivascular cuffing undoubtedly belongs to the spectrum of acute and

ongoing disease features, whether or not it truly represents the initial change is still debated (see Prineas, 1975).

Etiology. Since the earliest descriptions on MS, many agents and predisposing factors have been considered causal factors. Dawson (1916) raised the possibility of a "latent organism or an auto-toxin" to explain the remarkable association of lesions with brain vasculature. In recent years, a number of organisms have been ascribed as possibly being etiologically significant in MS, including certain bacteria, a rabieslike virus, and rod-shaped structures in glial cells within lesions. The last, described by Field *et al.* (1962), were later found to be centrioles (quoted by Périer and Grégoire, 1965). Epidemiological data have more or less established that high- and low-risk geographic areas exist and that persons moving from high- to low-risk areas after the age of 15 years carry with them the same high risk of acquiring the disease, which has a mean frequency in the United States of about 40 per 100,000. It has been suggested that exposure to the putative MS agent(s) occurs before the age of 15. It has been known since 1962 that elevated titers of antibody to measles virus are present in the sera of a significant number of MS patients in comparison to normal subjects, and since that time this finding has been confirmed on numerous occasions in tests on sera and CSF samples from MS cases (see review by Norrby *et al.,* 1974). Such immunological data raise the possibility that measles may be causally related to the disease. Antibody titers to a wide variety of viruses have now been tested in MS and

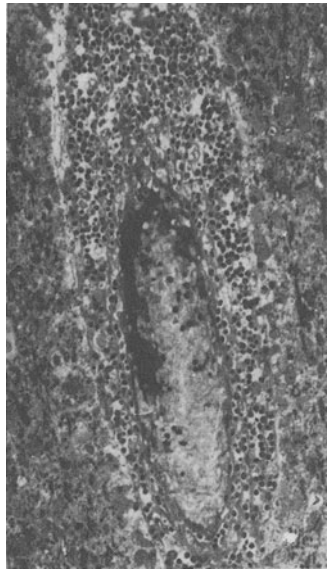

Fig. 5. Acute MS—toluidine blue stained 1-μm epon section. A typical perivascular cuff of hematogenous cells (mainly small lymphocytes) is seen rimmed by a narrow zone of recent demyelination. ×300.

control cases, but only measles remains consistently elevated in significant numbers. In 1972, ter Meulen *et al.* (1972*a*), using cell fusion techniques, reported on the isolation of a parainfluenza type I agent from brain cells grown out from two MS biopsy specimens. To date, this has not been confirmed, and there might exist some question as to whether or not the agent was a contaminant. Also in 1972, Prineas described "paramyxoviruslike" material in acute lesions from a patient with chronic relapsing MS. This observation has been confirmed by several groups who have prepared MS tissue for electron microscopy (EM) by a variety of techniques (reviewed by Raine *et al.,* 1975). On the basis of comparative studies with autopsy tissue from a number of unrelated diseases, it has now been established that this "paramyxoviruslike" material is not specific for MS and may be a by-product of cellular degeneration (Raine *et al.,* 1975). Thus, apart from the immunological data implicating a measleslike infection, there is no firm evidence that a virus is involved in MS, although indirect evidence from a number of conditions related to MS and a number of naturally occurring and experimental viral diseases suggests that a virus is the most likely candidate. Secondary to the putative infection in MS, it is hypothesized that an autoimmune (autoallergic) response to myelin antigens develops, akin to that produced in animals following sensitization to CNS myelin antigens (experimental allergic encephalomyelitis—EAE), thus accounting for the perivenular cuffing and demyelination. The latter are constant features in EAE, where a delayed hypersensitive reaction to myelin is well established. Attempts to demonstrate sensitization to myelin in MS have not yet been conclusive. Skin tests to myelin basic protein are negative in MS, unlike EAE, but as in EAE, although to a lesser degree, positive results have been obtained from *in vitro* tests for lymphokines and serum and CSF demyelinating factors (see Paterson, 1973; Raine, 1976). More recently, there is growing interest in the possibility that histocompatibility antigen (HLA) types may influence susceptibility to MS and some cases display a tendency for certain types to be linked (e.g., HLA-A3 and HLA-A7) (Lehrich *et al.,* 1974). See Chapters 11 and 12 for further discussion of relevant chemistry and immunology.

2. *Possible Variants of Multiple Sclerosis*

Although considered by some to represent separate disease entities, a small number of chronic demyelinating conditions of the CNS exist, which are most conveniently grouped together with MS. Devic's disease is such a condition, in which plaques are located in the optic tracts associated with necrotizing lesions in the spinal cord. Balo's concentric sclerosis is another, exceedingly rare, condition with some similarities to MS; in

some inexplicable way lesions develop concentrically with zones of apparently normal white matter alternating with grossly visible bands of demyelination.

3. *Acute Disseminated Encephalomyelitis*

Pathology. Acute disseminated encephalomyelitis (ADE) is a broad disease category embracing a number of relatively short-term, frequently fatal, fulminant inflammatory CNS conditions of varied etiology. The members may be spontaneous or iatrogenic. The most common disease in this group follows an exanthematous infection by a virus, e.g., measles, vaccinia, varicella, and influenza. These examples are also known as the postinfectious encephalomyelitides. Another form with identical lesions is seen after postrabies immunization, in which case the patient develops an autoallergic, EAE-type of response within the white matter, now known to be related to CNS tissue incorporated within the inoculum.

The neuropathology of these conditions, despite the variation in causal factors, is remarkably uniform. The lesions are often not visible grossly, but show well after myelin staining (Fig. 6). By light microscopy,

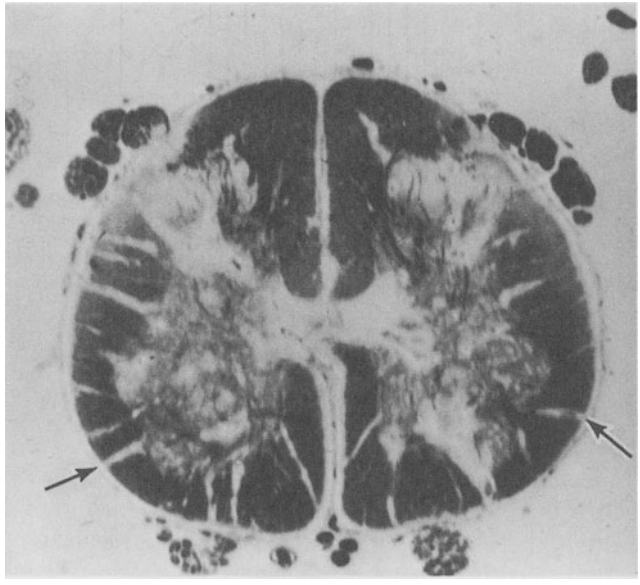

Fig. 6. Acute disseminated encephalomyelitis—myelin stain. This paraffin-embedded section of spinal cord displays linear radiating zones of demyelination (arrows) related to blood vessels penetrating from the meninges. ×10.

the white matter contains cuffs of lymphocytes, mononuclear cells, plasma cells, and occasional macrophages in relationship to the Virchow–Robin spaces of venules and small veins. Associated with the last are perivascular rims of demyelination and macrophages consisting of pleomorphic microgliocytes, histiocytes, and monocytes. In contrast to acute and chronic MS, this group of diseases displays inflammatory changes in the pai-arachnoid covering the brain stem, spinal cord, and optic nerves. This inflammation invariably overlies rims of subpial demyelination. Electron microscopic reports on these conditions are rare and add little to the histopathological picture.

Etiology. Examination of the clinical chart in most of the above cases will invariably reveal a recent exposure to a viral infection affecting either the patient or a close family member. However, specific viral isolation techniques have not been performed in most cases. Successful demonstration and isolation of virus material from cases of postinfectious encephalomyelitis are rare, examples being the observation of viral inclusions by Adams *et al.* (1966) and the rescue of a defective measles agent from one case by ter Meulen *et al.* (1972*b*). Because the pathology in these conditions does not conform to that usually associated with demyelination, it has been hypothesized that the disease is an immunological reaction to the virus or to brain constituents possibly altered during the infection course. Such a phenomenon would strengthen the significance of EAE to the study of ADE.

Indeed, *in vitro* tests for lymphokines to myelin basic protein as tested on lymphocytes undergoing blast cell formation have been positive in one case of postinfectious encephalomyelitis (Behan *et al.,* 1968). Postrabies inoculation encephalomyelitis today is a rare condition but was relatively common toward the end of the nineteenth century during the early trials with the Pasteur antirabies vaccine. The disease is analogous to a human form of EAE and was shown to be causally related to central nervous tissue incorporated into the vaccine during the culture of the virus in embryonic tissue.

4. *Acute Hemorrhagic Leukoencephalopathy (Weston–Hurst Disease)*

Pathology. Acute hemorrhagic leukoencephalopathy (Weston–Hurst disease) is rare, and the presence of hemorrhage and necrosis sometimes makes it difficult to recognize an inflammatory demyelinating state. In spite of these differences, however, it is generally regarded as a more severe form of postinfectious encephalomyelitis.

The lesions are large and grossly visible due to extravasation of red cells and infarction (Fig. 7). Microscopically, one sees that this disease differs from postinfectious encephalomyelitis by the presence of vascular

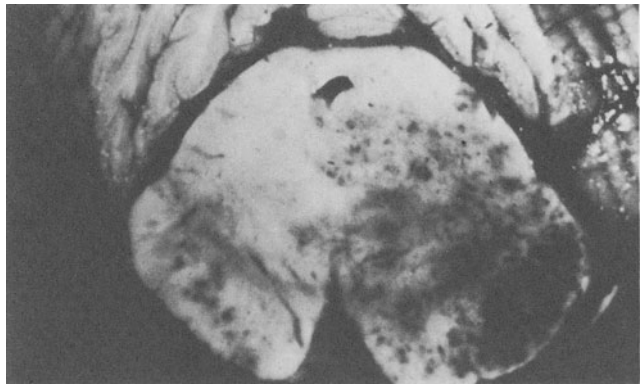

Fig. 7. Acute hemorrhagic leukoencephalopathy—gross specimen. The discoloration of white matter in this region of mesencephalon is due to severe hemorrhage, inflammation, and probably necrosis.

injury and fibrin thrombosis with infarction and abundant neutrophils in the vessel walls, lesions, and meninges. The major lesions are also accompanied by inflammatory foci and all changes are of the same age.

Etiology. Acute hemorrhagic leukoencephalopathy is usually preceded by an upper respiratory tract infection but can also follow an exanthem or vaccination (Johnson and Weiner, 1972). Also implicated in the disease process is an autoallergic response to myelin antigen, and recent work by Behan *et al.* (1968) has shown positive blast cell formation in the presence of myelin basic protein by lymphocytes from patients with this disease.

5. *Progressive Multifocal Leukoencephalopathy (PML)*

Pathology. A rare CNS condition, progressive multifocal leukoencephalopathy (PML) usually occurs in individuals with long-standing diseases of the reticuloendothelial system or neoplasms or in those receiving immunosuppressive therapy. Typically, death follows about 3–12 months after the onset of CNS symptoms. Coronal section of the fresh brain discloses multifocal, grossly visible lesions which by light microscopy are rimmed by bizarre astrocytes containing abnormal mitotic figures (Fig. 8 and 9). Large oligodendroglia lie toward the peripheries of the lesions, and many of these cells contain intranuclear inclusion bodies (Fig. 10). Myelin and oligodendroglia are absent within the lesions and it is not unusual to find a significant amount of axonal dropout. Variation in the topography and neuropathology of PML lesions has been surveyed by

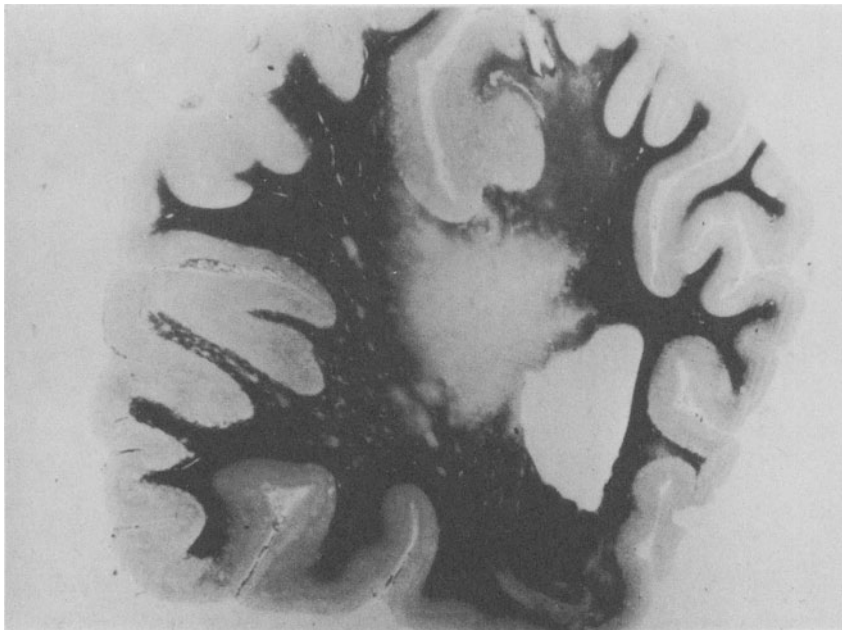

Fig. 8. Progressive multifocal leukoencephalopathy (PML)—myelin stain, whole mount. This section of occipital lobe shows a large white matter lesion with a puffball appearance, surrounded by several small lesions.

Brun *et al.* (1973). In contrast to other acquired demyelinating conditions, PML lesions are essentially noninflammatory. The mechanism of myelin degeneration is not known but it is speculated that the demyelination is a consequence of selective damage to oligodendroglia.

Etiology. A virus has been implicated in PML (for review, see Johnson and Weiner, 1972). Electron microscopy of PML lesions by Zu Rhein and Chou (1965) uncovered the presence of unequivocal viral particles within oligodendroglial nuclei, and the suggestion was made that these particles resembled a papovavirus. This finding was later confirmed by Zu Rhein (1969) in a study of more than 20 cases. Prompted by these EM observations, papovaviruses were successfully isolated from autopsy and biopsy PML brain tissue (for review, see Johnson and Weiner, 1972). The results from the serological and virological studies of Weiner *et al.* (1972) and Johnson and Weiner (1972) are consistent with there being more than one papovavirus with the ability to produce PML. The specificity of the infection for oligodendroglia supports the theory that myelin breakdown occurs subsequent to their death and the failure of the subjects to mount

an efficient inflammatory response is in accord with an immunological deficit and the absence of an immune-mediated process of demyelination.

6. *Idiopathic Polyneuritis*

Pathology. The term *idiopathic polyneuritis,* which embraces the various forms of the Landry–Guillain–Barré syndrome and postinfectious polyneuritis, represents a group of inflammatory demyelinating conditions specifically affecting the PNS. Lesions are not visible grossly but light microscope examination reveals a multifocal intense inflammation associated with primary demyelination (Figs. 11 and 12). The disease is most evident in radicular zones and ganglia, with the extremities less affected. While some forms display an acute, monophasic course, often with fatal outcome, some are chronic progressive or relapsing and display evidence of remyelination. The fine structure of LGBS and other idiopathic neuri-

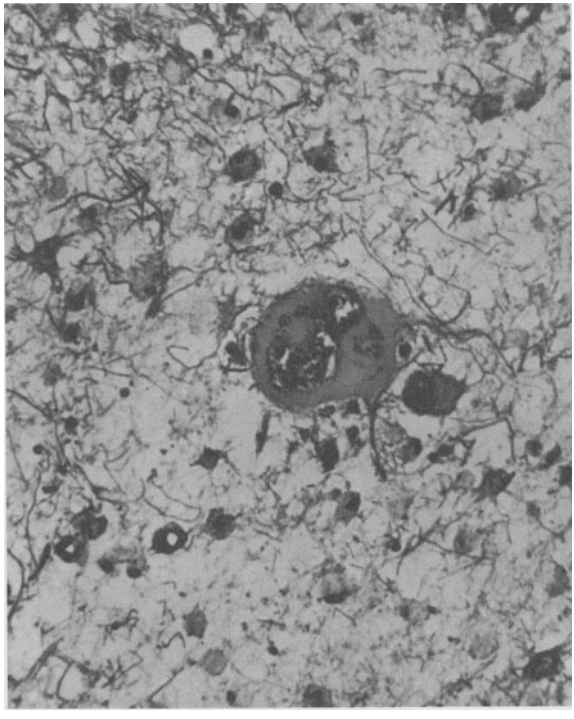

Fig. 9. PML—hematoxylin and eosin (H and E) stained paraffin section. A bizarre astrocyte is located within an area of demyelination. ×500.

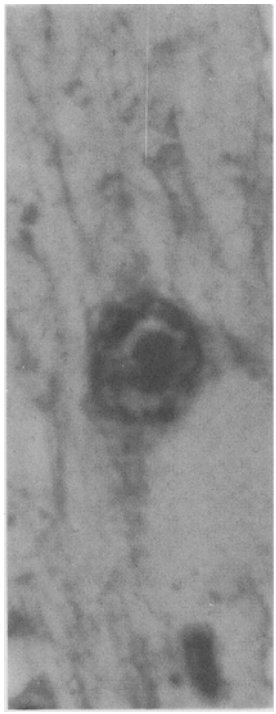

Fig. 10. PML—H and E preparation. An oligodendroglial cell nucleus contains a viral inclusion. ×1200.

tides has been extensively investigated (see Prineas, 1971), and a process of demyelination akin to that seen in the animal models of autoimmune demyelination—EAE and EAN—was the common pattern. As a general rule, there is little or no axonal degeneration in these diseases. Sometimes, in more severe cases where inflammation of the spinal nerve roots persists for several weeks after onset, there is secondary degeneration in the posterior columns of the spinal cord. Minor inflammatory changes are sometimes localized within the meninges. Even in cases with clinical recovery there may be long-standing foci of inflammation within peripheral nerves (Asbury *et al.,* 1969).

Etiology. While an autoallergic phenomenon has been accepted as the underlying cause of the PNS demyelination in these conditions, a conclusion heavily influenced by comparison with the animal model experimental allergic neuritis (EAN), many workers attribute the primary insult to an antecedent viral infection. Reports exist, for example, where idiopathic polyneuritis developed after a bout of measles, infectious hepatitis, respiratory tract infections, rabies infection, and infectious mononucleosis, although direct demonstrations or isolation of a virus are lacking. Recent serological tests on some cases of Landry–Guillain–Barré syndrome have

Fig. 11. Landry–Guillain–Barré syndrome (LGBS)—H and E section. This longitudinal section of spinal nerve roots demonstrates an increased cellularity due to inflammatory cells between the nerve fibers and related to blood vessels. ×200.

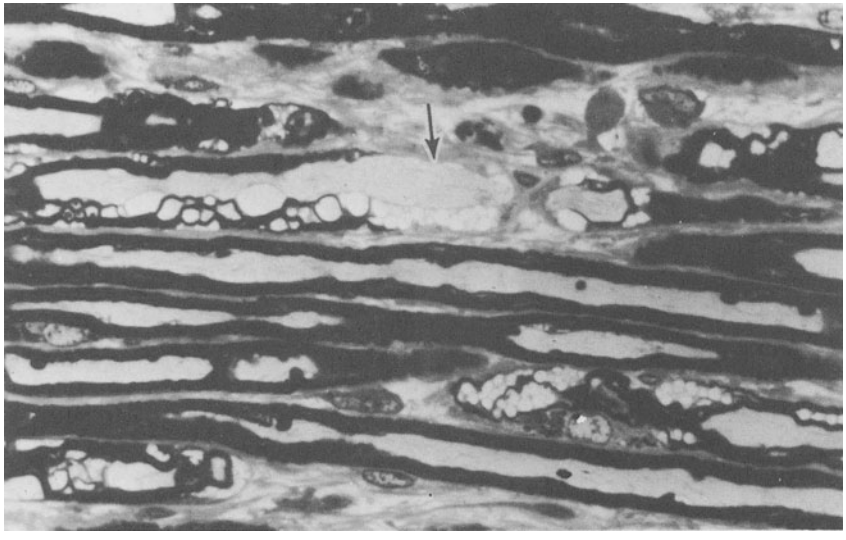

Fig. 12. LGBS—toluidine blue stained 1-μm epon section. Several longitudinally sectioned fibers demonstrate vacuolar changes in the myelin. A short segment of one fiber (arrow) is completely naked. ×600.

shown significantly higher titers of antibody against Epstein–Barr virus, a cell-associated herpesvirus. See Chapter 12 for further discussion of relevant immunology.

7. *Diphtheritic Neuropathy*

Pathology and Etiology. Diphtheritic neuropathy in man is the direct sequela of a bacterial infection, i.e., *Corynebacterium diphtheriae.* The myelin breakdown is caused by an exotoxin secreted by the organism and not by the bacterial invasion itself. Frequently, the disease is fatal and related to respiratory paralysis or disordered cardiac function. Primary demyelination occurs in the PNS and shows a predilection for the spinal nerve roots and proximal regions of nerves. There is remarkable preservation of axons and neurons. Myelin becomes fragmented and might be taken up locally by Schwann cells. A few mononuclear cells are also seen. The brain and spinal cord usually remain normal. The manner in which the toxin effects this demyelination has been attributed to its specific affinity toward membrane systems (Webster *et al.,* 1961). Biochemical data have shown the exotoxin to be a potent inhibitor of protein synthesis.

B. *Animal Examples*

A number of naturally occurring and experimental diseases exist which have marked similarities to the previous group of human diseases. In most of the spontaneous animal conditions, a viral etiology is either proven or highly likely. The experimental situations are induced by infection with a known virus or sensitization with nervous system antigen, the latter effecting a delayed hypersensitivity-type response within the CNS or PNS.

1. *Canine Distemper Encephalomyelitis*

Pathology and Etiology. Canine distemper encephalomyelitis, a usually fatal condition, occurs naturally in dogs and may be induced experimentally in a number of species, in particular, dogs and ferrets. Canine distemper virus, a paramyxovirus closely related to measles, usually produces a systemic infection and exanthem which precede the nervous system syndrome by 1–2 weeks. The disease has a number of forms—acute, chronic, and relapsing—and the pathology varies according to the persistence of the agent. At autopsy, the CNS of a distemper dog might display large visible plaques throughout, although it is not unusual to detect no abnormalities on gross examination. Microscopically, lesions are

inflammatory and purely demyelinative, although some burnt-out or severe plaques might show considerable axonal loss. Viral inclusions can be detected in a number of cell types. Ultrastructurally, the process of demyelination is associated with macrophages (Raine, 1972) and proceeds in the presence of local viral material. Whether there is active sensitization to myelin components or whether the demyelination is a sequela of specific infection of oligodendroglia, cross-reactivity between viral and myelin proteins, or accidental damage occurring in the midst of regions where lymphokines and hydrolytic enzymes might be synthesized against the agent, is not known. The infectious agent has been well characterized by a number of workers (see Appel and Gillespie, 1972), and *in vitro* tests have suggested depression of T cells and the possibility of specific myelinotoxic factors in the serum of infected animals.

2. *Visna*

Pathology and Etiology. Visna, a naturally occurring disease among Icelandic sheep until eradicated by an intensive killing program, exists today as an *in vitro* virus which is used to transmit the disease experimentally. CNS lesions are often grossly visible, and many display nonspecific necrosis. This necrotic feature may invalidate the inclusion of visna in the inflammatory group. However, some inflammatory demyelinating lesions can be found in the white matter. There is a predilection for lesions to affect subependymal regions. Since the disease progresses in the presence of an increase in spinal fluid protein and serum antibody and the observation that viral release *in vitro* is by a process of budding, it has been suggested that antibody–antigen reactions might occur on infected glial cell membranes, leading to cellular destruction and demyelination (for review, see Johnson and Weiner, 1972). However, definitive proof of the latter is lacking. Recent studies on the characterization of the agent suggests that visna may be related to the C-type RNA viruses (oncornaviruses).

3. *Coonhound Paralysis*

Pathology and Etiology. In coonhound paralysis, a naturally occurring condition of dogs, the PNS is specifically affected by an inflammatory disease process which renders the model highly suited for the study of the LGBS in man. Also, there are many similarities to EAN (see below). After the onset of limb weakness, the nerve roots and peripheral nerves display diffuse inflammation and concomitant segmental demyelination (Cummings and Haas, 1967). The disease is probably related to a viral infection, as yet not characterized, occurring after a coonhound (other breeds of

dog are also susceptible) is bitten by a racoon. The present consensus is that the disease might result from a combination of viral and autoimmune factors.

4. Marek's Disease

Pathology and Etiology. Among poultry breeders, Marek's disease provides a severe economic threat since it accounts for more deaths among chickens than does any other condition. Marek's disease is predominantly a malignant lymphomatous state related to infection by a herpesvirus. As a secondary complication, the PNS may become involved. This neurological complication is typified morphologically by the invasion of the PNS by inflammatory cells which destroy myelin in a manner similar to that seen in LGBS and EAN (Prineas and Wright, 1972). This suggests that autoimmune factors might play a role. Although it is assumed that the demyelination follows the viral infection, it is usually difficult to visualize virus particles in afflicted nerves.

5. Mouse Hepatitis Virus Encephalitis

Pathology and Etiology. In mice, an experimental viral encephalitis with some features reminiscent of acute disseminated encephalomyelitis and PML can be induced. The disease is caused by infection with a virus (JHM strain) isolated originally from the brain of a mouse (Cheever *et al.*, 1949). The agent has been since classified with the mouse hepatitis viruses among the coronaviruses. An affinity for myelin to be damaged was observed, and this was later confirmed by Waksman and Adams (1962). Ultrastructural study by Lampert *et al.* (1973*b*) has reported on the presence of virus in lesions and the occurrence of nonspecific demyelination related to mononuclear cells. The loss of myelin has been proposed to result from a specific infection of myelinating cells and not from an immune mechanism, a thesis supported by experiments on infected immunosuppressed animals which displayed myelin loss yet lacked inflammatory changes (see Johnson and Weiner, 1972).

6. Experimental Allergic Encephalomyelitis (EAE)

Pathology and Etiology. As the name suggests, experimental allergic encephalomyelitis (EAE) is an experimental disease. It is inducible in most laboratory species and generally involves the sensitization of animals with a single inoculation of white matter or myelin basic protein emulsified with complete Freund's adjuvant, although other protocols (*viz.*, the omission of complete Freund's adjuvant from the inoculum or the substitution for this component by other adjuvants) are capable of causing the disease.

About 2–3 weeks following the subcutaneous administration of the encephalitogenic emulsion, animals become paralyzed. This acute, monophasic disease is typified microscopically by foci of perivascular and meningeal inflammation which are invariably related to local demyelination (see Waksman and Adams, 1956). Acute lesions bear some morphological resemblances to those of acute disseminated encephalomyelitis and acute multiple sclerosis. The fine structure of the mechanism of demyelination has been shown to involve active stripping of myelin from axons by invading mononuclear cells (Lampert, 1967) and vesicular disruption of the myelin sheath (Raine *et al.*, 1974). In some species, the PNS is also affected. Manipulation of the induction protocol can cause a hyperacute disease which mimics acute hemorrhagic leukoencephalopathy. The amino acid sequence of myelin basic protein has been analyzed, and encephalitogenic sites on the molecule are now recognized for a number of species. The disease is T-cell mediated (Gonatas and Howard, 1974) and evidence for sensitization against myelin components is well known (see Paterson, 1973). Chronic forms of EAE also exist, some with relapsing disease courses (Raine, 1976). The latter have clinical and pathological stigmata resembling the human condition, MS, for which EAE is a possible experimental analogue. See the relevant discussions of immunology in Chapter 12 and metabolism in Chapter 13.

7. *Experimental Allergic Neuritis (EAN)*

Pathology and Etiology. Experimental allergic neuritis (EAN), the PNS counterpart of EAE, was originally described by Waksman and Adams (1956). Animals are sensitized against whole PNS tissue or PNS myelin basic protein in complete Freund's adjuvant and develop leg weakness in 2–3 weeks. Histologically, the PNS contains ongoing demyelination in the presence of inflammation, shown by Lampert (1969) to be effected by an active stripping process by invading mononuclear cells, analogous to the pattern described in EAE. Chronic and recurrent forms of EAN are also known. The disease is the standard laboratory model for the study of Landry–Guillain–Barré syndrome. See the relevant discussion in Chapter 12.

V. Class II: Hereditary Metabolic Diseases of Myelin

Diagnostic Criteria. The group of hereditary metabolic diseases of myelin covers a large number of conditions, each of which might have several variants, usually determined by age at onset. There are distinctive

clinical and morphological features which unify the various diseases in this group. Clinically, these diseases are reflections of inborn errors of metabolism which often become manifest in the first decade of life. Morphologically, the diseases (known collectively in most cases as the leukodystrophies) demonstrate a diffuse loss of both myelin and axons from large areas of white matter. Since the nervous system damage is more widespread than in the previous group, the term *leukodystrophy* is general and ignores the involvement of neurons and other organs in some instances and serves only to emphasize the severe destruction of white matter common to all members. It is becoming apparent that most leukodystrophies represent disorders of lipid metabolism and some are already classified amongst the lipidoses. With the exception of adrenoleukodystrophy, the conditions are noninflammatory and viral and immunological factors have not been implicated. All are extremely rare.

A. Human Examples

1. Metachromatic Leukodystrophy (MLD)—Sulfatide Lipidosis

Pathology. Metachromatic leukodystrophy (MLD), a rare familial disease, has its onset in most cases between the ages of 1 and 5 years and has a duration of 3–6 years. Adult forms exist but are rarer. The condition derives its name from the abnormal, metachromatically staining myelin degradation products (cerebroside sulfatide). Coronal section of the brain reveals extensive involvement of the entire white matter (Fig. 13), often in a symmetrical fashion, so that lesions have a butterfly configuration. Sometimes, in cases of long duration, the white matter is reduced to a narrow strip 1–2 cm in diameter, and the shrinkage of the white matter can lead to enlargement of the ventricles. The disease primarily affects myelin but the subsequent breakdown process invariably affects neurons and their axons. It is therefore not classed as a "demyelinating" disease but rather as a disorder of myelin. Early in the disease, myelin is completely lost from lesion areas, and this loss is followed by axonal degeneration. At the edge of affected areas and scattered throughout lesions are macrophages containing the specific degradation product (Fig. 14). Nerve cells throughout the brain show ballooning and swelling and presence of cytoplasmic inclusions containing cerebroside sulfatide. The most severe nerve cell changes occur in the mesencephalon, pons, medulla oblongata, and the spinal cord. Similarly, certain areas of white matter are more severely affected, mainly those which are myelinated late in ontogenesis. By light microscopy, oligodendroglia are absent from lesions. The specific

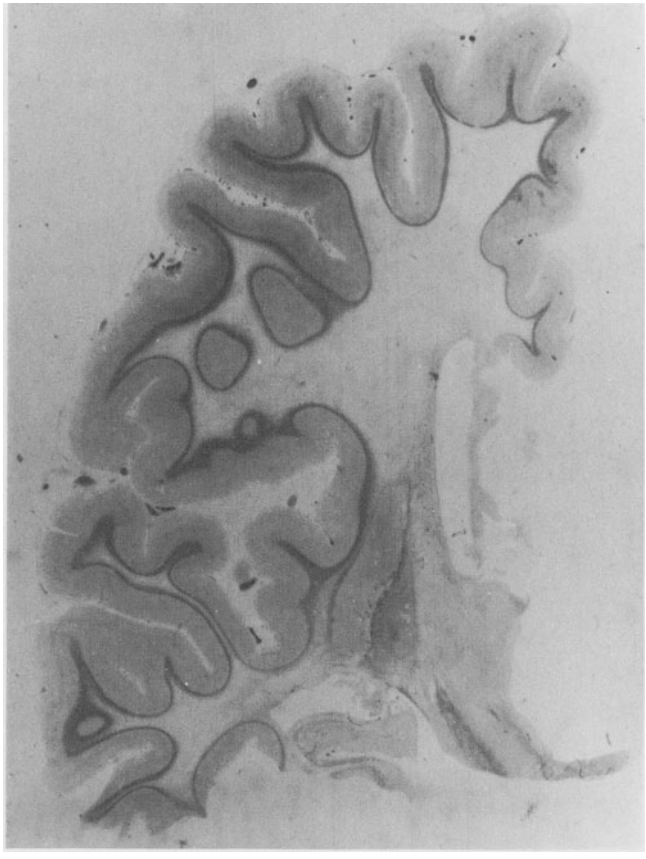

Fig. 13. Metachromatic leukodystrophy (MLD)—myelin stain, whole mount. This cerebral hemisphere demonstrates the severe involvement of white matter, the widespread loss of myelin, and the preservation of subcortical arcuate fibers.

inclusions (Fig. 15), which have a characteristic lamellated morphology (see Terry, 1970), are not only found in neurons and macrophages in the brain but also occur in Schwann cells of the PNS and in a variety of other organ systems, e.g., viscera (Wolfe and Pietra, 1964).

Etiology. Biochemical assays have determined that the myelin breakdown in MLD is due to a genetically determined deficiency of the enzyme cerebroside-3-sulfatase (arylsulfatase A) detectable in a number of tissues both pre- and postnatally (Peiffer, 1970; Moser, 1970; see Chapter 11 for details). Normal-appearing myelin from unaffected areas of white matter also shows biochemical abnormalities. It is hypothesized that myelination at first is normal despite the enzyme defect, but gradually sulfatide accumulates and the myelin becomes abnormal.

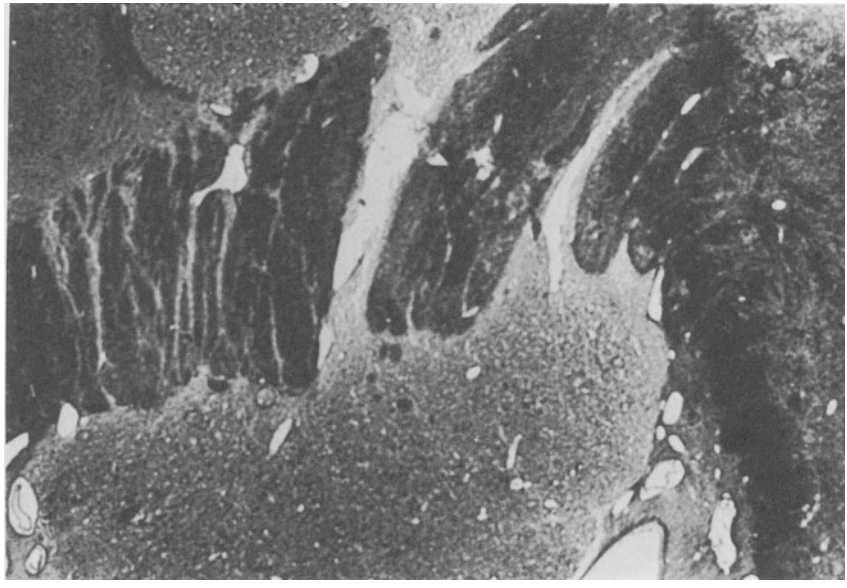

Fig. 14. MLD—acid cresyl violet stain. The degenerating fibers in the internal capsule have been stained darkly due to the presence of metachromatic material in contrast to the pale-staining, adjacent basal ganglia. ×100.

2. *Krabbe's Disease (Globoid Cell Leukodystrophy)*

Pathology. Krabbe's disease (globoid cell leukodystrophy) generally develops during the first 6 months of life and patients succumb in about 14 months. Examination of the gross brain reveals that it may be somewhat reduced in size. On coronal section (Fig. 16), it is seen that the cortex is relatively spared (except for occasional areas in the temporal and occipital lobes), but there is marked reduction in the amount of white matter, which shows a brown discoloration, more pronounced posteriorly. In the cerebral hemispheres, there is a tendency for arcuate fibers to be spared. Although loss of myelin occurs throughout the white matter, it is less pronounced in certain areas, e.g., frontal lobes. In grossly visible lesions, myelin and most axons are lost. Globoid cells, the pathognomonic feature of the disease, are apparent microscopically (Fig. 17). They are most common in less advanced lesions and may show a tendency to accumulate around blood vessels. These cells are multinucleated and contain specific crystalloid cytoplasmic inclusions (Yunis and Lee, 1969) (Fig. 18). Large rounded cells with single nuclei and a finely granular

cytoplasm also occur and may be precursors of globoid cells, since transitional forms between the two have been described (see Volk and Adachi, 1970). Neurons are relatively unaffected in this disease.

Etiology. It is well established that there is a familial trait in this disease. The defect has been found to be related to the deficient activity of galactocerebroside β-galactosidase detectable in a variety of tissues including white cells and fibroblasts (Suzuki and Suzuki, 1971; Suzuki *et al.*, 1971; see Chapter 11).

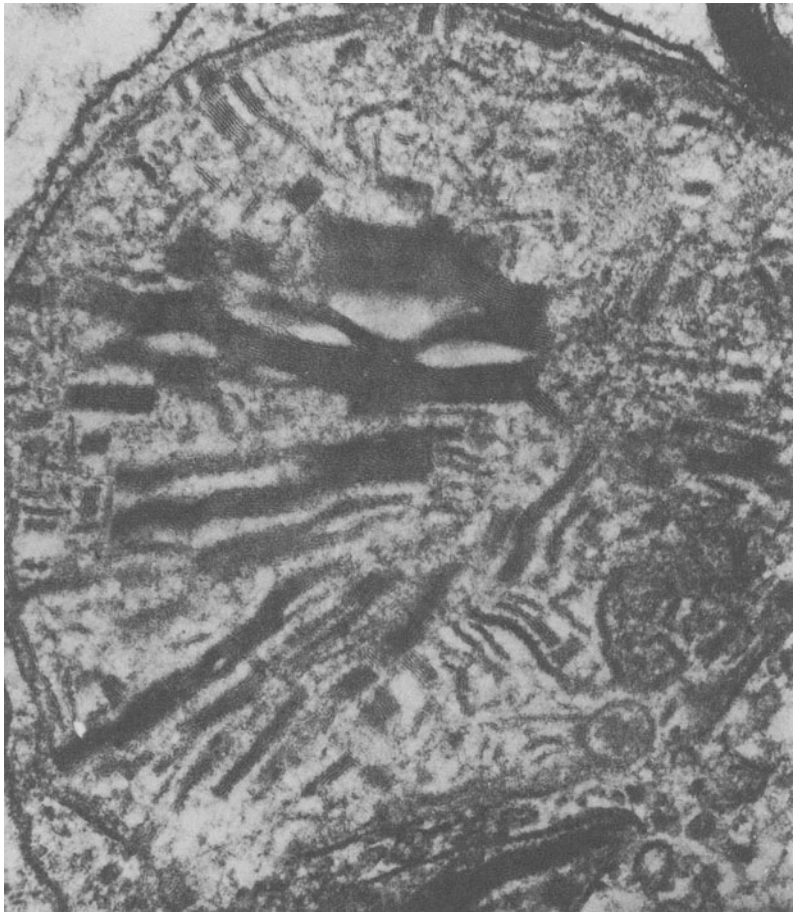

Fig. 15. MLD—electron micrograph. The macrophages containing the lipid storage product possess inclusions with a specific lamellated substructure. ×100,000.

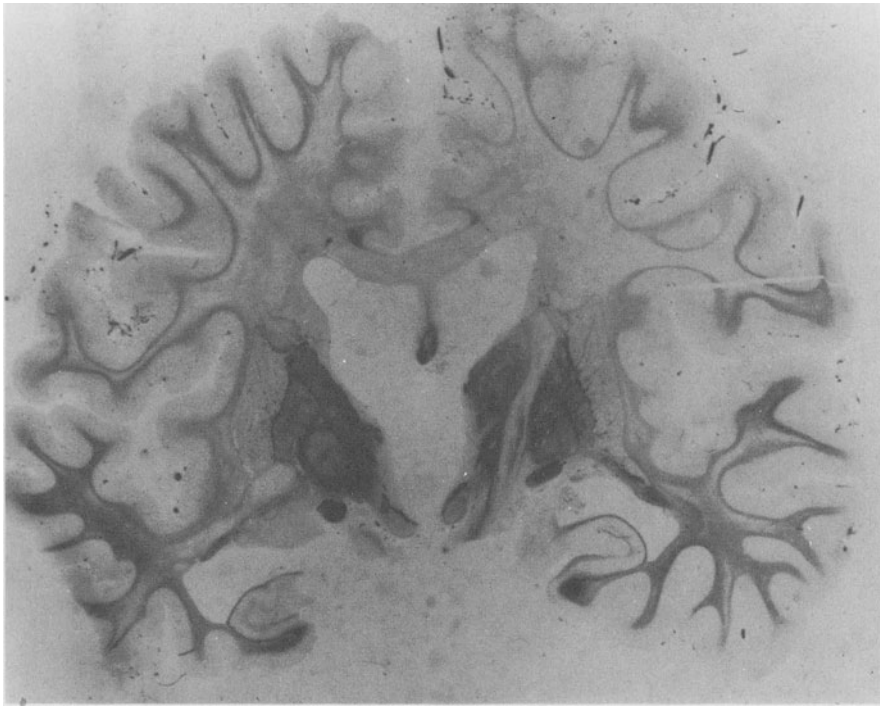

Fig. 16. Krabbe's disease—myelin stain, whole mount. Note the widespread involvement of myelin, the preservation of subcortical fibers, and the enlargement of the ventricles.

3. Adrenoleukodystrophy

Adrenoleukodystrophy (ALD), typically affecting males during late infancy, has a clinical course of about 2–4 years, although a few cases in older males (40–60 years) are known. CNS lesions are large and grossly visible. The lesions are often symmetrical and involve massive areas of white matter of the cerebral hemispheres with preservation of the subcortical arcuate fibers (Blaw, 1970). There is usually severe involvement of both occipital poles (Fig. 19). There is widespread loss of myelin with a subsequent loss of most axons. Unlike other metabolic disorders of myelin, there is an intense inflammatory response within lesions (Fig. 20), which has prompted some workers previously to classify this condition among the acquired inflammatory demyelinating diseases. This response appears to herald a secondary immunological problem. The changes in the adrenal glands are pathognomonic (Schaumburg, *et al.,* 1972).

Etiology. On the basis of familial traits and white matter involvement, this genetic leukodystrophy is thought to be due to an enzyme deficiency, as yet unknown. The presence of similar specific intracytoplasmic inclusions (Fig. 21) in the adrenal glands, CNS, PNS, and testis (Schaumburg *et al.,* 1975) indicates that the disease has a pathogenesis related to abnormal lipid storage. This hypothesis is further supported by the recent finding of a hitherto unrecognized long-chain fatty acid in the CNS and adrenal glands (Igarashi *et al.,* 1976; see Chapter 11).

4. *Refsum's Disease*

Pathology. In Refsum's disease, a genetically determined condition, the PNS is a major site of involvement. The clinical course is long, frequently with remissions, and the disease usually develops during adolescence, although adult cases have been reported. Pathologically, the

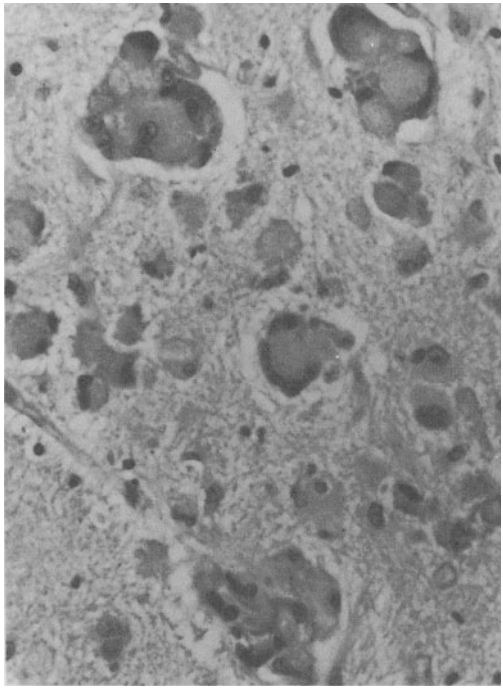

Fig. 17. Krabbe's disease—H and E preparation. Multinucleated globoid cells are located within the affected white matter. ×300.

Fig. 18. Krabbe's disease—electron micrograph. The specific crystalloid inclusions of the globoid cells are shown. ×40,000.

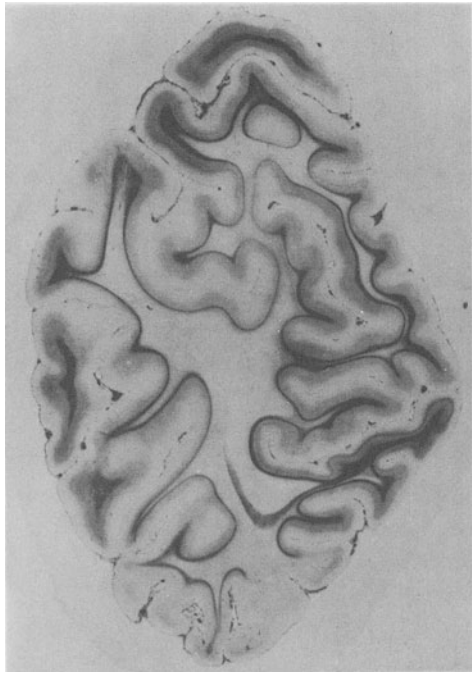

Fig. 19. Adrenoleukodystrophy (ALD)—myelin stain, whole mount, occipital pole. Note the total loss of myelin from the deeper white matter and the preservation of subcortical fibers in this section.

nerves are hypertrophied due to an increase in Schwann cell interstitial tissue. The aberrant Schwann cells form characteristic onion-bulb formations (Fardeau and Engel, 1969). This hypertrophy is brought about by repeated damage to nerve fibers. Loss of myelin and axons occurs and there is some remyelination. There is sometimes involvement of the CNS (Solcher, 1973).

Etiology. The disease is related to a specific deficit of lipid metabolism with high levels of blood and tissue phytanic acid. This inability to degrade phytanic acid is due to a deficiency in phytanic acid α-oxidase (Steinberg, 1972; see also Chapter 11).

The above four conditions are believed to reflect an enzyme deficiency expressing itself *after* the period of myelination. The following three conditions, on the other hand, are considered to represent an inborn metabolic disorder manifesting itself during or *before* the myelina-

tion period and consequently leading to a paucity of myelin formation. This "hypomyelination" might in future be used as a pathological feature to subdivide the leukodystrophies.

5. *Pelizaeus–Merzbacher Disease (Sudanophilic Leukodystrophy)*

Pathology. Pelizaeus–Merzbacher disease (sudanophilic leukodystrophy), which can develop congenitally or during the first 6 months of life, is characterized by a slow, progressive clinical course lasting for up to 30 years. Lesions in the congenital type show an almost total depletion of myelin with relative sparing of axons. In the later-onset form, the process of myelin loss is sometimes patchy, giving a "tigroid" appearance (see Seitelberger, 1970) (Fig. 22). Ultrastructural examination has revealed a lack of compaction of CNS myelin around axons and nonspecific crystalloid inclusions in a few hypertrophied astrocytes (Watanabe *et al.*, 1969, 1972).

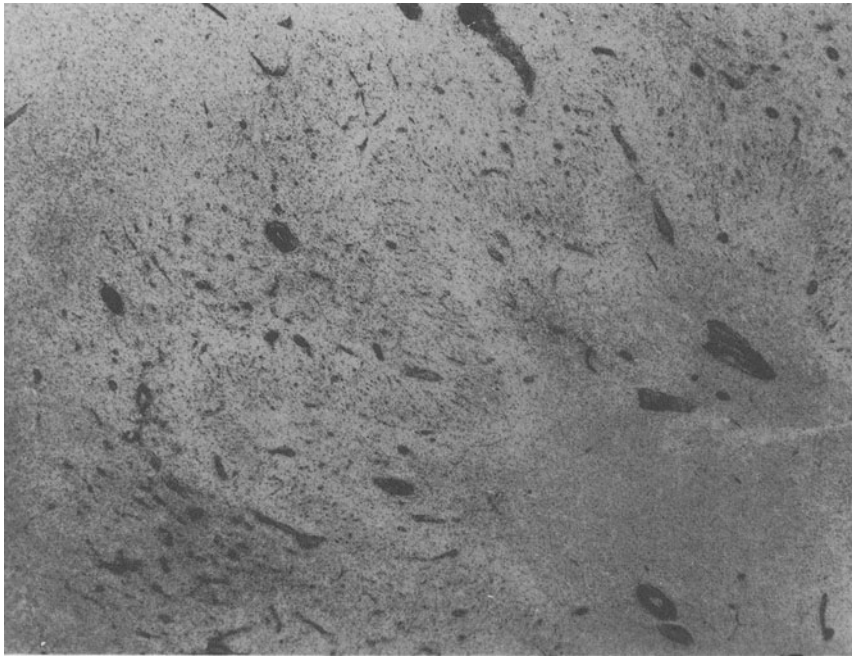

Fig. 20. ALD—H and E section. The centers of ALD lesions are totally devoid of myelin and invariably contain perivascular cuffs of lymphocytes and other hematogenous elements, seen here in low power. ×150.

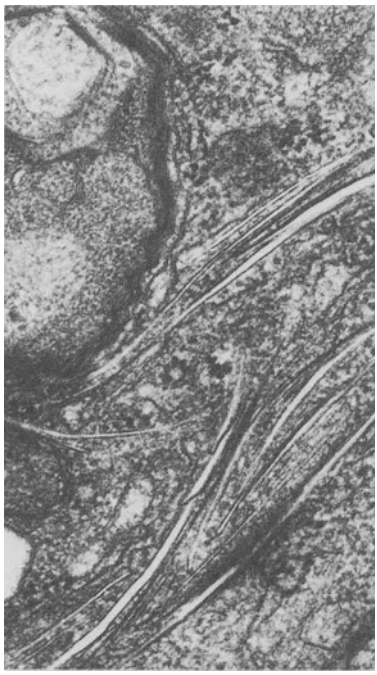

Fig. 21. ALD—electron micrograph. Macrophages within demyelinated areas contain crystalloid, spicular inclusions. ×53,000.

Etiology. The precise biochemical lesions corresponding to the two forms of the disorders have not been clarified (see Chapter 11).

6. *Alexander's Disease (Dysmyelinogenetic Leukodystrophy)*

Pathology. Alexander's disease usually manifests itself during the first year of life and has a variable course. Megalencephaly and hydrocephalus are not uncommon gross features (Fig. 23). Lesions are characterized by a lack of myelin, with widespread formation of Rosenthal fibers within astrocytes (Fig. 24), the pathological hallmark of this disease (see Herndon *et al.,* 1970; van Bogaert, 1970). Axons are relatively spared and there is an intense proliferation of astrocytes. EM examination has shown that the Rosenthal fibers are ill-defined, rodlike structures with an amorphous, granular matrix.

Etiology. The underlying biochemical defect is not known. Some workers consider that, on the basis of a lack of macrophage activity and the paucity of myelin, the disease might represent a genetically determined error in myelinogenesis, although precise evidence is lacking.

7. *Spongy Degeneration of White Matter (Canavan's Disease)*

Canavan's disease usually appears between 3 and 6 months after birth and is fatal in less than 2 years. Megalencephaly is typical, apparently due to increased intracellular water content, principally in the subcortical white matter (Fig. 25). There is marked vacuolation of myelin sheaths, with secondary degeneration of some fibers (Figs. 26 and 27). Alzheimer type II astrocytes are present in great numbers. There is a generalized hypertrophy of protoplasmic astrocytes (Figs. 28 and 29), which have

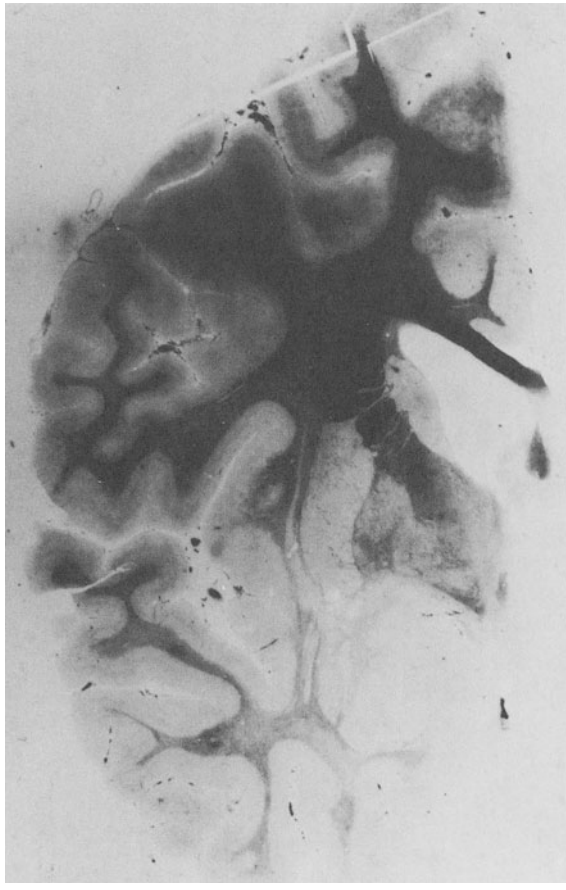

Fig. 22. Peliżaeus–Merzbacher disease—myelin stain, whole mount. This section shows the widespread depletion of myelin, particularly in the temporal lobe, below.

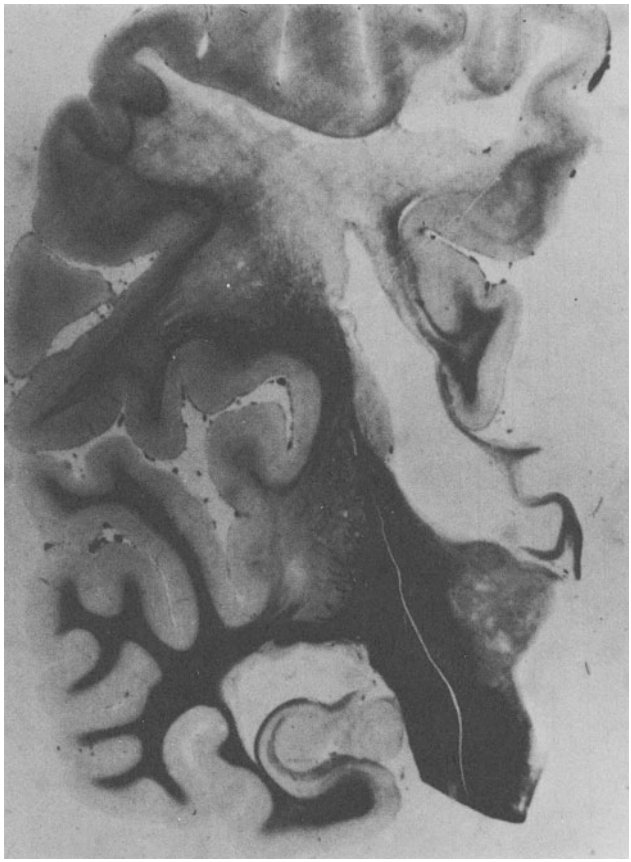

Fig. 23. Alexander's disease—myelin stain, whole mount. This section illustrates the degree of myelin involvement. Some hydrocephalus is also apparent.

been shown to contain bizarre, abnormally large mitochondria which have a crystalline substructure (Fig. 30).

Etiology. Although chemical investigations relevant to this disorder have been carried out (see Chapter 11), the metabolic defect is not known.

8. *Phenylketonuria*

Pathology and Etiology. Phenylketonuria, which occurs as both early- and late-onset forms, is known to have a familial pattern (Malamud, 1966). Clinically, the patients are mentally retarded. Repeated testing of the urine usually confirms the diagnosis of phenylketonuria, but in some

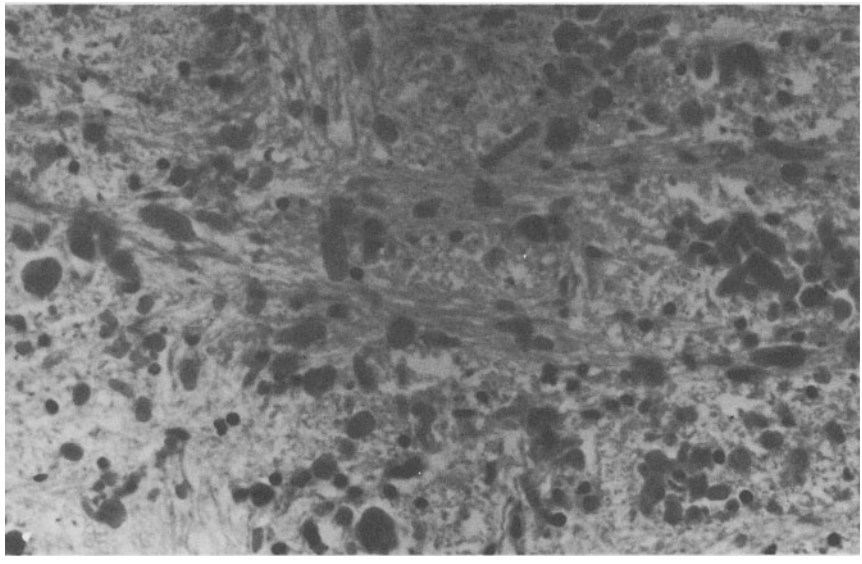

Fig. 24. Alexander's disease—H and E section. Darker-staining Rosenthal fibers, a striking feature of this disease, are present in large numbers. ×200.

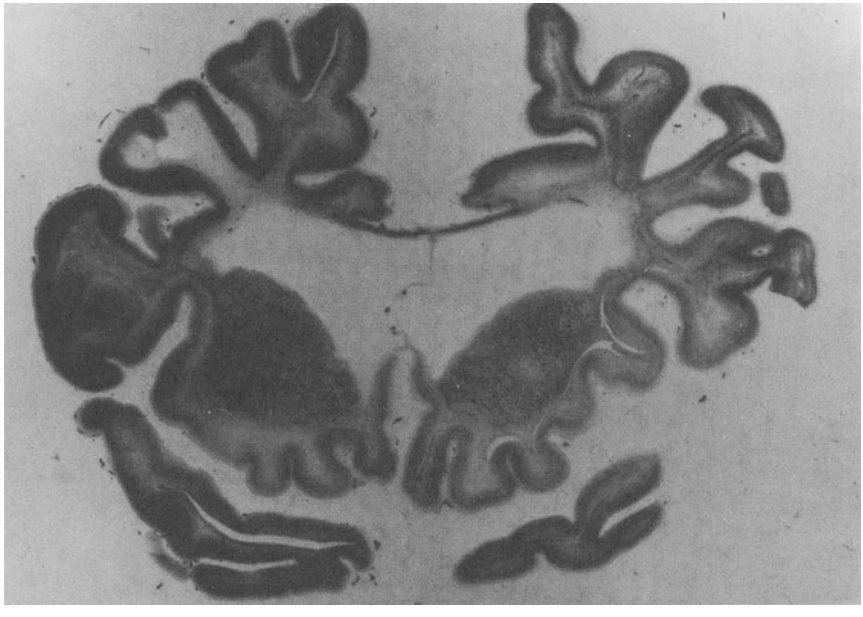

Fig. 25. Canavan's disease—myelin stain, whole mount. Note the generalized involvement of white matter with a striking accompanying enlargement of the ventricles.

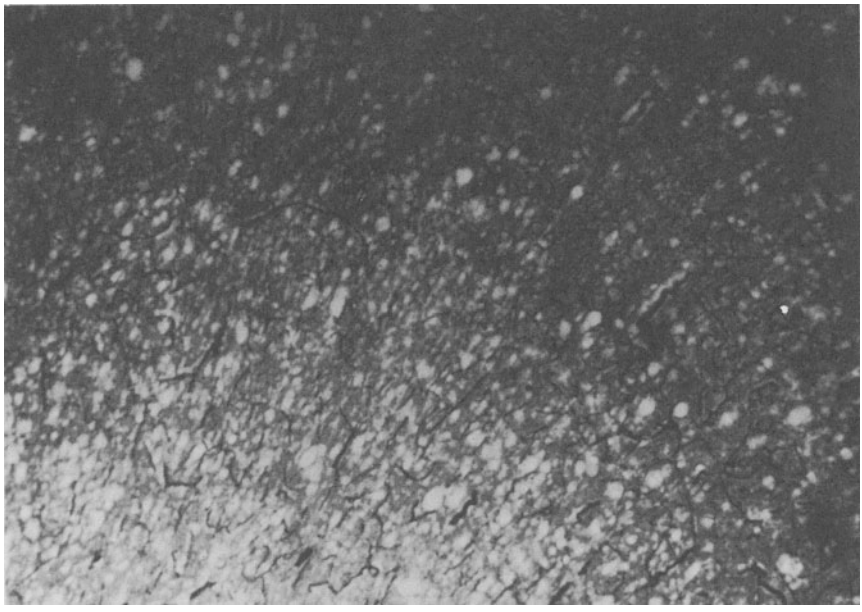

Fig. 26. Canavan's disease—H and E stain. The spongy degeneration of white matter is apparent at the edge of an affected area. ×100.

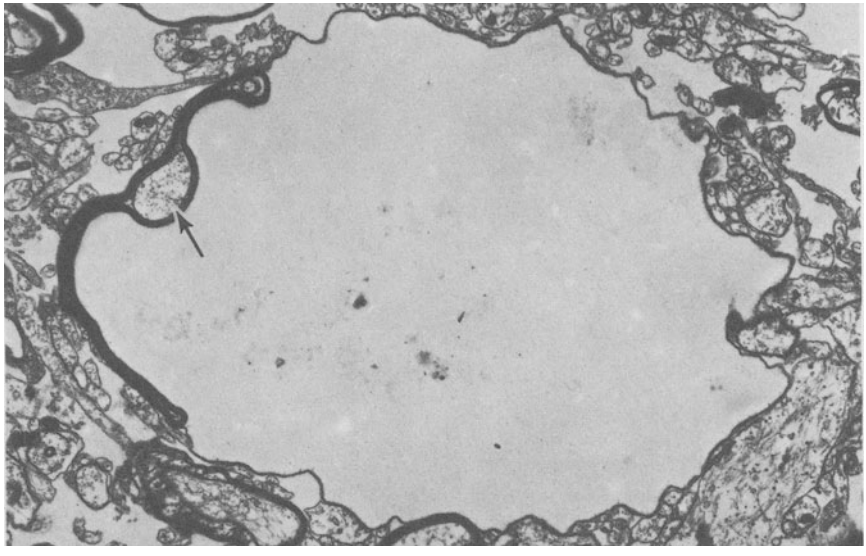

Fig. 27. Canavan's disease—electron micrograph. The spongy change is in part due to the dilatation of myelin sheaths while the axon (arrow) is pushed laterally. ×6500.

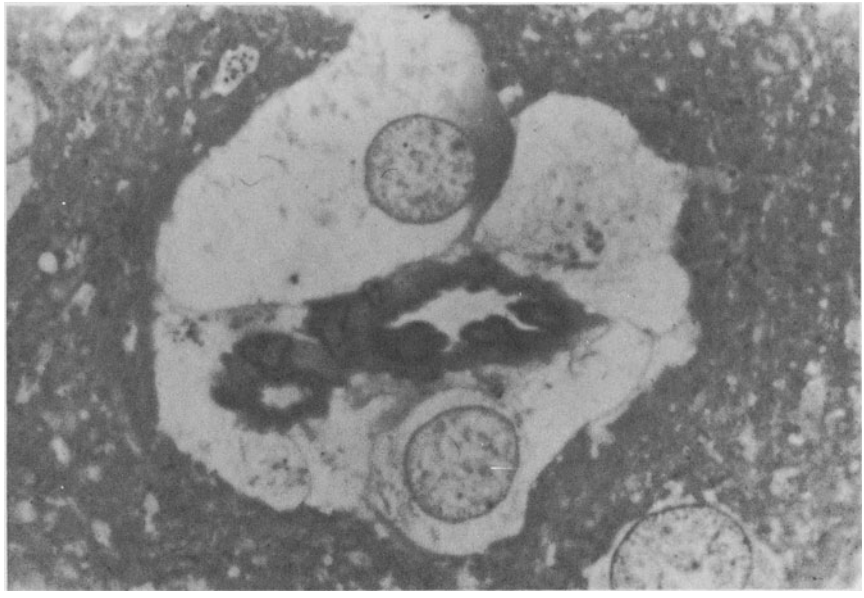

Fig. 28. Canavan's disease—toluidine blue stained 1-μm epon section. The spongy change is also related to hypertrophy of protoplasmic astrocytes, a group of which are seen here surrounding a blood vessel. ×1200.

instances it can be made on the phenylalanine content of the blood. Grossly, the brain is microcephalic. Spongy changes and a diffuse pallor in myelinated areas are common. Frank demyelination with sudanophilia is present in older patients (Jervis, 1963; Malamud, 1966). The disease is believed to be the result of a block in the oxidation of phenylalanine to tyrosine in the liver due to an inactive form of phenylalanine hydroxylase (Knox, 1972). Biochemical studies have also demonstrated an increased water content in the brain, a diminution in the cerebroside and free cholesterol content, and a rise in cholesterol esters, the last in accord with a process of demyelination (Crome *et al.,* 1962). See Chapter 11 for more details of this disorder and Chapter 13 for discussion of relevant animal models.

B. Animal Examples

The genetically determined metabolic diseases of myelin in man possess a number of animal analogues, both naturally occurring and experimental. Because of their ready availability and the rarity of the

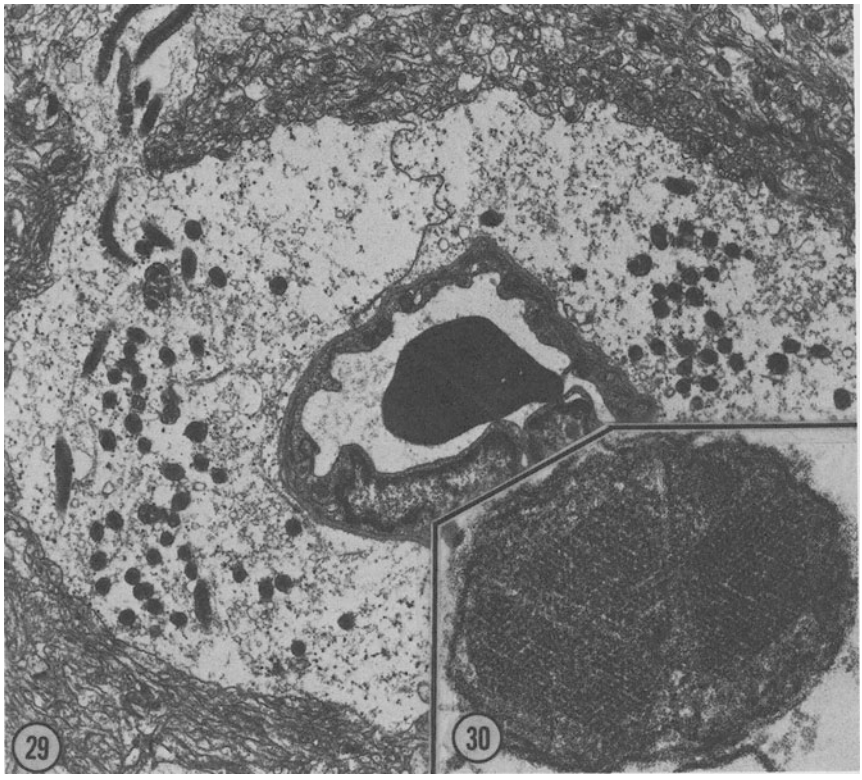

Fig. 29. Canavan's disease—electron micrograph. An area similar to Fig. 28 is seen. Note the central blood vessel containing a red blood corpuscle, the hypertrophied astrocytic end feet, and the multiple, bizarre, elongated mitochondria, shown in greater detail in the following figure. The surrounding neuropil seems relatively normal. ×7000. Fig. 30. Canavan's disease. This electron micrograph shows a single astrocytic mitochondrion which contains paracrystalline arrays of filamentous material commonly associated with this disease. ×70,000.

human conditions, these animal models have contributed considerably to our knowledge on genetic myelin disorders, particularly from a morphological and biochemical standpoint.

1. Globoid Cell Leukodystrophy

Pathology and Etiology. Certain breeds of dogs (e.g., Cairn and West Highland terriers) carry genes for globoid cell leukodystrophy, a disease which mimics human Krabbe's disease. Morphological similarities

between the respective CNS lesions are striking. Multinucleated globoid cells with tubular inclusions and diffuse destruction of cerebral white matter occur. The PNS is also affected and contains myelin changes and globoid macrophages. Experimental production of globoid cells is well known (Austin and Lehfeldt, 1965) and the cytoplasmic inclusions are believed to contain galactocerebroside (Suzuki, 1970). The inherited deficiency rests in a decrease in the catabolic enzyme galactocerebroside β-galactosidase, detectable in several tissues in addition to brain (Suzuki *et al.*, 1970).

2. *Jimpy Mice*

Pathology and Etiology. In jimpy mice, which have a genetically determined neurological disorder, recognized at the same time as "quaking" mice (see below) by Sidman *et al.* in 1964, there is a marked deficiency of CNS myelin occurring in the presence of sudanophilic deposits. This subsequently prompted Sidman and Hayes (1965) to refer to the disease as a murine form of inherited sudanophilic leukodystrophy. The neuropathology of jimpy mouse brain has been approached using both the light and electron microscope (Hirano *et al.*, 1969). The most striking anomaly was an almost total lack of myelination from large areas of the brain and the presence of lipid-laden macrophages. Abnormalities have been noted in oligodendroglia (Meier and Bischoff, 1974; Meier *et al.*, 1974). Axonal changes were also prominent but less specific, being observed in both jimpy mice and their apparently normal litter mates. See Chapter 14 for details of the relevant biochemistry, which includes demonstration of multiple enzyme deficits.

3. *Quaking Mice*

Pathology and Etiology. Quaking, another murine mutant first recognized by Sidman *et al.* (1964), is a condition in which CNS myelin formation commences apparently normally but the process is not completed, so that at time points when unaffected littermates display abundant CNS myelin, quaking animals show large numbers of axons with only a few lamellae. This microanatomical impediment appears to be related to aberrant oligodendroglial cell activity since these cells fail to deposit myelin correctly and produce instead immature lamellar arrangements which frequently never become compacted. The fine structure of the myelination problem has been described by several workers (e.g., Berger, 1971; Wisniewski and Morell, 1971). Chapter 14 presents details of the observed compositional abnormalities and enzyme deficits.

4. *Murine Muscular Dystrophy*

Pathology and Etiology. Among the murine mutants with myelination defects, perhaps one of the most enigmatic occurs in the 129 Re *dy/dy* mouse, and the related mutant, *dy2J,* of the Bar Harbor strain. This model, utilized for some years as a major tool for research into muscular dystrophy, was recently reported to possess profound abnormalities in PNS myelination (Bradley and Jenkison, 1973). The PNS lesion was microscopic, was most marked in the proximal regions (spinal nerve roots), and consisted of a near total lack of Schwann cells and myelin from nerve fascicles. This amyelination resulted in adult *dy/dy* animals displaying areas of the PNS with organizations reminiscent of the fetal state. More recent studies have shown the affected PNS in these animals to contain oligodendroglia and CNS myelin, a feature known in no other neuropathological condition (Weinberg *et al.,* 1975). The myelin defect is genetically determined and affected animals are double recessives. Biochemical analyses of the PNS of these animals have not yet revealed significant data.

5. *Border Disease*

Pathology and Etiology. In border disease, a naturally occurring disease of sheep, the CNS is affected in a manner akin to that encountered in quaking mice. The condition was first recognized in the border counties between England and Wales, hence the name. The anomaly, known also as hypomyelinogenesis congenita, microscopically consists of a retardation of myelination evinced by lack of compaction of oligodendroglial cytoplasm around axons, thin myelin sheaths, and oligodendroglia containing lipid deposits. Large areas of spinal cord white matter can be affected in this way (Barlow and Dickinson, 1965). Genetic factors are implicated in the disease but recent data have also shown that the disease may have an infectious etiology since inoculation of pregnant ewes with brain suspensions from animals with Border disease transmits the disease to the offspring. The nature of this putative agent is as yet uncharacterized. See Chapter 14 for references to biochemical investigations.

VI. Class III: Acquired Toxic–Metabolic Disorders of Myelin

A. Human Examples

Diagnostic Criteria. The third group of primary disorders of myelin—the acquired toxic–metabolic disorders—is represented by a collection of

diseases all secondary to the action of exogenous myelinotoxic compounds. Most are exceedingly rare complications but are nevertheless important since they serve to demonstrate the exquisite sensitivity of myelin to certain foreign compounds. The few examples presented here are representative of a much larger collection. Two are chosen since they best illustrate the variation in the action of myelin toxins. These diseases exist both as naturally occurring human diseases and as experimental models. Lesions are noninflammatory, and in cases where myelin is broken down, phagocytosis is usually accomplished by cells of local origin.

1. Hexachlorophene Intoxication

Pathology and Etiology. Hexachlorophene is a compound widely used in hospitals, particularly in newborn nurseries, for the control of bacterial colonization of the skin. Over the past few years, neuropathological examination of the nervous system of premature infants has uncovered changes believed specifically related to hexachlorophene exposure. The central nervous tissue demonstrates extensive edema of white matter caused by intramyelinic splits and vacuoles (e.g., Powell *et al.,* 1973), akin to triethyl tin sulfate intoxication. This spongiform encephalopathy has appeared in a number of premature infants with a birth weight below 1400 g who were given topical application of pHisoHex. The number of dermal exposures to the compound is also significant (usually more than four), as is the presence of skin lesions. Dermal absorption has been documented, together with high levels of the drug in the blood. The manner in which hexachlorophene causes the CNS changes is not known. It has been speculated that it may be related to its ability to chelate copper, a mechanism believed to effect damage to the bacterial cell walls, or, based on laboratory tests, to its being a potent uncoupler of phosphorylation.

2. Hypoxic Encephalopathy—Anoxic Anoxia and Anemic Anoxia (Carbon Monoxide Poisoning)

The CNS complications of anoxic anoxia fall heavily upon neurons as well as upon myelin. The tissue destruction occurs when insufficient oxygen reaches the blood so that both the arterial oxygen content and tension are low. The selective neuronal loss following anoxic anoxia (e.g., Purkinje cells, hippocampal neurons, and cortical neurons) represents a common and classical finding in neuropathology. Less appreciated are the neuropathological findings which accompany the clinical syndrome of delayed postanoxic encephalopathy (Plum *et al.,* 1962). In these rare cases, there is relatively little neuronal damage. However, there is massive

destruction of myelin. This appears as a diffuse, severe, and bilateral myelin destruction in both cerebral hemispheres with sparing of the immediate subcortical nerve fibers and the brain stem.

In anemic anoxia, the amount of available hemoglobin is insufficient to transport enough oxygen to tissues. In carbon monoxide poisoning, a classic example of anemic anoxia, the hemoglobin is bound as carboxyhemoglobin and is not available for oxygenation of tissues. In addition to the well-known neuronal involvement associated with anoxic anoxia, carbon monoxide poisoning may produce selective necrosis of the globus pallidus. In rare cases, there may be a delayed, widespread, focally accentuated degeneration of the myelin of the cerebral hemispheres, with relative sparing of axis cylinders (Fig. 31).

The mechanisms of myelin destruction in hypoxic encephalopathy remain obscure.

B. Animal Examples

1. Diphtheritic Neuropathy

Experimental diphtheritic neuropathy is inducible in a number of species by injection of either crude toxoid or incompletely neutralized toxin from *Corynebacterium diphtheriae.* Although as a human condition, diphtheritic neuropathy is considered infectious because of its association with a bacterium, in the laboratory this disease is classed as a toxic disease since the toxin alone is sufficient to induce the lesions. About 1 week after injection, animals show limb weakness and usually die due to respiratory involvement. Peripheral nervous tissue shows marked demyelinative changes (Webster *et al.,* 1961; Weller, 1965). Myelin fragments and is apparently taken up by Schwann cells. The CNS is usually not involved, but demyelinating lesions can be induced in the CNS by local infusion (Wisniewski and Raine, 1971). It was also found that the PNS and CNS remyelination occurred in chronic lesions. The toxin is specific for myelin or other membrane systems (Webster *et al.,* 1961). Relevant metabolic studies have been carried out.

2. Hexachlorophene Intoxication

In experiments involving the incorporation of hexachlorophene into the diets of laboratory rats it was found that both an encephalopathy (e.g., Lampert *et al.,* 1973*a*) and a neuropathy (Pleasure *et al.,* 1974) could be induced. The morphological picture is one indistinguishable in many

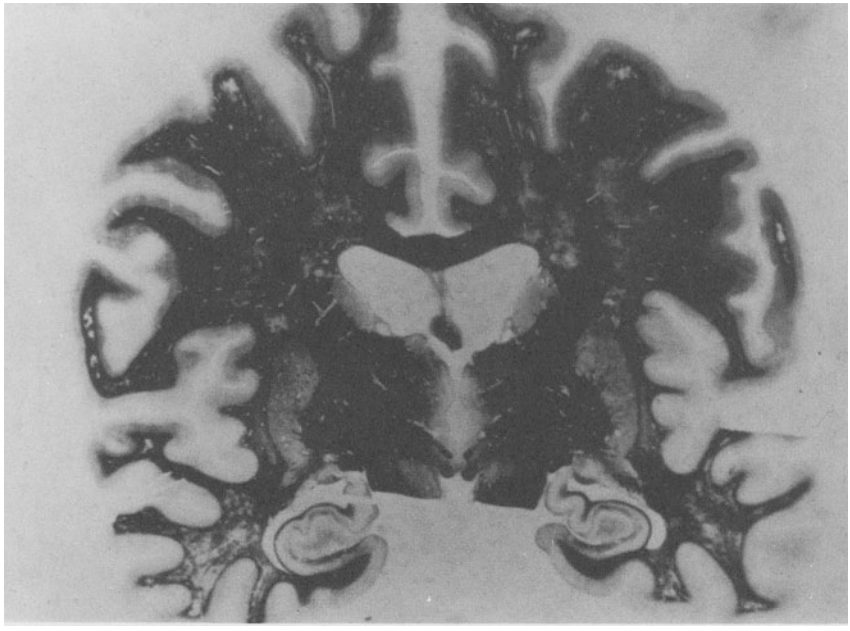

Fig. 31. Carbon monoxide intoxication—myelin stain, whole mount. Note the widely scattered small punctate areas of demyelination.

regards from that produced by triethyl tin sulfate. A white matter spongiform encephalopathy was typical, caused by the severe dilatation of myelin sheaths by splits occurring at the intraperiod line and the filling of the vacuoles with fluid. Biochemical assays have shown that hexachlorophene inhibits protein and lipid synthesis in nerves, and also that during incubation the nerve content of adenosine triphosphate decreased, causing a diminution in the rate of activation of 3′-phosphoadenosine 5′-phosphosulfate (Pleasure *et al.*, 1974).

The pattern of myelin breakdown in the above experiments also bears striking similarities to CNS changes seen after intoxication with other compounds, among them isonicotinic acid hydrazide (INH) (Lampert and Schochet, 1968) and cuprizone (bicyclohexanone oxalyldihydrazone) (Suzuki and Kikkawa, 1969) which, like hexachlorophene, are active chelaters of copper. Unlike hexachlorophene and INH, cuprizone has been demonstrated to cause extensive loss of myelin in some areas (Blakemore, 1973) which remyelinate when animals are allowed to recover. Several biochemical investigations have been conducted (Chapter 13).

3. Triethyl Tin (TET) Intoxication

Pathology and Etiology. TET intoxication as a human condition is now virtually unknown. Today it exists as an experimentally induced spongy condition of white matter and has been studied in detail at the level of the light and electron microscope by Aleu *et al.* (1963). There is a selective edema of CNS white matter related to the dilatation of myelin sheaths. After intraperitoneal injections of this compound, animals develop generalized muscle weakness, become immobilized within a day of the onset of signs, and frequently die. The edematous change in CNS white matter involves separation of lamellae along intraperiod lines and the formation of large, fluid-filled intramyelinic splits. Other elements appear unaffected. The lesion is specific for CNS myelin although some workers have demonstrated minor, later changes in PNS myelin. The myelin vacuolation is reversible in animals which recover from the initial intoxication. There is a dramatic increase in water content in TET animals (91% over controls) (Katzman *et al.,* 1963). Using ^{35}S as a marker, it was found that there was no significant increase in extracellular space, thus correlating with the EM evidence that the edema is intramyelinic. Biochemical analysis and relevant metabolic studies are detailed in Chapter 13.

Fig. 32. Vitamin B_{12} deficiency—thoracic spinal cord, myelin stain. In this combined system disease, note the large-scale involvement of several myelinated tracts.

4. *AY 9944 Intoxication*

Pathology and Etiology. AY 9944 is a hypocholesterolemic drug with a known affinity to retard both CNS and PNS myelination in developing animals and to cause selective damage to myelinating cells (Rawlins and Uzman, 1970*a,b;* Suzuki and Zagoren, 1974). The retardation of myelination is manifested morphologically by the formation of thinner-than-normal or uncompacted sheaths. Myelinating cells accumulate abnormal lipid inclusions, describe bizarre configurations around axons, and occasionally undergo frank degeneration. This experimentally induced hypomyelination may be relevant to the study of Pelizaeus–Merzbacher disease and some animal mutants, e.g., quaking mouse. Biochemical studies on animals treated with AY 9944 during the period of rapid myelination have shown that myelin cholesterol is largely replaced by its precursors, and as a consequence, the yield of myelin is reduced (see discussion and references in Section IIIB of Chapter 7).

VII. Class IV: Nutritional Diseases of Myelin

A. Human Examples

1. *Vitamin B_{12} Deficiency*

Patients lacking intrinsic factor necessary for the passage of vitamin B_{12} across the gastric mucosa frequently develop central and peripheral nervous system complications. In the CNS, the degeneration is first and largely manifested in the myelin sheath. The major involvement in the spinal cord occurs in the large fiber tracts, i.e., corticospinal pathways and dorsal columns, although in severe cases all tracts are affected (Pant *et al.,* 1968) (Fig. 32). The thoracic spinal cord appears particularly vulnerable. Rarely, multiple punctate areas of myelin loss are found in the centrum semiovale. A peripheral neuropathy is commonly observed and optic nerve degeneration has been occasionally reported.

2. *Central Pontine Myelinolysis*

Central pontine myelinolysis, first described by Adams (1959) in alcoholics and undernourished individuals, has now been reported in association with a number of other conditions, often with hepatic and other organ disease. Traditionally, most investigators have attributed the condition to nutritional deprivation, although the precise etiology remains

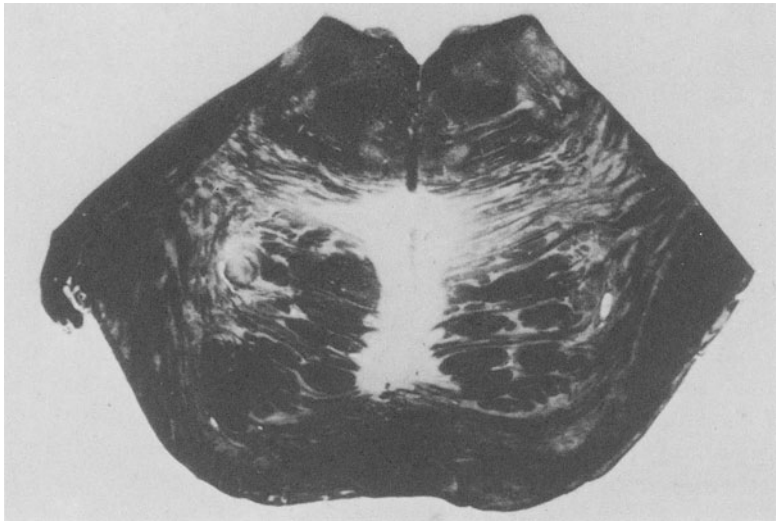

Fig. 33. Central pontine myelinolysis—myelin stain. A large zone of myelin loss is seen in the center of the pons.

obscure. Morphologically, there is a single, symmetrical focus of demyelination in the center of the basis pontis (Fig. 33). Histologically, there is a dissolution of myelin with relative sparing of axons occurring in the absence of inflammation.

3. Marchiafava–Bignami Disease

Marchiafava–Bignami disease, an extremely rare complication of alcoholism, is usually found in Italian males who drink red wine to excess (Merritt and Weisman, 1945). The striking feature of this condition is the symmetrical degeneration of myelin often restricted to the corpus callosum and the anterior commissure (Figs. 34 and 35). Axons are also involved, but to a lesser degree. There is scant evidence of inflammation and only moderate capillary endothelial proliferation. Lesions have also been found in the long association bundles and the cerebellar peduncles.

B. Animal Models

1. Malnutrition

There exist no animal models which precisely mimic the above conditions. Studies on undernourished animals, carried out mainly from

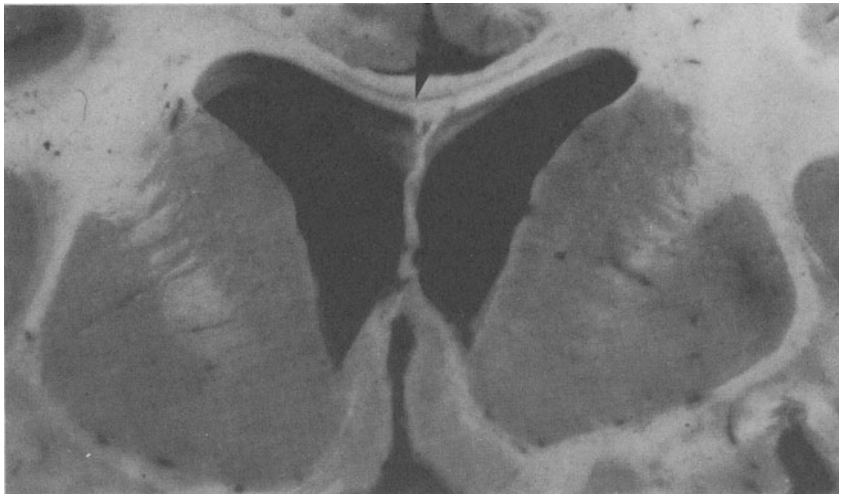

Fig. 34. Marchiafava–Bignami disease—gross specimen. Note the narrow zone of demyelination in the corpus callosum (arrow).

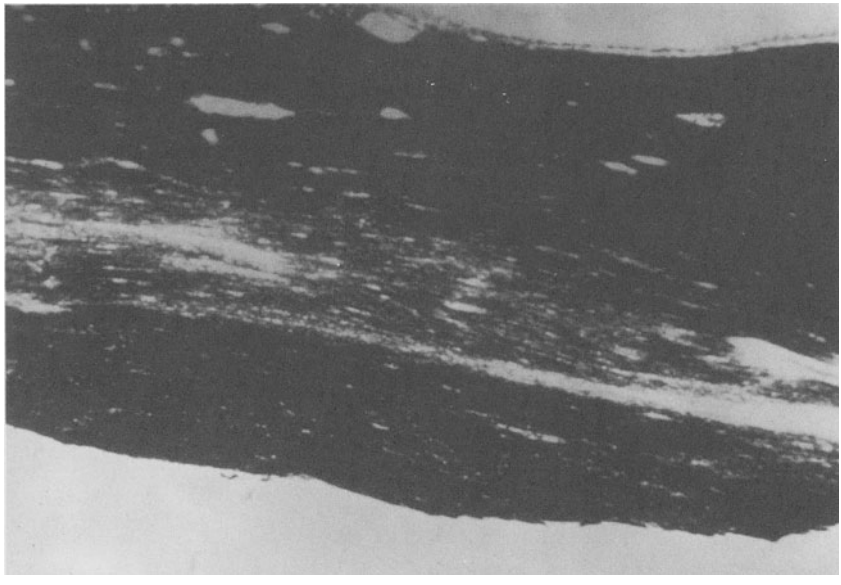

Fig. 35. Marchiafava–Bignami disease—myelin stain from Fig. 34. The corpus callosum shows a discrete area of myelin loss. ×30.

the biochemical standpoint, have demonstrated that if rats are deprived of food during certain vulnerable periods of CNS development they show a preferential reduction in the amount of myelin synthesized (Dobbing, 1968). Nutritional deprivation can have a permanent effect if the most proliferative period of myelination is included in the period of starvation, thus suggesting that once myelinating glial cells have passed the time of active division they are incapable of later extensive proliferation. There is also some evidence indicating that the developmental program for myelinogenesis may be retarded by starvation. Discussion and references to correlated ultrastructural and biochemical studies are given in Chapter 13.

VIII. Class V: Traumatic Diseases of Myelin

A. Human and Animal Examples

1. Edema

It is well known that edema secondary to tumors, trauma, etc., can cause myelin sheaths to be diffusely affected. The underlying reasons for this degeneration are multiple and usually involve a local disturbance of electrolytes and nonspecific degeneration of the myelinating cells. The pattern of demyelination has received little scrutiny.

2. Compression

If mechanical pressure is applied for prolonged periods to a myelinated peripheral nerve or area of central white matter, a common sequela is the loss of myelin from the affected areas. The myelin becomes fragmented and is taken up by local macrophages. Following the loss of myelin, the surviving axons frequently remyelinate. Examples in man include white matter adjacent to tumors and nerves compressed by tourniquets or in the carpal tunnel syndrome. Extensive experimental work on pressure effects upon myelinated fibers has been done utilizing tourniquet lesions (Ochoa *et al.*, 1972).

3. Barbotage

As a very rare complication of repeated removal and exchange of cerebrospinal fluid, an extensive rim of subpial demyelination may

develop which completely encircles the spinal cord. An identical situation can be produced in the spinal cord of animals (e.g., cats) by repeated exchange of CSF. Myelin is rapidly lost and local macrophages have been shown to participate in myelin removal, (Bunge *et al.,* 1960). In animals which survive, remyelination ensues within a month.

4. Pressure Release

It has been known for many years that a local interruption of the perineurium can lead to a herniation of the contents of a nerve, therefore suggesting that nerve fibers exist in an environment which is under a positive pressure. By creating a window in the perineurium of the peroneal nerves of rats, Spencer *et al.* (1975) have reported the occurrence of exquisitely focal demyelination and remyelination of those segments of nerve fibers extruded into the herniated bleb. This implies that the integrity of myelin–axon relationships is in part dependent on the maintenance of a constant endoneurial pressure.

IX. Conclusions

The preceding paragraphs have described in detail the varied neuropathology of those conditions in which the myelin sheath is apparently the primary target. A number of types of myelin diseases have been highlighted: *viz.,* myelin degeneration precipitated by a viral infection; an immune response; a genetic defect manifesting itself after or prior to the formation of myelin; a lytic effect of a toxic factor; or a metabolic or mechanical insult to the myelinating cell. That axons frequently degenerate in the examples cited should not detract from the specificity of the disease process, since in most if not all cases the *primary* lesion is to the myelin sheath. In some cases, biochemical data have permitted precise categorization of diseases. However, in those cases where *widespread* myelin degeneration occurs, it has been found that degraded myelin is biochemically similar to conditions showing secondary involvement of myelin, e.g., during Wallerian degeneration.

The schema presented above is fairly complete. Further clarification is needed in those acquired inflammatory demyelinating diseases where the etiology is unknown and in the genetic and metabolic disorders where a biochemical defect has not been recognized. It is suspected that ultimately the unifying character of the acquired inflammatory group is going to be a viral etiology. That such a putative infection is also governed

by immunogenetic factors appears highly likely, although this alone will not explain the geographic distribution of diseases like multiple sclerosis. Another enigmatic issue is the florid inflammatory component in adrenoleukodystrophy, a disease belonging to a group in which immunological events have not been implicated. Retrospectively, it is now easy to understand how adrenoleukodystrophy, previously called Schilder's disease, was for many years considered to belong to the multiple sclerosis group.

Several positive contributions to the neuropathology of the human disorders of myelin have emanated from the field of experimental neuropathology by the development and exploitation of appropriate animal models. It is possible that no human disease of myelin lacks a valid animal analogue. The major problem in some of the human diseases has rested in their chronicity and fluctuating picture, and in the animal diseases in the relatively short life span of the laboratory animals used. Nevertheless, recent experimentation with different species and strains (e.g., in the case of EAE) has uncovered some animal models with disease patterns more akin to the human conditions.

Finally, neuropathology is no longer dependent solely on the pathologist but also depends very heavily on a multidisciplinary approach encompassing clinicians, neuroscientists, virologists, immunologists, geneticists, and biochemists. It is as a direct result of the close collaboration of these diverse disciplines that the present comprehensive classification of the myelin diseases has been made.

Acknowledgments

The authors thank Drs. Robert D. Terry, John W. Prineas, and Kinuko Suzuki for their valued discussion. The excellent technical assistance of Everett Swanson, Miriam Pakingan, and Howard Finch is gratefully acknowledged. We thank Mrs. Mary Palumbo and Mrs. Violet Hantz for secretarial assistance.

This work was supported by USPHS Grants No. NS 08952 and NS 03356, a grant from the Alfred P. Sloan Foundation, and Grant No. 1001-A-1 from the National Multiple Sclerosis Society. Dr. Raine is the recipient of a Research Career Development Award from the USPHS—Grant No. NS 70265.

We thank Dr. Kinuko Suzuki for Figs. 18 and 27–30, Dr. Robert D. Terry for Fig. 15, and Dr. E. P. Richardson for many of the gross illustrations.

X. *References*

Adams, J. M., Baird, C., and Filloy, L., 1966, Inclusion bodies in measles encephalitis, *J. M. A. Med. Assoc.* **195**:290.

Adams, R. D., 1959, Nutritional diseases of the nervous system in the alcoholic patient, *Trans. Am. Clin. Climatol. Assoc.* **71**:59.

Adams, R. D., and Kubik, C. S., 1952, The morbid anatomy of the demyelinative diseases, *Am. J. Med.* **12**:510.

Adams, R. D., and Sidman, R. L., 1968, *Introduction to Neuropathology,* McGraw-Hill, New York.

Aleu, F. P., Katzman, R., and Terry, R. D., 1963, Fine structure and electrolyte analyses of cerebral edema induced by alkyl tin intoxication, *J. Neuropathol. Exp. Neurol.* **22**:403.

Appel, M. J. G., and Gillespie, J. H., 1972, Canine distemper virus, *Virol. Mongr.* **10**:1.

Asbury, A. K., Arnason, B. W. G., and Adams, R. D., 1969, The inflammatory lesion in idiopathic polyneuritis, *Medicine* **48**:173.

Austin, J. H., and Lehfeldt, D., 1965, Studies in globoid (Krabbe) leucodystrophy. III. Significance of experimentally produced globoid-like elements in rat white matter and spleen, *J. Neuropathol. Exp. Neurol.* **24**:265.

Baker, A. B., and Baker, L. H., 1974, *Clinical Neurology,* Harper and Row, New York.

Barlow, R. M., and Dickinson, A. G., 1965, On the pathology and histochemistry of the central nervous system in Border disease of sheep, *Res. Vet. Sci.* **6**:230.

Behan, P. O., Geschwind, N., Lamarche, J. B., Lisak, R. P., and Kies, M. W., 1968, Delayed hypersensitivity to encephalitogenic protein in disseminated encephalomyelitis, *Lancet* **II**:1009.

Berger, B., 1971, Quelques aspects ultrastructuraux de la substance blanche chez la souris quaking, *Brain Res.* **25**:35.

Blackwood, W., McMenemey, W. H., Meyer, A., Norman, R. M., and Russel, D. S., 1971, *Greenfield's Neuropathology,* E. Arnold, London.

Blakemore, W. F., 1973, Demyelination of the superior cerebellar peduncle in the mouse induced by cuprizone, *J. Neurol. Sci.* **20**:63.

Blaw, M. E., 1970, Melanodermic type leukodystrophy (adreno-leukodystrophy), in: *Handbook of Clinical Neurology,* Vol. 10 (P. J. Vinken and G. W. Bruyn, eds.), pp. 128–133, North-Holland, Amsterdam.

Bradley, W. G., and Jenkison, M., 1973, Abnormalities of peripheral nerves in murine muscular dystrophy, *J. Neurol. Sci.* **18**:227.

Brain, L., and Walton, J. N., 1969, in: *Brain's Disease of the Nervous System,* Oxford University Press, London.

Brun, A., Nordenfeldt, E., and Kjellen, L., 1973, Aspects on the variability of progressive multifocal leukoencephalopathy, *Acta Neuropathol.* **24**:232.

Bunge, R. P., Bunge, M. B., and Ris, H., 1960, Electron microscopic study of demyelination in an experimentally induced lesion in adult cat spinal cord, *J. Biophys. Biochem. Cytol.* **7**:685.

Cheever, F. S., Daniels, J. B., Pappenheimer, A. M., and Bailey, O. T., 1949, A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. I. Isolation and biological properties of the virus, *J. Exp. Med.* **90**:181.

Crome, L., Tymms, V., and Woolf, L. I., 1962, A chemical investigation of myelination in phenylketonuria, *J. Neurol. Neurosurg. Psychiat.* **25**:143.

Cummings, J. F., and Haas, D. C., 1967, Coonhound paralysis: An acute idiopathic polyradiculoneuritis in dogs resembling the Landry-Guillain-Barré syndrome, *J. Neurol. Sci.* **4**:51.

Dawson, J. W., 1916, The Histology of Disseminated Sclerosis, Vol. 50, *Trans. R. Soc. Edinb.*

Dobbing, J., 1968, Vulnerable periods of developing brain, in: *Applied Neurochemistry* (A. N. Davison and J. Dobbing, eds.), pp. 287–316, F. A. Davis, Philadelphia.

Fardeau, M., and Engel, W. K., 1969, Ultrastructural study of a peripheral nerve biopsy in Refsum's disease, *J. Neuropathol. Exp. Neurol.* **28**:278.

Field, E. J., Miller, H., and Russell, D. S., 1962, Observations on glial inclusion bodies in a case of acute disseminated sclerosis, *J. Clin. Pathol.* **15**:278.

Gonatas, N. K., and Howard, J. C., 1974, Inhibition of experimental allergic encephalomyelitis in rats severely depleted of T cells, *Science* **186**:839.

Herndon, R. M., Rubinstein, L. J., Freedman, J. M., and Methieson, G., 1970, Light and electron microscopic observations on Rosenthal fibers in Alexander's disease and in multiple sclerosis, *J. Neuropathol. Exp. Neurol.* **29**:524.

Hirano, A., Sax, D. S., and Zimmerman, H. M., 1969, The fine structure of the cerebella of jimpy mice and their "normal" litter mates, *J. Neuropathol. Exp. Neurol.* **28**:388.

Igarashi, M., Schaumburg, H. H., Powers, J. M., Kishimoto, Y., Kolodny, E. H., and Suzuki, K., 1976, Unusual fatty-acid abnormality in adrenoleukodystrophy, *J. Neurochem.* **26**:851–860.

Jervis, G. A., 1963, Pathology of phenylketonuria, in: *Phenylketonuria* (F. Lyman, ed.), pp. 96–100, Charles C Thomas, Springfield, Ill.

Johnson, R. T., and Weiner, L. P., 1972, The role of viral infections in demyelinating diseases, in: *Multiple Sclerosis: Immunology, Virology and Ultrastructure* (F. Wolfgram, G. W. Ellison, J. G. Stevens, and J. M. Andrews, eds.), pp. 245–274, Academic Press, New York.

Katzman, R., Aleu, F., and Wilson, C., 1963, Further observations on triethyl tin edema, *Arch. Neurol.* **9**:178.

Knox, W. E., 1972, Phenylketonuria, in: *The Metabolic Basis of Inherited Disease* (J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, eds.), McGraw Hill, New York.

Lampert, P. W., 1967, Electron microscopic studies on ordinary and hyperacute experimental allergic encephalomyelitis, *Acta Neuropathol.* **9**:99.

Lampert, P. W., 1969, Mechanism of demyelination in experimental allergic neuritis, *Lab. Invest.* **20**:127.

Lampert, P. W., and Schochet, S., 1968, Electron microscopic observations on experimental spongy degeneration of the cerebellar white matter, *J. Neuropathol. Exp. Neurol.* **27**:210.

Lampert, P., O'Brien, J., and Garrett, R., 1973*a*, Hexachlorophene encephalopathy, *Acta Neuropathol.* **23**:326.

Lampert, P. W., Sims, J. K., and Kniazeff, A. J., 1973*b*, Mechanism of demyelination in JHM virus encephalomyelitis, *Acta Neuropathol.* **24**:76.

Lehrich, J. L., Arnason, G. G. W., Fuller, T. C., and Wray, S. H., 1974, Parainfluenza, histocompatibility and multiple sclerosis, *A.M.A. Arch. Neurol.* **30**:327.

Malamud, N., 1966, Neuropathology of phenylketonuria, *J. Neuropathol. Exp. Neurol.* **25**:254.

Meier, C., and Bischoff, A., 1974, Dysmyelination in "jimpy" mouse: Electron microscopic study, *J. Neuropathol. Exp. Neurol.* **33**:343.

Meier, C., Herschkowitz, N., and Bischoff, A., 1974, Morphological and biochemical observations in the jimpy spinal cord, *Acta Neuropathol.* **27**:349.

Merritt, H. H., and Weisman, A. D., 1945, Primary degeneration of the corpus callosum (Marchiafava–Bignami's disease), *J. Neuropathol. Exp. Neurol.* **4**:155.

Morell, P., Bornstein, M. B., and Raine, C. S., 1976, Diseases involving myelin, in: *Basic Neurochemistry,* 2nd ed. (R. W. Albers, G. J. Siegel, R. Katzman, and B. W. Agranoff, eds.), pp. 581–604, Little, Brown, Boston.

Moser, H. W., 1970, Sulfatide lipidosis: Metachromatic leukodystrophy, in: *The Metabolic Basis*

of Inherited Disease (J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, eds.), pp. 688–729, McGraw-Hill, New York.

Norrby, E., Link, H., and Olsson, J. E., 1974, Measles virus antibodies in multiple sclerosis, *Arch. Neurol.* **30**:285.

Ochoa, J., Fowler, T. J., and Gilliatt, R. W., 1972, Anatomical changes in peripheral nerves compressed by a pneumatic tourniquet, *J. Anat.* **113**:433.

Padgett, B. L., Walker, D. L., ZuRhein, G. M., Eckroade, R. J., and Dessel, B. H., 1971, Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy, *Lancet* **1**:1257.

Pant, S. S., Asbury, K., and Richardson, E. P., 1968, The myelopathy of pernicious anemia, *Acta Neurol. Scand.* **44**: Suppl. 35.

Paterson, P. Y., 1973, Multiple sclerosis: An immunologic reassessment, *J. Chronic Dis.* **26**:119.

Peiffer, J., 1970, Metachromatic leucodystrophy, in: *Handbook of Clinical Neurology,* Vol. 10 (P. J. Vinken and G. W. Bruyn, eds.), pp. 43–66, North-Holland, Amsterdam.

Périer, O., and Grégoire, A., 1965, Electron microscopic features of multiple sclerosis lesions, *Brain* **88**:937.

Pleasure, D., Towfighi, J., Silberberg, D., and Parris, J., 1974, The pathogenesis of hexachlorophene neuropathy: *In vivo* and *in vitro* studies, *Neurology* **24**:1068.

Plum, F., Posner, J. B., and Hain, R. F., 1962, Delayed neurological deterioration after anoxia, *Arch. Intern. Med.* **110**:18.

Powell, H., Swarner, O., Gluck, L., and Lampert, P., 1973, Hexachlorophene myelinopathy in premature infants, *J. Pediatr.* **82**:976.

Prineas, J. W., 1971, Acute idiopathic polyneuritis: An electron microscope study, *Lab. Invest.* **26**:34.

Prineas, J. W., 1972, Paramyxovirus-like particles associated with acute demyelination in chronic relapsing multiple sclerosis, *Science* **178**:760.

Prineas, J. W., 1975, Pathology of the early lesions in multiple sclerosis, *Hum. Pathol.* **6**:531.

Prineas, J. W., and Wright, R. T., 1972, The fine structure of peripheral nerve lesions in a virus-induced demyelinating disease in fowl (Marek's disease), *Lab. Invest.* **26**:548.

Raine, C. S., 1972, Viral infections of nervous tissue and their relevance to multiple sclerosis, in: *Multiple Sclerosis: Immunology, Virology and Ultrastructure* (F. Wolfgram, G. W. Ellison, J. G. Stevens, and J. M. Andrews, eds.), pp. 91–118, Academic Press, New York.

Raine, C. S., 1976, Experimental allergic encephalomyelitis and related conditions, in: *Progress in Neuropathology,* Vol. 3 (H. Zimmerman, ed.), Grune and Stratton, New York.

Raine, C. S., Snyder, D. H., Valsamis, M. P., and Stone, S. H., 1974, Chronic experimental allergic encephalomyelitis in inbred guinea pigs—An ultrastructural study, *Lab. Invest.* **31**:369.

Raine, C. S., Schaumburg, H. H., Snyder, D. H., and Suzuki, K., 1975, Intranuclear "paramyxovirus-like" material in multiple sclerosis, adrenoleukodystrophy and Kuf's disease, *J. Neurol. Sci.* **25**:29.

Rawlins, F. A., and Uzman, B. G., 1970*a,* Retardation of peripheral nerve myelination in mice treated with inhibitors of cholesterol biosynthesis: A quantitative electron microscopic study, *J. Cell Biol.* **46**:505.

Rawlins, F. A., and Uzman, B. G., 1970*b,* Effect of AY-9944, a cholesterol biosynthesis inhibitor, on peripheral nerve myelination, *Lab Invest.* **23**:184.

Schaumburg, H. H., Richardson, E. P., Johnson, P. C., Cohen, R. B., Powers, J. M., and Raine, C. S., 1972, Schilder's disease: Sex-linked recessive transmission with specific adrenal changes, *Arch. Neurol.* **27**:458.

Schaumburg, H. H., Powers, J. M., Raine, C. S., Suzuki, K., and Richardson, E. P. Jr., 1975, Adreno-leukodystrophy, a clinical and pathological study of seventeen cases, *Arch. Neurol.* **33**:577–591.

Seitelberger, F., 1970, Pelizaeus–Merzbacher disease, in: *Handbook of Clinical Neurology,* Vol. 10 (P. J. Vinken and G. W. Bruyn, eds.) pp. 128–133, North-Holland, Amsterdam.

Sidman, R. L., and Hayes, R., 1965, Jimpy: A mouse with inherited sudanophilic leukodystrophy, *J. Neuropathol. Exp. Neurol.* **24**:172.

Sidman, R. L., Dickie, M. M., and Appel, S. H., 1964, Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system, *Science* **144**:309.

Solcher, H., 1973, Ueber Hirnveranderungen bei Heredopathia atactica polyneuritiformis (Refsum), *Acta Neuropathol.* **24**:92.

Spencer, P. S., Weinberg, H. J., Raine, C. S., and Prineas, J. W., 1975, The perineurial window—A new model to study demyelination and remyelination, *Brain Res.* **96(2)**:323.

Steinberg, D., 1972, Phytanic acid storage disease: Refsum's syndrome, in: *The Metabolic Basis of Inherited Disease* (J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, eds.), pp. 833–853, McGraw-Hill, New York.

Suzuki, K., 1970, Ultrastructural study of experimental globoid cells, *Lab. Invest.* **23**:612.

Suzuki, K., and Kikkawa, Y., 1969, Status spongiosus of CNS and hepatic changes induced by Cuprizone (biscyclohexanone oxalyldihydrazone), *Am. J. Pathol.* **54**:307.

Suzuki, K., and Zagoren, J. C., 1974, Degeneration of oligodendroglial cells in the central nervous system of rats treated with AY-9944 or triparanol, *Lab. Invest.* **31**:503.

Suzuki, K., Suzuki, Y., and Eto, Y., 1971, Deficiency of galactocerebroside β-galactosidase in Krabbe's globoid cell leucodystrophy, in: *Lipid Storage Diseases,* pp. 111–136, Academic Press, New York.

Suzuki, Y., and Suzuki, K., 1971, Krabbe's globoid cell leukodystrophy: Deficiency of galactocerebrosidase in serum, leukocytes and fibroblasts, *Science* **171**:73.

Suzuki, Y., Austin, J., Armstrong, D., Suzuki, K., Schlenker, J., and Fletcher, T., 1970, Studies in globoid leukodystrophy: Enzymatic and lipid findings in the canine form, *Exp. Neurol.* **29**:65.

ter Meulen, V., Koprowski, H., Iwasaki, H., Kackell, Y. M., and Muller, D., 1972*a,* Fusion of cultured multiple-sclerosis brain cells with indicator cells: Presence of nucleocapsids and virions and isolation of parainfluenza-type I virus, *Lancet* **II**:1.

ter Meulen, V., Muller, D., Kackel, Y., Katz, M., and Meyermann, R., 1972*b,* Isolation of infectious measles virus in measles encephalitis, *Lancet* **1**:1172.

Terry, R. D., 1970, Electron microscopy of selected neurolipidoses, in: *Handbook of Clinical Neurology,* Vol. 10 (P. J. Vinken and G. W. Bruyn, eds.), pp. 362–384, North-Holland, Amsterdam.

van Bogaert, L., 1970, Spongy degeneration of the brain, in: *Handbook of Clinical Neurology,* Vol. 10 (P. J. Vinken and G. W. Bruyn, eds.), pp. 203–211, North-Holland, Amsterdam.

Volk, B. W., and Adachi, M., 1970, Diffuse cerebral sclerosis—Krabbe type, in: *Handbook of Clinical Neurology,* Vol. 10 (P. J. Vinken and G. W. Bruyn, eds.), pp. 67–93, North Holland, Amsterdam.

Waksman, B. H., and Adams, R. D., 1956, Comparative study of experimental allergic neuritis in the rabbit, guinea pig, and mouse, *J. Neuropathol. Exp. Neurol.* **15**:293.

Waksman, B. H., and Adams, R. D., 1962, Infectious leukoencephalitis: A critical comparison of certain experimental and naturally-occurring viral leukoencephalitides with experimental allergic encephalomyelitis, *J. Neuropathol. Exp. Neurol.* **21**:491.

Watanabe, I., McCaman, R., Dyken, P., and Zeman, W., 1969, Absence of cerebral myelin

sheaths in a case of presumed Pelizaeus–Merzbacher disease, *J. Neuropathol. Exp. Neurol.* **243**:256.

Watanabe, I., Patel, V., Goebel, H. H., Siakotos, A. N., Zeman, W., DeMyer, W., and Dyer, J. S., 1972, Early lesion of Pelizaeus–Merzbacher disease: Electron microscopic and biochemical study, *J. Neuropathol. Exp. Neurol.* **32**:313.

Webster, H. DeF., Spiro, D., Waksman, B., and Adams, R. D., 1961, Phase and electron microscope studies of experimental demyelination. 2. Schwann cell changes in guinea pig sciatic nerves during experimental diphtheritic neuritis, *J. Neuropathol. Exp. Neurol.* **20**:5.

Weinberg, H. J., Spencer, P. S., and Raine, C. S., 1975, Aberrant PNS development in dystrophic mice, *Brain Res.* **88**:532.

Weiner, L. P., Herndon, R. M., Narayan, O., Johnson, R. T., Shah, K., Rubinstein, L. J., Preziosi, T. J., and Conley, F. K., 1972, Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy, *N. Engl. J. Med.* **286**:385.

Weller, R. D., 1965, Diphtheritic neuropathy in the chicken: An E.M. study, *J. Pathol. Bacteriol.* **89**:591.

Wisniewski, H., and Morell, P., 1971, Quaking mouse: Ultrastructural evidence for arrest of myelinogenesis, *Brain Res.* **29**:63.

Wisniewski, H., and Raine, C. W., 1971, An ultrastructural study of experimental demyelination and remyelination. V. Central and peripheral nervous system lesions caused by diphtheria toxin, *Lab. Invest.* **25**:73.

Wisniewski, H., Raine, C. S., and Kay, W. J., 1972, Observations on viral demyelinating encephalomyelitis: Canine distemper, *Lab. Invest.* **26**:589.

Wolfe, H. J., and Pietra, G. G., 1964, The visceral lesions of metachromatic leukodystrophy, *Am. J. Pathol.* **44**:921.

Yunis, E. J., and Lee, R. E., 1969, The ultrastructure of globoid (Krabbe) leukodystrophy, *Lab. Invest.* **21**:415.

Zu Rhein, G. M., 1969, Association of papova-virions with a human demyelinating disease (progressive multifocal leukoencephalopathy), *Progr. Med. Virol.* **11**:185.

Zu Rhein, G. M., and Chou, S. M., 1965, Particles resembling papovaviruses in human cerebral demyelinating disease, *Science* **148**:1477.